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# A phylogenetic re-evaluation of *Dothideomycetes*

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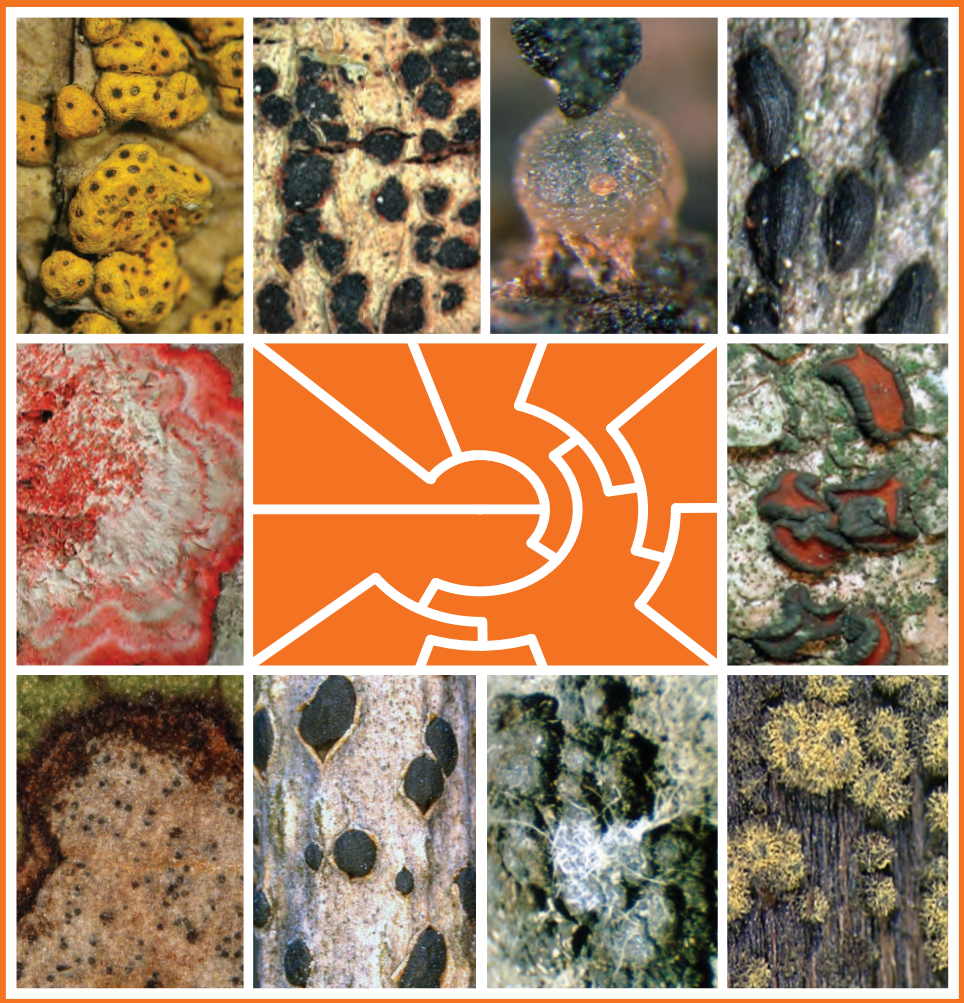

CBS-KNAW Fungal Biodiversity Centre,
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# *Studies in Mycology*

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Cover, centre: Simplified phylogeny of select orders of *Dothideomycetes*. Photos clockwise from top left (not scaled equivalently): *Trypetheliales* - *Trypethelium platystomum*, *Botryosphaeriales* - *Botryosphaeria dothidea*, *Jahnulales* - *Jahnula potamophila*, *Mytilinidiales* - *Mytilinidion thujarum*, *Hysteriales* - *Rhytidhysteron hysterinum*, *Pleosporales* - *Pseudotrichia mutabilis*, *Myriangiales* - *Myriangium* sp., *Dothideales* - *Dothidea sambuci*, *Capnodiales* - *Mycosphaerella marksii*, *Arthoniomycetes* (outgroup) - *Herpothallon rubrocinctum*.

# A phylogenetic re-evaluation of *Dothideomycetes*

Conrad L. Schoch
National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, U.S.A.

Joseph W. Spatafora
Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331 U.S.A.

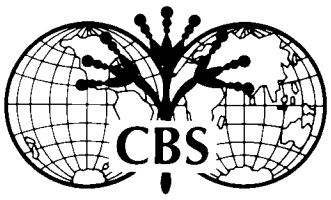
H. Thorsten Lumbsch
Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.

Sabine M. Huhndorf
Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.

Kevin D. Hyde
School 17 of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand
International Fungal Research Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan, P.R. China

Johannes Z. Groenewald
CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, Netherlands

Pedro W. Crous
CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, Netherlands

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## DEDICATION

This volume of *Studies in Mycology* is dedicated to the memory of Josef Adolf von Arx (1922–1988), Emil Müller (1920–2008) and Margaret Elizabeth Barr Bigelow (1923–2008), each of whom spent a significant part of their careers doing research on "pyrenomycetous" and "loculoascomycetous" fungi. Their numerous publications brought these fungi from long obscurity, and in the process they proposed taxonomic hypotheses that provide the basis for "modern" phylogenetic analyses. "*Die Gattungen der amerosporen Pyrenomyceten* (*Genera of the amerosporous Pyrenomycetes*, 1954)" followed by "*Die Gattungen der didymosporen Pyrenomyceten* (*Genera of didymosporous Pyrenomycetes*, 1962)," from Müller and von Arx are unparalleled in scope and completeness. Of special significance to the present volume of *Studies in Mycology* is their treatment of the bitunicate ascomycetes, "*A re-evaluation of the bitunicate ascomycetes with keys to families and genera*" (von Arx & Müller 1975). Margaret Barr did not always agree with her friends, "Ascus" and von Arx. But, like them her knowledge of ascomycetous fungi was vast and during the last quarter of her career she summarised some of that knowledge in two major and provocative publications, the self published "*Prodromus to class Loculoascomycetes*" (Barr 1987), followed by others on *Melanommatales*, *Pleosporales* and *Hymenoascomycetes* (Barr 1990a–c), to name but a few.

As a result of being born and raised in Switzerland, von Arx studied natural sciences at the Swiss Federal Institute of Technology (ETH), Zürich. After receiving his diploma as a biologist he started a PhD at the ETH on the ascomycete genus *Mycosphaerella* under the supervision of Prof. Ernst Gäumann and worked there as a research assistant. In the summer of 1948 Emil Müller started a PhD, also under prof. Gäumann's supervision at the ETH on *Leptosphaeria*. During part of that time he worked under the direct supervision by von Arx, who was already an advanced PhD student. Both students also spent some time with Dr F. Petrak (then editor of *Sydowia*), who further encouraged their interest in ascomycetous fungi (see Müller 1989, Petrini *et al*. 2009).

Josef Adolf von Arx (1922–1988). Photo from the CBS Archive.

Emil Müller (1920–2008). Photo by Orlando Petrini.

Von Arx and Müller became close friends, and remained so after Dr von Arx took up a position in 1949 at the Phytopathological Institute "Willie Commelin Scholten" in Baarn, under the directorship of Prof. Johanna Westerdijk. Prof. Westerdijk was also the director of the Centraalbureau voor Schimmelcultures (CBS), which was housed on the same premises at Baarn. Several years later, in 1963, Dr von Arx became director of CBS, which by then was an independent institute. In the meanwhile Dr Müller became curator of the herbaria at ETH, and dedicated himself fully to taxonomic research.

Margaret E. Barr Bigelow grew up in western Canada and spent her mycological career in the United States. She was a Ph.D. student of L.E. Wehmeyer at the University of Michigan, and oddly enough, like von Arx, also did her doctorate on the genus *Mycosphaerella*. She was appointed as instructor at the University of Massachusetts in 1957, as part of a "women's auxiliary" fund, which allowed her to teach and do research for many years for a modest compensation. Eventually she progressed to the Ray Ethan Torrey Professorship. Her numerous books and other publications on the loculoascomycetes and pyrenomycetes continue to be important references for others working on these groups of fungi as will be evident from papers in this volume. In addition to her research Barr volunteered time and money to mycology, serving as programme chairwoman for the MSA and AIBS meetings and establishing several endowments. The bulk of her extensive collections were transferred to the New York Botanical Garden and her unpublished notes are at the Field Museum in Chicago (see Blackwell *et al*. 2008). After her retirement she returned to live and work in her home on Vancouver Island, British Columbia, Canada.

Late in 1949, von Arx visited Müller in Switzerland to discuss future collaboration, which eventually led to the publication of the two major works on ascomycetous fungi (von Arx & Müller 1954, Müller & von Arx 1962). This collaboration, together with the works

Margaret Elizabeth Barr Bigelow (1923–2008). Photo by Meredith Blackwell.

published by Barr provided much of what we know to date about loculoascomycetous fungi. We have used their work as the crucial hypotheses to be tested with molecular phylogenetic data. We hope that our work, using the firm base provided by the three earlier mycologists we honour here, will extend our understanding of these fascinating fungi.

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C.L. Schoch[1*], P.W. Crous[2], J.Z. Groenewald[2], E.W.A. Boehm[3], T.I. Burgess[4], J. de Gruyter[2, 5], G.S. de Hoog[2], L.J. Dixon[6], M. Grube[7], C. Gueidan[2], Y. Harada[8], S. Hatakeyama[8], K. Hirayama[8], T. Hosoya[9], S.M. Huhndorf[10], K.D. Hyde[11, 33], E.B.G. Jones[12], J. Kohlmeyer[13], Å. Kruys[14], Y.M. Li[33], R. Lücking[10], H.T. Lumbsch[10], L. Marvanová[15], J.S. Mbatchou[10, 16], A.H. McVay[17], A.N. Miller[18], G.K. Mugambi[10, 19, 27], L. Muggia[7], M.P. Nelsen[10, 20], P. Nelson[21], C A. Owensby[17], A.J.L. Phillips[22], S. Phongpaichit[23], S.B. Pointing[24], V. Pujade-Renaud[25], H.A. Raja[26], E. Rivas Plata[10, 27], B. Robbertse[1], C. Ruibal[28], J. Sakayaroj[12], T. Sano[8], L. Selbmann[29], C.A. Shearer[26], T. Shirouzu[30], B. Slippers[31], S. Suetrong[12, 23], K. Tanaka[8], B. Volkmann-Kohlmeyer[13], M.J. Wingfield[31], A.R. Wood[32], J.H.C.Woudenberg[2], H. Yonezawa[8], Y. Zhang[24], J.W. Spatafora[17]

[1]*National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, U.S.A.;* [2]*CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, Netherlands;* [3]*Department of Biological Sciences, Kean University, 1000 Morris Ave., Union, New Jersey 07083, U.S.A.;* [4]*Biological Sciences, Murdoch University, Murdoch, 6150, Australia;* [5]*Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;* [6]*USDA-ARS Systematic Mycology and Microbiology Laboratory, Beltsville, MD 20705, U.S.A.;* [7]*Institute of Plant Sciences, Karl-Franzens-University of Graz, Austria;* [8]*Faculty of Agriculture and Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan;* [9]*National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan;* [10]*Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.;* [11]*School 17 of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand;* [12]*Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Paholyothin Road, Khlong 1, Khlong Luang, Pathum Thani, 12120, Thailand;* [13]*Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557, U.S.A.;* [14]*Department of Systematic Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-752 36 Uppsala, Sweden;* [15]*Czech Collection of Mircroorganisms, Institute of Experimental Biology, Faculty of Science, Masaryk University, Tvrdého 14, Brno CZ-602 00, Czech Republic;* [16]*College of Liberal Arts and Sciences, DePaul University, 1 E. Jackson Street, Chicago, Illinois 60604, U.S.A.;* [17]*Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331 U.S.A.;* [18]*Illinois Natural History Survey, University of Illinois, 1816 South Oak St., Champaign, IL, 61820, U.S.A.;* [19]*National Museums of Kenya, Botany Dept., P.O. Box 45166, 00100, Nairobi, Kenya;* [20]*Committee on Evolutionary Biology, University of Chicago, 1025 E. 57th Street, Chicago, Illinois 60637, U.S.A.;* [21]*University of Minnesota, Ecology, Evolution, and Behavior, 100 Ecology Building, St. Paul, MN 55108, U.S.A.;* [22]*Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal;* [23]*Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand;* [24]*Division of Microbiology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China;* [25]*CIRAD/PIAF, Université Blaise Pascal, Bâtiment Biologie Végétale Recherche, 24 avenue des Landais, BP 80026, 63177 Aubière, France;* [26]*Department of Plant Biology, University of Illinois, 505 S. Goodwin Ave., Urbana, IL 61801, U.S.A.;* [27]*Department of Biological Sciences, University of Illinois-Chicago, 845 West Taylor Street (MC 066), Chicago, Illinois 60607, U.S.A.;* [28]*Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (UPM), José Gutiérrez Abascal 2, 28006 Madrid, Spain;* [29]*DECOS, Università degli Studi della Tuscia, Largo dell'Università, Viterbo, Italy;* [30]*Fungus/Mushroom Resource and Research Center, Tottori University, Minami 4-101, Koyama, Tottori, Tottori 680-8553 Japan;* [31]*Forestry and Agricultural Biotechnology Institute (FABI), Centre of Excellence in Tree Health Biotechnology, Department of Genetics, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa;* [32]*ARC – Plant Protection Research Institute, P. Bag X5017, Stellenbosch, 7599, South Africa;* [33]*International Fungal Research Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan, P.R. China*

**Correspondence*: Conrad L. Schoch, schoch2@ncbi.nlm.nih.gov

**Abstract:** We present a comprehensive phylogeny derived from 5 genes, nucSSU, nucLSU rDNA, *TEF1*, *RPB1* and *RPB2*, for 356 isolates and 41 families (six newly described in this volume) in *Dothideomycetes*. All currently accepted orders in the class are represented for the first time in addition to numerous previously unplaced lineages. Subclass *Pleosporomycetidae* is expanded to include the aquatic order *Jahnulales.* An ancestral reconstruction of basic nutritional modes supports numerous transitions from saprobic life histories to plant associated and lichenised modes and a transition from terrestrial to aquatic habitats are confirmed. Finally, a genomic comparison of 6 dothideomycete genomes with other fungi finds a high level of unique protein associated with the class, supporting its delineation as a separate taxon.

**Key words:** *Ascomycota*, *Pezizomycotina*, Dothideomyceta, fungal evolution, lichens, multigene phylogeny, phylogenomics, plant pathogens, saprobes, Tree of Life.

## INTRODUCTION

Multi laboratory collaborative research in various biological disciplines is providing a high level of interaction amongst researchers with diverse interests and backgrounds. For the mycological community, the "Assembling the Fungal Tree of Life" project (AFTOL) provided the first DNA-based comprehensive multigene phylogenetic view of the fungal Kingdom (Lutzoni *et al.* 2004, James *et al.* 2006). This has also made it possible to revise the classification of the fungi above the ordinal level (Hibbett *et al.* 2007). Subsequent work is focused on elucidating poorly resolved nodes that were highlighted in the initial DNA-based phylogeny (McLaughlin *et al.* 2009).

At the other end of the scale from the tree of life projects, taxon sampling with relatively small numbers of sequence characters are also progressing in various barcoding projects (Seifert *et al.* 2007, Chase *et al.* 2009, Seifert 2009). It remains important to link these two ends of the spectrum by also sampling intensively at foci of interest between barcoding and the tree of life. With this in mind it is the aim of this paper and subsequent ones in this volume to provide a broadly sampled phylogeny at class level and below for *Dothideomycetes.* This result is combined efforts and data from a diverse group of researchers to focus on systematic sampling, therefore developing a more robust fungal class wide phylogeny of *Dothideomycetes*. This is especially important as a framework

for comprehending how fungi have evolved as they shift ecological habitats and adapt to new environments and nutritional modes.

It is apparent that the assemblage of fungi, now defined as *Dothideomycetes,* exemplifies a dynamic evolutionary history. This is by far the largest and arguably most phylogenetically diverse class within the largest fungal phylum, *Ascomycota* (Kirk *et al.* 2008). It contains a heterogeneous group of fungi that subsist in the majority of the niches where fungi can be found. The best-known members of the group are plant pathogens that cause serious crop losses. Species in the genera *Cochliobolus*, *Didymella, Phaeosphaeria, Pyrenophora, Venturia, Mycosphaerella* and *Leptosphaeria,* or their anamorphs, are major pathogens of corn, melons, wheat, barley, apples, bananas and brassicas respectively, in most areas of the world where they are cultivated. Other species are important pathogens in forestry *e.g.* species in the genera *Botryosphaeria* and *Mycosphaerella* and their anamorphs that attack economically important tree species.

Despite a large body of work containing taxonomic, phytopathological, genetic and genomic research, the majority of fungi hypothesised to be members of *Dothideomycetes* remain under-sampled within a systematic framework. Several studies performed during the course of the last four years have advanced our understanding of these fungi, but phylogenetic relationships of the saprobes, aquatic, asexual and lichenised species remain particularly poorly studied. Indeed, their conspicuous absence in phylogenetic analyses frustrates a broader understanding of dothideomycete evolution.

*Dothideomycetes* share a number of morphological characters with other fungal classes. It was recently formally described (Eriksson & Winka 1997) replacing in part the long-recognised loculoascomycetes (Luttrell 1955). This redefinition of the loculoascomycetes was mainly prompted by DNA sequencing comparisons of ribosomal RNA genes (Berbee & Taylor 1992, Spatafora *et al.* 1995) that was subsequently expanded and confirmed (Berbee 1996, Silva-Hanlin & Hanlin 1999, Lindemuth *et al.* 2001, Lumbsch & Lindemuth 2001). These early phylogenetic studies demonstrated that loculoascomycetes, as it was defined, is not monophyletic, although contrary views exist (Liu & Hall 2004). Nevertheless the majority of analyses have shown that some loculoascomycete taxa, such as the "black yeasts" in *Chaetothyriales* as well as the lichenised *Verrucariales*, reside within *Eurotiomycetes* as subclass *Chaetothyriomycetidae* (Spatafora *et al.* 1995, Winka *et al.* 1998, Geiser *et al.* 2006, Gueidan *et al.* 2008). The majority of the remaining loculoascomycete species are now placed in *Dothideomycetes*. Although finer morphological distinctions between the distantly related members of loculoascomycetes can be made, their synapomorphies remain elusive (Lumbsch & Huhndorf 2007). These findings all point to the fact that a number of loculoascomycete morphological characters are either retained ancestral traits or that they exhibit convergence due to similar selection pressures.

Traditionally the most important morphological characters used to define major groups in *Ascomycota* were the type of ascus, septation of ascospores, the morphology and development of the ascoma, as well as the structure and organisation of the centrum. *Dothideomycetes* (and previously, loculoascomycetes) have fissitunicate (or functionally bitunicate) asci, that emerge from ascolocular development in preformed locules within vegetative tissue, that represents the ascoma. The reproductive structures in ascolocular development are derived from cells before fusion of opposing mating types occurs and can contain one or several locules. This form of ascolocular development is in contrast to the ascohymenial development found in most other fungal classes. During ascohymenial development asci are generated in a hymenium and the reproductive structure is derived from cells after fusion of opposing mating types. The fissitunicate ascus has been described for more than a century, but the importance of ascolocular development was first emphasised in 1932 (Nannfeldt 1932). Importantly Nannfeldt's concepts were also the basis for the Santesson's integration of lichens into the fungal classification (Santesson 1952). In fissitunicate asci, generally, the ascospores are dispersed by the rupture of the thick outer layers (ectotunica) at its apex, allowing the thinner inner layer (endotunica) to elongate similar to a "jack in a box". The elongated endotunica ruptures apically and releases the ascospores forcefully through the ascoma opening. The spores are then released in the air, or in aquatic species, under water. Building on this work and that of others (Miller 1949), Luttrell proposed Loculoascomycetes, synonymous to Nannfeldt's "Ascoloculares" (Luttrell 1955). Importantly, he proposed a correlation between fissitunicate asci and ascolocular development, also emphasising the importance of ascus morphology and dehiscence as well as the development of surrounding elements within the ascoma.

Although the concept of a group of fungi (including the *Dothideomycetes*) with fissitunicate asci and ascolocular development has been accepted by several authors, much less agreement could be found on ordinal definitions in the era before molecular characters. This ranged from proposing a single order (von Arx & Müller 1975) to three (Müller & von Arx 1962), five (Luttrell 1951, 1955) six (Barr 1979), or seven (Barr 1987). Luttrell initially described a number of important development types centered on descriptions of all tissues inside the ascoma (the centrum concept) and combined this with ascoma structure to define his five orders (Luttrell 1951, 1955). Of Luttrell's initial centrum concepts three are applicable to the *Dothideomycetes* as they are presently defined. Thus, the *Pleospora* type, the *Dothidea* type and the *Elsinoë* type centra correspond to the dothideomycete orders *Pleosporales*, *Dothideales* and *Myriangiales*, respectively. An important refinement to Luttrell's ideas was introduced with the concept of the hamathecium by Eriksson (Eriksson 1981). This is defined as a neutral term for sterile hyphae or other tissues between the asci in the ascoma (Kirk *et al.* 2008). For example, hamathecial types can include the presence or absence of pseudoparaphyses, which are sterile cells that extend down from the upper portion of the ascomatal cavity. They become attached at both ends, although the upper part may become free at maturity. Other important concepts introduced by Müller and von Arx (Müller & von Arx 1962) focused on the morphology of the ascoma opening and ascus shape. The *Dothidea* type centrum in the type species of *Dothidea*, *D. sambuci* illustrates several typical dothideomycete morphologies (Fig. 1). These include the thick-walled fissitunicate asci produced within a multilocular stroma.

The most recent dothideomycete class-wide morphological assessments were carried out by Barr (Barr 1979, 1987). Her subclasses were determined based on characters in the centrum, including the absence, presence and types of hamathecial tissues. Consistent with several earlier authors, Barr's ordinal classifications were based on ascomatal shape (perithecioid or apothecioid) and manner in which nutrients are obtained by the fungus (Barr 1987). In addition to these characters she emphasised the importance of finer distinctions in the hamathecium such as the shape and structure of the pseudoparaphyses (Barr 1979, 1987).

The introduction of molecular phylogenies for *Dothideomycetes* (Berbee 1996) provided an opportunity to verify the significance

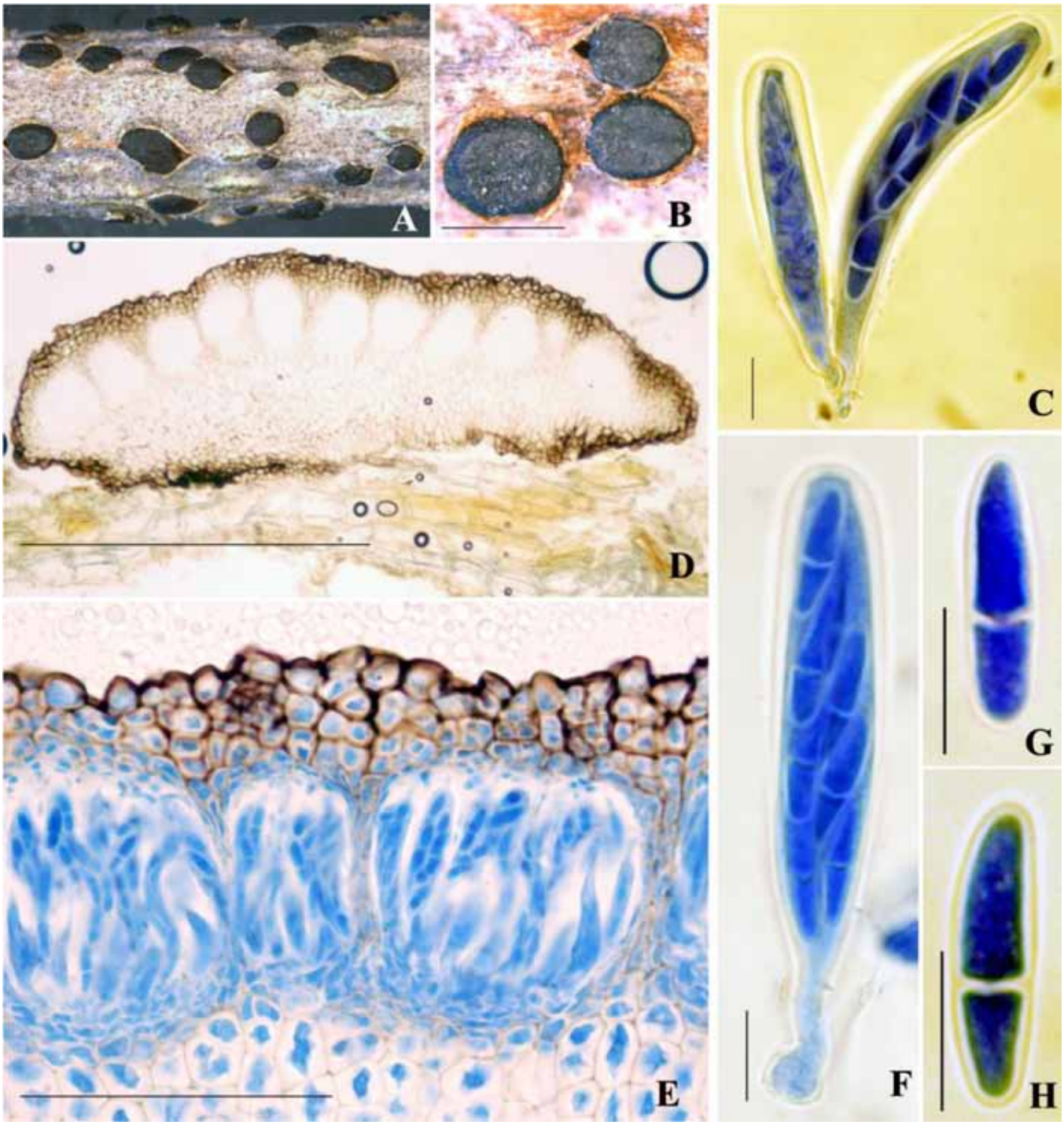


**Fig. 1.** *Dothidea sambuci.* A–B. Appearance of ascomata on the host surface. C, F. Asci in cotton blue reagent. D. Vertical section through ascomata illustrating the mutilocule at the upper layer. E. Vertical section through ascomata in cotton blue reagent illustrating the locule. G–H. Ascospores in cotton blue reagent. Scale bars: B = 1000 µm; C = 500 µm; E = 100 µm; F–H = 10 µm.

of various morphological characters used in the aforementioned classifications. The clearest correlation with a DNA sequence-based phylogeny was for the presence or absence of pseudoparaphyses, largely agreeing with the first orders proposed by Luttrell (Liew *et al.* 2000, Lumbsch & Lindemuth 2001). Barr's concept of applying the shape of the pseudoparaphyses to define orders was rejected by molecular phylogenies (Liew *et al.* 2000). This set the stage for more comprehensive analyses incorporating protein data, and resulted in the definition of two subclasses, *Pleosporomycetidae* (pseudoparaphyses present) and the *Dothideomycetidae* (pseudoparaphyses absent; Schoch *et al.* 2006). Numerous orders and other taxa remained unresolved outside of these two subclasses.

The most recent class level phylogenetic analyses combining sequences from protein coding genes with ribosomal RNA sequences fortified the view that *Dothideomycetes* is a monophyletic group (Schoch *et al.* 2009a, b). Furthermore, strong support was found for a sister relationship between *Dothideomycetes* and the lichenised class *Arthoniomycetes* (Lumbsch *et al.* 2005, Spatafora *et al.* 2006, Schoch *et al.* 2009a). This clade was recently defined as a rankless taxon "Dothideomyceta" (Schoch *et al.* 2009a, b). The *Arthoniomycetes* consists of a single order (*Arthoniales*) of lichens and lichenicolous fungi (Ertz *et al.* 2009) that produce bitunicate asci in ascohymenial apothecia and was proposed as an intermediate group or "Zwischengruppe" (Henssen & Thor 1994). This placement raises intriguing questions regarding the origins of

ascolocular development and further illustrates the importance of including lichen-forming fungi in dothideomycete phylogenies.

While considerable progress has been made in defining these fungi the placement of *Dothideomycetes* in relation to the majority of other *Ascomycota* classes remains unresolved. Here, greater clarity would likely require a huge increase of characters from genome projects. In this regard, the first phylogenomic studies have shown low resolution for this relationship (Fitzpatrick *et al.* 2006, Kuramae *et al.* 2006, Robbertse *et al.* 2006). This could indicate a rapid radiation event, but more likely suggests taxon sampling bias. This latter view is supported by the fact that none of these studies has included lichenised species that represent about 25 % of the number of species in *Ascomycota*.

The authors of this volume have focused on two primary goals. These are to considerably expand the taxon sampling of existing orders by including saprobes, asexual species and other poorly sampled groups. Secondly we aim to sample widely within specific environmental niches and present a multigene phylogeny that exposes the highly diverse nature of *Dothideomycetes*.

## MATERIAL AND METHODS

### DNA extraction, amplification and sequencing

The majority of fungal cultures were obtained from the CBS culture collection and additional sources mentioned in other papers of this volume. DNA was also provided by authors of several papers presented in this volume and the reader is referred to Boehm *et al*. (2009a), Crous *et al*. (2009a), Suetrong *et al*. (2009) and Zhang *et al*. (2009). For additional details see Table 1 - see online Supplementary Information. Fungal genomic DNA was obtained by scraping mycelium from PDA plates. Samples were subsequently pulverised and the DNA was extracted using the FastDNA® kit and the FastPrep® instrument from MPI Biochemicals (Irvine, CA, U.S.A.). DNA amplifications were completed using *Taq* polymerase (GenScript, Piscataway, NJ, U.S.A.), with FailSafe™ PCR 2× PreMix E (Epicentre, San Diego, CA, U.S.A.). Primers were used as noted in the Assembling the Fungal Tree of Life project (AFTOL; Schoch *et al.* 2009a). This resulted in DNA sequence data obtained from the small and large subunits of the nuclear ribosomal RNA genes (SSU, LSU) and three protein coding genes, namely the translation elongation factor-1 alpha (*TEF1*) and the largest and second largest subunits of RNA polymerase II (*RPB1, RPB2*). Primer sets used for these genes were as follows: SSU: NS1/NS4; LSU: LR0R/LR5; *TEF1* 983/2218R (initially obtained from S. Rehner: ocid.nacse.org/research/deephyphae/EF1primer.pdf); *RPB2*: fRPB2-SF/fRPB2-7cR; *RPB1*: RPB1-Ac/RPB1-Cr (obtained from V. Hofstetter). Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). PCRs for these genes were performed in various laboratories of the coauthors mentioned but the majority of reactions were run under conditions described previously (Lutzoni *et al.* 2004, Schoch *et al.* 2009a). Two duplicate sets of sequences were inadvertently included in the analysis (indicated in Table 1).

### Sequence alignment and phylogenetic analyses

Sequences were obtained from WASABI (Kauff *et al.* 2007) as well as from previous publications (*e.g.* Lutzoni *et al*. 2004, Schoch *et al*. 2009a). Introns were removed and an initial core set of 171 taxa were aligned by using default options for a simultaneous method of estimating alignments and tree phylogenies, SATé (Liu *et al.* 2009). In order to consider codons without the insertion of unwanted gaps, protein coding fragments were translated in BioEdit v. 7.0.1 (Hall 2004) and aligned within SATé as amino acids. These were then realigned with their respective DNA sequences using the RevTrans 1.4 Server (Wernersson & Pedersen 2003). After the removal of intron sequences the alignment was examined manually in BioEdit with a shade threshold of 40 % and regions with high amounts of gap characters were excluded. This resulted in a reduction of 99 columns in the LSU data set, 118 in *RPB1* and 162 in *RPB2*, for a total of 379. Nothing was removed for *TEF1*. In order to allow for the extension of our alignment as newly generated sequences became available from other studies in this volume, these were subsequently added to this core alignment with MAFFT v. 6.713 (Katoh *et al.* 2009). The E-INS-i setting, focused on high accuracy with a high percentage of unalignable regions such as introns, was applied and the SATé alignment was used as a seed. This resulted in a supermatrix of five genes (LSU, SSU *TEF1*, *RPB1*, *RPB2*) consisting of 52 % gaps and undetermined characters out of a total of 6 582 characters. GenBank accession numbers are shown in Table 1.

#### Conflict tests

Conflict tests on the initial core set of 204 taxa were conducted by selecting single gene data sets and doing comparisons on a gene by gene basis. This was done using the "bootstopping" criterion in RAxML v. 7.0.4 (Stamatakis *et al*. 2008) under the CIPRES v. 2.1 webportal to produce trees of comparative gene sets where all taxa have the gene present. Comparisons between all potential sets of gene trees with no missing taxa were done using a script (Kauff & Lutzoni 2002) obtained through the Lutzoni lab website and to detect present or absent taxa within clades with a cut-off bootstrap value of 70 %. This is described in more detail elsewhere (Miadlikowska *et al.* 2006, Schoch *et al.* 2009a).

#### Phylogeny

A phylogenetic analysis was performed using RAxML v. 7.0.4 (Stamatakis 2006) applying unique model parameters for each gene and codon. The dataset was divided in 11 partitions as previously described in Schoch *et al.* (2009a). A general time reversible model (GTR) was applied with a discrete gamma distribution and four rate classes following procedures laid out in Schoch *et al*. (2009). Ten thorough maximum likelihood (ML) tree searches were done in RAxML v. 7.0.4 under the same model, each one starting from a randomised tree. Bootstrap pseudo replicates were performed 2000 times using the fast bootstrapping option and the best scoring tree form 10 separate runs were selected. The resulting trees were printed with TreeDyn v. 198.3 (Chevenet *et al.* 2006). All alignments are deposited in TreeBASE. Additionally, the data sets were analyzed in GARLI v. 0.96 (Zwickl 2006) using the GTR-gamma-invariant model. In this case 200 bootstraps were run under default conditions.

**Fig. 2A–C**. (Page 5–7). Best scoring ML tree with RAxML and GARLI bootstrap values respectively above (green) and below (red) the nodes. Values below 50 % were removed and branches with more than 90 % bootstrap for both methods are thickened without values. Environmental sources relevant to the papers in this volume are indicated in the key (R-Rock; M-Marine; F-Freshwater; D-Dung; B-Bamboo). Nutritional characters are indicated by colour as per the key.

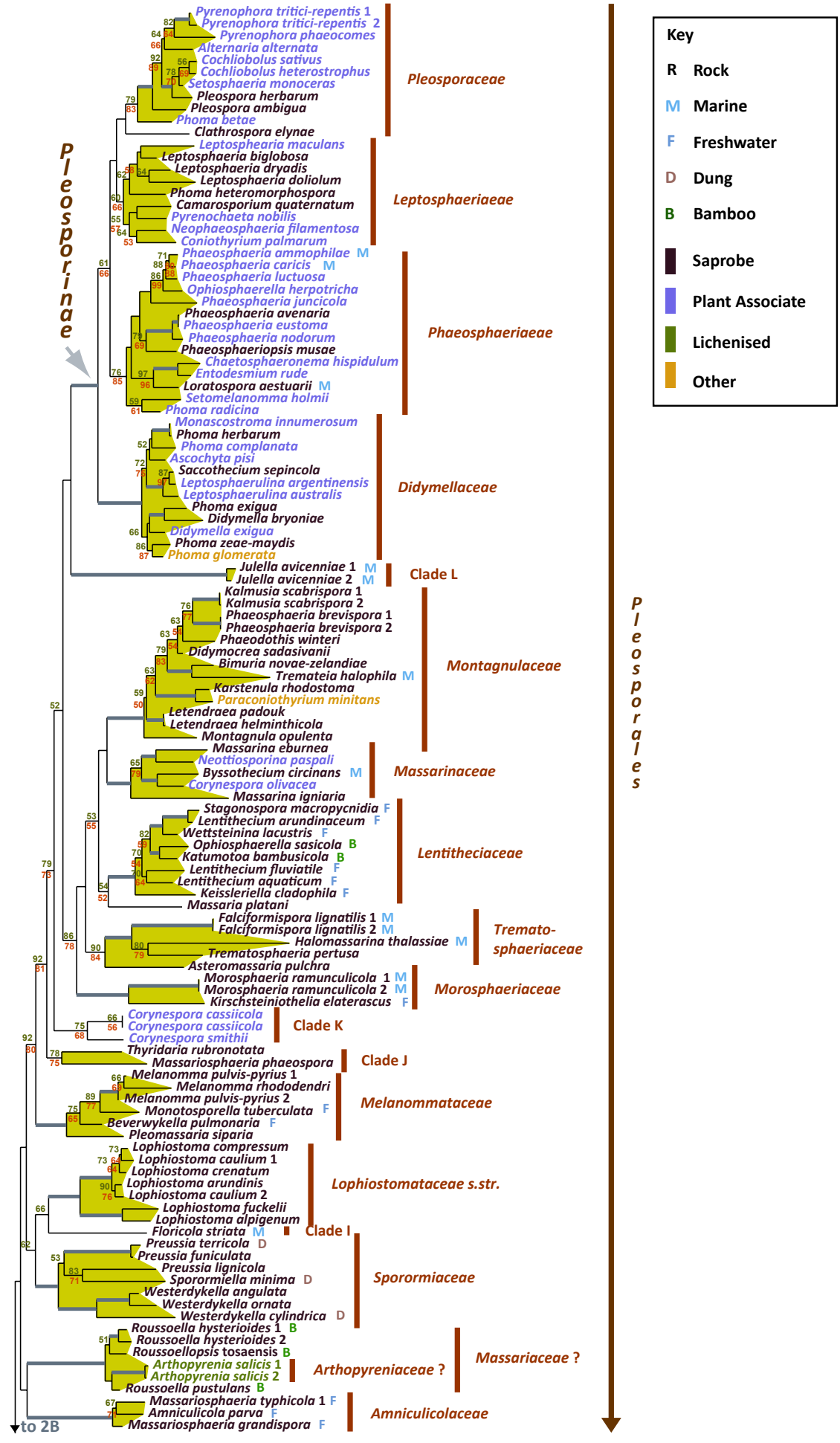
Pyrenophora tritici-repentis 1
Pyrenophora tritici-repentis 2
Pyrenophora phaeocomes
Alternaria alternata
Cochliobolus sativus
Cochliobolus heterostrophus
Setosphaeria monoceras
Pleospora herbarum
Pleospora ambigua
Phoma betae
Clathrospora elynae
Pleosporaceae
Leptosphearia maculans
Leptosphaeria biglobosa
Leptosphaeria dryadis
Leptosphaeria doliolum
Phoma heteromorphospora
Camarosporium quaternatum
Pyrenochaeta nobilis
Neophaeosphaeria filamentosa
Coniothyrium palmarum
Leptosphaeriaeae
Phaeosphaeria ammophilae M
Phaeosphaeria caricis M
Phaeosphaeria luctuosa
Ophiosphaerella herpotricha
Phaeosphaeria juncicola
Phaeosphaeria avenaria
Phaeosphaeria eustoma
Phaeosphaeria nodorum
Phaeosphaeriopsis musae
Chaetosphaeronema hispidulum
Entodesmium rude
Loratospora aestuarii M
Setomelanomma holmii
Phoma radicina
Phaeosphaeriaeae
Monascostroma innumerosum
Phoma herbarum
Phoma complanata
Ascochyta pisi
Saccothecium sepincola
Leptosphaerulina argentinensis
Leptosphaerulina australis
Phoma exigua
Didymella bryoniae
Didymella exigua
Phoma zeae-maydis
Phoma glomerata
Didymellaceae
Julella avicenniae 1 M
Julella avicenniae 2 M
Clade L
Kalmusia scabrispora 1
Kalmusia scabrispora 2
Phaeosphaeria brevispora 1
Phaeosphaeria brevispora 2
Phaeodothis winteri
Didymocrea sadasivanii
Bimuria novae-zelandiae
Trematheia halophila M
Karstenula rhodostoma
Paraconiothyrium minitans
Letendraea padouk
Letendraea helminthicola
Montagnula opulenta
Montagnulaceae
Massarina eburnea
Neottiosporina paspali
Byssothecium circinans M
Corynespora olivacea
Massarina igniaria
Massarinaceae
Stagonospora macropycnidia F
Lentithecium arundinaceum F
Wettsteinina lacustris F
Ophiosphaerella sasicola B
Katumotoa bambusicola B
Lentithecium fluviatile F
Lentithecium aquaticum F
Keissleriella cladophila F
Massaria platani
Lentitheciaceae
Falciformispora lignatilis 1 M
Falciformispora lignatilis 2 M
Halomassarina thalassiae M
Trematosphaeria pertusa
Asteromassaria pulchra
Trematosphaeriaceae
Morosphaeria ramunculicola 1 M
Morosphaeria ramunculicola 2 M
Kirschsteiniothelia elaterascus F
Morosphaeriaceae
Corynespora cassiicola
Corynespora cassiicola
Corynespora smithii
Clade K
Thyridaria rubronotata
Massariosphaeria phaeospora
Clade J
Melanomma pulvis-pyrius 1
Melanomma rhododendri
Melanomma pulvis-pyrius 2
Monotosporella tuberculata F
Beverwykella pulmonaria F
Pleomassaria siparia
Melanommataceae
Lophiostoma compressum
Lophiostoma caulium 1
Lophiostoma crenatum
Lophiostoma arundinis
Lophiostoma caulium 2
Lophiostoma fuckelii
Lophiostoma alpigenum
Lophiostomataceae s.str.
Floricola striata M
Clade I
Preussia terricola D
Preussia funiculata
Preussia lignicola
Sporormiella minima D
Westerdykella angulata
Westerdykella ornata
Westerdykella cylindrica D
Sporormiaceae
Roussoella hysterioides 1 B
Roussoella hysterioides 2
Roussoellopsis tosaensis B
Arthopyrenia salicis 1
Arthopyrenia salicis 2
Arthopyreniaceae ?
Roussoella pustulans B
Massariaceae ?
Massariosphaeria typhicola 1 F
Amniculicola parva F
Massariosphaeria grandispora F
Amniculicolaceae
to 2B
Pleosporinae
Pleosporales
Key
R Rock
M Marine
F Freshwater
D Dung
B Bamboo
Saprobe
Plant Associate
Lichenised
Other

**Fig. 2A.**

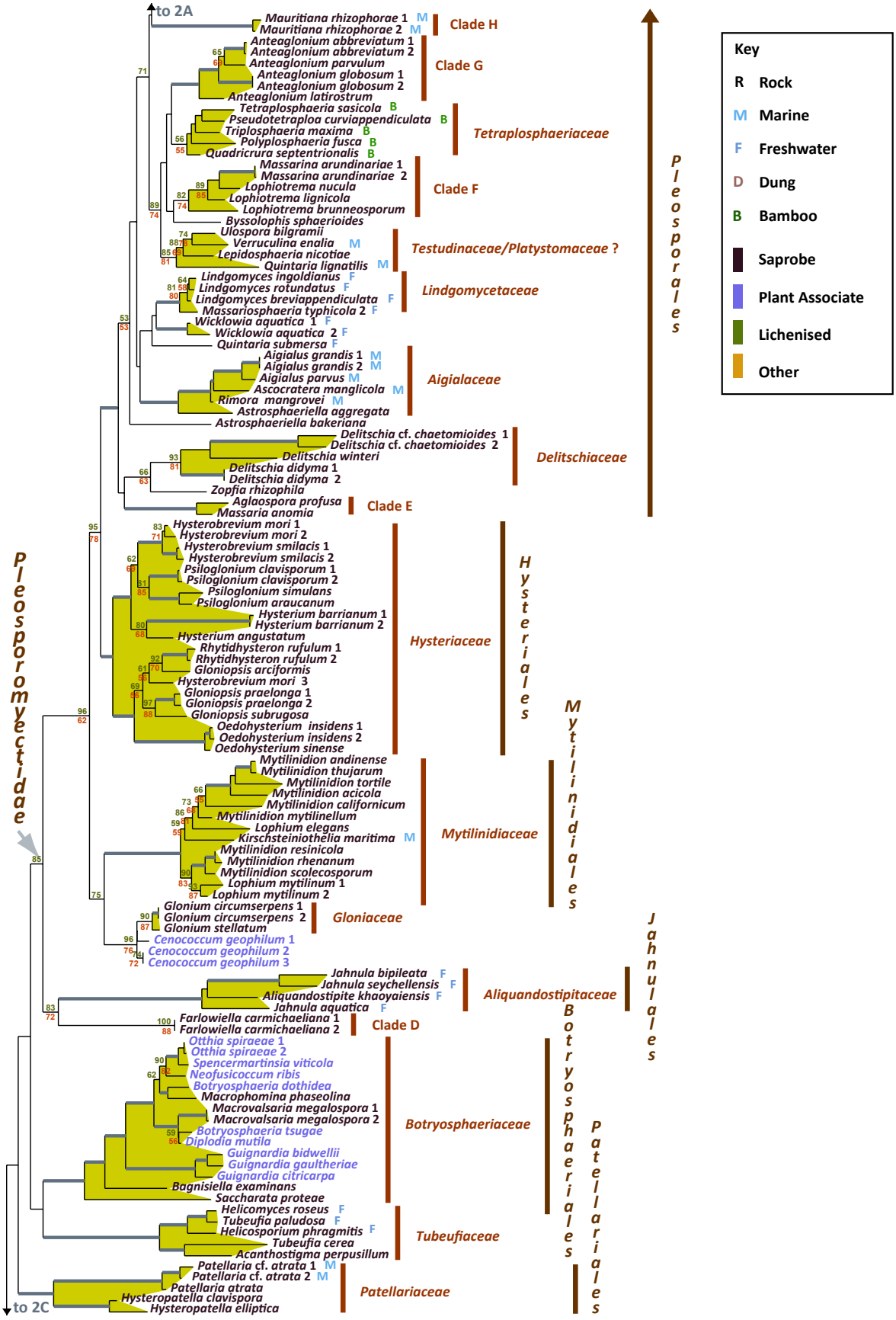
to 2A
Mauritiana rhizophorae 1 M
Mauritiana rhizophorae 2 M
Clade H
Anteaglonium abbreviatum 1
Anteaglonium abbreviatum 2
Anteaglonium parvulum
Anteaglonium globosum 1
Anteaglonium globosum 2
Anteaglonium latirostrum
Clade G
Tetraplosphaeria sasicola B
Pseudotetraploa curviappendiculata B
Triplosphaeria maxima B
Polyplosphaeria fusca B
Quadricrura septentrionalis B
Tetraplosphaeriaceae
Massarina arundinariae 1
Massarina arundinariae 2
Lophiotrema nucula
Lophiotrema lignicola
Lophiotrema brunneosporum
Clade F
Byssolophis sphaerioides
Ulospora bilgramii
Verruculina enalia M
Lepidosphaeria nicotiae
Quintaria lignatilis M
Testudinaceae/Platystomaceae ?
Lindgomyces ingoldianus F
Lindgomyces rotundatus F
Lindgomyces breviappendiculata F
Massariosphaeria typhicola 2 F
Lindgomycetaceae
Wicklowia aquatica 1 F
Wicklowia aquatica 2 F
Quintaria submersa F
Aigialus grandis 1 M
Aigialus grandis 2 M
Aigialus parvus M
Ascocratera manglicola M
Rimora mangrovei M
Astrosphaeriella aggregata
Aigialaceae
Astrosphaeriella bakeriana
Delitschia cf. chaetomioides 1
Delitschia cf. chaetomioides 2
Delitschia winteri
Delitschia didyma 1
Delitschia didyma 2
Delitschiaceae
Zopfia rhizophila
Aglaospora profusa
Massaria anomia
Clade E
Pleosporales
Pleosporomycetidae
Hysterobrevium mori 1
Hysterobrevium mori 2
Hysterobrevium smilacis 1
Hysterobrevium smilacis 2
Psiloglonium clavisporum 1
Psiloglonium clavisporum 2
Psiloglonium simulans
Psiloglonium araucanum
Hysterium barrianum 1
Hysterium barrianum 2
Hysterium angustatum
Rhytidhysteron rufulum 1
Rhytidhysteron rufulum 2
Gloniopsis arciformis
Hysterobrevium mori 3
Gloniopsis praelonga 1
Gloniopsis praelonga 2
Gloniopsis subrugosa
Oedohysterium insidens 1
Oedohysterium insidens 2
Oedohysterium sinense
Hysteriaceae
Hysteriales
Mytilinidion andinense
Mytilinidion thujarum
Mytilinidion tortile
Mytilinidion acicola
Mytilinidion californicum
Mytilinidion mytilinellum
Lophium elegans
Kirschsteiniothelia maritima M
Mytilinidion resinicola
Mytilinidion rhenanum
Mytilinidion scolecosporum
Lophium mytilinum 1
Lophium mytilinum 2
Mytilinidiaceae
Mytilinidiales
Glonium circumserpens 1
Glonium circumserpens 2
Glonium stellatum
Gloniaceae
Cenococcum geophilum 1
Cenococcum geophilum 2
Cenococcum geophilum 3
Jahnula bipileata F
Jahnula seychellensis F
Aliquandostipite khaoyaiensis F
Jahnula aquatica F
Aliquandostipitaceae
Jahnulales
Farlowiella carmichaeliana 1
Farlowiella carmichaeliana 2
Clade D
Otthia spiraeae 1
Otthia spiraeae 2
Spencermartinsia viticola
Neofusicoccum ribis
Botryosphaeria dothidea
Macrophomina phaseolina
Macrovalsaria megalospora 1
Macrovalsaria megalospora 2
Botryosphaeria tsugae
Diplodia mutila
Guignardia bidwellii
Guignardia gaultheriae
Guignardia citricarpa
Bagnisiella examinans
Saccharata proteae
Botryosphaeriaceae
Helicomyces roseus F
Tubeufia paludosa F
Helicosporium phragmitis F
Tubeufia cerea
Acanthostigma perpusillum
Tubeufiaceae
Botryosphaeriales
Patellaria cf. atrata 1 M
Patellaria cf. atrata 2 M
Patellaria atrata
Hysteropatella clavispora
Hysteropatella elliptica
Patellariaceae
Patellariales
to 2C
Key
R Rock
M Marine
F Freshwater
D Dung
B Bamboo
Saprobe
Plant Associate
Lichenised
Other

**Fig. 2B**.

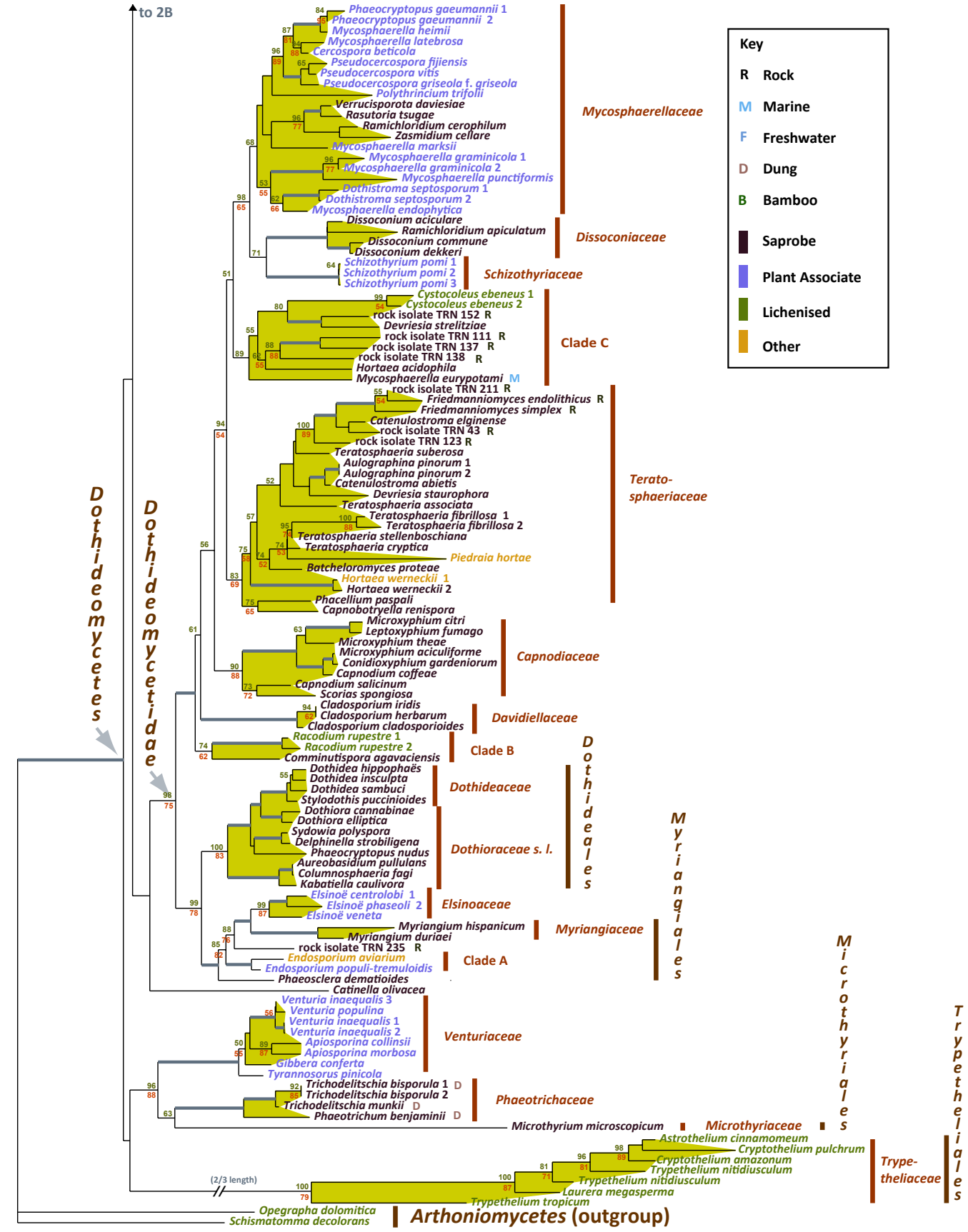
to 2B
Key
R Rock
M Marine
F Freshwater
D Dung
B Bamboo
Saprobe
Plant Associate
Lichenised
Other
Dothideomycetes
Dothideomycetidae
Phaeocryptopus gaeumannii 1
Phaeocryptopus gaeumannii 2
Mycosphaerella heimii
Mycosphaerella latebrosa
Cercospora beticola
Pseudocercospora fijiensis
Pseudocercospora vitis
Pseudocercospora griseola f. griseola
Polythrincium trifolii
Verrucisporota daviesiae
Rasutoria tsugae
Ramichloridium cerophilum
Zasmidium cellare
Mycosphaerella marksii
Mycosphaerella graminicola 1
Mycosphaerella graminicola 2
Mycosphaerella punctiformis
Dothistroma septosporum 1
Dothistroma septosporum 2
Mycosphaerella endophytica
Mycosphaerellaceae
Dissoconium aciculare
Ramichloridium apiculatum
Dissoconium commune
Dissoconium dekkeri
Dissoconiaceae
Schizothyrium pomi 1
Schizothyrium pomi 2
Schizothyrium pomi 3
Schizothyriaceae
Cystocoleus ebeneus 1
Cystocoleus ebeneus 2
rock isolate TRN 152 R
Devriesia strelitziae
rock isolate TRN 111 R
rock isolate TRN 137 R
rock isolate TRN 138 R
Hortaea acidophila
Mycosphaerella eurypotami M
Clade C
rock isolate TRN 211 R
Friedmanniomyces endolithicus R
Friedmanniomyces simplex R
Catenulostroma elginense
rock isolate TRN 43 R
rock isolate TRN 123 R
Teratosphaeria suberosa
Aulographina pinorum 1
Aulographina pinorum 2
Catenulostroma abietis
Devriesia staurophora
Teratosphaeria associata
Teratosphaeria fibrillosa 1
Teratosphaeria fibrillosa 2
Teratosphaeria stellenboschiana
Teratosphaeria cryptica
Piedraia hortae
Batcheloromyces proteae
Hortaea werneckii 1
Hortaea werneckii 2
Phacellium paspali
Capnobotryella renispora
Teratosphaeriaceae
Microxyphium citri
Leptoxyphium fumago
Microxyphium theae
Microxyphium aciculiforme
Conidioxyphium gardeniorum
Capnodium coffeae
Capnodium salicinum
Scorias spongiosa
Capnodiaceae
Cladosporium iridis
Cladosporium herbarum
Cladosporium cladosporioides
Davidiellaceae
Racodium rupestre 1
Racodium rupestre 2
Comminutispora agavaciensis
Clade B
Dothidea hippophaës
Dothidea insculpta
Dothidea sambuci
Stylodothis puccinioides
Dothideaceae
Dothiora cannabinae
Dothiora elliptica
Sydowia polyspora
Delphinella strobiligena
Phaeocryptopus nudus
Aureobasidium pullulans
Columnosphaeria fagi
Kabatiella caulivora
Dothioraceae s. l.
Dothideales
Elsinoë centrolobi 1
Elsinoë phaseoli 2
Elsinoë veneta
Elsinoaceae
Myriangium hispanicum
Myriangium duriaei
Myriangiaceae
Myriangiales
rock isolate TRN 235 R
Endosporium aviarium
Endosporium populi-tremuloidis
Clade A
Phaeosclera dematioides
Catinella olivacea
Venturia inaequalis 3
Venturia populina
Venturia inaequalis 1
Venturia inaequalis 2
Apiosporina collinsii
Apiosporina morbosa
Gibbera conferta
Tyrannosorus pinicola
Venturiaceae
Trichodelitschia bisporula 1 D
Trichodelitschia bisporula 2
Trichodelitschia munkii D
Phaeotrichaceae
Phaeotrichales
Microthyrium microscopicum
Microthyriaceae
Microthyriales
Astrothelium cinnamomeum
Cryptothelium pulchrum
Cryptothelium amazonum
Trypethelium nitidiusculum
Trypethelium nitidiusculum
Laurera megasperma
Trypethelium tropicum
Trype-theliaceae
Trypetheliales
(2/3 length)
Opegrapha dolomitica
Schismatomma decolorans
Arthoniomycetes (outgroup)

**Fig. 2C**.

### Ancestral reconstruction

Ancestral reconstructions were performed in Mesquite v. 2.6 with character states traced over 2000 bootstrapped trees obtained with RAxML-MPI v. 7.0.4 (Stamatakis 2006). Following the phylogeny presented (Fig. 2) this reconstruction was performed with a maximum-likelihood criterion using the single parameter Mk1 model. Ancestral states were assigned to a node if the raw likelihood was higher by at least 2 log units than the likelihood value of the other ancestral state(s) according to default settings. Character states were also mapped using TreeDyn v. 198.3 (Chevenet *et al.* 2006), shown in Fig. 3. This is presented as a clockwise circular tree, starting with outgroup taxa. Only clades with more than two taxa of the same state are shown and bootstrap recovery was not considered in assigning character states. In applying the character states of saprobes (including rock heterotrophs), plant associated fungi (including pathogens, endophytes and mycorrhizae) and lichenised fungi the broad concepts presented were followed as laid out in Schoch *et al.* (2009a). Some character assessments were taken from Zhang *et al*. (2009; this volume). Ecological characters of sampling sources, terrestrial, fresh water and marine were assessed based on papers elsewhere in this volume (Suetrong *et al*. 2009*,* Shearer *et al*. 2009*)*.

### Genome analyses

A MCL (Markov Cluster Algorithm) protein analysis of 52 fungi and one metazoan (*Drosophila melanogaster*) (Table 2 - see online Supplementary Information) and the phylogenetic placement of these species was used to characterise the phylogenetic profile of each cluster. *Chytridiomycota* and *Mucoromycotina* each were represented by one and two species, respectively. In *Dikarya*, *Basidiomycota* and *Ascomycota* were represented by 8 and 40 species respectively. The *Pezizomycotina* (filamentous ascomycetes) was presented by 26 species in four classes [*Sordariomycetes* (12), *Leotiomycetes* (2), *Dothideomycetes* (6) and *Eurotiomycetes* (6)].

## RESULTS AND DISCUSSION

### Taxon sampling

The phylogram presented in Fig. 2 represents the largest ever phylogenetic assessment of *Dothideomycetes* to date. Here the focus has been on expanding taxon diversity in the class while specifically avoiding a small number of taxa that other analyses suggest reside on long unstable branches. This still allowed for an extensive sweep of dothideomycete taxon diversity; in doing so we followed the premise of allowing for missing data in our supermatrix (Wiens 2006). An effort was made to intersperse taxa with poor character sampling amongst those having better sampling throughout the tree, but the inclusion of missing characters could still have unanticipated effects on phylogenetic assessments (Lemmon *et al.* 2009). While recognising this caveat, a recent expansive data set covering all of *Ascomycota* noted very little changes in major nodes even after the removal of taxa with high proportions of missing characters (Schoch *et al.* 2009a). The phylogeny presented here agrees well with broad phylogenies in this volume and elsewhere (Schoch *et al.* 2006, Crous *et al.* 2007a, Zhang *et al.* 2008, Crous *et al.* 2009b). After all introns and 379 ambiguous character positions were removed, the matrix consisted of 52 % missing and indeterminate characters. This maximum-likelihood analysis had 5 069 distinct alignment patterns and produced a best known likely tree with a log likelihood of -207247.761117.

### Evolution of nutritional modes

The ancestral reconstructions in Fig. 3 indicate that phytopathogenicity can be confined to a number of terminal clades throughout the tree and that these always reside within saprobic lineages. A maximum of seven transitions likely occurred in several lineages of the orders *Pleosporales, Capnodiales* and singular lineages in *Myriangiales*, *Botryosphaeriales* and *Venturiaceae* (also see in this volume; Crous *et al.* 2009a, Zhang *et al.* 2009). Several transitions to lichenisation have also occurred, although phylogenetic uncertainty may limit this to a minimum of two. Due to the use of lichenised *Arthoniomycetes* as outgroup a broader assessment is required to determine whether the *Dothideomycetes* evolved from a lichenised ancestor. Previous studies suggested that the saprobic habit is an ancestral trait but only with marginal support (Schoch *et al.* 2009a). Similar conclusions can be reached for the aquatic ecological characters – the majority of fresh water and marine clades reside within terrestrial clades as has been shown previously *e.g.* (Spatafora *et al.* 1998, Vijaykrishna *et al.* 2006). Transitions from a terrestrial life style to fresh water likely occurred at least three times and transitions to marine environments up to six times. Phylogenetic uncertainty for the placement of some marine clades can limit this to a minimum of four times (Fig. 2). Reversions from aquatic to terrestrial environments are rare, with one possible exception in the *Lentitheciaceae* where bambusicolous saprobes reside, nested within several fungi occurring in freshwater habitats (for additional details see Zhang *et al.* 2009; this volume). Phylogenetic resolution will have to improve to test this further.

An analysis of recently released genomes was compared to consider whether genome composition reinforces phylogenetic support for *Dothideomycetes* (Fig. 4). Relative to a clustering analysis of proteins from 52 sequenced fungi and *Drosophila melanogaster*, about 5 515 protein coding genes from *Dothideomycetes* shared protein clusters with proteins from other dothideomycete fungi only. This comprises roughly 8–11 % of the protein coding genes in each of six sequenced *Dothideomycetes*. The species profile of each protein cluster was used to assign a phylogenetically informed designation. The profiles most frequently seen were those of the most conserved proteins, namely clusters designated as having a shared Ophistokont phylogenetic profile. Among the more derived nodes of the *Dothideomycetes*, protein clusters were observed that had a species composition that could reflect the result of selection pressure on more distantly related fungi that share the same niche.

A phylogenomic profile (Fig. 4) of the proteins from six *Dothideomycetes* from the two largest orders seen in Fig. 1 is presented (*Mycosphaerella graminicola, Mycosphaerella fijiensis, Phaeosphaeria nodorum, Alternaria brassicicola, Pyrenophora tritici-repentis, Cochliobolus heterostrophus*). The highest percentage of proteins (excluding species specific proteins) were conserved outside kingdom *Fungi* (Ophistokont node, 23 %), followed by proteins specific for the *Dikarya* (14 %) and the *Pezizomycotina* (13 %). This breakdown was also prevalent within other *Pezizomycotina* classes. Approximately 8 % of the proteins from the six *Dothideomycetes* were conserved across and within derived nodes in this class. Relative to this analysis 28 % of the proteins were specific to the *Dothideomycetes* (including species specific proteins). The other class containing loculoascomyetes, *Eurotiomycetes,* had 19.5 % proteins characterised as class specific. This means the percentage dothideomycete specific proteins were about 8.5 % more. *Eurotiomycetes* in the analysis were mostly human pathogens, with most having no known sexual state whereas the *Dothideomycetes* in the analysis were all plant

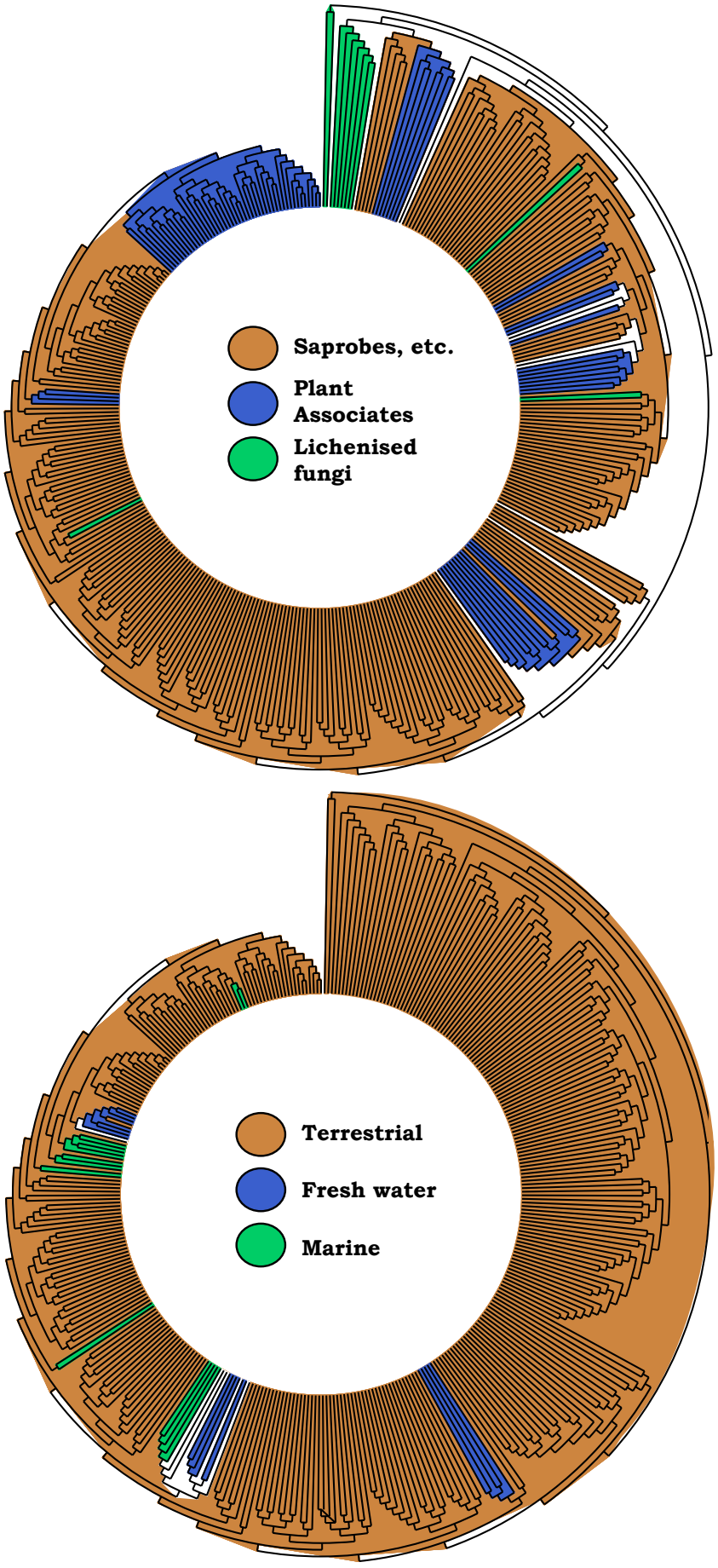


**Fig. 3.** Simplified ancestral state reconstructions, showing potential transitions between character states. The same phylogeny as in Fig. 2A–C is shown, with the outgroups positioned at twelve o' clock and subsequent clades arranged in a clockwise manner. Characters were traced over 2 000 bootstrap trees and those that were recovered in the majority are coloured on the nodes. In the case of equivocal construction no colour was used (white). To simplify the figure, only clades with two or more neighbouring character states are shown.

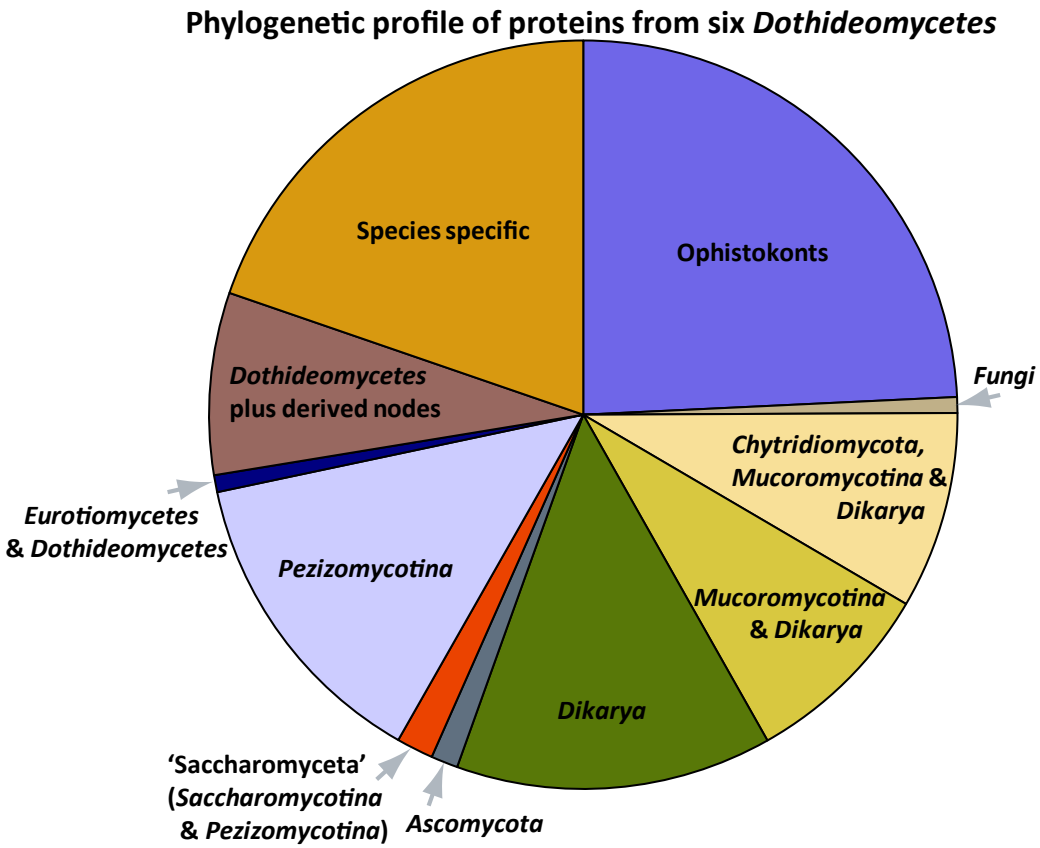


**Fig. 4.** Pie chart showing relative numbers of unique proteins per genome according to taxonomic classification.

pathogens and mostly with known sexual states. This breakdown of nutritional modes, although not comprehensive for these two classes, is somewhat representative. In *Eurotiomycetes* human pathogens are more diverse and plant pathogens uncommon, with the converse being true for *Dothideomycetes*. Both classes contain melanised species with similar morphologies and more comprehensive comparative studies need to expand sampling to incorporate species from the different nutritional modes for both classes.

## Phylogenetic relationships

In the phylogram presented (Fig. 2) the two dothideomycete subclasses previously described based on presence or absence of pseudoparaphyses (Schoch *et al.* 2006) could be recovered with varying levels of bootstrap representation. Subclass *Pleosporomycetidae* previously included *Pleosporales* plus a single species, representing *Mytilinidiaceae*, namely *Lophium mytilinum* (Schoch *et al.* 2006). Taxon sampling for the *Mytilinidiaceae* was considerably expanded by Boehm *et al*. (2009b), with the addition of a number of new taxa, leading to the establishment of the *Mytilinidiales*. Likewise, extensive taxon sampling for the family *Hysteriaceae* led to a newly redefined *Hysteriales* also included in this subclass (Boehm *et al.* 2009a; this volume). It appears that persistent, hysteriaceous carbonaceous ascomata that dehisce via a longitudinal slit (*e.g.*, hysterothecia) have evolved multiple times within *Pleosporomycetidae* (Mugambi & Huhndorf 2009). *Pleosporomycetidae* can be expanded to tentatively include *Jahnulales* (Fig. 2B) based on strong bootstrap support from RAxML analyses and morphology. Perithecioid ascomata and a hamathecium of wide cellular pseudoparaphyses are characteristic of *Jahnulales* (Inderbitzin *et al.* 2001, Pang *et al.* 2002; Shearer *et al.* 2009; this volume) and agree with diagnostic features for *Pleosporomycetidae*. We also recommend that the definition of the subclass be reassessed with more inclusive character sets. Also, *Leptosphaerulina* species characterised by the absence of pseudoparaphyses reside within the pseudoparaphysate *Pleosporales* (Fig. 2C; Silva-Hanlin & Hanlin 1999, Kodsueb *et al.* 2006), indicating that pseudoparaphyses could have been lost multiple times. It should be noted that the maturity of ascomata may play an important role in these assessments. Immature specimens may contain pseudoparaphyses that dehisce when mature and these characteristics need to be evaluated with more complete sampling of the numerous aparaphysate taxa still listed as *incertae sedis*. The second subclass, *Dothideomycetidae,* previously circumscribed based on the absence of pseudoparaphyses remains well supported (Fig. 2C).

The results of this study provided continued support for ten orders within class *Dothideomycetes*, namely *Pleosporales*, *Hysteriales*, *Mytilinidiales*, *Patellariales*, *Botryosphaeriales*, *Jahnulales*, *Dothideales*, *Capnodiales, Myriangiales* and *Trypetheliales*. The latter order was recently proposed (Aptroot *et al.* 2008) and represents the largest lichen forming clade in *Dothideomycetes*. Another recently proposed order, *Botryosphaeriales* includes only the single family, *Botryosphaeriaceae*. The analysis (Fig. 2B), however, shows strong support for a narrower interpretation of the *Botryosphaeriaceae,* typified by *Botryosphaeria dothidea* and related genera, excluding a separate clade of species residing in *Guignardia* (with *Phyllosticta* anamorphs). *Bagnisiella examinens* and *Saccharata protea* did not reside in either of the above clades, placed on early diverging branches. A more extensive taxon sampling is required to address the diversity in this order, which most likely will validate the separation of additional families. Another currently accepted order, *Microthyriales*, consisting of

species occurring as saprobes or epiphytes on stems and leaves is represented in this study by only a single sample, *Microthyrium microscopicum* (Fig. 2C). Members of this order are poorly represented in culture and have unusual thyrothecial ascomata that have a scutate covering comprising a thin layer of radiating cells. This structure is generally lacking a basal layer and is quite unlike any morphologies in other orders. This positioning adjacent to the plant parasitic *Venturiaceae* and coprophilic *Phaeotrichaceae*, is unexpected but since the single representative of the *Microthyriales* is on a long branch this is a relationship that will require more intensive taxon sampling.

Additional families that could not be placed in an order are *Tubeufiaceae* and *Gloniaceae* (Fig. 2B). Species in *Tubeufiaceae* have superficial clustered ascomata and characteristic bitunicate asci with relatively long ascospores, often with helicosporous anamorphs (Kodsueb *et al.* 2008). Members of *Tubeufiaceae*, which frequently occur in freshwater habitats include anamorph genera, such as *Helicoon* and *Helicodendron,* and are ecologically classified as aeroaquatic species. A few teleomorph taxa such as *Tubeufia asiana* occur on submerged wood (Tsui *et al*. 2007), and *Tubeufia paludosa* occur on herbaceous substrates in wet habitats (Webster 1951). The *Gloniaceae* are saprobic, have dichotomously branched, laterally anastomosed pseudothecia that form radiating pseudo-stellate composites and dehisce by an inconspicuous, longitudinal, but evaginated slit. They reside sister to the saprobic *Mytilinidiales* but due to conspicuous morphological differences and moderate statistical support they are placed in *Pleosporomycetidae incertae sedis* (Boehm *et al.* 2009a, this volume).

Several other well supported clades representing families were evident in this study (Fig. 2). These include several families in *Pleosporales,* treated elsewhere (Zhang *et al.* 2009; this volume). Other clades have lower levels of support. For example *Leptosphaeriaceae* (Fig. 2A) have moderate bootstrap support and it is treated in the very broad sense here. There was also support for several newly described families treated in different papers within this volume. In *Pleosporales* these include *Amniculicolaceae* and *Lentitheciaceae* (Zhang *et al.* 2009; this volume). The *Lindgomycetaceae* (Shearer *et al.* 2009; this volume, Hirayama *et al.* 2010) encompassing a majority of species isolated from fresh water habitats. Two other novel families, *Aigialaceae* and *Morosphaeriaceae* include mainly marine species (Suetrong *et al.* 2009; this volume). In addition to these, the sampling of a wide diversity of fungi on bamboo yielded the description of *Tetraplosphaeriaceae* (Tanaka *et al.* 2009; this volume). Another novel family, *Dissoconiaceae,* is proposed by Crous *et al.* 2009 (this volume) for foliicolous commensalists on *Eucalyptus* leaves, some of which are putative hyper parasites and reside in *Capnodiales.*

Results of this study suggest that sampling within existing families also requires continued expansion as familial definitions in *Dothideomycetes* remains problematic. A paper focused on two families, with poor representation in molecular data sets, *Melanommataceae* and *Lophiostomataceae* addresses this in more detail (Mugambi & Huhndorf 2009; this volume). Numerous other clades in our tree remain without familial placement. This includes a diverse group in *Capnodiales* (Fig. 2C, clade C) a newly described group of hysteriaceous fungi in *Pleosporales* (Fig. 2A, clade G) and additional marine lineages (clades H, L, Fig. 2A). An interesting clade tentatively circumdescribed by Zhang *et al*. (2009; this volume) as *Massariaceae* contains bambusicolous fungi and appears related to the lichenised *Arthopyreniaceae* (Fig. 2A).

Finally, a clade including *Corynespora* anamorphs (clade K, Fig. 2A) is placed for the first time, but without clear relationship to any other currently defined families. The genus *Corynespora* includes anamorphic fungi with tretic, percurrent, and acropetal conidiogenesis. The melanised, pseudoseptate conidia have a pronounced hilum from which the conidial germ tube emerges and are borne apically from solitary, melanised conidiophores. Though nearly 100 species are described based on differences in morphology, considerable phenotypic plasticity within individual isolates complicates species recognition, and molecular analyses that may result in taxonomic clarification have not been done. *Corynespora* species fill a diversity of roles as saprobes, pathogens, and endophytes on and in woody and herbaceous plants, other fungi, nematodes, and human skin (Dixon *et al.* 2009). One of the species represented here, *C. cassiicola* is an important pathogen of rubber. The teleomorphic fungi *Pleomassaria swidae* (*Pleomassariaceae*; Tanaka *et al.* 2005) and *Corynesporasca caryotae* (*Corynesporascaceae*; Sivanesan 1996) have unnamed *Corynespora* species as anamorphs. In this study, species currently placed in *Corynespora* are not monophyletic and are positioned in at least two families: *Massarinaceae* and Clade K (Fig. 2A).

## Anamorph taxa

The previously mentioned *Dissoconiaceae* relies on taxonomic descriptions based on anamorph characters. This is a theme that is expected to continue for mitosporic taxa in *Dothideomycetes* as molecular data accelerates their integration. The artificial nature of the "higher" taxa of anamorphs *e.g.*, deuteromycetes (Kirk *et al.* 2001) is now well recognised, but the integration of anamorphs into the phylogenetic classification of teleomorphs remains a significant challenge in fungal systematics (Shenoy *et al.* 2007). The correlation of teleomorphs and anamorphs (Seifert *et al.* 2000) is not always predictive but it has been applied in some genera within *Dothideomycetes*, *e.g. Botryosphaeria* and *Mycosphaerella* (Crous *et al.* 2006, 2009b). However, numerous examples underscoring anamorph convergence can be found throughout the class *e.g. Dictyosporium* (Tsui *et al.* 2006, Kodsueb *et al.* 2008), *Sporidesmium* (Shenoy *et al.* 2006), *Cladosporium* (Crous *et al.* 2007b) and *Phoma* (Fig. 2A; Aveskamp *et al.* 2009, de Gruyter *et al.* 2009, Woudenberg *et al.* 2009) as well as *Fusicoccum* and *Diplodia* (Crous *et al.* 2006, Phillips *et al.* 2008). The use of large multigene phylogenies will be essential to bring taxonomic order to cryptic anamorph lineages.

## Ecological diversity

Besides the unclassified diversity found in anamorphic genera, numerous ecological niches contain diverse lineages of fungi lacking systematically sampled molecular characters. Several examples of this knowledge gap can be found in papers in this volume. In this regard, the rock inhabiting fungi are amongst the least understood. These fungi exist ubiquitously as melanised, slow growing colonies and that usually do not produce generative structures. They subsist on bare rock surfaces and are consequently highly tolerant of the environmental stresses induced by lack of nutrients, water and extremes in radiation and temperature (Palmer *et al.* 1990, Sterflinger 1998, Ruibal 2004, Gorbushina *et al.* 2008). Members of this ecological guild are diverse and occur in two classes – *Eurotiomycetes* and *Dothideomycetes*. Ruibal *et al.* 2009 (this volume) present the results of an expanded sampling of rock-inhabiting fungi that include lineages residing within *Dothideomycetes* and sister class *Arthoniomycetes*. These rock inhabiting fungi can be placed in

*Capnodiales*, *Pleosporales*, *Dothideales* and *Myriangiales*, as well as some unclassified lineages of *Dothideomycetes*. Interestingly, some associated lineages were without clear placement within either *Arthoniomycetes* or *Dothideomycetes*. The rock isolates included in Fig. 2C illustrate a subsection of genetic diversity seen in these extremophiles, in particular for the *Capnodiales*, with two rock isolates-rich lineages *Teratosphaeriaceae* and Clade C (Fig. 2C). A more detailed analysis (Ruibal *et al.* 2009; this volume) allows for the presentation of hypotheses related to evolution of pathogenicity and lichenisation because these modes of nutrition are often found in close proximity of rock inhabiting fungal lineages.

The lichenised fungi allied with the *Dothideomycetes* represent another poorly sampled group of fungi. Several lichenised species remain enigmatically placed after they were confirmed as members of *Dothideomycetes* based on DNA sequence data (Lumbsch *et al.* 2005, Del Prado *et al.* 2006). Although the number of species is comparatively small, their placement can play an important link in determining how transitions to and from lichenisation influenced dothideomycete evolution. *Trypetheliaceae* known for its anastomosing, branched pseudoparaphyses was until very recently still placed within *Pyrenulales*, an ascohymenial order in *Eurotiomycetes*, based on bitunicate asci and lense-shaped lumina in the ascospores (Del Prado *et al.* 2006). Attempts to resolve members of this family remain challenging as they tend to occur on long, rapidly evolving branches in our phylogenetic analyses, which often lead to artifacts. Nelsen *et al.* 2009 (this volume) demonstrate the occurrence of two additional lichen-forming lineages within *Dothideomycetes* representing the families *Strigulaceae* and *Monoblastiaceae*. The delineation of lichenised family *Arthopyreniaceae* should continue to be assessed given their placement with a clade containing bambusicolous fungi (Tanaka *et al.* 2009; this volume) and their non monophyly is also confirmed elsewhere (Nelsen *et al.* 2009; this volume). The relationship between the lichenised groups and bambusicolous genera *Roussoella* and *Roussoellopsis* (*Didymosphaeriaceae;* Ju *et al.* 1996, Lumbsch & Huhndorf 2007) is strongly supported, but their affinity is not fully understood due to their considerable morphological differences.

The fungi collected from marine and freshwater habitats contain yet more varied species that have not been assessed well within a molecular based framework. Their diversity is supported by the fact that whole orders (*Jahnulales*) and several families, already mentioned, almost exclusively consist of species collected from these environments. A recent assessment of marine fungi tallied a number of more than 500 species with more than a fifth of these suggested to reside in *Dothideomycetes* (Jones *et al.* 2009). The number for fungi from fresh water habitats is somewhat lower (about 170 taxa).

Despite similarities in their preferred medium for spore dispersal (water) an examination of phylogenetic diversity within *Dothideomycetes* indicates that these groups of fungi tend to reside in divergent parts of the tree (Figs 2, 3). However, some exceptions may occur: For example, members of *Aigialaceae* are weakly supported to share ancestry with members of freshwater clade *Lindgomycetaceae* (Raja *et al.* 2010). The *Jahnulales* represents another recently delineated aquatic lineage with an interesting mixture of fresh water and marine taxa. It was delineated based on molecular and morphological data (Inderbitzin *et al.* 2001, Pang *et al.* 2002) and now contains four genera and several species (Campbell *et al.* 2007). Previously, two anamorphic species in the *Jahnulales, Xylomyces rhizophorae* (described from mangrove wood of *Rhizophora)* and *X. chlamydosporus* have been reported from mangroves and thus saline habitats (Kohlmeyer & Volkmann-Kohlmeyer 1998). It has further been documented that *X. chlamydosporus* is the anamorph of *Jahnula aquatica,* a freshwater species (Sivichai, pers. comm.).

Marine *Dothideomycetes* generally exist in association with algae and plants in marine and brackish environments, usually with intertidal or secondary marine plants (*e.g.*, mangroves). The majority of these fungi have been classified in families and genera that comprise mostly terrestrial species (*e.g.*, *Pleospora*) and no definitive clades of marine *Dothideomycetes* have been identified. Here we find support for diverse aquatic lineages similar to the situation in *Sordariomycetes*. Papers by Suetrong *et al.* 2009 (this volume) and Shearer *et al.* 2009 (this volume) continue to address this disparity by using multigene phylogenies to describe several lineages within a class wide context. In contrast, many marine members of the *Dothideomycetes* await interrogation at the DNA sequence level, especially the genera *Belizeana, Thalassoascus, Lautospora* and *Loratospora,* all exclusively marine taxa.

The final environmentally defined group sampled in this volume is the bambusicolous fungi. More than 1 100 fungal species have been described or recorded worldwide from bamboo (Hyde *et al.* 2002). Furthermore, their ecological specialisation as pathogens, saprophytes, and endophytes has been relatively well documented (*e.g.* Hino 1961). However, relatively few studies based on DNA sequence comparisons have been undertaken for many bambusicolous fungi. Several unique lineages, *e.g.* the *Katumotoa bambusicola-Ophiosphaerella sasicola* clade in a freshwater lineage (*Lentitheciaceae*) and the *Roussoella-Roussoellopsis* clade close to lichen-forming families could be found (Fig. 2). Particularly, a new family *Tetraplosphaeriaceae* including five new genera characterised by a *Tetraploa* anamorph *s. l.* is introduced as a lineage of fungi with bamboo habitat (Tanaka *et al.* 2009; this volume). It is clear that much additional diversity within this group of fungi remains to be sampled using DNA sequence data

A number of other niches remain poorly discussed in this volume. Coprophilous fungi occur in three families *Delitschiaceae*, *Phaeotrichaceae*, and *Sporormiaceae* (Figs 2A, C). These families are not closely related and it is clear that the fimicolous life style has arisen more than once in the *Dothideomycetes*. Also, many species from these groups are not strictly dung-inhabiting, but can be found on other substrates like soil, wood, and plant-debris. Interestingly, some are human pathogens, plant endophytes and lichenicolous fungi. As is true throughout the *Ascomycota,* a change in substrate is apparently not a substantial evolutionary step in these taxa (Kruys & Wedin 2009).

## Additional observations

Several orders *e.g. Dothideales*, *Myriangiales* and *Microthyriales* have not been treated using the extensive systematic sampling that is true for studies treated in this volume. However, individual smaller studies continue to provide interesting and surprising results. One such example is the first described meristematic and endoconidial species residing in *Myriangiales* (Fig. 2C) reported by Tsuneda *et al.* (2008). These *Endosporium* species were isolated from very different substrates such as: poplar twigs and a dead bird. They also have a close relationship to a single lineage of rock inhabiting fungi. The nutritional shifts represented by these closely related species correlate well with scenarios described by Ruibal *et al.* (2009; this volume) for rock inhabiting fungi. Another melanised meristematic fungus, *Sarcinomyces crustaceus*, isolated from pine trees appears in a similar position in a phylogeny presented in the aforementioned paper (Ruibal *et al.* 2009; this volume).

Another unusual species, *Catinella olivacea* is included in Fig. 2C, but without any clearly resolved position, diverging early to *Dothideomycetidae*. This species was initially placed in *Leotiomycetes,* due to their flattened apothecia, found on the underside of moist, well-decayed logs of hardwood. Asci are unitunicate but they appear to form after ascolocular development. As in the previous analysis, it was not possible to identify relationships between this species and any known order, although there are indications of a close relationship with the *Dothideomycetidae* (Greif *et al.* 2007).

The placement of the single asexual mycorrhizal lineage representing *Cenococcum geophilum* in the *Dothideomycetes* (LoBuglio *et al.* 1996), allied to members of the saprobic *Gloniaceae* is intriguing (Fig. 2B; Boehm *et al.* 2009a; this volume). No resolved placement for this species in *Dothideomycetes* has been possible in the past. The results of this study were also unexpected because no biological data suggest a connection to the family. *Cenococcum* is a fungus that is intensively used in environmental studies and this could suggest a very interesting biology for members of the ostensibly saprobic *Gloniaceae*. Results of this study advocate a more expansive sampling of *Cenococcum* in order to confirm this intriguing result.

## CONCLUSIONS

One of the major obstacles in dothideomycete systematics remains the lack of a clear understanding of what species are members of the class based on morphology alone. Throughout most of the 20th Century, comparative morphological studies have been the only character on which to base phylogenetic relationships. The advent of large DNA-sequence data sets should allow for a substantially improved interpretation of morphological characters for this class of fungi. Studies in this volume and elsewhere have provided a clear understanding that many of the characters classically used in taxonomy and systematics of the group are homoplastic and not helpful for reconstructing phylogenetic relationships. Dothideomycete taxonomy also needs to keep pace with the rapid advances being made in phylogenetics, genomics and related fields. The important principle here is that our classification should communicate diversity accurately and allow dothideomycete biologists from disparate fields to have access to an agreed upon set of taxonomic names to aid communication. In addition, it should allow for a focus on under-sampled groups and clades (i.e. poorly sampled saprobes and others). A major task ahead will be to add asexual genera to present phylogenetic schemes, and integrate these into the existing familial and ordinal classification. As most of these asexual genera are in fact poly- and paraphyletic, their type species will need to be recollected to clarify their phylogenetic position. In addition to this, it appears that even some concepts of teleomorphic taxa will require extensive reconsideration. Finally, we should attempt to incorporate valuable biological information from past workers, such as the three mycologists to which this volume is dedicated, by reliably assessing culture and sequence identity. It is hoped that the papers in this volume will make a meaningful contribution towards these goals.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Isolates of *Dothideomycetes* included in this study. Newly deposited sequences are shown in bold.

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Acanthostigma perpusillum* | UAMH | AY856937 | AY856892 | | | |
| *Aglaospora profusa* | CBS 123109 | **GU296130** | **GU301792** | | | **GU349062** |
| *Aigialus grandis* 1 | 2Q | **GU296132** | **GU301794** | | | **GU349063** |
| *Aigialus grandis* 2 | JK 5244A | **GU296131** | **GU301793** | | **GU371762** | |
| *Aigialus parvus* | A6 | **GU296133** | **GU301795** | | **GU371771** | **GU349064** |
| *Aliquandostipite khaoyaiensis* | CBS 118232 | AF201453 | **GU301796** | | FJ238360 | **GU349048** |
| *Alternaria alternata* | CBS 916.96 | DQ678031 | DQ678082 | | DQ677980 | DQ677927 |
| *Amniculicola parva* | CBS 123092 | **GU296134** | FJ795497 | | | **GU349065** |
| *Anteaglonium abbreviatum* 1 | ANM 925.1 | | GQ221877 | | | GQ221924 |
| *Anteaglonium abbreviatum* 2 | GKM 1029 | | GQ221878 | | | GQ221915 |
| *Anteaglonium globosum* 1 | SMH 5283 | | GQ221911 | | | GQ221919 |
| *Anteaglonium globosum* 2 | ANM 925.2 | | GQ221879 | | | GQ221925 |
| *Anteaglonium latirostrum* | L100N 2 | | GQ221876 | | | GQ221938 |
| *Anteaglonium parvulum* | SMH 5210 | | GQ221907 | | | GQ221917 |
| *Apiosporina collinsii* | CBS 118973 | **GU296135** | **GU301798** | **GU357778** | | **GU349057** |
| *Apiosporina morbosa* | dimosp | | EF114694 | | | |
| *Arthopyrenia salicis* 1 | 1994 Coppins | | AY607730 | AY607742 | | |
| *Arthopyrenia salicis* 2 | CBS 368.94 | AY538333 | AY538339 | **GU371814** | | |
| *Ascochyta pisi* | CBS 126.54 | DQ678018 | DQ678070 | | DQ677967 | DQ677913 |
| *Ascocratera manglicola* | JK 5262C | **GU296136** | **GU301799** | | **GU371763** | |
| *Asteromassaria pulchra* | CBS 124082 | **GU296137** | **GU301800** | | **GU371772** | **GU349066** |
| *Astrosphaeriella aggregata* | MAFF 239486 | AB524450 | AB524591 | | AB539105 | AB539092 |
| *Astrosphaeriella bakeriana* | CBS 115556 | | **GU301801** | **GU357752** | | **GU349015** |
| *Astrothelium cinnamomeum* | DUKE 0000007 | | AY584652 | | | DQ782896 |
| *Aulographina pinorum* 1 | CBS 302.71 | | | | **GU371766** | |
| *Aulographina pinorum* 2 | CBS 174.90 | **GU296138** | **GU301802** | **GU357763** | **GU371737** | **GU349046** |
| *Aureobasidium pullulans* | CBS 584.75 | DQ471004 | DQ470956 | DQ471148 | DQ470906 | DQ471075 |
| *Bagnisiella examinans* | CBS 551.66 | **GU296139** | **GU301803** | **GU357776** | **GU371746** | **GU349056** |
| *Batcheloromyces proteae* | CBS 110696 | AY251102 | EU019247 | | | |
| *Beverwykella pulmonaria* | CBS 283.53 | | **GU301804** | | **GU371768** | |
| *Bimuria novae-zelandiae* | CBS 107.79 | AY016338 | AY016356 | DQ471159 | DQ470917 | DQ471087 |
| *Botryosphaeria dothidea* | CBS 115476 | DQ677998 | DQ678051 | **GU357802** | DQ677944 | DQ767637 |
| *Botryosphaeria tsugae* | CBS 418.64 | AF271127 | DQ767655 | | DQ767644 | DQ677914 |
| *Byssolophis sphaerioides* | IFRDCC2053 | **GU296140** | **GU301805** | | GU456348 | GU456263 |
| *Byssothecium circinans* | CBS 675.92 | AY016339 | AY016357 | | DQ767646 | **GU349061** |
| *Camarosporium quaternatum* | CBS 483.95 | **GU296141** | **GU301806** | **GU357761** | | **GU349044** |
| *Capnobotryella renispora* | CBS 215.90 | AY220613 | GQ852582 | | | |
| *Capnodium coffeae* | CBS 147.52 | DQ247808 | DQ247800 | DQ471162 | DQ247788 | DQ471089 |
| *Capnodium salicinum* | CBS 131.34 | DQ677997 | DQ678050 | | | DQ677889 |
| *Catenulostroma abietis* (as *Trimmatostroma abietis*) | CBS 459.93 | DQ678040 | DQ678092 | **GU357797** | | DQ677933 |
| *Catenulostroma elginense* | CBS 111030 | GU214517 | EU019252 | | | |
| *Catinella olivacea* | UAMH 10679 | DQ915484 | EF622212 | | | |
| *Cenococcum geophilum* 1 | HUNT A1 | L76616 | | | | |
| *Cenococcum geophilum* 2 | CGMONT | L76617 | | | | |
| *Cenococcum geophilum* 3 | 10 | L76618 | | | | |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Cercospora beticola* | CBS 116456 | DQ678039 | DQ678091 | | | DQ677932 |
| *Chaetosphaeronema hispidulum* | CBS 216.75 | EU754045 | EU754144 | **GU357808** | **GU371777** | |
| *Cladosporium cladosporioides* | CBS 170.54 | DQ678004 | DQ678057 | **GU357790** | DQ677952 | DQ677898 |
| *Cladosporium iridis* (teleomorph *Davidiella macrospora*) | CBS 138.40 | | DQ008148 | | | |
| *Clathrospora elynae* | CBS 196.54 | **GU296142** | **GU323214** | | | |
| *Cochliobolus heterostrophus* | CBS 134.39 | AY544727 | AY544645 | | DQ247790 | DQ497603 |
| *Cochliobolus sativus* | DAOM 226212 | DQ677995 | DQ678045 | | DQ677939 | |
| *Columnosphaeria fagi* | CBS 171.93 | AY016342 | AY016359 | | DQ677966 | |
| *Comminutispora agavaciensis* | CBS 619 95 | Y18699 | EU981286 | | | |
| *Conidioxyphium gardeniorum* | CPC 14327 | **GU296143** | **GU301807** | **GU357774** | **GU371743** | **GU349054** |
| *Coniothyrium palmarum* | CBS 400.71 | DQ678008 | DQ767653 | | DQ677956 | DQ677903 |
| *Corynespora cassiicola* 1 | CBS 100822 | **GU296144** | **GU301808** | **GU357772** | **GU371742** | **GU349052** |
| *Corynespora cassiicola* 2 | CCP | **GU296145** | | | | |
| *Corynespora olivacea* | CBS 114450 | | **GU301809** | | | **GU349014** |
| *Corynespora smithii* | CABI 5649b | | **GU323201** | **GU371804** | **GU371783** | **GU349018** |
| *Cryptothelium amazonum* | 47 | | GU327713 | | | GU327731 |
| *Cryptothelium pulchrum* | 63C | | GU327714 | | | |
| *Cystocoleus ebeneus* 1 | L348 | EU048573 | EU048580 | | | |
| *Cystocoleus ebeneus* 2 | L315 | EU048572 | | | | |
| *Davidiella tassiana* | CBS 399.80 | DQ678022 | DQ678074 | **GU357793** | DQ677971 | DQ677918 |
| *Delitschia* cf. *chaetomioides* 1 | GKM 3253.2 | | **GU390656** | | | |
| *Delitschia* cf. *chaetomioides* 2 | GKM 1283 | | **GU385172** | | | |
| *Delitschia didyma* 1 (duplicate) | UME 31411 | | **DQ384090** | | | |
| *Delitschia didyma* 2 | UME 31411 | AF242264 | DQ384090 | | | |
| *Delitschia winteri* | CBS 225.62 | DQ678026 | DQ678077 | | DQ677975 | DQ677922 |
| *Delphinella strobiligena* | CBS 735.71 | | DQ470977 | DQ471175 | DQ677951 | DQ471100 |
| *Devriesia staurophora* | CBS 375.81 | EF137359 | DQ008151 | | | |
| *Devriesia strelitziae* | CBS 122379 | **GU296146** | **GU301810** | | **GU371738** | **GU349049** |
| *Didymella bryoniae* (as *Phoma cucurbitacearum)* | CBS 133.96 | | **GU301863** | | **GU371767** | |
| *Didymella exigua* | CBS 183.55 | **GU296147** | | **GU357800** | **GU371764** | |
| *Didymocrea sadasivanii* | CBS 438 65 | DQ384066 | DQ384103 | | | |
| *Diplodia mutila* (teleomorph *Botryosphaeria stevensii*) | CBS 431.82 | DQ678012 | DQ678064 | | DQ677960 | DQ677907 |
| *Dissoconium aciculare* | CBS 204.89 | GU214523 | GQ852587 | | | |
| *Dissoconium commune* (teleomorph *Mycosphaerella communis*) | CBS 110747 | GU214525 | GQ852589 | | | |
| *Dissoconium dekkeri* (teleomorph *Mycosphaerella lateralis*) | CBS 111282 | GU214531 | GU214425 | | | |
| *Dothidea hippophaës* | CBS 188.58 | U42475 | DQ678048 | **GU357801** | DQ677942 | DQ677887 |
| *Dothidea insculpta* | CBS 189.58 | DQ247810 | DQ247802 | DQ471154 | AF107800 | DQ471081 |
| *Dothidea sambuci* | DAOM 231303 | AY544722 | AY544681 | | DQ522854 | DQ497606 |
| *Dothiora cannabinae* | CBS 737.71 | DQ479933 | DQ470984 | DQ471182 | DQ470936 | DQ471107 |
| *Dothiora elliptica* | CBS 736.71 | | **GU301811** | | | **GU349013** |
| *Dothistroma septosporum* 1 ( teleomorph *Mycosphaerella pini*) | CBS 543 74 | | **GU301853** | | **GU371730** | |
| *Dothistroma septosporum* 2 | CBS 112498 | GU214533 | GQ852597 | | | |
| *Elsinoë centrolobi* | CBS 222.50 | DQ678041 | DQ678094 | **GU357798** | | DQ677934 |
| *Elsinoë phaseoli* | CBS 165.31 | DQ678042 | DQ678095 | **GU357799** | | DQ677935 |
| *Elsinoë veneta* | CBS 150.27 | DQ767651 | DQ767658 | | | DQ767641 |
| *Endosporium aviarium* | UAMH 10530 | EU304349 | EU304351 | | | |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Endosporium populi-tremuloidis* | UAMH 10529 | EU304346_ | EU304348 | | | |
| *Entodesmium rude* | CBS 650.86 | | **GU301812** | | | **GU349012** |
| *Falciformispora lignatilis* 1 | BCC 21118 | **GU371835** | **GU371827** | | | **GU371820** |
| *Falciformispora lignatilis* 2 | BCC 21117 | **GU371834** | **GU371826** | | | **GU371819** |
| *Farlowiella carmichaeliana* 2 | CBS 179.73 | **GU296148** | | | | |
| *Farlowiella carmichealiana* 1 (as anamorph *Acrogenospora sphaerocephala*) | CBS 164.76 | **GU296129** | **GU301791** | **GU357780** | **GU371748** | **GU349059** |
| *Floricola striata* | JK 5678I | **GU296149** | **GU301813** | | **GU371758** | |
| *Friedmanniomyces endolithicus* | CCFEE 522 | DQ066715 | | | | |
| *Friedmanniomyces simplex* | CBS 116775 | DQ066716 | | | | |
| *Gibbera conferta* | CBS 191.53 | **GU296150** | **GU301814** | **GU357758** | | **GU349041** |
| *Gloniopsis arciformis* | GKM L166A | **GU323180** | **GU323211** | | | |
| *Gloniopsis praelonga* 1 | CBS 112415 | FJ161134 | FJ161173 | | FJ161113 | FJ161090 |
| *Gloniopsis praelonga* 2 | CBS 123337 | FJ161154 | FJ161195 | FJ161103 | | FJ161103 |
| *Gloniopsis subrugosa* | CBS 123346 | FJ161170 | FJ161210 | **GU371808** | FJ161131 | |
| *Glonium circumserpens* 1 | CBS 123342 | FJ161168 | FJ161208 | | | |
| *Glonium circumserpens* 2 | CBS 123343 | FJ161160 | FJ161200 | **GU371806** | FJ161126 | FJ161108 |
| *Glonium stellatum* | CBS 207.34 | FJ161140 | FJ161179 | | | FJ161095 |
| *Guignardia bidwellii* | CBS 237.48 | DQ678034 | DQ678085 | **GU357794** | DQ677983 | |
| *Guignardia citricarpa* | CBS 102374 | **GU296151** | **GU301815** | **GU357773** | | **GU349053** |
| *Guignardia gaultheriae* | CBS 447.70 | | DQ678089 | **GU357796** | DQ677987 | |
| *Halomassarina ramunculicola* 1 (as *Massarina ramunculicola*) | BCC 18404 | GQ925838 | GQ925853 | | | |
| *Halomassarina ramunculicola* 2 (as *Massarina ramunculicola*) | BCC 18405 | GQ925839 | GQ925854 | | | |
| *Halomassarina thalassiae* (as *Massarina thalassia*) | JK 5262D | | **GU301816** | | | **GU349011** |
| *Helicomyces roseus* | CBS 283.51 | DQ678032 | DQ678083 | | DQ677981 | DQ677928 |
| *Hortaea acidophila* | CBS 113389 | | **GU323202** | **GU357768** | | |
| *Hortaea werneckii* | CBS 708.76 | **GU296153** | **GU301818** | **GU357779** | **GU371747** | **GU349058** |
| *Hortaea werneckii* | CBS 100496 | **GU296152** | **GU301817** | | **GU371739** | **GU349050** |
| *Hysterium angustatum* | CBS 123334 | FJ161167 | FJ161207 | | FJ161129 | FJ161111 |
| *Hysterium barrianum* 1 | ANM 1495 | **GU323182** | GQ221885 | | | |
| *Hysterium barrianum* 2 | ANM 1442 | **GU323181** | GQ221884 | | | |
| *Hysterobrevium mori* 1 | CBS 123336 | FJ161164 | FJ161204 | | | |
| *Hysterobrevium mori* 2 | SMH 5273 | | **GU301820** | | | GQ221936 |
| *Hysterobrevium mori* 3 | GKM 1013 | | **GU301819** | | | **GU397338** |
| *Hysterobrevium smilacis* 1 | CBS 114601 | FJ161135 | FJ161174 | **GU357806** | FJ161114 | FJ161091 |
| *Hysterobrevium smilacis* 2 | SMH 5280 | **GU323183** | GQ221912 | **GU371810** | **GU371784** | |
| *Hysteropatella clavispora* | CBS 247.34 | DQ678006 | AY541493 | | DQ677955 | DQ677901 |
| *Hysteropatella elliptica* | CBS 935.97 | EF495114 | DQ767657 | | DQ767647 | DQ767640 |
| *Jahnula aquatica* | R68-1 | EF175633 | EF175655 | | | |
| *Jahnula bipileata* | F49-1 | EF175635 | EF175657 | | | |
| *Jahnula seychellensis* | SS2113.1 | EF175644 | EF175665 | | | |
| *Julella avicenniae* 1 | BCC 18422 | **GU371831** | **GU371823** | | **GU371787** | **GU371816** |
| *Julella avicenniae* 2 | BCC 20173 | **GU371830** | **GU371822** | | **GU371786** | **GU371815** |
| *Kabatiella caulivora* | CBS 242.64 | EU167576 | EU167576 | **GU357765** | | |
| *Kalmusia scabrispora* 1 | MAFF 239517 | AB524452 | AB524593 | | AB539093 | AB539106 |
| *Kalmusia scabrispora* 2 | NBRC 106237 | AB524453 | AB524594 | | AB539094 | AB539107 |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Karstenula rhodostoma* | CBS 690.94 | **GU296154** | **GU301821** | | **GU371788** | **GU349067** |
| *Katumotoa bambusicola* | MAFF 239641 | AB524454 | AB524595 | | AB539095 | AB539108 |
| *Keissleriella cladophila* | CBS 104.55 | **GU296155** | **GU301822** | | **GU371735** | **GU349043** |
| *Kirschsteiniothelia elaterascus* | A22-5A / HKUCC7769 | AF053727 | AY787934 | | | |
| *Kirschsteiniothelia maritima* | CBS 221.60 | | **GU323203** | | | **GU349001** |
| *Laurera megasperma* | AFTOL 2094 | | FJ267702 | | | |
| *Lentithecium aquaticum* | CBS 123099 | **GU296156** | **GU301823** | | **GU371789** | **GU349068** |
| *Lentithecium arundinaceum* | CBS 619.86 | **GU296157** | **GU301824** | | FJ795473 | |
| *Lentithecium fluviatile* | CBS 122367 | **GU296158** | **GU301825** | | | **GU349074** |
| *Lepidosphaeria nicotiae* | CBS 101341 | | DQ678067 | | DQ677963 | DQ677910 |
| *Leptosphaeria biglobosa* | CBS 303.51 | | **GU301826** | | | **GU349010** |
| *Leptosphaeria doliolum* | CBS 505.75 | **GU296159** | **GU301827** | | | **GU349069** |
| *Leptosphaeria dryadis* | CBS 643.86 | | **GU301828** | | **GU371733** | **GU349009** |
| *Leptosphaerulina argentinensis* | CBS 569.94 | | **GU301829** | **GU357759** | | **GU349008** |
| *Leptosphaerulina australis* | CBS 317.83 | **GU296160** | **GU301830** | | **GU371790** | **GU349070** |
| *Leptosphearia maculans* | DAOM 229267 | DQ470993 | DQ470946 | DQ471136 | DQ470894 | DQ471062 |
| *Leptoxyphium fumago* | CBS 123.26 | **GU296161** | **GU301831** | **GU357771** | **GU371741** | **GU349051** |
| *Letendraea helminthicola* | CBS 884.85 | AY016345 | AY016362 | | | |
| *Letendraea padouk* | CBS 485.70 | **GU296162** | AY849951 | | | |
| *Lindgomyces breviappendiculata* | HHUF 28193 | AB521733 | AB521748 | | | |
| *Lindgomyces ingoldianus* | ATCC_200398 | AB521719 | AB521736 | | | |
| *Lindgomyces rotundatus* | HHUF_27999 | AB521723 | AB521740 | | | |
| *Lophiostoma alpigenum* | GKM 1091b | | **GU385193** | | | |
| *Lophiostoma arundinis* | CBS 621.86 | DQ782383 | DQ782384 | | DQ782386 | DQ782387 |
| *Lophiostoma caulium* 1 | CBS 623.86 | **GU296163** | **GU301833** | | **GU371791** | |
| *Lophiostoma caulium* 2 | CBS 624.86 | | **GU301832** | | | **GU349007** |
| *Lophiostoma compressum* | IFRD 2014 | **GU296164** | **GU301834** | | FJ795457 | |
| *Lophiostoma crenatum* | CBS 629.86 | DQ678017 | DQ678069 | | DQ677965 | DQ677912 |
| *Lophiostoma fuckelii* | GKM 1063 | | **GU385192** | | | |
| *Lophiotrema brunneosporum* | CBS 123095 | **GU296165** | **GU301835** | | | **GU349071** |
| *Lophiotrema lignicola* | CBS 122364 | **GU296166** | **GU301836** | | | **GU349072** |
| *Lophiotrema nucula* | CBS 627.86 | **GU296167** | **GU301837** | | **GU371792** | **GU349073** |
| *Lophium elegans* | EB 0366 | **GU323184** | **GU323210** | | | |
| *Lophium mytilinum* 1 | CBS 114111 | EF596819 | EF596819 | | | |
| *Lophium mytilinum* 2 | CBS 269.34 | DQ678030 | DQ678081 | | DQ677979 | DQ677926 |
| *Loratospora aestuarii* | JK 5535B | **GU296168** | **GU301838** | | **GU371760** | |
| *Macrophomina phaseolina* | CBS 227.33 | DQ678037 | DQ678088 | | DQ677986 | DQ677929 |
| *Macrovalsaria megalospora* 1 | 178150 | FJ215707 | FJ215701 | | | |
| *Macrovalsaria megalospora* 2 | 178149 | FJ215706 | FJ215700 | | | |
| *Massaria anomia* | CBS 591.78 | **GU296169** | **GU301839** | | **GU371769** | |
| *Massaria platani* | CBS 221.37 | DQ678013 | DQ678065 | | DQ677961 | DQ677908 |
| *Massarina arundinariae* 1 | MAFF 239461 | AB524455 | AB524596 | | AB539096 | AB524817 |
| *Massarina arundinariae* 2 | NBRC 106238 | AB524456 | AB524597 | | AB539097 | AB524818 |
| *Massarina eburnea* | CBS 473.64 | **GU296170** | **GU301840** | **GU357755** | **GU371732** | **GU349040** |
| *Massarina igniaria* | CBS 845.96 | **GU296171** | **GU301841** | | **GU371793** | |
| *Massariosphaeria grandispora* | CBS 613 86 | **GU296172** | **GU301842** | **GU357747** | **GU371725** | **GU349036** |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Massariosphaeria phaeospora* | CBS 611.86 | **GU296173** | **GU301843** | | **GU371794** | |
| *Massariosphaeria typhicola* 1 | CBS 123126 | **GU296174** | **GU301844** | | **GU371795** | |
| *Massariosphaeria typhicola* 2 | KT 797 | AB521730 | AB521747 | | | |
| *Mauritiana rhizophorae* 1 | BCC 28866 | **GU371832** | **GU371824** | | **GU371796** | **GU371817** |
| *Mauritiana rhizophorae* 2 | BCC 28867 | **GU371833** | **GU371825** | | **GU371797** | **GU371818** |
| *Melanomma pulvis-pyrius* 1 | SMH 3291 | | **GU385197** | | | |
| *Melanomma pulvis-pyrius* 2 | CBS 371.75 | | **GU301845** | | **GU371798** | **GU349019** |
| *Melanomma rhododendri* | ANM 73 | | **GU385198** | | | |
| *Microthyrium microscopicum* | CBS 115976 | **GU296175** | **GU301846** | | **GU371734** | **GU349042** |
| *Microxyphium aciculiforme* | CBS 892.73 | **GU296176** | **GU301847** | **GU357762** | **GU371736** | **GU349045** |
| *Microxyphium citri* | CBS 451.66 | **GU296177** | **GU301848** | **GU357750** | **GU371727** | **GU349039** |
| *Microxyphium theae* | CBS 202.30 | **GU296178** | **GU301849** | **GU357781** | | **GU349060** |
| *Monascostroma innumerosum* | CBS 345.50 | **GU296179** | **GU301850** | | | **GU349033** |
| *Monotosporella tuberculata* | CBS 256.84 | | **GU301851** | | | **GU349006** |
| *Montagnula opulenta* | CBS 168.34 | AF164370 | DQ678086 | | DQ677984 | |
| *Mycosphaerella endophytica* | CBS 114662 | GU214538 | DQ246255 | | | |
| *Mycosphaerella eurypotami* | JK 5586J | | **GU301852** | | **GU371722** | |
| *Mycosphaerella graminicola* 1 | CBS 292.38 | DQ678033 | DQ678084 | | DQ677982 | |
| *Mycosphaerella graminicola* 2 | CBS 115943 | GU214540 | GU214436 | | | |
| *Mycosphaerella heimii* | CBS 110682 | GU214541 | GQ852604 | | | |
| *Mycosphaerella latebrosa* | CBS 687.94 | DQ848331 | GU214444 | | | |
| *Mycosphaerella marksii* | CBS 110942 | GU214549 | GQ852612 | | | |
| *Mycosphaerella punctiformis* (anamorph *Ramularia endophylla*) | CBS 113265 | DQ471017 | DQ470968 | DQ471165 | DQ470920 | DQ471092 |
| *Myriangium duriaei* | CBS 260.36 | AY016347 | DQ678059 | | DQ677954 | DQ677900 |
| *Myriangium hispanicum* | CBS 247.33 | **GU296180** | **GU301854** | **GU357775** | **GU371744** | **GU349055** |
| *Mytilinidion acicola* | EB 0349 | **GU323185** | **GU323209** | | **GU371757** | |
| *Mytilinidion andinense* | CBS 123562 | FJ161159 | FJ161199 | | FJ161125 | FJ161107 |
| *Mytilinidion californicum* | EB 0385 | **GU323186** | **GU323208** | | | |
| *Mytilinidion mytilinellum* | CBS 303.34 | FJ161144 | FJ161184 | **GU357810** | FJ161119 | FJ161100 |
| *Mytilinidion resinicola* | CBS 304.34 | FJ161145 | FJ161185 | FJ161101 | FJ161101 | FJ161120 |
| *Mytilinidion rhenanum* | EB 0341 | **GU323187** | **GU323207** | | | |
| *Mytilinidion scolecosporum* | CBS 305.34 | FJ161146 | FJ161186 | **GU357811** | FJ161121 | FJ161102 |
| *Mytilinidion thujarum* | EB 0268 | **GU323188** | **GU323206** | | | |
| *Mytilinidion tortile* | EB 0377 | **GU323189** | **GU323205** | | | |
| *Neofusicoccum ribis* (teleomorph *Botryosphaeria ribis*) | CBS 115475 | DQ678000 | DQ678053 | **GU357789** | DQ677947 | DQ677893 |
| *Neophaeosphaeria filamentosa* | CBS 102202 | GQ387516 | GQ387577 | **GU357803** | **GU371773** | **GU349084** |
| *Neottiosporina paspali* | CBS 331.37 | EU754073 | EU754172 | **GU357812** | **GU371779** | **GU349079** |
| *Oedohysterium insidens* 1 | CBS 238.34 | FJ161142 | FJ161182 | | FJ161118 | FJ161097 |
| *Oedohysterium insidens* 2 | ANM 1443 | **GU323190** | GQ221882 | **GU371811** | **GU371785** | |
| *Oedohysterium sinense* | CBS 123345 | FJ161169 | FJ161209 | **GU371807** | FJ161130 | |
| *Opegrapha dolomitica* | DUKE 0047528 | DQ883706 | | DQ883717 | DQ883714 | DQ883732 |
| *Ophiosphaerella herpotricha* | CBS 620.86 | DQ678010 | DQ678062 | | DQ677958 | DQ677905 |
| *Ophiosphaerella sasicola* | MAFF 239644 | AB524458 | AB524599 | | AB539098 | AB539111 |
| *Otthia spiraeae* 1 | CBS 114124 | EF204515 | EF204498 | | | |
| *Otthia spiraeae* 2 | CBS 113091 | EF204516 | EF204499 | **GU357777** | | |
| *Paraconiothyrium minitans* | CBS 122788 | EU754074 | EU754173 | **GU357807** | **GU371776** | **GU349083** |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Patellaria atrata* | CBS 958.97 | **GU296181** | **GU301855** | **GU357749** | **GU371726** | **GU349038** |
| *Patellaria* cf. *atrata* 1 | BCC 28876 | **GU371836** | **GU371828** | | | |
| *Patellaria* cf. *atrata* 2 | BCC 28877 | **GU371837** | **GU371829** | | | |
| *Phacellium paspali* | CBS 113093 | GU214669 | GQ852627 | | | |
| *Phaeocryptopus gaeumannii* 1 | CBS 244.38 | | | **GU357766** | **GU371740** | |
| *Phaeocryptopus gaeumannii* 2 | CBS 267.37 | EF114722 | EF114698 | **GU357770** | | |
| *Phaeocryptopus nudus* | CBS 268.37 | **GU296182** | **GU301856** | **GU357745** | | **GU349034** |
| *Phaeodothis winteri* | CBS 182.58 | **GU296183** | **GU301857** | | | **DQ677917** |
| *Phaeosclera dematioides* | CBS 157.81 | **GU296184** | **GU301858** | **GU357764** | | **GU349047** |
| *Phaeosphaeria ammophilae* | CBS 114595 | **GU296185** | **GU301859** | **GU357746** | **GU371724** | **GU349035** |
| *Phaeosphaeria avenaria* | DAOM 226215 | AY544725 | AY544684 | | DQ677941 | DQ677885 |
| *Phaeosphaeria brevispora* 1 | NBRC 106240 | AB524460 | AB524601 | | AB539100 | AB539113 |
| *Phaeosphaeria brevispora* 2 | MAFF 239276 | AB524459 | AB524600 | | AB539099 | AB539112 |
| *Phaeosphaeria caricis* | CBS 120249 | | **GU301860** | | | **GU349005** |
| *Phaeosphaeria eustoma* | CBS 573.86 | DQ678011 | DQ678063 | | DQ677959 | DQ677906 |
| *Phaeosphaeria juncicola* | CBS 595.86 | | | | | **GU349016** |
| *Phaeosphaeria luctuosa* | CBS 308.79 | | **GU301861** | | | **GU349004** |
| *Phaeosphaeria nodorum* | Broad | Genome | Genome | Genome | Genome | Genome |
| *Phaeosphaeriopsis musae* | CBS 120026 | **GU296186** | **GU301862** | **GU357748** | | **GU349037** |
| *Phaeotrichum benjaminii* | CBS 541.72 | AY016348 | AY004340 | **GU357788** | DQ677946 | DQ677892 |
| *Phoma betae* | CBS 109410 | EU754079 | EU754178 | **GU357804** | **GU371774** | **GU349075** |
| *Phoma complanata* | CBS 268.92 | EU754081 | EU754180 | **GU357809** | **GU371778** | **GU349078** |
| *Phoma exigua* | CBS 431.74 | EU754084 | EU754183 | **GU357813** | **GU371780** | **GU349080** |
| *Phoma glomerata* | CBS 528.66 | EU754085 | EU754184 | | **GU371781** | **GU349081** |
| *Phoma herbarum* | CBS 276.37 | DQ678014 | DQ678066 | **GU357792** | DQ677962 | DQ677909 |
| *Phoma heteromorphospora* | CBS 115.96 | EU754089 | EU754188 | | **GU371775** | **GU349077** |
| *Phoma radicina* | CBS 111.79 | EU754092 | EU754191 | **GU357805** | | **GU349076** |
| *Phoma zeae-maydis* | CBS 588.69 | EU754093 | EU754192 | **GU357814** | **GU371782** | **GU349082** |
| *Piedraia hortae* | CBS 480.64 | AY016349 | AY016366 | | DQ677990 | |
| *Pleomassaria siparia* | CBS 279.74 | DQ678027 | DQ678078 | | DQ677976 | DQ677923 |
| *Pleospora ambigua* | CBS 113979 | | AY787937 | **GU357760** | | |
| *Pleospora herbarum* | CBS 191.86 | DQ247812 | DQ247804 | DQ471163 | DQ247794 | DQ471090 |
| *Polyplosphaeria fusca* | MAFF 239685 | AB524463 | AB524604 | | | |
| *Polythrincium trifolii* (as *Cymadothea trifolii*) | 133 | EU167612 | EU167612 | | | |
| *Preussia funiculata* | CBS 659.74 | **GU296187** | **GU301864** | | **GU371799** | **GU349032** |
| *Preussia lignicola* (as *Sporormia lignincola*) | CBS 264.69 | **GU296197** | **GU301872** | | **GU371765** | **GU349027** |
| *Preussia terricola* | DAOM 230091 | AY544726 | AY544686 | DQ471137 | DQ470895 | DQ471063 |
| *Pseudocercospora fijiensis* (teleomorph *Mycosphaerella fijiensis*) | OSC 100622 | DQ767652 | DQ678098 | | DQ677993 | |
| *Pseudocercospora griseola* f. *griseola* | CPC 10461 | **GU323191** | **GU348997** | | | |
| *Pseudocercospora vitis* | CPC 11595 | DQ289864 | GU214483 | | | |
| *Pseudotetraploa curviappendiculata* | MAFF 239495 | AB524467 | AB524608 | | | |
| *Psiloglonium araucanum* | CBS 112412 | FJ161133 | FJ161172 | **GU357743** | FJ161112 | FJ161089 |
| *Psiloglonium clavisporum* 1 | CBS 123338 | FJ161156 | FJ161197 | | FJ161123 | |
| *Psiloglonium clavisporum* 2 | GKM L172A | **GU323192** | **GU323204** | | | |
| *Psiloglonium simulans* | CBS 206.34 | FJ161139 | FJ161178 | | FJ161116 | FJ161094 |
| *Pyrenochaeta nobilis* | CBS 407.76 | | DQ678096 | | DQ677991 | DQ677936 |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Pyrenophora phaeocomes* | DAOM 222769 | DQ499595 | DQ499596 | | DQ497614 | DQ497607 |
| *Pyrenophora tritici-repentis* 1 | OSC 100066 | | AY544672 | | | DQ677882 |
| *Pyrenophora tritici-repentis* 2 | CBS 328.53 | | | | | **GU349017** |
| *Quadricrura septentrionalis* | CBS 125429 | AB524474 | AB524615 | | | |
| *Quintaria lignatilis* | CBS 117700 | **GU296188** | **GU301865** | | **GU371761** | |
| *Quintaria submersa* | CBS 115553 | | **GU301866** | **GU357751** | | **GU349003** |
| *Racodium rupestre* 1 | L423 | EU048576 | EU048581 | | | |
| *Racodium rupestre* 2 | L424 | EU048577 | EU048582 | | | |
| *Ramichloridium apiculatum* | CBS 156.59 | **GU296189** | | | **GU371770** | |
| *Ramichloridium cerophilum* | CBS 103.59 | **GU296190** | EU041855 | | | |
| *Rasutoria tsugae* | ratstk | EF114730 | EF114705 | **GU371809** | | |
| *Rhytidhysterium rufulum* 2 | CBS 306.38 | **GU296191** | FJ469672 | FJ238444 | | **GU349031** |
| *Rhytidhysteron rufulum* 1 | GKM 361A | **GU296192** | **GU301867** | | | |
| *Rimora mangrovei* | JK 5246A | **GU296193** | **GU301868** | | **GU371759** | |
| rock isolate TRN 111 | CBS 118294 | **GU323193** | **GU323220** | **GU357783** | **GU371751** | **GU349088** |
| rock isolate TRN 123 | CBS 117932 | **GU323194** | **GU323219** | **GU357784** | **GU371753** | |
| rock isolate TRN 137 | CBS 118300 | **GU323195** | **GU323218** | **GU357782** | **GU371749** | |
| rock isolate TRN 138 | CBS 118301 | **GU323196** | **GU323217** | | **GU371750** | |
| rock isolate TRN 152 | CBS 118346 | **GU323197** | **GU323223** | | **GU371752** | |
| rock isolate TRN 211 | CBS 117937 | **GU323198** | **GU323222** | **GU357785** | **GU371754** | |
| rock isolate TRN 235 | CBS 118605 | **GU323199** | | **GU357787** | **GU371756** | **GU349087** |
| rock isolate TRN 43 | CBS 117950 | **GU323200** | **GU323221** | **GU357786** | **GU371755** | **GU349086** |
| *Roussoella hysterioides* 1 | MAFF 239636 | AB524480 | AB524621 | | AB539101 | AB539114 |
| *Roussoella hysterioides* 2 | CBS 125434 | AB524481 | AB524622 | | AB539102 | AB539115 |
| *Roussoella pustulans* | MAFF 239637 | AB524482 | AB524623 | | AB539103 | AB539116 |
| *Roussoellopsis tosaensis* | MAFF 239638 | | AB524625 | | AB539104 | AB539117 |
| *Saccharata proteae* | CBS 115206 | **GU296194** | **GU301869** | **GU357753** | **GU371729** | **GU349030** |
| *Saccothecium sepincola* | CBS 278.32 | **GU296195** | **GU301870** | | **GU371745** | **GU349029** |
| *Schismatomma decolorans* | DUKE 0047570 | AY548809 | AY548815 | | DQ883715 | DQ883725 |
| *Schizothyrium pomi* 1 | CBS 406.61 | EF134949 | EF134949 | | | |
| *Schizothyrium pomi* 2 | CBS 486.50 | EF134948 | EF134948 | | | |
| *Schizothyrium pomi* 3 | CBS 228.57 | EF134947 | EF134947 | | | |
| *Scorias spongiosa* | CBS 325.33 | DQ678024 | DQ678075 | | DQ677973 | DQ677920 |
| *Setomelanomma holmii* | CBS 110217 | **GU296196** | **GU301871** | | **GU371800** | **GU349028** |
| *Setosphaeria monoceras* | AY016368 | | AY016368 | | | |
| *Spencermartinsia viticola* (teleomorph *Botryosphaeria viticola*) | CBS 117009 | DQ678036 | DQ678087 | **GU357795** | DQ677985 | |
| *Sporormiella minima* | CBS 524.50 | DQ678003 | DQ678056 | | DQ677950 | DQ677897 |
| *Stagonospora macropycnidia* | CBS 114202 | **GU296198** | **GU301873** | | | **GU349026** |
| *Stylodothis puccinioides* | CBS 193.58 | | AY004342 | FJ238427 | | DQ677886 |
| *Sydowia polyspora* | CBS 116.29 | DQ678005 | DQ678058 | **GU357791** | DQ677953 | DQ677899 |
| *Teratosphaeria associata* (as *Teratosphaeria jonkershoekensis*) | CBS 112224 | **GU296200** | **GU301874** | **GU357744** | **GU371723** | **GU349025** |
| *Teratosphaeria cryptica* (as *Mycosphaerella cryptica*) | CBS 110975 | GU214602 | GQ852682 | | | |
| *Teratosphaeria fibrillosa* 1 | CBS 121707 | **GU296199** | **GU323213** | **GU357767** | | |
| *Teratosphaeria fibrillosa* 2 | CPC 1876 | | GU214506 | | | |
| *Teratosphaeria stellenboschiana* (as *Colletogloeopsis stellenboschiana*) | CBS 116428 | GU214583 | EU019295 | | | |

**Table 1.** (Continued).

| Taxon | voucher/culture[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|
| *Teratosphaeria suberosa* (as *Mycosphaerella suberosa*) | CPC 11032 | GU214614 | GQ852718 | | | |
| *Tetraplosphaeria sasicola* | MAFF 239677 | AB524490 | AB524631 | | | |
| *Thyridaria rubronotata* | CBS 419.85 | | **GU301875** | | **GU371728** | **GU349002** |
| *Tremateia halophila* | JK 5517J | **GU296201** | | | **GU371721** | |
| *Trematosphaeria pertusa* | CBS 122371 | **GU348999** | **GU301876** | | **GU371801** | **GU349085** |
| *Trichodelitschia bisporula* 1 | CBS 262.69 | **GU349000** | **GU348996** | **GU371812** | **GU371802** | **GU349020** |
| *Trichodelitschia bisporula* 2 (duplicate) | CBS 262.69 | **GU296202** | | | | |
| *Trichodelitschia munkii* | Kruys201 | DQ384070 | DQ384096 | | | |
| *Triplosphaeria maxima* | MAFF 239682 | AB524496 | AB524637 | | | |
| *Trypethelium nitidiusculum* 1 | 139 | | GU327728 | | | GU327732 |
| *Trypethelium nitidiusculum* 2 | AFTOL 2099 | | FJ267701 | | | |
| *Trypethelium tropicum* | 25 | | GU327730 | | | |
| *Tubeufia cerea* | CBS 254.75 | DQ471034 | DQ470982 | DQ471180 | DQ470934 | DQ471105 |
| *Tubeufia paludosa* | CBS 120503 | **GU296203** | **GU301877** | **GU357754** | **GU371731** | **GU349024** |
| *Tubeufia paludosa* (as anamorph *Helicosporium phragmitis*) | CBS 245.49 | DQ767649 | DQ767654 | | DQ767643 | DQ767638 |
| *Tyrannosorus pinicola* | CBS 124.88 | DQ471025 | DQ470974 | DQ471171 | DQ470928 | DQ471098 |
| *Ulospora bilgramii* | CBS 110020 | DQ678025 | DQ678076 | | DQ677974 | DQ677921 |
| *Venturia inaequalis* 1 | CBS 594.70 | **GU296205** | **GU301879** | **GU357757** | | **GU349022** |
| *Venturia inaequalis* 2 | CBS 815.69 | **GU296204** | **GU301878** | **GU357756** | | **GU349023** |
| *Venturia inaequalis* 3 (as *Spilocaea pomi*) | CBS 176.42 | | **GU348998** | | | **GU349089** |
| *Venturia populina* | CBS 256.38 | **GU296206** | **GU323212** | **GU357769** | | |
| *Verrucisporota daviesiae* | CBS 116002 | **GU296207** | GQ852730 | | | |
| *Verruculina enalia* | JK 5253A | DQ678028 | DQ678079 | | DQ677977 | DQ677924 |
| *Westerdykella angulata* (as *Eremodothis angulata*) | CBS 610.74 | DQ384067 | DQ384105 | **GU371805** | | **GU371821** |
| *Westerdykella cylindrica* | CBS 454.72 | AY016355 | AY004343 | DQ471168 | DQ470925 | DQ497610 |
| *Westerdykella ornata* | CBS 379.55 | **GU296208** | **GU301880** | | **GU371803** | **GU349021** |
| *Wettsteinina lacustris* | CBS 618.86 | DQ678023 | | | DQ677972 | DQ677919 |
| *Wicklowia aquatica* | AF289-1 | | GU045446 | | | |
| *Wicklowia aquatica* | CBS 125634 | GU266232 | GU045445 | **GU371813** | | |
| *Zasmidium cellare* | CBS 146.36 | EF137362 | EU041878 | | | |
| *Zopfia rhizophila* | CBS 207.26 | DQ384086 | DQ384104 | | | |

[1]BCC: Belgian Coordinated Collections of Microorganisms; CABI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DUKE: Duke University Herbarium, Durham, North Carolina, U.S.A.; HHUF: Herbarium of Hirosaki University, Japan; IFRDCC: Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Japan; NBRC: NITE Biological Resource Centre, Japan; OSC: Oregon State University Herbarium, U.S.A.; UAMH: University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada; UME: Herbarium of the University of Umeå, Umeå, Sweden; Culture and specimen abbreviations: ANM: A.N. Miller; CPC; P.W. Crous; EB: E.W.A. Boehm; EG: E.B.G. Jones; GKM: G.K. Mugambi; JK: J. Kohlmeyer; KT: K. Tanaka; SMH: S.M. Huhndorf.

## SUPPLEMENTARY INFORMATION

**Table 2.** Genomes used for phylogenetic profile. All are opisthokonts; remaining classifications used in Fig. 4 are indicated in columns: Do – *Dothideomycetes,* ED - *Eurotiomycetes* & *Dothideomycetes*, S – Saccharomyceta, A – *Ascomycota,* Di – *Dikarya,* MD - *Mucoromycotina & Dikarya*, CMD - *Chytridiomycota,* F - *Fungi*.

| **Genomes** | **Classifications** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Alternaria brassicicola* | Do | ED | S | A | Di | MD | CMD | F |
| *Cochliobolus heterostrophus* | Do | ED | S | A | Di | MD | CMD | F |
| *Mycosphaerella fijiensis* | Do | ED | S | A | Di | MD | CMD | F |
| *Mycosphaerella graminicola* | Do | ED | S | A | Di | MD | CMD | F |
| *Pyrenophora tritici-repentis* | Do | ED | S | A | Di | MD | CMD | F |
| *Stagonospora nodorum* | Do | ED | S | A | Di | MD | CMD | F |
| *Aspergillus fumigatus* | | ED | S | A | Di | MD | CMD | F |
| *Aspergillus nidulans* | | ED | S | A | Di | MD | CMD | F |
| *Aspergillus terreus* | | ED | S | A | Di | MD | CMD | F |
| *Coccidioides immitis* | | ED | S | A | Di | MD | CMD | F |
| *Histoplasma capsulatum* | | ED | S | A | Di | MD | CMD | F |
| *Uncinocarpus reesii* | | ED | S | A | Di | MD | CMD | F |
| *Ashbya gossypii* | | | S | A | Di | MD | CMD | F |
| *Botrytis cinerea* | | | S | A | Di | MD | CMD | F |
| *Candida albicans* | | | S | A | Di | MD | CMD | F |
| *Candida glabrata* | | | S | A | Di | MD | CMD | F |
| *Candida guilliermondii* | | | S | A | Di | MD | CMD | F |
| *Candida lusitaniae* | | | S | A | Di | MD | CMD | F |
| *Chaetomium globosum* | | | S | A | Di | MD | CMD | F |
| *Debaryomyces hansenii* | | | S | A | Di | MD | CMD | F |
| *Fusarium graminearum* | | | S | A | Di | MD | CMD | F |
| *Fusarium oxysporum* | | | S | A | Di | MD | CMD | F |
| *Fusarium verticillioides* | | | S | A | Di | MD | CMD | F |
| *Kluyveromyces lactis* | | | S | A | Di | MD | CMD | F |
| *Laccaria bicolor* | | | S | A | Di | MD | CMD | F |
| *Lodderomyces elongisporus* | | | S | A | Di | MD | CMD | F |
| *Magnaporthe grisea* | | | S | A | Di | MD | CMD | F |
| *Nectria haematococca* | | | S | A | Di | MD | CMD | F |
| *Neurospora crassa* | | | S | A | Di | MD | CMD | F |
| *Pichia stipitis* | | | S | A | Di | MD | CMD | F |
| *Podospora anserina* | | | S | A | Di | MD | CMD | F |
| *Saccharomyces cerevisiae* | | | S | A | Di | MD | CMD | F |
| *Sclerotinia sclerotiorum* | | | S | A | Di | MD | CMD | F |
| *Sporobolomyces roseus* | | | S | A | Di | MD | CMD | F |
| *Trichoderma atroviride* | | | S | A | Di | MD | CMD | F |
| *Trichoderma reseei* | | | S | A | Di | MD | CMD | F |
| *Trichoderma virens* | | | S | A | Di | MD | CMD | F |
| *Verticillium dahliae* | | | S | A | Di | MD | CMD | F |
| *Yarrowia lipolytica* | | | S | A | Di | MD | CMD | F |
| *Schizosaccharomyces japonicus* | | | | A | Di | MD | CMD | F |
| *Schizosaccharomyces octosporus* | | | | A | Di | MD | CMD | F |
| *Schizosaccharomyces pombe* | | | | A | Di | MD | CMD | F |
| *Coprinus cinereus* | | | | | Di | MD | CMD | F |
| *Cryptococcus neoformans* | | | | | Di | MD | CMD | F |
| *Phanerochaete chrysosporium* | | | | | Di | MD | CMD | F |

**Table 1.** (Continued).

| Genomes | Classifications | | | |
|---|---|---|---|---|
| *Postia placenta* | Di | MD | CMD | F |
| *Puccinia graminis* f. *sp. tritici* | Di | MD | CMD | F |
| *Ustilago maydis* | Di | MD | CMD | F |
| *Phycomyces blakesleeanus* | | MD | CMD | F |
| *Rhizopus oryzae* | | MD | CMD | F |
| *Batrachochytrium dendrobatidis* | | | CMD | F |
| *Encephalitozoon cuniculi* | | | | F |
| *Drosophila melanogaster* | | | | |

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# Phylogenetic lineages in the *Capnodiales*

P.W. Crous[1, 2*], C.L. Schoch[3], K.D. Hyde[4], A.R. Wood[5], C. Gueidan[1], G.S. de Hoog[1] and J.Z. Groenewald[1]

[1]*CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands;* [2]*Wageningen University and Research Centre (WUR), Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands;* [3]*National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, U.S.A.;* [4]*School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand;* [5]*ARC – Plant Protection Research Institute, P. Bag X5017, Stellenbosch, 7599, South Africa*

**Correspondence*: Pedro W. Crous, p.crous@cbs.knaw.nl

**Abstract:** The *Capnodiales* incorporates plant and human pathogens, endophytes, saprobes and epiphytes, with a wide range of nutritional modes. Several species are lichenised, or occur as parasites on fungi, or animals. The aim of the present study was to use DNA sequence data of the nuclear ribosomal small and large subunit RNA genes to test the monophyly of the *Capnodiales*, and resolve families within the order. We designed primers to allow the amplification and sequencing of almost the complete nuclear ribosomal small and large subunit RNA genes. Other than the *Capnodiaceae* (sooty moulds), and the *Davidiellaceae*, which contains saprobes and plant pathogens, the order presently incorporates families of major plant pathological importance such as the *Mycosphaerellaceae*, *Teratosphaeriaceae* and *Schizothyriaceae*. The *Piedraiaceae* was not supported, but resolves in the *Teratosphaeriaceae*. The *Dissoconiaceae* is introduced as a new family to accommodate *Dissoconium* and *Ramichloridium*. Lichenisation, as well as the ability to be saprobic or plant pathogenic evolved more than once in several families, though the taxa in the upper clades of the tree lead us to conclude that the strictly plant pathogenic, nectrotrophic families evolved from saprobic ancestors (*Capnodiaceae*), which is the more primitive state.

**Key words:** *Ascomycetes*, *Brunneosphaerella*, *Capnodiales*, DNA sequence comparisons, *Mycosphaerella*, novel primers, systematics.
**Taxonomic novelties:** *Brunneosphaerella* Crous, gen. nov., *B. jonkershoekensis* (Marinc., M.J. Wingf. & Crous) Crous, comb. nov., *B. protearum* (Syd. & P. Syd.) Crous, comb. nov., *Devriesia hilliana* Crous & U. Braun, sp. nov., *D. lagerstroemiae* Crous & M.J. Wingf., sp. nov., *D. strelitziicola* Arzanlou & Crous, sp. nov., *Dissoconiaceae* Crous & de Hoog, fam. nov., *Hortaea thailandica* Crous & K.D. Hyde, sp. nov., *Passalora ageratinae* Crous & A.R. Wood, sp. nov., *P. armatae* Crous & A.R. Wood, sp. nov., *Rachicladosporium cboliae* Crous, sp. nov.

## INTRODUCTION

The *Dothideomycetes* encompasses plant and human pathogens, endophytes, saprobes and epiphytes. The class presently contains two subclasses, namely *Pleosporomycetidae* and *Dothideomycetidae* (Schoch *et al*. 2006, 2009a). Although the main orders, *Pleosporales* and *Dothideales* correlate with the presence or absence of pseudoparaphyses and other centrum characteristics, many orders remain unresolved. The *Dothideomycetidae* include the orders *Dothideales*, *Capnodiales* and *Myriangiales*, which lack paraphyses, pseudoparaphyses and periphysoids. Based on a multi-gene phylogeny, and the presence of ostiolar periphyses as possible synapomorphy, the *Capnodiales* were recognised as the order incorporating the *Capnodiaceae*, *Davidiellaceae*, *Mycosphaerellaceae* and *Piedraiaceae* (Schoch *et al*. 2006). However, several studies (Hunter *et al.* 2006, Crous *et al*. 2007a, b) showed the *Mycosphaerellaceae* to be polyphyletic, and to contain additional variation at the familial level, leading to the circumscriptions of the *Teratosphaeriaceae* and *Schizothyriaceae*. Crous *et al.* (2009b, c) again revealed *Teratosphaeriaceae* to be too widely defined, including some further unresolved families.

The present study focuses on the *Capnodiales*, which is based on the *Capnodiaceae*, representing a group of leaf epiphytes associated with honeydew of insects, usually visible as a black growth on leaf surfaces, fruit and twigs. Members of the *Capnodiaceae* form superficial ascomata with fasciculate asci, and hyaline to dark, septate ascospores. Anamorphs are dematiaceous, and include mycelial (phragmo- to dictyoconidia), spermatial and pycnidial synanamorphs (Hughes 1976, Cheewangkoon *et al.* 2009).

The *Mycosphaerellaceae* was treated as a family in the *Dothideales* by Hawksworth *et al*. (1995), while Kirk *et al*. (2001) introduced a separate order, the *Mycosphaerellales* for this family, and Kirk *et al*. (2008) again placed it in the *Capnodiales*. The *Mycosphaerellaceae* is recognised by having characteristic pseudothecial ascomata that can be immersed or superficial, embedded in host tissue or erumpent, having ostiolar periphyses, but lacking interascal tissue at maturity. Ascospores are hyaline, but in some cases slightly pigmented (Barr 1987), and predominantly 1-septate, although some taxa with 3-septate ascospores have been recorded (Crous *et al.* 2003). Although up to 30 anamorph genera have been linked to *Mycosphaerella* (Crous *et al*. 2000, 2001, 2007a–c, 2009a–c, Crous 2009), recent studies have shown this to be incorrect, and that the family in fact consists of numerous genera with morphologically conserved *Mycosphaerella*-like teleomorphs, and distinct anamorphs (Crous *et al*. 2007a, b, 2009b, c).

Families tentatively placed in the *Capnodiales* (Lumbsch & Huhndorf 2007, Kirk *et al*. 2008) include epiphytes (*Antennulariellaceae, Capnodiaceae*, *Metacapnodiaceae*) (Hughes 1976), saprobes and plant pathogens (*Davidiellaceae*, *Dissoconiaceae*, *Mycosphaerellaceae*, *Schizothyriaceae, Teratosphaeriaceae*) (Aptroot 2006, Crous 2009), and colonisers or hair shafts of mammals (*Piedraiaceae*) (de Hoog *et al*. 2000). To address the status of the *Capnodiales* as an order, and the intrafamilial relationships within this order, DNA sequences of

the 18S, 5.8S and 28S nrRNA genes were generated for a set of specifically selected taxa. A further aim was to clarify genera within these families, and resolve anamorph-teleomorph relationships for the taxa investigated.

## MATERIALS AND METHODS

### Isolates

Isolates were selected (Table 1 - see online Supplementary Information) that are representative of the *Mycosphaerellaceae* (Crous 1998, Crous *et al*. 2004a, c, 2006a, b, 2007a), *Schizothyriaceae* (Batzer *et al.* 2005, 2007), *Teratosphaeriaceae* (Crous *et al.* 2007a, 2008b, c, 2009a–c), *Piedraiaceae* (Kruys *et al.* 2006), *Davidiellaceae* (Braun *et al*. 2003, Schubert *et al*. 2007a, b), *Capnodiaceae* (Schoch *et al*. 2006), as well as numerous other genera for which the familial relationships have remained unclear, such as the *Phaeophleospora* complex (Crous *et al.* 1997, 2007a, 2009b, c, Andjic *et al.* 2007), *Polythrincium* (Simon *et al.* 2009), the *Dissoconium* complex (Crous *et al.* 2004c, 2007c, 2008b, Arzanlou *et al*. 2008b), and several less well-known genera represented by one or two species only. For fresh material excised leaf spots bearing ascomata were soaked in water for approximately 2 h, after which they were placed in the bottom of Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA; Crous *et al*. 2009d). Ascospore germination patterns were examined after 24 h, and single-ascospore and conidial cultures established as described by Crous *et al*. (1991). Colonies were sub-cultured onto synthetic nutrient-poor agar (SNA), potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Crous *et al.* 2009d), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Other cultures were obtained from the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW) in Utrecht, the Netherlands or the working collection of Pedro Crous (CPC).

### DNA isolation, amplification and molecular phylogeny

Genomic DNA was extracted from mycelium taken from fungal colonies on MEA using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, U.S.A.). A part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bp at the 5' end of the 28S rRNA gene (LSU) was amplified and sequenced as described by Cheewangkoon *et al*. (2008) standard for all strains included (Table 1). For selected strains (see Table 1), the almost complete SSU and LSU (missing the first and last 20–30 nucleotides) were amplified and sequenced using novel and previously published primers (Table 2; see below).

Novel primers were designed using a variety of complete SSU and LSU sequences obtained from the GenBank sequence database (www.ncbi.nlm.nih.gov/). The selection was not limited only to fungi belonging to the *Dothideomycetes* but encompassed as many as possible full sequences in order to make the primers as robust as possible. We aimed to keep the melting temperature (Tm) of the novel primers at 40–45 °C and the GC content to approximately 50 % to keep them as compatible as possible to existing published primers. Primer parameters were calculated using the OligoAnalyzer tool on the web site of Integrated DNA Technologies (http://eu.idtdna.com/analyzer/Applications/OligoAnalyzer/) with the "Oligo Conc" parameter set at 0.2 mM and the "Na+ Conc" parameter set at 16 mM. A framework of existing and novel primers was then aligned onto the sequence of *Magnaporthe grisea* (GenBank accession AB026819) to derive primer positions (Table 2) and evaluate coverage over the gene regions. These primers were amplified and sequenced in the following overlapping sections to cover the almost complete SSU and LSU for the selected strains (Table 2): SSU1Fd or SSU6Fm with SSU2Rd, SSU2Fd with SSU3Rd, SSU7Fm with SSU4Rd or SSU6Rm, SSU4Fd with 5.8S1Rd, V9G or LSU1Fd with LSU3Rd, LSU8Fd with LSU8Rd, LSU4Fd with LSU5Rd, and LSU5Fd with LSU7Rd. For some strains (Table 3) it was necessary to add an additional overlap for SSU4Fd with 5.8S1Rd (using SSU4Fd with SSU7Rm and SSU8Fm with 5.8S1Rd), for LSU8Fd with LSU8Rd (using LSU8Fd with LSU3Rd and LSU3Fd with LSU8Rd), and for LSU5Fd with LSU7Rd (using LSU5Fd with LSU6Rd and LSU6Fd with LSU7Rd) to complete the gaps due to large insertions.

The internal transcribed spacer regions, as well as all insertions (Table 3) were excluded from all analyses. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org). Two separate analyses were performed: The first using only partial LSU data due to the limited number of complete LSU sequences available and the second using the almost complete SSU, 5.8S nrDNA and LSU alignment.

Maximum likelihood analyses (ML) were conducted in RAxML v. 7.0.4 (Stamatakis 2006) for the partial LSU alignment. A general time reversible model (GTR) with a discrete gamma distribution and four rate classes was applied. A tree was obtained by simultaneously running a fast bootstrap search of 1000 pseudoreplicates (Stamatakis *et al*. 2008) followed by a search for the most likely tree. Maximum Likelihood bootstrap value (MLBP) equal or greater than 70 % are given at the nodes (Fig. 1).

Maximum likelihood analyses (ML) were conducted in RAxML v. 7.0.4 (Stamatakis 2006) for the almost complete SSU, 5.8S nrDNA and LSU alignment. A general time reversible model (GTR) with a discrete gamma distribution and four rate classes was applied to each partition (SSU, 5.8S nrDNA and LSU). A tree was obtained by simultaneously running a fast bootstrap search of 500 pseudoreplicates (Stamatakis *et al*. 2008) followed by a search for the most likely tree. Maximum Likelihood bootstrap value (MLBP) equal or greater than 70 % are given at the nodes (Fig. 2).

### Taxonomy

Fungal structures were mounted in lactic acid, and 30 measurements (× 1000 magnification) obtained per structure type. The range obtained is presented, except for spore measurements, where the 95 % confidence intervals are given with the extremes in parentheses. Colony colours (surface and reverse) were assessed after 1–2 wk on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS-KNAW) in Utrecht, the Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al*. 2004b). Names for which the taxonomy has not been resolved, but need to be allocated to another genus, are placed in inverted commas, *e.g.* "*Mycosphaerella*" *iridis*.

**Table 2.** Details of primers used for this study and their relation to selected published primers. Primer names ending with a "d" denotes a degenerate primer whereas those ending with a "m" denotes specific primers designed based on the partial novel sequences generated. The start and end positions of the primers are derived using *Magnaporthe grisea* GenBank accession AB026819 as reference in the 5'–3' direction.

| Name | Sequence (5' – 3') | Orientation | %GC | Tm (°C) | Start | End | Reference |
|---|---|---|---|---|---|---|---|
| 5.8S1Fd | CTC TTG GTT CBV GCA TCG | Forward | 57.4 | 49.8 – 54.2 – 56.8 | 2333 | 2350 | This study |
| 5.8S1Rd | WAA TGA CGC TCG RAC AGG CAT G | Reverse | 52.3 | 57.6 – 58.9 – 60.2 | 2451 | 2472 | This study |
| F377 | AGA TGA AAA GAA CTT TGA AAA GAG AA | Forward | 26.9 | 40.3 | 3005 | 3030 | www.lutzonilab.net/primers/page244.shtml |
| ITS1 | TCC GTA GGT GAA CCT GCG G | Forward | 63.2 | 49.5 | 2162 | 2180 | White *et al*. (1990) |
| ITS1F | CTT GGT CAT TTA GAG GAA GTA A | Forward | 36.4 | 39.0 | 2124 | 2145 | Gardes & Bruns (1993) |
| ITS1Fd | CGA TTG AAT GGC TCA GTG AGG C | Forward | 54.5 | 48.0 | 2043 | 2064 | This study |
| ITS1Rd | GAT ATG CTT AAG TTC AGC GGG | Reverse | 47.6 | 43.1 | 2671 | 2691 | This study |
| ITS4 | TCC TCC GCT TAT TGA TAT GC | Reverse | 45.0 | 41.6 | 2685 | 2704 | White *et al*. (1990) |
| ITS4S | CCT CCG CTT ATT GAT ATG CTT AAG | Reverse | 41.7 | 42.9 | 2680 | 2703 | Kretzer *et al*. (1996) |
| ITS5 | GGA AGT AAA AGT CGT AAC AAG G | Forward | 40.9 | 40.8 | 2138 | 2159 | White *et al*. (1990) |
| LR0R | GTA CCC GCT GAA CTT AAG C | Forward | 52.6 | 43.2 | 2668 | 2686 | Rehner & Samuels (1994) |
| LR2 | TTT TCA AAG TTC TTT TC | Reverse | 23.5 | 28.5 | 3009 | 3025 | www.lutzonilab.net/primers/page244.shtml |
| LR2R | AAG AAC TTT GAA AAG AG | Forward | 29.4 | 30.4 | 3012 | 3028 | www.lutzonilab.net/primers/page244.shtml |
| LR3 | GGT CCG TGT TTC AAG AC | Reverse | 52.9 | 40.5 | 3275 | 3291 | Vilgalys & Hester (1990) |
| LR3R | GTC TTG AAA CAC GGA CC | Forward | 52.9 | 40.5 | 3275 | 3291 | www.lutzonilab.net/primers/page244.shtml |
| LR5 | TCC TGA GGG AAA CTT CG | Reverse | 52.9 | 41.0 | 3579 | 3595 | Vilgalys & Hester (1990) |
| LR5R | GAA GTT TCC CTC AGG AT | Forward | 47.1 | 37.8 | 3580 | 3596 | www.biology.duke.edu/fungi/mycolab/primers.htm |
| LR6 | CGC CAG TTC TGC TTA CC | Reverse | 58.8 | 43.5 | 3756 | 3772 | Vilgalys & Hester (1990) |
| LR7 | TAC TAC CAC CAA GAT CT | Reverse | 41.2 | 35.3 | 4062 | 4078 | Vilgalys & Hester (1990) |
| LR8 | CAC CTT GGA GAC CTG CT | Reverse | 58.8 | 44.3 | 4473 | 4489 | www.lutzonilab.net/primers/page244.shtml |
| LR8R | AGC AGG TCT CCA AGG TG | Forward | 58.8 | 44.3 | 4473 | 4489 | www.lutzonilab.net/primers/page244.shtml |
| LR9 | AGA GCA CTG GGC AGA AA | Reverse | 52.9 | 43.6 | 4799 | 4815 | www.lutzonilab.net/primers/page244.shtml |
| LR10 | AGT CAA GCT CAA CAG GG | Reverse | 52.9 | 41.6 | 5015 | 5031 | www.lutzonilab.net/primers/page244.shtml |
| LR10R | GAC CCT GTT GAG CTT GA | Forward | 52.9 | 41.6 | 5013 | 5029 | www.lutzonilab.net/primers/page244.shtml |
| LR11 | GCC AGT TAT CCC TGT GGT AA | Reverse | 50.0 | 43.9 | 5412 | 5431 | www.lutzonilab.net/primers/page244.shtml |
| LR12 | GAC TTA GAG GCG TTC AG | Reverse | 52.9 | 39.4 | 5715 | 5731 | Vilgalys & Hester (1990) |
| LR12R | CTG AAC GCC TCT AAG TCA GAA | Forward | 47.6 | 43.7 | 5715 | 5735 | www.biology.duke.edu/fungi/mycolab/primers.htm |
| LR13 | CAT CGG AAC AAC AAT GC | Reverse | 47.1 | 38.8 | 5935 | 5951 | www.lutzonilab.net/primers/page244.shtml |
| LR14 | AGC CAA ACT CCC CAC CTG | Reverse | 61.1 | 47.6 | 5206 | 5223 | www.lutzonilab.net/primers/page244.shtml |
| LR15 | TAA ATT ACA ACT CGG AC | Reverse | 35.3 | 32.5 | 2780 | 2796 | www.lutzonilab.net/primers/page244.shtml |
| LR16 | TTC CAC CCA AAC ACT CG | Reverse | 52.9 | 42.1 | 3311 | 3327 | Moncalvo *et al*. (1993) |
| LR17R | TAA CCT ATT CTC AAA CTT | Forward | 27.8 | 31.2 | 3664 | 3681 | www.lutzonilab.net/primers/page244.shtml |
| LR20R | GTG AGA CAG GTT AGT TTT ACC CT | Forward | 43.5 | 43.6 | 5570 | 5592 | www.lutzonilab.net/primers/page244.shtml |
| LR21 | ACT TCA AGC GTT TCC CTT T | Reverse | 42.1 | 41.7 | 3054 | 3072 | www.lutzonilab.net/primers/page244.shtml |
| LR22 | CCT CAC GGT ACT TGT TCG CT | Reverse | 55.0 | 46.8 | 2982 | 3001 | www.lutzonilab.net/primers/page244.shtml |

**Table 2.** (Continued).

| Name | Sequence (5' – 3') | Orientation | %GC | Tm (°C) | Start | End | Reference |
|---|---|---|---|---|---|---|---|
| LSU1Fd | GRA TCA GGT AGG RAT ACC CG | Forward | 55.0 | 41.8 – 44.0 – 46.3 | 2655 | 2674 | This study |
| LSU1Rd | CTG TTG CCG CTT CAC TCG C | Reverse | 63.2 | 49.6 | 2736 | 2754 | This study |
| LSU2Fd | GAA ACA CGG ACC RAG GAG TC | Forward | 57.5 | 45.5 – 46.5 – 47.6 | 3280 | 3299 | This study |
| LSU2Rd | ATC CGA RAA CWT CAG GAT CGG TCG | Reverse | 52.1 | 48.3 – 49.0 – 49.8 | 3379 | 3402 | This study |
| LSU3Fd | GTT CAT CYA GAC AGC MGG ACG | Forward | 57.1 | 44.7 – 47.4 – 50.2 | 3843 | 3863 | This study |
| LSU3Rd | CAC ACT CCT TAG CGG ATT CCG AC | Reverse | 56.5 | 49.1 | 3876 | 3898 | This study |
| LSU4Fd | CCG CAG CAG GTC TCC AAG G | Forward | 68.4 | 51.2 | 4469 | 4487 | This study |
| LSU4Rd | CGG ATC TRT TTT GCC GAC TTC CC | Reverse | 54.3 | 47.4 – 48.7 – 50.0 | 4523 | 4545 | This study |
| LSU5Fd | AGT GGG AGC TTC GGC GC | Forward | 70.6 | 51.6 | 3357 / 5072 | 3373 / 5088 | This study |
| LSU5Rd | GGA CTA AAG GAT CGA TAG GCC ACA C | Reverse | 52.0 | 48.3 | 5355 | 5379 | This study |
| LSU6Fd | CCG AAG CAG AAT TCG GTA AGC G | Forward | 54.5 | 48.1 | 5499 | 5520 | This study |
| LSU6Rd | TCT AAA CCC AGC TCA CGT TCC C | Reverse | 54.5 | 48.6 | 5543 | 5564 | This study |
| LSU7Fd | GTT ACG ATC TRC TGA GGG TAA GCC | Forward | 52.1 | 46.0 – 47.4 – 48.8 | 5943 | 5966 | This study |
| LSU7Rd | GCA GAT CGT AAC AAC AAG GCT ACT CTA C | Reverse | 46.4 | 47.9 | 5927 | 5954 | This study |
| LSU8Fd | CCA GAG GAA ACT CTG GTG GAG GC | Forward | 60.9 | 51.2 | 3469 | 3491 | This study |
| LSU8Rd | GTC AGA TTC CCC TTG TCC GTA CC | Reverse | 56.5 | 48.9 | 4720 | 4742 | This study |
| LSU9Fm | GGT AGC CAA ATG CCT CGT CAT C | Forward | 54.5 | 47.9 | 4882 | 4903 | This study |
| LSU9Rm | GAT TYT GCS AAG CCC GTT CCC | Reverse | 59.5 | 49.2 – 50.0 – 50.9 | 4979 | 4999 | This study |
| LSU10Fm | GGG AAC GTG AGC TGG GTT TAG A | Forward | 54.5 | 48.6 | 5543 | 5564 | This study |
| LSU10Rm | CGC TTA CCG AAT TCT GCT TCG G | Reverse | 54.5 | 48.1 | 5499 | 5520 | This study |
| LSU11Fm | TTTGGTAAGCAGAACTGGCGATGC | Forward | 50.0 | 49.4 | 3753 | 3776 | This study |
| LSU12Fd | GTGTGGCCTATCGATCCTTTAGTCC | Forward | 52.0 | 48.3 | 5355 | 5379 | This study |
| NS1 | GTA GTC ATA TGC TTG TCT C | Forward | 42.1 | 36.9 | 413 | 431 | White *et al*. (1990) |
| NS1R | GAG ACA AGC ATA TGA CTA C | Reverse | 42.1 | 36.9 | 413 | 431 | www.lutzonilab.net/primers/page244.shtml |
| NS2 | GGC TGC TGG CAC CAG ACT TGC | Reverse | 66.7 | 53.8 | 943 | 963 | White *et al*. (1990) |
| NS3 | GCAAGTCTGGTGCCAGCAGCC | Forward | 66.7 | 53.8 | 943 | 963 | White *et al*. (1990) |
| NS4 | CTT CCG TCA ATT CCT TTA AG | Reverse | 40.0 | 38.2 | 1525 | 1544 | White *et al*. (1990) |
| NS5 | AAC TTA AAG GAA TTG ACG GAA G | Forward | 36.4 | 40.1 | 1523 | 1544 | White *et al*. (1990) |
| NS6 | GCA TCA CAG ACC TGT TAT TGC CTC | Reverse | 50.0 | 47.5 | 1806 | 1829 | White *et al*. (1990) |
| NS7 | GAG GCA ATA ACA GGT CTG TGA TGC | Forward | 50.0 | 47.5 | 1806 | 1829 | White *et al*. (1990) |
| NS8 | TCC GCA GGT TCA CCT ACG GA | Reverse | 60.0 | 50.4 | 2162 | 2181 | White *et al*. (1990) |
| NS17 | CAT GTC TAA GTT TAA GCA A | Forward | 31.6 | 34.2 | 447 | 465 | Gargas & Taylor (1992) |
| NS18 | CTC ATT CCA ATT ACA AGA CC | Reverse | 40.0 | 38.0 | 887 | 906 | Gargas & Taylor (1992) |
| NS19 | CCG GAG AAG GAG CCT GAG AAA C | Forward | 59.1 | 49.3 | 771 | 792 | Gargas & Taylor (1992) |
| NS20 | CGT CCC TAT TAA TCA TTA CG | Reverse | 40.0 | 37.3 | 1243 | 1262 | Gargas & Taylor (1992) |
| NS21 | GAA TAA TAG AAT AGG ACG | Forward | 33.3 | 30.5 | 1193 | 1210 | Gargas & Taylor (1992) |
| NS22 | AAT TAA GCA GAC AAA TCA CT | Reverse | 30.0 | 36.4 | 1687 | 1706 | Gargas & Taylor (1992) |
| NS23 | GAC TCA ACA CGG GGA AAC TC | Forward | 55.0 | 45.5 | 1579 | 1598 | Gargas & Taylor (1992) |
| NS24 | AAA CCT TGT TAC GAC TTT TA | Reverse | 30.0 | 36.2 | 2143 | 2162 | Gargas & Taylor (1992) |
| SR11R | GGA GCC TGA GAA ACG GCT AC | Forward | 60.0 | 47.8 | 779 | 798 | Spatafora *et al*. (1995) |
| SR1R | TAC CTG GTT GAT TCT GC | Forward | 47.1 | 38.5 | 394 | 410 | Vilgalys & Hester (1990) |
| SR3 | GAA AGT TGA TAG GGC T | Reverse | 43.8 | 34.8 | 696 | 711 | www.biology.duke.edu/fungi/mycolab/primers.htm |

**Table 2.** (Continued).

| Name | Sequence (5' – 3') | Orientation | %GC | Tm (°C) | Start | End | Reference |
|---|---|---|---|---|---|---|---|
| SSU1Fd | CTG CCA GTA GTC ATA TGC TTG TCT C | Forward | 48.0 | 46.5 | 407 | 431 | This study |
| SSU1Rd | CTT TGA GAC AAG CAT ATG AC | Reverse | 40.0 | 48.7 | 416 | 435 | This study |
| SSU2Fd | GAA CAA YTR GAG GGC AAG | Forward | 50.0 | 47.8 – 50.7 – 53.5 | 930 | 947 | This study |
| SSU2Rd | TAT ACG CTW YTG GAG CTG | Reverse | 47.2 | 48.4 – 49.9 – 51.2 | 974 | 991 | This study |
| SSU3Fd | ATC AGA TAC CGT YGT AGT C | Forward | 44.7 | 48.4 – 49.5 – 50.5 | 1389 | 1407 | This study |
| SSU3Rd | TAY GGT TRA GAC TAC RAC GG | Reverse | 47.5 | 49.0 – 52.5 – 56.0 | 1397 | 1416 | This study |
| SSU4Fd | CCG TTC TTA GTT GGT GG | Forward | 52.9 | 50.0 | 1670 | 1686 | This study |
| SSU4Rd | CAG ACA AAT CAC TCC ACC | Reverse | 50.0 | 50.3 | 1682 | 1699 | This study |
| SSU5Fd | TAC TAC CGA TYG AAT GGC | Forward | 47.2 | 48.9 – 50.1 – 51.2 | 2037 | 2054 | This study |
| SSU5Rd | CGG AGA CCT TGT TAC GAC | Reverse | 55.6 | 52.5 | 2148 | 2165 | This study |
| SSU6Fm | GCT TGT CTC AAA GAT TAA GCC ATG CAT GTC | Forward | 43.3 | 49.0 | 423 | 452 | This study |
| SSU6Rm | GCA GGT TAA GGT CTC GTT CGT TAT CGC | Reverse | 51.9 | 50.1 | 1707 | 1733 | This study |
| SSU7Fm | GAG TGT TCA AAG CAG GCC TNT GCT CG | Forward | 55.8 | 51.0 – 52.2 – 53.3 | 1153 | 1178 | This study |
| SSU7Rm | CAA TGC TCK ATC CCC AGC ACG AC | Reverse | 58.7 | 49.5 – 50.8 – 52.1 | 1921 | 1943 | This study |
| SSU8Fm | GCA CGC GCG CTA CAC TGA C | Forward | 68.4 | 52.2 | 1848 | 1866 | This study |
| V9G | TTA CGT CCC TGC CCT TTG TA | Forward | 45.0 | 42.8 | 2002 | 2021 | de Hoog & Gerrits van den Ende (1998) |

**Table 3.** Isolates containing group I intron sequences. The insertion positions of these introns are derived using *Magnaporthe grisea* GenBank accession AB026819 as reference in the 5'–3' direction.

| Isolate | Insertion between | 18S or 28S nrDNA | Intron size (bp) | Blast result |
|---|---|---|---|---|
| *Batcheloromyces leucadendri* CBS 110892 | 1559 – 1560 | 18S nrDNA | 350 | No significant similarity |
| | 1820 – 1821 | 18S nrDNA | 399 | 190/252 of AY545722 *Hydropisphaera erubescens* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 328 | 211/264 of DQ246237 *Teratosphaeria mexicana* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 538 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 383 | 218/283 of EU181458 *Trichophyton soudanense* 28S nrDNA |
| *Batcheloromyces proteae* CBS 110696 | 1559 – 1560 | 18S nrDNA | 325 | No significant similarity |
| | 1820 – 1821 | 18S nrDNA | 399 | 191/254 of AY545722 *Hydropisphaera erubescens* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 328 | 211/263 of DQ246237 *Teratosphaeria mexicana* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 535 | 75/90 of DQ442697 *Arxula adeninivorans* 26S nrDNA |
| | 5538 – 5539 | 28S nrDNA | 372 | 34/36 of GQ120133 Uncultured marine fungus 18S nrDNA |
| *Catenulostroma macowanii* CBS 110756 | 1559 – 1560 | 18S nrDNA | 395 | 297/379 of DQ848302 *Mycosphaerella latebrosa* 18S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 914 | No significant similarity |
| *Catenulostroma macowanii* CBS 111029 | 1559 – 1560 | 18S nrDNA | 395 | 303/379 of DQ848302 *Mycosphaerella latebrosa* 18S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 914 | No significant similarity |
| *Cercospora apii* CBS 118712 | 1820 – 1821 | 18S nrDNA | 733 | 288/363 of EU167577 *Mycosphaerella milleri* 18S nrDNA |
| *Cercospora capsici* CPC 12307 | 1820 – 1821 | 18S nrDNA | 732 | 287/363 of EU167577 *Mycosphaerella milleri* 18S nrDNA |
| *Cercospora janseana* CBS 145.37 | 1820 – 1821 | 18S nrDNA | 350 | 295/365 of EU167577 *Mycosphaerella milleri* 18S nrDNA |
| *Devriesia staurophora* CBS 375.81 | 3560 – 3561 | 28S nrDNA | 309 | No significant similarity |
| *Miuraea persicae* CPC 10069 | 1820 – 1821 | 18S nrDNA | 603 | 399/443 of DQ848342 *Mycosphaerella populorum* 18S nrDNA |
| *Mycosphaerella latebrosa* CBS 652.85 | 1559 – 1560 | 18S nrDNA | 370 | 234/296 of DQ848311 *Septoria betulae* 18S nrDNA |
| | 1820 – 1821 | 18S nrDNA | 933 | Matches same species |
| | 2168 – 2169 | 18S nrDNA | 494 | 377/449 of DQ848326 *Septoria alnifolia* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 481 | No significant similarity |
| | missing 5018 – 5019 | 28S nrDNA | Not present | Not present |

**Table 3.** (Continued).

| Isolate | Insertion between | 18S or 28S nrDNA | Intron size (bp) | Blast result |
|---|---|---|---|---|
| | 5424 – 5425 | 28S nrDNA | 680 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 471 | No significant similarity |
| *Mycosphaerella latebrosa* CBS 687.94 | 1559 – 1560 | 18S nrDNA | 370 | 231/295 of DQ848310 *Septoria betulae* 18S nrDNA |
| | 1820 – 1821 | 18S nrDNA | 918 | Matches same species |
| | 2168 – 2169 | 18S nrDNA | 494 | 377/449 of DQ848326 *Septoria alnifolia* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 480 | No significant similarity |
| | 5018 – 5019 | 28S nrDNA | 417 | 144/181 of AF430703 *Beauveria bassiana* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 680 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 471 | No significant similarity |
| *Mycosphaerella marksii* CBS 110942 | 1559 – 1560 | 18S nrDNA | 341 | 332/355 of DQ848296 *Mycosphaerella musae* 18S nrDNA |
| *Mycosphaerella marksii* CPC 11222 | 1559 – 1560 | 18S nrDNA | 341 | 332/355 of DQ848296 *Mycosphaerella musae* 18S nrDNA |
| *Passalora*-like genus CPC 11876 | 5538 – 5539 | 28S nrDNA | 580 | No significant similarity |
| *Passalora bellynckii* CBS 150.49 | 1559 – 1560 | 18S nrDNA | 409 | 147/191 of DQ848296 *Mycosphaerella musae* 18S nrDNA |
| *Passalora dodonaea* CPC 1223 | 5424 – 5425 | 28S nrDNA | 738 | No significant similarity |
| *Phacellium paspali* CBS 113093 | 4875 – 4876 | 28S nrDNA | 340 | 161/197 of DQ248314 *Symbiotaphrina kochii* 28S nrDNA |
| *Phaeophleospora eugeniicola* CPC 2557 | missing 5424 – 5425 | 28S nrDNA | Not present | Not present |
| | 5538 – 5539 | 28S nrDNA | 744 | No significant similarity |
| *Phaeophleospora eugeniicola* CPC 2558 | 5424 – 5425 | 28S nrDNA | 1846 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 744 | No significant similarity |
| *Pseudocercospora angolensis* CBS 112933 | 5018 – 5019 | 28S nrDNA | 379 | No significant similarity |
| *Pseudocercospora angolensis* CBS 149.53 | 5018 – 5019 | 28S nrDNA | 379 | No significant similarity |
| *Pseudocercospora punctata* CBS 113315 | 5424 – 5425 | 28S nrDNA | 723 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 725 | 67/73 of AF430699 *Beauveria bassiana* 28S nrDNA |
| *Pseudocercospora punctata* CPC 10532 | 5424 – 5425 | 28S nrDNA | 731 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 725 | 67/73 of AF430699 *Beauveria bassiana* 28S nrDNA |
| *Ramularia coleosporii* CPC 11516 | 1559 – 1560 | 18S nrDNA | 445 | No significant similarity |
| *Ramularia grevilleana* CPC 656 | 5538 – 5539 | 28S nrDNA | 546 | No significant similarity |
| *Septoria apiicola* CBS 400.54 | 5424 – 5425 | 28S nrDNA | 763 | No significant similarity |
| *Septoria obesa* CBS 354.58 | 1820 – 1821 | 18S nrDNA | 575 | No significant similarity |
| | 2168 – 2169 | 18S nrDNA | 548 | 394/454 of DQ848326 *Septoria alnifolia* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 430 | No significant similarity |
| *Septoria pyricola* CBS 222.31 | 5424 – 5425 | 28S nrDNA | 723 | No significant similarity |
| *Septoria quercicola* CBS 663.94 | 1559 – 1560 | 18S nrDNA | 334 | 241/308 of DQ848303 *Mycosphaerella latebrosa* 18S nrDNA |
| | 1820 – 1821 | 18S nrDNA | 442 | 379/452 of DQ848335 *Mycosphaerella latebrosa* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 345 | No significant similarity |
| | 5018 – 5019 | 28S nrDNA | 367 | 122/155 of DQ518980 *Lipomyces spencermartinsiae* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 526 | No significant similarity |
| | 5538 – 5539 | 28S nrDNA | 603 | No significant similarity |
| *Septoria rosae* CBS 355.58 | 1820 – 1821 | 18S nrDNA | 496 | No significant similarity |
| *Sonderhenia eucalypticola* CPC 11252 | 1559 – 1560 | 18S nrDNA | 408 | 339/404 of DQ848314 *Mycosphaerella populorum* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 337 | 229/289 of AB044641 *Cordyceps* sp. 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 705 | No significant similarity |
| *Stigmina platani* CBS 110755 | 1559 – 1560 | 18S nrDNA | 379 | 40/44 of AB007686 *Exophiala calicioides* 18S nrDNA |
| | 5018 – 5019 | 28S nrDNA | 376 | No significant similarity |
| *Stigmina* synanamorph CPC 11721 | 5018 – 5019 | 28S nrDNA | 371 | No significant similarity |
| *Teratosphaeria* aff. *nubilosa* CBS 114419 | 4871 – 4872 | 28S nrDNA | 141 | No significant similarity; high identity to *Teratosphaeria nubilosa* |
| | 5538 – 5539 | 28S nrDNA | 580 | No significant similarity; high identity to *Teratosphaeria nubilosa* |

**Table 3.** (Continued).

| Isolate | Insertion between | 18S or 28S nrDNA | Intron size (bp) | Blast result |
|---|---|---|---|---|
| *Teratosphaeria* aff. *nubilosa* CBS 116283 | 4871 – 4872 | 28S nrDNA | 141 | No significant similarity; high identity to *Teratosphaeria nubilosa* |
| | 5538 – 5539 | 28S nrDNA | 580 | No significant similarity; high identity to *Teratosphaeria nubilosa* |
| *Teratosphaeria juvenalis* CBS 110906 | 1559 – 1560 | 18S nrDNA | 403 | 52/61 of DQ471010 *Rutstroemia firma* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 345 | 224/290 of EF115309 *Cordyceps bassiana* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 478 | 47/50 of EF115313 *Cordyceps bassiana* 28S nrDNA |
| | 5538 – 5539 | 28S nrDNA | 402 | No significant similarity |
| *Teratosphaeria juvenalis* CBS 111149 | 1559 – 1560 | 18S nrDNA | 403 | 52/61 of DQ471010 *Rutstroemia firma* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 345 | 224/290 of EF115309 *Cordyceps bassiana* 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 478 | 47/50 of EF115313 *Cordyceps bassiana* 28S nrDNA |
| | 5538 – 5539 | 28S nrDNA | 402 | No significant similarity |
| *Teratosphaeria mexicana* CBS 110502 | 954 – 955 | 18S nrDNA | 316 | 129/158 of DQ518980 *Lipomyces spencermartinsiae* 26S nrDNA |
| | 1559 – 1560 | 18S nrDNA | 360 | No significant similarity |
| | 1820 – 1821 | 18S nrDNA | 388 | 128/168 of AF281670 *Cryptendoxyla hypophloia* 18S nrDNA |
| | 3560 – 3561 | 28S nrDNA | 383 | 124/151 of EF647754 *Thecaphora thlaspeos* 28S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 327 | 99/114 of L81104 *Gaeumannomyces graminis* var. *tritici* 28S nrDNA |
| | 5018 – 5019 | 28S nrDNA | 315 | No significant similarity |
| | 5424 – 5425 | 28S nrDNA | 553 | No significant similarity |
| *Teratosphaeria mexicana* CBS 120744 | 954 – 955 | 18S nrDNA | 318 | 130/158 of DQ518980 *Lipomyces spencermartinsiae* 26S nrDNA |
| | 1559 – 1560 | 18S nrDNA | 360 | No significant similarity |
| | 1820 – 1821 | 18S nrDNA | 389 | 85/109 of AF281670 *Cryptendoxyla hypophloia* 18S nrDNA |
| | 3560 – 3561 | 28S nrDNA | 378 | 119/155 of AY298780 *Lentinellus castoreus* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 327 | 162/200 of AB033530 *Penicillium sabulosum* 18S nrDNA |
| | 5018 – 5019 | 28S nrDNA | 309 | No significant similarity |
| | 5424 – 5425 | 28S nrDNA | 659 | No significant similarity |
| *Teratosphaeria nubilosa* CBS 115669 | 4871 – 4872 | 28S nrDNA | 141 | No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa* |
| | 5538 – 5539 | 28S nrDNA | 580 | No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa* |
| *Teratosphaeria nubilosa* CBS 116005 | 4871 – 4872 | 28S nrDNA | 141 | No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa* |
| | 5538 – 5539 | 28S nrDNA | 580 | No significant similarity; high identity to *Teratosphaeria* aff. *nubilosa* |
| *Teratosphaeria ohnowa* CBS 112896 | 954 – 955 | 18S nrDNA | 325 | 28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA |
| | 3560 – 3561 | 28S nrDNA | 294 | 168/227 of FJ358267 *Chaetothyriales* sp. 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 607 | 47/48 of EF115313 *Cordyceps bassiana* 28S nrDNA |
| *Teratosphaeria ohnowa* CBS 112973 | 954 – 955 | 18S nrDNA | 324 | 28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA |
| | 3560 – 3561 | 28S nrDNA | 294 | 168/227 of FJ358267 *Chaetothyriales* sp. 28S nrDNA |
| | 5424 – 5425 | 28S nrDNA | 607 | 47/48 of EF115313 *Cordyceps bassiana* 28S nrDNA |
| *Teratosphaeria pseudosuberosa* CBS 118911 | 3560 – 3561 | 28S nrDNA | 324 | 28/28 of DQ848329 *Botryosphaeria quercuum* 18S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 364 | No significant similarity |
| *Teratosphaeria* sp. CBS 208.94 | 954 – 955 | 18S nrDNA | 342 | No significant similarity |
| | 3560 – 3561 | 28S nrDNA | 309 | 59/70 of AY207244 *Mycena pura* 28S nrDNA |
| | 4875 – 4876 | 28S nrDNA | 296 | 44/51 of EF551317 *Tremella globispora* 28S nrDNA |
| *Teratosphaeria suberosa* CPC 11032 | 5424 – 5425 | 28S nrDNA | 313 | 159/197 of AB033529 *Penicillium oblatum* 18S nrDNA |
| | 5538 – 5539 | 28S nrDNA | 596 | 80/99 of AB044639 *Cordyceps kanzashiana* 28S nrDNA |
| *Thedgonia*-like genus CPC 12304 | 1820 – 1821 | 18S nrDNA | 444 | 262/331 of EU167577 *Mycosphaerella milleri* 18S nrDNA |

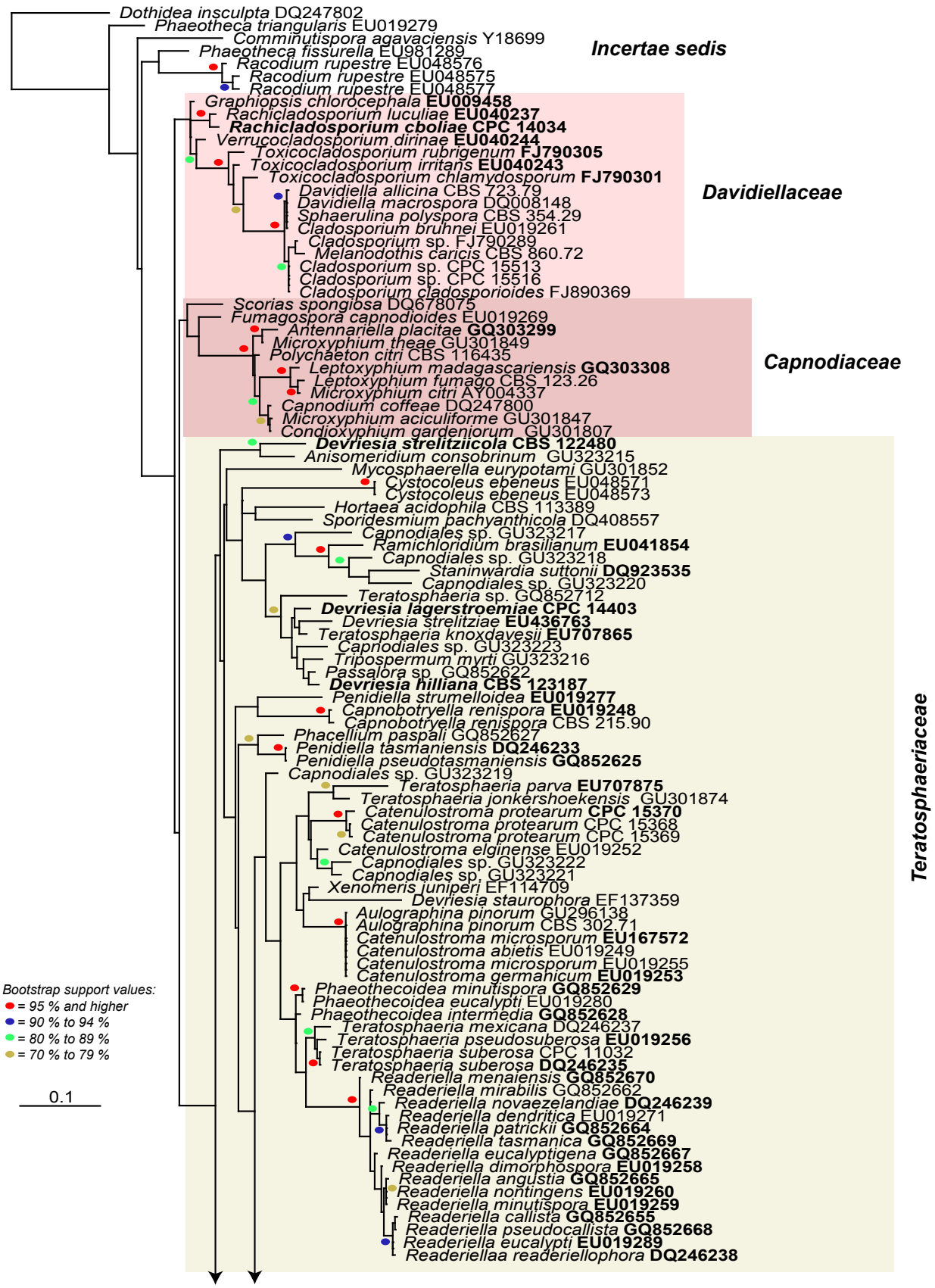



**Fig. 1.** RAxML tree using only the partial LSU alignment with bootstrap values after 1 000 pseudorepetitions on the nodes. Type strains and novel species described in this study are indicated in **bold**.

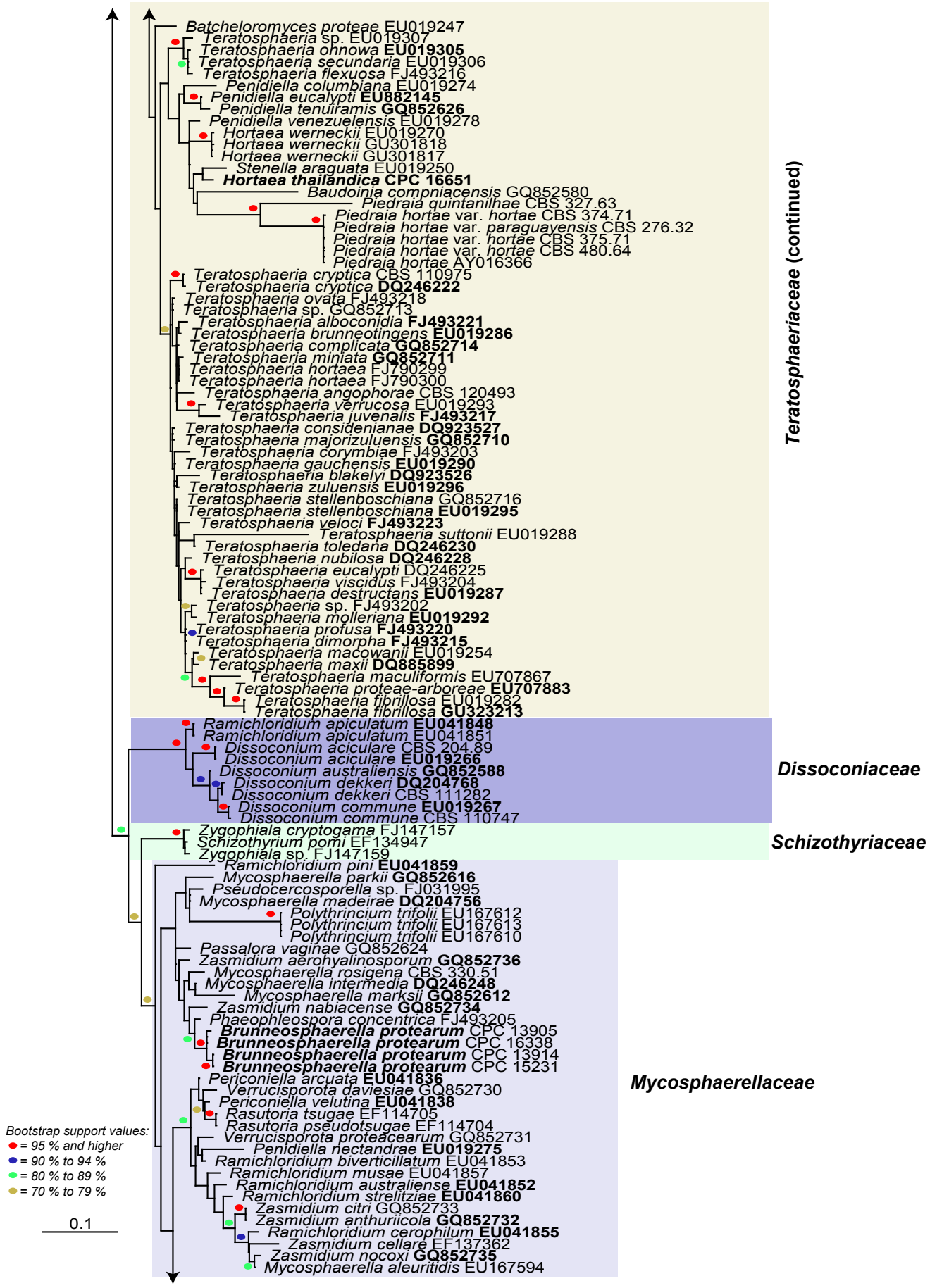



**Fig. 1.** (Continued).

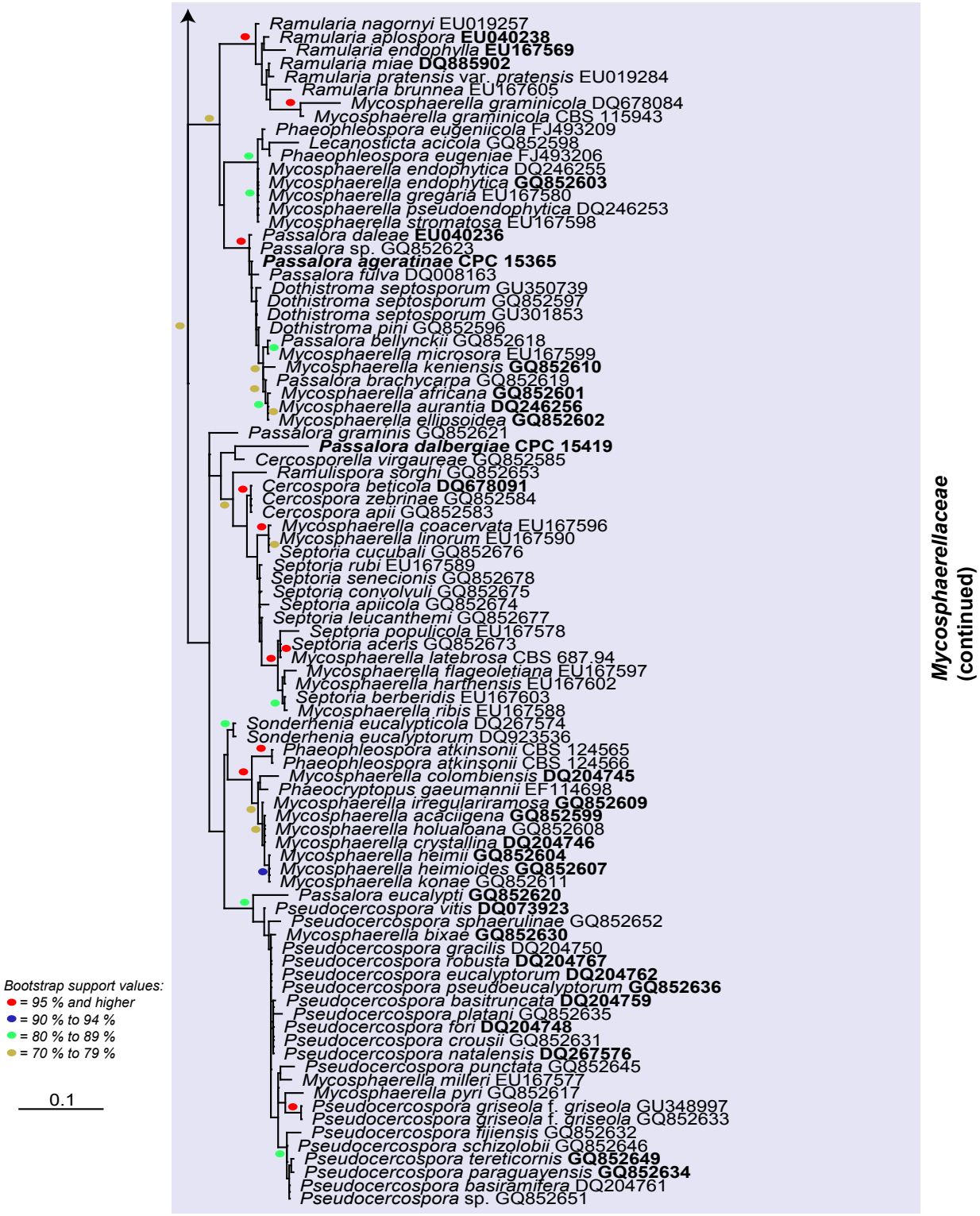



**Fig. 1.** (Continued).


**Fig. 2.** RAxML tree using the SSU, 5.8S nrDNA and LSU alignment with bootstrap values after 500 pseudorepetitions on the nodes.

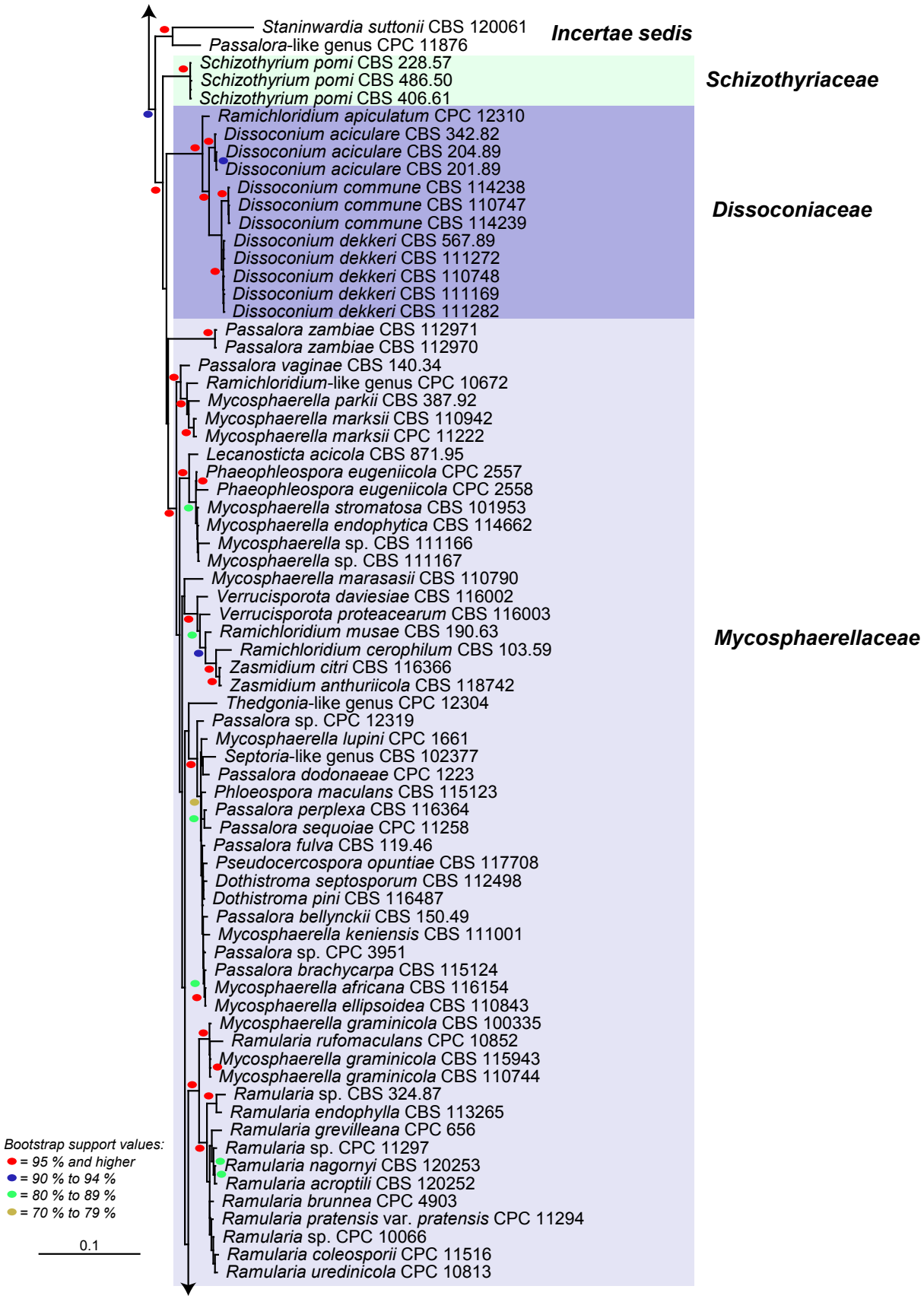
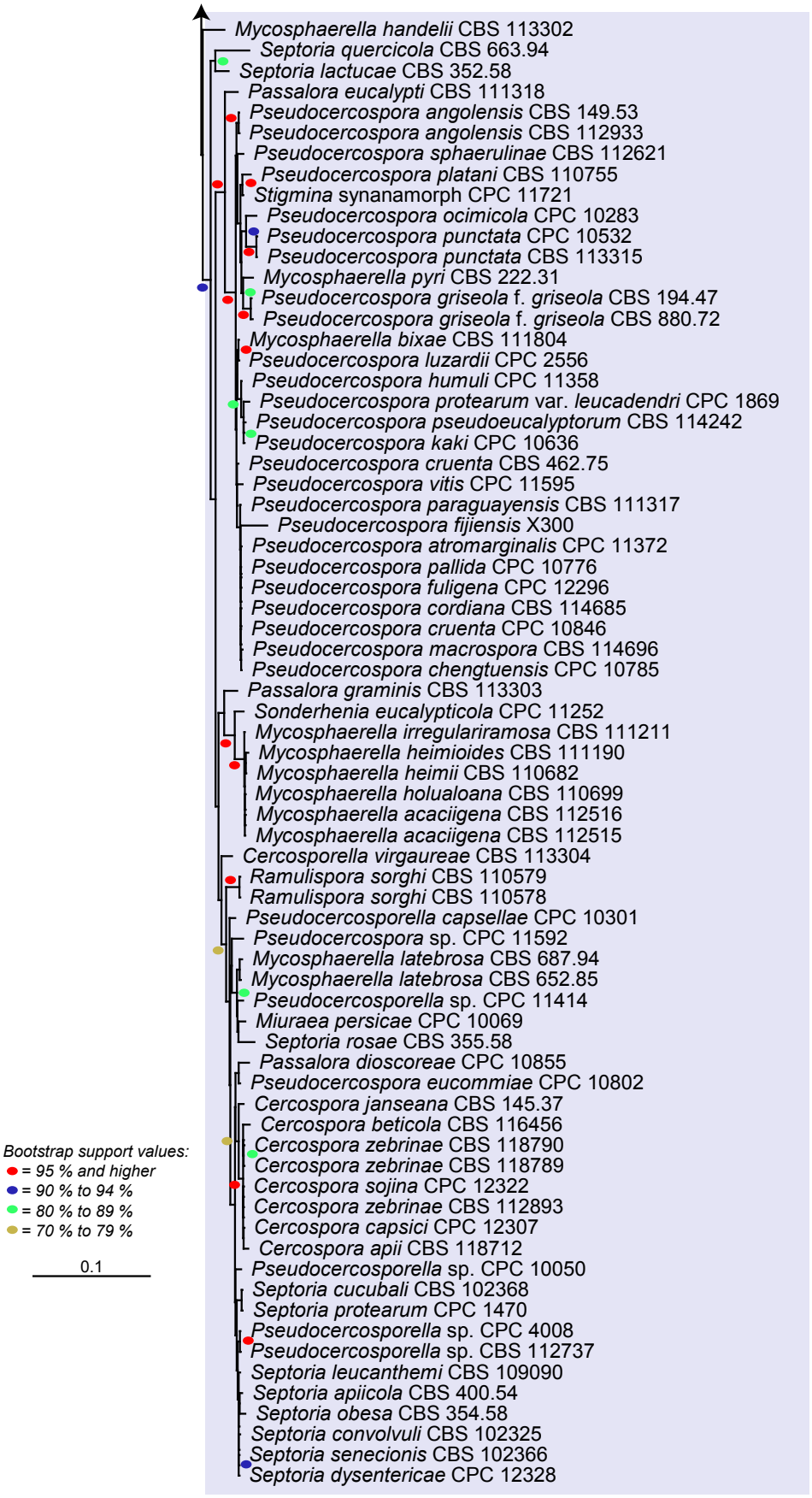



**Fig. 2.** (Continued).




***Mycosphaerellaceae*** **(continued)**

**Fig. 2.** (Continued).

## RESULTS

### DNA amplification and phylogeny

Amplification products of approximately 1 700 bases were obtained for the standard amplification of the isolates listed in Table 1. The LSU region of these sequences was used to obtain additional sequences from GenBank that were added to the partial LSU alignment. We expected a total size of approximately 5 500 bp for the concatenated SSU, ITS1, 5.8S nrDNA, ITS2 and LSU at the start of the study; however, our alignment totalled about 12 000 bp due to numerous insertions (most likely group 1 introns) encountered for several strains (Table 3). These insertions frequently resulted in products too large to amplify or sequence effectively and sometimes required us to design additional novel primers in extra overlapping steps to complete these gaps (see Materials and Methods for details). Searching the GenBank database using these insertions had varied success (Table 3). Sequences of the 18S nrDNA are more abundant in the database whereas sequences of the second half to two-thirds of the 28S nrDNA are mostly absent. This also evident in Table 3, where insertions in the SSU more frequently found with similarity sequences in the database and insertions in the LSU (*e.g.* those between positions 5018–5019 and 5424–5425) frequently did not retrieve any significant similarity. Although there were some exceptions (*e.g.* the insertion between 1820 and 1821 in the SSU of *Batcheloromyces leucadendri*), most of the insertions in the SSU obtained hits with SSU sequences of species of *Capnodiales* in the database. In one case, between 954 and 955 for the SSU sequence of *Teratosphaeria mexicana* (both strains), a partial hit was obtained with an LSU sequence of *Lipomyces spencermartinsiae* (GenBank DQ518980). Many of the insertions in the LSU sequences did not retrieve significant hits in the database and those that did were with unrelated taxa. It is quite possible that this is an artifact of the poor representation of full-length LSU sequences in the database, especially for members of the *Capnodiales*. In some cases, an LSU insertion retrieved a hit with SSU sequences in the database, *e.g.* the insertion between 5538 and 5539 in *Batcheloromyces proteae* and between 3560 and 3561 and 4875 and 4876 in *Teratosphaeria mexicana* strain CBS 120744. In two cases (*Mycosphaerella latebrosa* and *Phaeophleospora eugeniicola*), an insertion was either lost or gained between two strains of the same species. The primers designed in this study allowed us to effectively amplify and sequence the SSU and LSU for the selected isolates. Althought these primers were not tested on taxa outside of the *Capnodiales* (except for one of the outgroups, *Neofusicoccum australe*), we attempted to design them as robust as possible using degeneracy if needed. We therefore expect that these primers will have wider applicability than just the *Capnodiales* in cases where other published primers fail to amplify or amplify poorly.

The RAxML search of the partial LSU alignment yielded a most likely tree (Fig. 1) with a log likelihood -13397.994021. The matrix had 395 distinct alignment patterns, with 6 % completely undetermined characters in the alignment. The manually adjusted alignment contained 295 sequences (including the outgroup sequence, *Dothidea insculpta* GenBank DQ247802) and 763 characters including alignment gaps. The RAxML search of the almost complete SSU, 5.8S nrDNA and LSU alignment yielded a most likely tree (Fig. 2) with a log likelihood -39022.881140. The matrix had 1211 alignment patterns with 0.01 % of the characters consisting of gaps or undetermined characters. The manually adjusted alignment contained 205 sequences (including the outgroup sequences, *Neofusicoccum australe* CPC 10899 and *Magnaporthe grisea* GenBank AB026819) and 5110 characters including alignment gaps. The obtained phylogenies (Figs 1–2) are discussed in the Taxonomy section below.

### Taxonomy

Several well-supported clades could be distinguished in the present study (Figs 1–2), correlating to families in the *Capnodiales*. These families, and several new genera and species, are treated below.

### Treatment of phylogenetic clades

***Capnodiales*** Woron. Ann. Mycol. 23: 177. 1925.

Data obtained from multi-gene phylogenies prompted Schoch *et al*. (2006) to merge *Mycosphaerellales* with *Capnodiales*. Although the present study included numerous additional isolates, the orders remain problematic. Although there is support for the *Mycosphaerellales* as an order, additional families such as the *Schizothyriaceae* and *Dissoconiaceae* (see below) would have to also be elevated to order level, which would result in orders containing a single family, while *Teratosphaeriaceae* appears to comprise unresolved lineages. For this reason it was decided to retain these families within *Capnodiales*, but noting that as more families are added and better circumscribed, it is quite possible that the *Mycosphaerellales* would again be resurrected.

***Mycosphaerellaceae*** Lindau, In: Engler & Prantl, Nat. Pflanzenfamilien 1(1): 421. 1897.

*Type species*: *Mycosphaerella punctiformis* (Pers. : Fr.) Starbäck, Bih. Kongl. Svenska Vetensk.-Akad. Handl. 15(3, 2): 9. 1889.

*Notes*: The *Mycosphaerellaceae* contains numerous genera, 20 of which are listed by Crous (2009), with many names under consideration (Crous *et al*. 2009b, c). From these data it is clear that genera such as *Passalora, Pseudocercospora, Pseudocercosporella, Septoria, Zasmidium* and *Ramichloridium* are paraphyletic (Hunter *et al*. in prep.). Well-resolved genera include *Cercospora*, *Cercosporella, Ramularia*, *Ramulispora*, *Sonderhenia* and *Polythrincium*. One particularly problematic clade contains *Periconiella*, *Ramichloridium, Verrucisporota* and *Zasmidium,* along with "*Mycosphaerella*" and *Rasutoria* teleomorphs. Barr (1987) erected *Rasutoria* for species with brown ascospores occurring on *Gymnospermae*. *Rasutoria* clusters in a clade adjacent to "*Mycosphaerella*" species with hyaline ascospores, such as *M. aleuritidis* and *Mycosphaerella daviesiicola* (*Verrucisporota daviesiae*) (Beilharz & Pascoe 2002).

The genus *Phaeophleospora* (1916) clusters with *Lecanosticta acicola*. The genus *Lecanosticta* (1922) has typical *Phaeophleospora*-like conidia, except that its conidiomata are acervular, and not pycnidial. If the type of *Lecanosticta, L. pini* also clusters in this clade, the generic concept *Phaeophleospora* may have to be widened to include *Lecanosticta*, as was done with *Kirramyces* to include *Colletogloeopsis* (Cortinas *et al*. 2006a, b).

Considerable controversy has surrounded the coelomycetes that Crous *et al*. (1997) placed in *Phaeophleospora*. Based on DNA phylogenetic data, it has now been shown that *Kirramyces* anamorphs (Walker *et al*. 1992), including those accommodated in *Colletogloeopsis* (Crous & Wingfield 1996, Crous *et al.* 2004c, 2006c, Cortinas *et al*. 2006a, b), are linked to *Teratosphaeria* (Andjic *et al*. 2007, Crous *et al*. 2009b, c). Crous *et al*. (2007a)

showed *Phaeophleospora* to reside in the *Mycosphaerellaceae* and *Kirramyces* in the *Teratosphaeriaceae*, respectively. However, most taxa investigated to date were collected from *Eucalyptus*. As shown in the present study, *Phaeophleospora atkinsonii*, a pathogen of *Hebes* spp. (Wu *et al*. 1996, Pennycook & McKenzie 2002), clusters distant from *Phaeophleospora s. str.*, while the same is true for *Phaeophleospora concentrica*, which is a pathogen of *Protea* spp. (Taylor *et al*. 2001a), and *Phaeophleospora stonei*, a pathogen of *Eucalyptus* (Crous *et al*. 2007c, 2009c). These taxa thus clearly represent yet another two genera in the *Phaeophleospora* complex. An older name that would potentially be available is *Scoleciasis*. However, when B. Sutton examined exsiccati of the type species, *S. aquatica*, only ascomata of a *Leptosphaeria* species were found (Crous *et al.* 1997). The association of *S. aquatica* with the *Leptosphaeria* was also noted in the original description, and this may indicate that *Scoleciasis* is allied to taxa in the *Phaeosphaeriopsis/ Phaeoseptoria* complex (Arzanlou & Crous 2006). Both *P. atkinsonii* and *P. concentrica* have a typical *Kirramyces* morphology, namely brown, percurrently proliferating conidiogenous cells, and brown, obclavate, verruculose, transversely euseptate conidia. Further species thus need to be included in analyses before these generic concepts can be clarified.

During the course of this study several fresh collections of *Leptosphaeria protearum* were obtained. *Leptosphaeria protearum* is a major leaf spot and blight pathogen of *Protea* spp. (Knox-Davies *et al.* 1987), and causes severe losses in plantations of South African *Protea* spp. in Hawaii, and has been recorded in many countries where South African proteas are cultivated (Taylor & Crous 1998, Taylor *et al*. 2001b, Crous *et al*. 2004a). Cultures of this pathogen were found to cluster in the *Mycosphaerellaceae*, where they represent an undescribed genus, characterised by having bitunicate asci without pseudoparaphyses, brown, 3-septate ascospores, and a *Coniothyrium*-like anamorph. Its close phylogenetic relationship to *Phaeophloeospora concentrica* (Fig. 1) suggests that they could be congeneric, and that in future more *Phaeophloeospora*-like anamorphs may be found to cluster in this clade. We propose a new genus to accommodate *Leptosphaeria protearum* below.

***Brunneosphaerella*** Crous, **gen. nov.** MycoBank MB514694.

*Etymology*: *Brunneus* + *Sphaerella* = is after its brown ascospores and *Sphaerella*-like morphology.

*Mycosphaerellae* similis, sed ascosporis brunneis, 3-septatis.

*Ascomata* amphigenous, immersed to semi-immersed, black, single, gregarious, substomatal, pyriform or globose with a papillate, periphysate ostiole. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense. *Pseudoparaphyses* absent. *Ascospores* biseriate, fusiform, broader at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum.

*Type species*: *Brunneosphaerella protearum* (Syd. & P. Syd.) Crous, comb. nov.

***Brunneosphaerella jonkershoekensis*** (Marinc., M.J. Wingf. & Crous) Crous, **comb. nov.** MycoBank MB514695. Fig. 3.

*Basionym*: *Leptosphaeria jonkershoekensis* Marinc., M.J. Wingf. & Crous, In: Marincowitz *et al*., *Microfungi occurring on Proteaceae in the fynbos*: 62. 2008.

*Ascomata* pseudothecial, subepidermal, immersed, obpyriform, papillate, 180–205 × 160–235 µm. *Peridium* 20–30 µm thick, composed of relatively large cells, 11–15 × 2.5–5.5 µm; cells arranged in three strata; outer stratum consisting of 3–5 layers of dark brown, very thick-walled cells; middle stratum transient, consisting of a few layers of pale brown, thick-walled, compressed cells; inner stratum consisting of 1–2 layers of thin-walled, very compressed cells. *Pseudoparaphyses* absent. *Asci* bitunicate, inflated cylindrical to clavate, 81–95 × 13–15 µm, ocular chamber dome-shaped, indistinct. *Ascospores* pale brown, fusoid to ellipsoidal, tapering towards the base, (25–)29–34(–36) × (5–)6–7(–9) µm (av. 31.4 × 6.7 µm), apical cell the shortest, upper hemispore slightly larger than lower, at times slightly curved, 3-septate, smooth, guttulate (adapted from Marincowitz *et al*. 2008).

*Host range and geographic distribution*: *Protea repens* (South Africa, Western Cape) (Marincowitz *et al*. 2008).

*Specimen examined*: **South Africa**, Western Cape Province, Jonkershoek Nature Reserve, leaf litter of *Protea repens*, 6 Jun. 2000, S. Marincowitz, PREM 59447 **holotype**.

*Notes*: Although no culture is presently available for this species, it clearly represents a species of *Brunneosphaerella*, characterised by its bitunicate asci, and brown, 3-septate ascospores, as well as the absence of pseudoparaphyses. *Brunneosphaerella jonkershoekensis* can easily be distinguished from *B. protearum* based on its much larger ascospores (Crous *et al*. 2004a).

***Brunneosphaerella protearum*** (Syd. & P. Syd.) Crous, **comb. nov.** MycoBank MB514696. Fig. 4.

*Basionym*: *Leptosphaeria protearum* Syd. & P. Syd., Ann. Mycol. 10: 441. 1912.

*Anamorph*: "*Coniothyrium" protearum* Joanne E. Taylor & Crous, IMI Descriptions of Fungi and Bacteria No. 1343. 1998.

*Leaf spots* circular to irregular, discrete to confluent, variable in size, under conditions favourable to disease symptoms more similar to a blight than a leaf spot, necrotic, sunken with a raised dark brown margin and with conspicuous black ascomata in the dead tissue, 4–30 mm diam. *Ascomata* pseudothecial, substomatal, amphigenous, immersed to semi-immersed, not erumpent, black, single, gregarious, 180–320 µm diam; in section, substomatal, subepidermal, pyriform or globose with a papillate, periphysate ostiole, immersed in a stroma consisting of deteriorated host mesophyll cells filled with fungal hyphae, (210–)230–264(–288) µm high, (180–)200–255(–300) µm diam. *Peridium* consisting of three strata of slightly compressed *textura angularis*, an outer stratum of dark brown, thick-walled cells, becoming paler in the central stratum, and hyaline, thin-walled in the inner stratum, altogether (20–)24.5–37.5(–50) µm thick. *Asci* clavate to cylindro-clavate, often curved, tapering to a pedicel, narrowing slightly to a rounded apex with an indistinct ocular chamber, 8-spored, bitunicate with fissitunicate dehiscense, (70–)80–87.5(–105) × (13.5–)14.5–16(–21.5) µm. *Pseudoparaphyses* absent. *Ascospores* biseriate, fusiform, broader

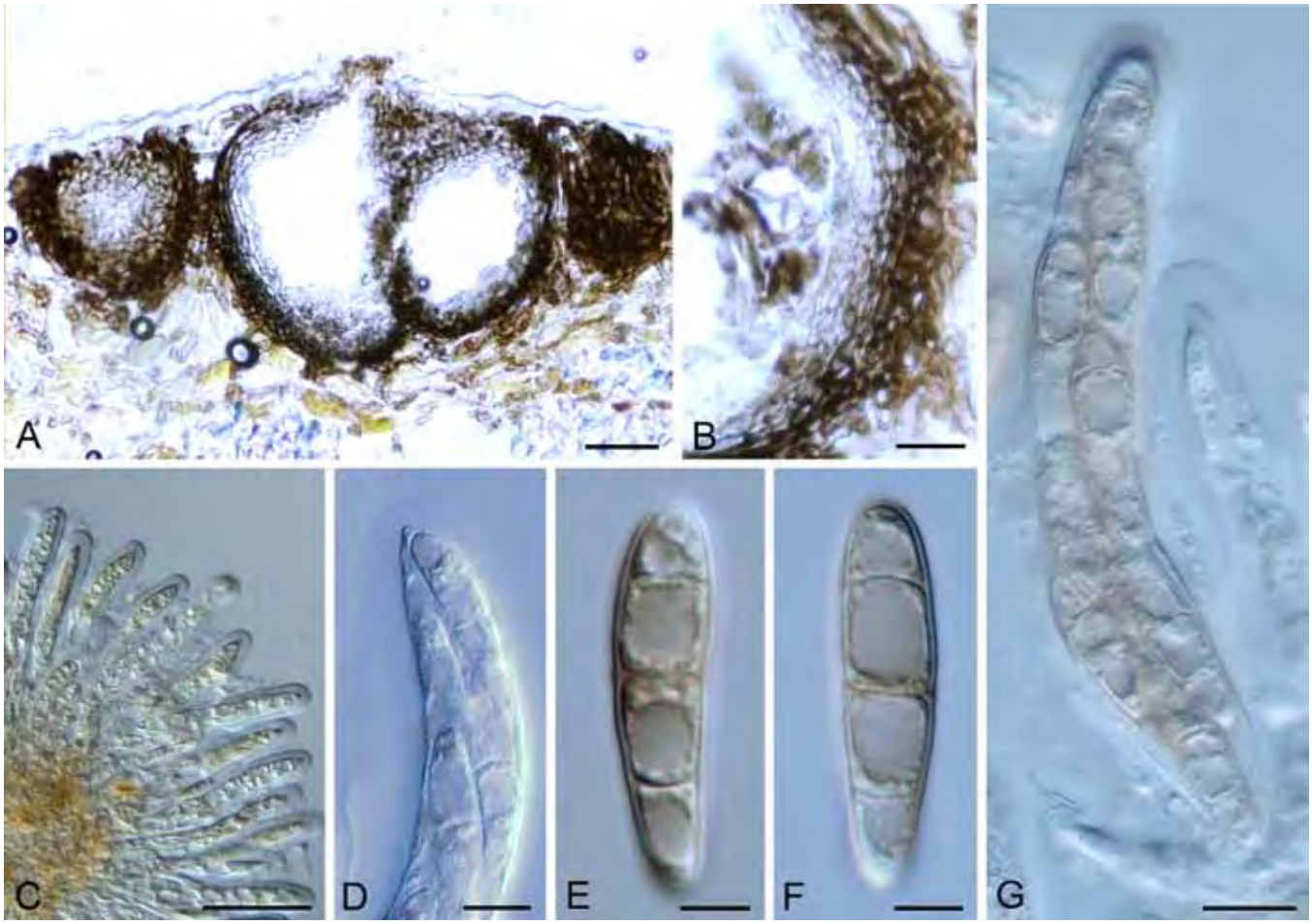



**Fig. 3.** *Brunneosphaerella jonkershoekensis*. A–B. Vertical sections through ascomata showing wall structure. C–D, G. Bitunicate asci. E–F. Ascospores. Scale bars: A, C = 50 µm, B = 20 µm, D, G = 10 µm, E–F = 5 µm (from Marincowitz *et al*. 2008).

at the apical end, initially hyaline and 1-septate, becoming yellow-brown and 3-septate at maturity, slightly constricted at median to supra-median septum, (21.5–)27.5–29.5(–37.5) × (6.3–)7.5–8(–10) µm in water mounts, (21–)25.5–27.5(–31) × (5.5–)6–7(–8) µm in lactophenol. *Conidiomata* barely visible and interspersed between ascomata, pycnidial, subepidermal, substomatal, separate, globose to pyriform, occasionally with well-developed papilla, dark brown, < 200 µm diam. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* discrete, smooth, hyaline, doliiform to ampulliform, holoblastic, proliferating 1–2 times percurrently, 4–6 × 3–4 µm. *Conidia* pale brown to medium brown, thick-walled on maturity, smooth to finely verruculose, eguttulate, ellipsoidal to globose, often truncate at one end, 5–10 × 3–7 µm (adapted from Crous *et al.* 2004a).

*Host range and geographic distribution*: *Protea cynaroides*, *P.* 'Susara' (Portugal, Madeira) (Moura & Rodrigues 2001); *P. caffra*, *P. compacta, P. cynaroides*, *P. gaguedi, P. grandiceps, P. lacticolor, P. laurifolia, P. lepidocarpodendron, P. lorifolia*, *P. magnifica, P. nitida, P. punctata, P. repens, P.* 'Sheila', *Protea* spp. (South Africa); *P. cynaroides*, *P. laurifolia*, *P. neriifolia*, *P.* 'Ivory Musk', *P.* 'Mink', *P.* 'Pink Ice', *P.* 'Rose Mink', *P. susannae, Protea* sp. (U.S.A., Hawaii) (Taylor *et al*. 2001b); *P. cynaroides*, *P. gaguedi, P. neriifolia, Protea* sp. (Zimbabwe, Inyanga) (Masuka *et al*. 1998).

*Specimens examined*: **South Africa**, Western Cape Province, Bettys' Bay, leaf litter of *Protea magnifica*, 11 Jul. 2000, S. Marincowitz, PREM 59448; Helderberg Nature Reserve, leaf litter of *Protea laurifolia*, 14 Aug. 2000, S. Marincowitz, PREM 59482; Helderberg Nature Reserve, leaf litter of *Protea obtusifolia*, 14 Aug. 2000, S. Marincowitz, PREM 59495; Jonkershoek Nature Reserve, leaf litter of *Protea nitida*, 6 Jun. 2000, S. Marincowitz, PREM 59442; Jonkershoek Nature Reserve, leaf litter of *Protea repens*, 6 Jun. 2000, S. Marincowitz, PREM 59450; Jonkershoek Nature Reserve, S33°59'11.2" E18°57'14.7" leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20330, cultures CPC 13914–13916; Jonkershoek Nature Reserve, S33°59'26.1" E18°57'59.5" leaves of *Protea repens*, 1 Apr. 2007, P.W. Crous, CBS H-20331, cultures CPC 13911–13913; Jonkershoek Nature Reserve, leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20332, cultures CPC 13908–13910; Jonkershoek Nature Reserve, "Tweede Waterval", leaves of *Protea* sp., 1 Apr. 2007, P.W. Crous, CBS H-20333, cultures CPC 13902–13907; Jonkershoek Nature Reserve, leaves of *Protea nitida*, 12 Apr. 2008, L. Mostert, CBS H-20334, cultures CPC 15231–15233; Kirstenbosch Botanical Garden, leaves of *Protea* sp., 13 Jan. 2009, P.W. Crous, CBS H-20335, culture CPC 16338.

*Notes*: Although Taylor & Crous (1998) reported a *Coniothyrium*-like anamorph to develop in culture, none of the cultures examined in the present study on MEA, PDA or OA could be induced to sporulate, though spermatogonia and ascomatal initials were commonly observed.

The fact that cultures of *Leptosphaeria protearum*, which represents a well-known and serious pathogen of *Proteaceae*, clustered in the *Mycosphaerellaceae,* was totally unexpected. A further surprise was the fact that this species appears to represent a complex of several cryptic taxa. Whether these taxa can be correlated with differences in host range and geographic distribution can only be resolved once more collections have been obtained for study. Although the genus *Sphaerulina,* which represents *Mycosphaerella*-like taxa with 3-septate, hyaline ascospores, is part of the *Mycosphaerellaceae* (Crous *et al*., unpubl data), the type species, *S. myriadea*, clusters in the *Septoria* clade, and is thus unavailable for the species occurring on *Proteaceae*. Morphologically *Brunneosphaerella* is also distinct in

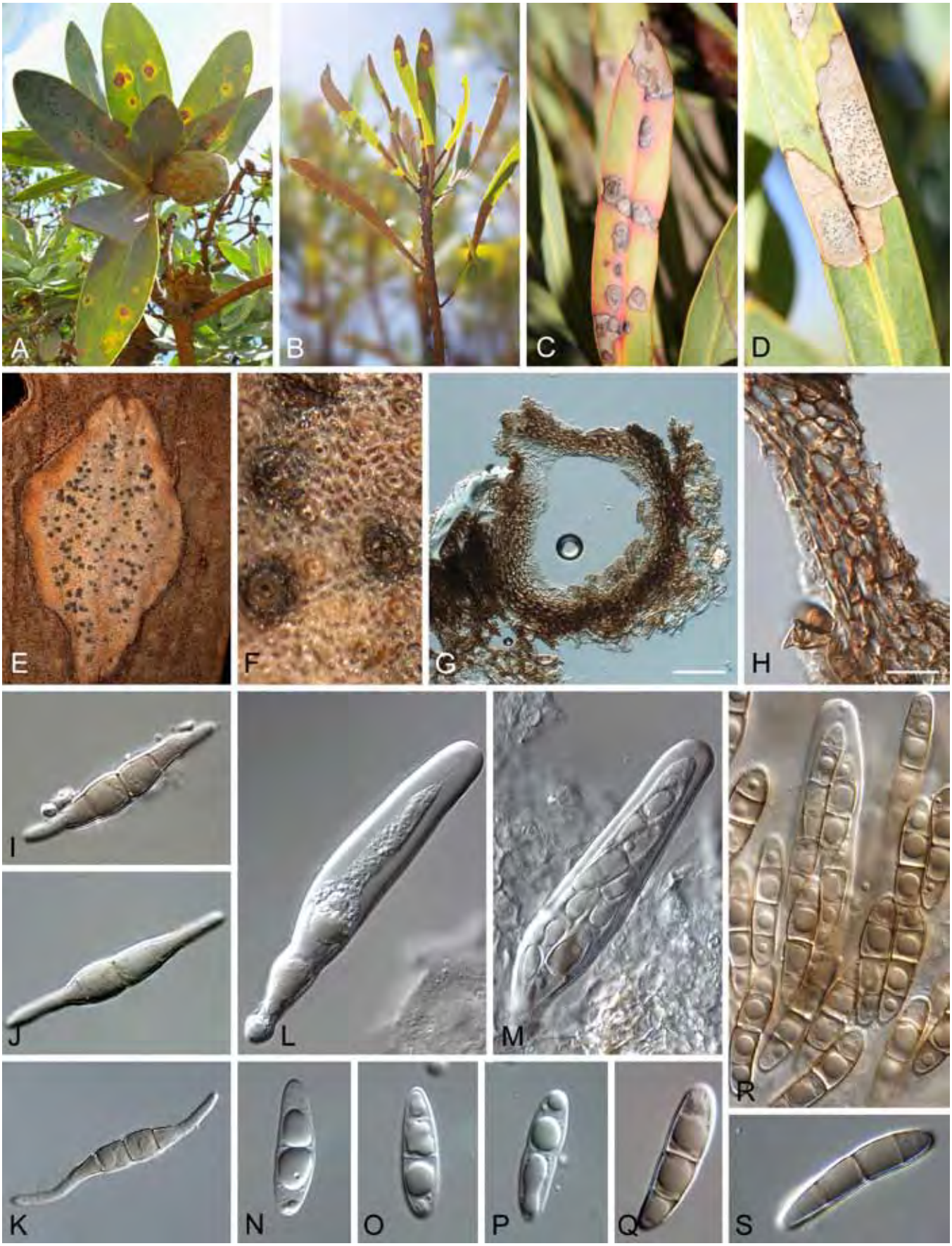



**Fig. 4.** *Brunneosphaerella protearum*. A–D. Leaf spots on different *Protea* spp. E. Close up of leaf spot showing ascomata. F. Substomatal ascomata. G–H. Vertical sections though ascomata, showing wall structure. I–K. Germinating ascospores on MEA. L–M, R. Bitunicate asci. N–Q, S. Juvenile to mature ascospores. Scale bars: G = 75 µm, H = 10 µm.

that ascospores are always brown at maturity, and anamorphs have brown, percurrently proliferating conidiogenous cells, appearing *Phaeophleospora*-like. The recognition of *Brunneosphaerella* as a distinct genus in the *Mycosphaerellaceae* also raises the intriguing possibility that many phytopathogenic species of the *Leptosphaeria*-complex with brown, 3-septate ascospores, but lacking paraphyses, actually belong to *Brunneosphaerella*.

***Passalora ageratinae*** Crous & A.R. Wood, **sp. nov.** MycoBank MB514697. Fig. 5.

*Etymology*: Named after the host on which it occurs, *Ageratina adenophora.*

*Passalorae assamensis* similis, sed coloniis amphigenis, sine mycelio externo, conidiophoris brevioribus, 15–40 × 3–4.5 µm.

*Leaf spots* amphigenous, angular to irregular, 2–8 mm diam, medium brown, frequently with pale to grey-brown central part, and raised, dark brown border; pale to medium brown in reverse, with raised, dark brown border. *Mycelium* internal, consisting of smooth, branched, pale brown, 2–3 µm wide hyphae. *Caespituli* fasciculate, amphigenous, medium brown, arising from a brown, erumpent stroma, up to 80 µm wide, 40 µm high. *Conidiophores* subcylindrical, straight to geniculous-sinuous, unbranched, medium brown, finely verruculose, 1–3-septate, 15–40 × 3–4.5 µm. *Conidiogenous cells* terminal, pale to medium brown, finely verruculose with terminal, sympodial conidiogenous loci that are 1–2 µm diam, slightly thickened, darkened and refractive, 10–20 × 3–4 µm. *Conidia* in unbranched chains, pale brown, smooth, finely to prominently guttulate, subcylindrical to narrowly obclavate, apex obtuse, base long obconically subtruncate, (0–)1–3(–5)-septate, (20–)30–60(–80) × (3–)4(–4.5) µm; hila 1–1.5 µm wide, somewhat thickened, darkened and refractive.

*Culture characteristics*: On MEA erumpent, with uneven, folded surface, lobate margin, and moderate aerial mycelium; centre pale mouse-grey with patches of cinnamon, outer margin olivaceous-grey; reverse olivaceous-grey with patches of cinnamon; reaching 15 mm diam; on PDA spreading, with cinnamon to cream patches in centre, becoming umber towards smooth margins, with diffuse red pigment in agar; reverse olivaceous-grey, with patches of red, reaching 15 mm diam; on OA flat, spreading, up to 30 mm diam, iron-grey, with white, solitary mycelia strands, though aerial mycelium generally absent, reaching 30 mm diam.

*Host range and geographic distribution*: *Ageratina adenophora*, Australia, South Africa.

*Specimen examined*: **South Africa**, KwaZulu-Natal Province, Hilton, on leaves of *Ageratina adenophora*, 28 May 2008, A.R. Wood, CBS H-20336 **holotype**, cultures ex-type CPC 15365 = CBS 125419, CPC 15366, 15367.

*Notes*: *Ageratina adenophora* (crofton weed; *Asteraceae*), which is indigenous to Mexico, has invaded many countries as a rapidly growing weed, forming dense thickets (Morris 1989, Parsons & Cuthbertson 1992, Wagner *et al.* 1999, Zhu *et al.* 2007, Muniappan *et al*. 2009). It is considered a serious weed in agriculture and forestry (Bess & Haramoto 1958, Sharma & Chhetri 1977, Kluge 1991), often replacing more-desired vegetation or native species.

A leaf spot pathogen, originally misidentified as *Cercospora eupatorii* (this species is currently known as *Pseudocercospora eupatorii*), was found to infect plants in Australia where a stem galling fly (*Procecidochares utilis*; *Tephritidae*) was introduced from Hawaii as a biological control agent (Dodd 1961). Presumably the fungus was introduced together with the flies originally from Mexico to Hawaii and then to Australia. Subsequently this same fungus was obtained from Australia and released in South Africa after host specificity testing indicated it was restricted to *A. adenophora*

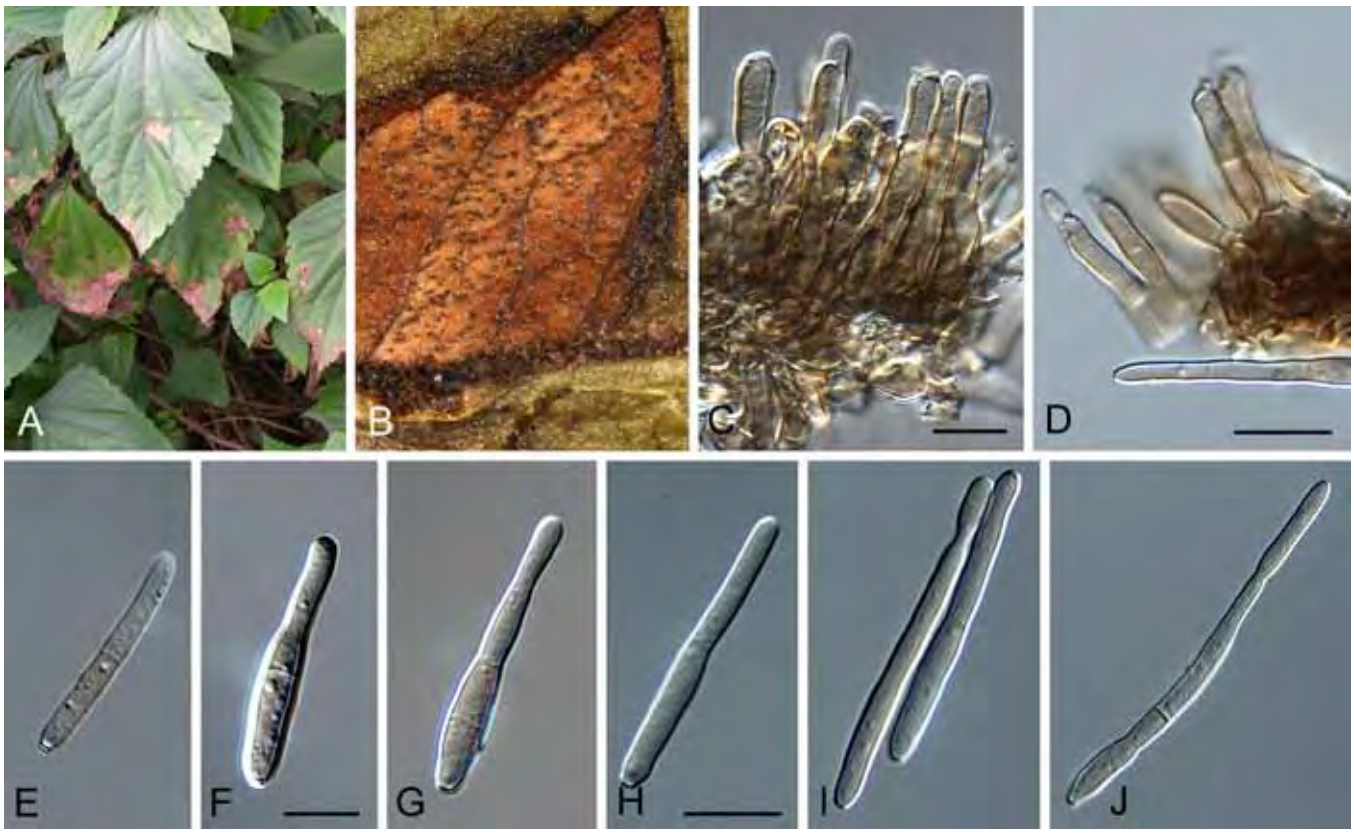



**Fig. 5.** *Passalora ageratinae*. A. Leaf spots. B. Close up of leaf spot with fruiting structures. C–D. Conidiophores. E–J. Conidia. Scale bars = 10 µm.

(Morris 1989). The fungus causes partial defoliation of mature plants (Dodd 1961, Auld 1969), though the impact depends on environmental conditions (Dodd 1961). Seedlings are however killed rapidly (Wang *et al*. 1997).

This fungus, which has hitherto been known simply as "*Phaeoramularia*" sp., still lacks a name and proper description. The genus *Phaeoramularia* is treated as a synonym of *Passalora* (Crous & Braun 2003), and hence the species is named in the latter genus as *P. ageratinae*. Interestingly, this species appears to be closely related to *Passalora fulva*, which is a serious pathogen of tomato (*Solanaceae*) (Thomma *et al*. 2005).

***Passalora armatae*** Crous & A.R. Wood, **sp. nov.** MycoBank MB514698. Fig. 6.

*Etymology*: Named after the host on which it occurs, *Dalbergia armata.*

*Passaloraea dalbergiicolae* similis, sed conidiophoris in synnematibus densis, conidiis ad basim obconice truncatis, apice rostrato.

*Leaf spots* amphigenous, on upper surface visible as red-brown, irregular to subcircular spots with indistinct margins, 0.5–2 mm diam; in reverse indistinct, chlorotic to medium or red-brown. *Mycelium* internal, consisting of smooth, branched, pale brown, 2–3 µm wide hyphae. *Caespituli* hypophyllous, fasciculate to synnematous, up to 200 µm high and 250 µm wide, situated on a prominently erumpent, pale brown stroma, up to 100 µm high and wide. *Conidiophores* subcylindrical, unbranched, flexuous, guttulate, pale to medium brown, smooth, 120–180 × 4–6 µm, 2–6-septate. *Conidiogenous cells* terminal, subcylindrical, guttulate, pale to medium brown, finely verruculose, becoming somewhat swollen, appearing slightly clavate, 25–70 × 6–8 µm; conidiogenous loci 4–20 per conidiogenous cell, sympodial, round, darkened, thickened, refractive, prominent, 2–3 µm wide, up to 1 µm high. *Conidia* (27–)30–40(–45) × 9–10(–12) µm, pale to medium brown, smooth to finely verruculose, granular to guttulate, thin-walled, ellipsoidal to obovoid, transversely 2–4-euseptate, widest in middle of basal cell, or middle of conidium, tapering to an obconically truncate base; hilum thickened, darkened and refractive; apical cell conical, elongating to an apical beak up to 20 µm long. When cultivated conidia remain attached to conidiogenous cells, giving conidiophores the appearance of small tufts which is very characteristic, and not commonly observed in *Passalora*.

*Culture characteristics*: On MEA slow growing, erumpent, with dense white aerial mycelium, which becomes mouse-grey, reaching 5 mm diam after 1 wk; on PDA mouse-grey (surface), iron-grey (reverse), with diffuse red pigment in agar; on OA similar to PDA, also with diffuse red pigment in agar.

*Host range and geographic distribution*: *Dalbergia armata*, South Africa.

*Specimen examined*: **South Africa**, KwaZulu-Natal Province, South Coast, Mpenjati Nature Reserve, between Ramsgate and Port Edward, on leaves of *Dalbergia armata*, 28 May 2008, A.R. Wood, CBS H-20337 **holotype**, cultures ex-type CPC 15419 = CBS 125420, CPC 15420, 15421.

*Notes*: *Passalora dalbergiae*, which occurs on *Dalbergia sissoo* (*Fabaceae*) in India, is distinct from *P. armatae* in having superficial mycelium and solitary conidiophores (Hernández-Gutiérrez &

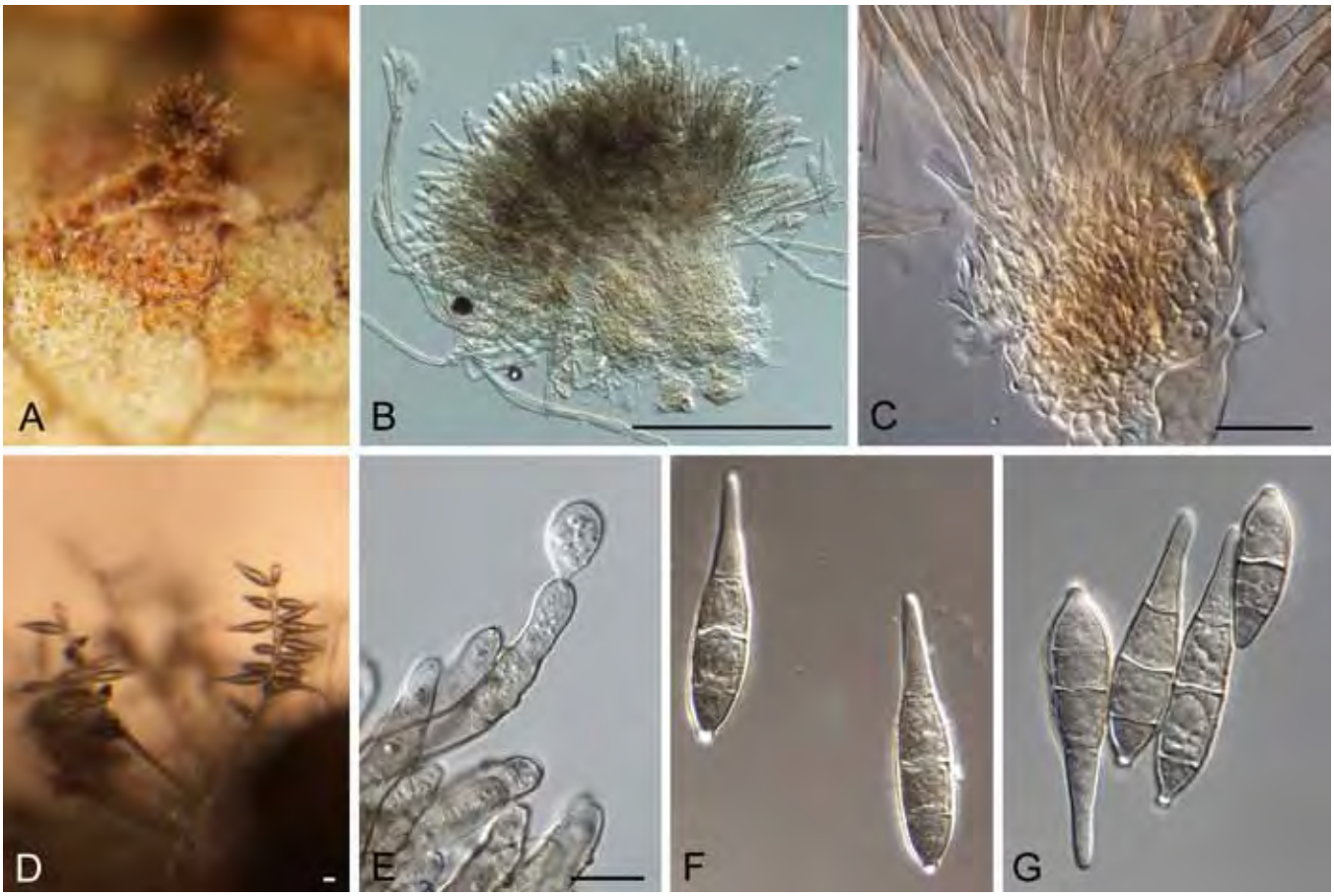



**Fig. 6.** *Passalora armatae*. A. Fruiting *in vivo*. B–C. Caespituli with prominent basal stroma. D. Sporulation on MEA. E. Conidiogenous cells giving rise to conidia. F–G. Conidia. Scale bars: B = 125 µm, C–E = 10 µm.

Dianese 2009). The previously described *Passalora dalbergiicola* is similar to *P. armatae* in conidial dimensions (3-septate, 25–45 × 7–10 µm; Ellis 1976), but distinct in that conidiophores are not in dense synnemata, conidiogenous cells can have single apical loci, and conidia have a less prominent basal taper, and lack the apical beaks typical of *P. armatae* (*in vivo* and *in vitro*).

***Schizothyriaceae*** Höhn. ex Trotter, Sacc., D. Sacc. & Traverso, In: Saccardo, Syll. Fung. 24(2): 1254. 1928.

*Type species*: *Schizothyrium acerinum* Desm., Ann. Sci. Nat. Bot. 11: 360. 1849.

*Notes*: Members of the *Schizothyriaceae* are associated with flyspeck symptoms on apples and pear fruit. The fungi grow superficially on the epicuticular wax, thereby reducing the marketability of the fruit, but do not penetrate the cuticle (Belding *et al*. 2000). Batzer *et al.* (2005, 2007) reported a range of diverse fungi to be associated with flyspeck symptoms on apples, the most prominent being species of *Schizothyrium*.

***Dissoconiaceae*** Crous & de Hoog, **fam. nov.** MycoBank MB514699.

Ascomata pseudotheciales, immerse, globosa, uniloculares. Sine pseudoparaphysibus. Asci fasciculati, octospori, bitunicati. Ascosporae ellipsoideae-fusiformes, 1-septatae, hyalinae. Conidiophora separata, ex hyphis oriunda, subcylindrica, subulata, lageniformia vel cylindrica, apicem versus attenuata, apice obtuse rotundato vel truncate, recta vel semel geniculata, laevia, modice brunnea, 0–pluriseptata, locis terminalibus vel lateralibus, rhachidi cum cicatricibus leniter incrassates, fuscatis. Conidia solitaria, pallide olivaceo-brunnea, laevia, ellipsoidea, obclavata vel globosa, 0–1-septata, hilis aliquantum fuscatis. Conidia secundaria nulla vel formata ad conidia primaria, pallide olivacea vel subhyalina, aseptata, pyriformia; conidiis impigre vel passive emittentibus.

*Ascomata* pseudothecial, immersed, globose, unilocular, papillate, ostiolate, canal periphysate; wall consisting of 3–4 layers of brown *textura angularis*; inner layer of flattened, hyaline cells. *Pseudoparaphyses* absent. *Asci* fasciculate, 8-spored, bitunicate. *Ascospores* ellipsoid-fusoid, 1-septate, hyaline, with or without mucoid sheath. *Mycelium* internal and external, consisting of branched, septate, smooth, hyaline to pale brown hyphae. *Conidiophores* separate, arising from hyphae, subcylindrical, subulate or lageniform to cylindrical, tapering to a bluntly rounded or truncate apex, straight to once geniculate, smooth, medium brown, 0–multi-septate; loci terminal and lateral, visible as slightly thickened, darkened scars on a rachis. *Conidia* solitary, pale olivaceous-brown, smooth, ellipsoid to obclavate or globose, 0–1-septate; hila somewhat darkened. *Secondary conidia* present or absent; developing adjacent to primary conidia, pale olivaceous to subhyaline, aseptate, pyriform; conidium discharge active or passive.

*Type species*: *Dissoconium aciculare* de Hoog, Oorschot & Hijwegen, Proc. K. Ned. Akad. Wet., Ser. C, Biol. Med. Sci. 86(2): 198. 1983.

*Notes*: Species of *Dissoconium* have *Mycosphaerella*-like teleomorphs (Crous *et al.* 2004c). The genus is characterised by forming conidia in pairs that are forcefully discharged, which is quite unique in the *Capnodiales* (de Hoog *et al*. 1983). Although *D. aciculare*, the type species of *Dissoconium*, was originally assumed to be hyperparasitic on powdery mildew (de Hoog *et al*. 1983), Jackson *et al*. (2004) revealed that another species, *D. dekkeri*, could act as a foliar pathogen of *Eucalyptus*. *Dissoconium dekkeri* is, however, most commonly found in leaf spots in association with other species of *Teratosphaeria* and *Mycosphaerella*. Species of *Dissoconium* remain commensalists, and frequently occur asexually on lesions associated with pathogenic species of *Capnodiales* (Crous *unpubl. data*). They are ecologically and morphologically quite distinct from other members of the *Capnodiales*, and hence a separate family, the *Dissoconiaceae*, is herewith introduced to accommodate them. *Ramichloridium* forms brown, solitary conidiophores with a rachis and apical loci similar to that observed on *Dissoconium*, and primary conidia that are pale brown, 0–1-septate, with slightly thickened hila, but lacks secondary conidia (Arzanlou *et al*. 2008b). Both *Dissoconium* and *Ramichloridium* have in the past been reported as hyperparasitic on powdery mildews on various hosts (Hijwegen & Buchenauer 1984), which suggests that they share a similar ecology.

***Teratosphaeriaceae*** Crous & U. Braun, Stud. Mycol. 58: 8. 2007.

*Type species*: *Teratosphaeria fibrillosa* Syd. & P. Syd., Ann. Mycol. 10: 40. 1912.

*Notes*: Since the family was established by Crous *et al*. (2007a) it has been shown to be too widely defined, incorporating many diverse genera (Crous *et al*. 2009b, c), and even families such as the *Piedraiaceae* (Fig. 1). The node as such is not well supported, suggesting that as more taxa are added, further families remain to be separated from the *Teratosphaeriaceae*. Presently it incorporates diverse elements, and even lichens such as *Cystocoleus ebeneus* and *Anisomeridium consobrinum*. The identity of the latter strain (CBS 101364) needs to be confirmed, as its position in the tree appears doubtful.

The genus *Catenulostroma*, which is associated with numerous diverse substrates and habitats (Crous *et al*. 2007a), is typified by *C. protearum*, for which an epitype is designated in the present study. Several strains isolated from rock surfaces (Guiedan *et al*. 2008, Ruibal *et al*. 2008, 2009, this volume) cluster with *Catenulostroma* (Fig. 1), and appear to represent undescribed species of the latter. Of interest is the fact that the type species of *Aulographina, A. pinorum* (CBS 302.71, 174.90), which has hysterothecia, clusters in a clade with *Catenulostroma microsporum*, which has a *Teratosphaeria*-like teleomorph with pseudothecia (Taylor & Crous 2000, Crous *et al*. 2004a, 2007a). Isolates of *A. pinorum* were found to produce a *Catenulostroma* anamorph in culture. This raises two possibilities, namely that either the incorrect fungus was originally isolated from pine needles (namely *Catenulostroma abietis*), or that this is a species complex, in which *A. pinorum* resides. If these strains are indeed confirmed to represent *A. pinorum*, then it reveals the genus *Aulographina* to be heterogeneous, as *A. eucalypti*, which is a major leaf spot pathogen of *Eucalyptus* (Crous *et al.* 1989, Park *et al.* 2000, Carnegie & Keane 2003), clusters distant from *A. pinorum*. The taxonomy of these taxa is currently being addressed, and will be reported on elsewhere (Cheewangkoon *et al*., in prep.). During the course of this study some new members of the *Teratosphaeriaceae* were collected, which are described below:


**Fig. 7.** *Catenulostroma protearum*. A. Colony on OA. B–G. Sporulating colony, with variable muriform to transversely septate conidia. Scale bars = 10 µm.

***Catenulostroma protearum*** (Crous & M.E. Palm) Crous & U. Braun, Stud. Mycol. 58: 17. 2007. Fig. 7.
*Basionym*: *Trimmatostroma protearum* Crous & M.E. Palm, Mycol. Res. 103: 1303. 1999.

*Culture characteristics*: On MEA spreading, erumpent, with folded surface, and unevenly lobed, smooth margins; aerial mycelium sparse; surface iron-grey to greenish black, reverse greenish black; reaching 15 mm diam after 2 wk; similar on PDA and OA.

*Host range and geographic distribution*: *Protea, Leucadendron* and *Hakea* spp., South Africa.

*Specimens examined*: **South Africa**, on leaves of *Protea grandiceps*, L. Schroeder, 15 Sept. 1986, **holotype** BPI 1107849; **South Africa**, Western Cape Province, Stellenbosch, Assegaibos, on leaves of *Leucadendron tinctum*, F. Roets, 16 Apr. 2008, **epitype designated here** CBS H-20338, culture ex-epitype, CPC 15369, 15370 = CBS 125421; *ditto*, on leaves of *Hakea sericea*, CBS H-20339, single ascospore culture CPC 15368.

*Notes*: *Catenulostroma protearum* was originally described from dead leaves of *Protea grandiceps* collected in South Africa (Crous & Palm 1999). Unfortunately the cultures died before they could be deposited, and hence the phylogenetic position of *Catenulostroma* remained uncertain. This proved to be problematic, as the genus was later shown to be heterogeneous (Crous *et al*. 2007a). The designation of the epitype in the present study clarifies the phylogenetic position of the genus, and reveals *Catenulostroma s. str.* to represent species that occur in extreme environments, on rocks, or on hard, leathery leaves such as *Proteaceae* and *Gymnospermae*.

***Devriesia hilliana*** Crous & U. Braun, **sp. nov.** MycoBank MB514700. Fig. 8.

*Etymology*: Named in fond memory of Dr C.F. Hill. "Frank" collected numerous fungi over the years, and sent them to the various international colleagues he knew to be working on these groups. The present species was one of a batch of novel taxa that Frank collected and sent to us for treatment shortly before he had a relapse. Frank's friendship and mycological expertise will be sorely missed.

*Devriesiae strelitziae* similis, sed conidiis minoribus, (5–)7–10(–12) × (2–)2.5(–3) µm.

*Colonies* sporulating on MEA. *Mycelium* consisting of branched, septate, pale brown, smooth, 2–3 µm wide hyphae. *Conidiophores* solitary, erect on creaping hyphae, unbranched, medium brown, smooth, flexuous, thick-walled, 15–50 × 2–3 µm, 3–11-septate. *Conidiogenous cells* terminal, medium brown, subcylindrical, smooth, 5–20 × 2–3 µm; proliferating sympodially; hila flattened, unthickened, somewhat darkened, 1–1.5 µm wide. *Conidia* medium brown, smooth, subcylindrical to narrowly fusoid-ellipsoidal or obclavate, apical conidium with obtuse apex, additional conidia with truncate ends, somewhat darkened, 1–1.5 µm wide; conidia straight to irregularly bent, mostly in unbranched chains, (5–)7–10(–12) × (2–)2.5(–3) µm.

*Culture characteristics*: On MEA erumpent, spreading, with folded surface, and smooth margins with sparse aerial mycelium; surface mouse-grey, with thin, olivaceous-grey margin; reverse iron-grey, reaching 8 mm diam; on PDA similar, up to 8 mm diam, centre mouse-grey, margin and reverse iron-grey; on OA erumpent with moderate mouse-grey aerial mycelium, and iron-grey margin.

*Host range and geographic distribution*: *Macrozamia communis*, Auckland, New Zealand.

*Specimen examined*: **New Zealand**, Auckland, Auckland University Campus, Princes Street, on *Macrozamia communis,* C.F. Hill, 20 Apr. 2008, CBS H-20340 **holotype**, culture ex-type CPC 15382 = CBS 123187.

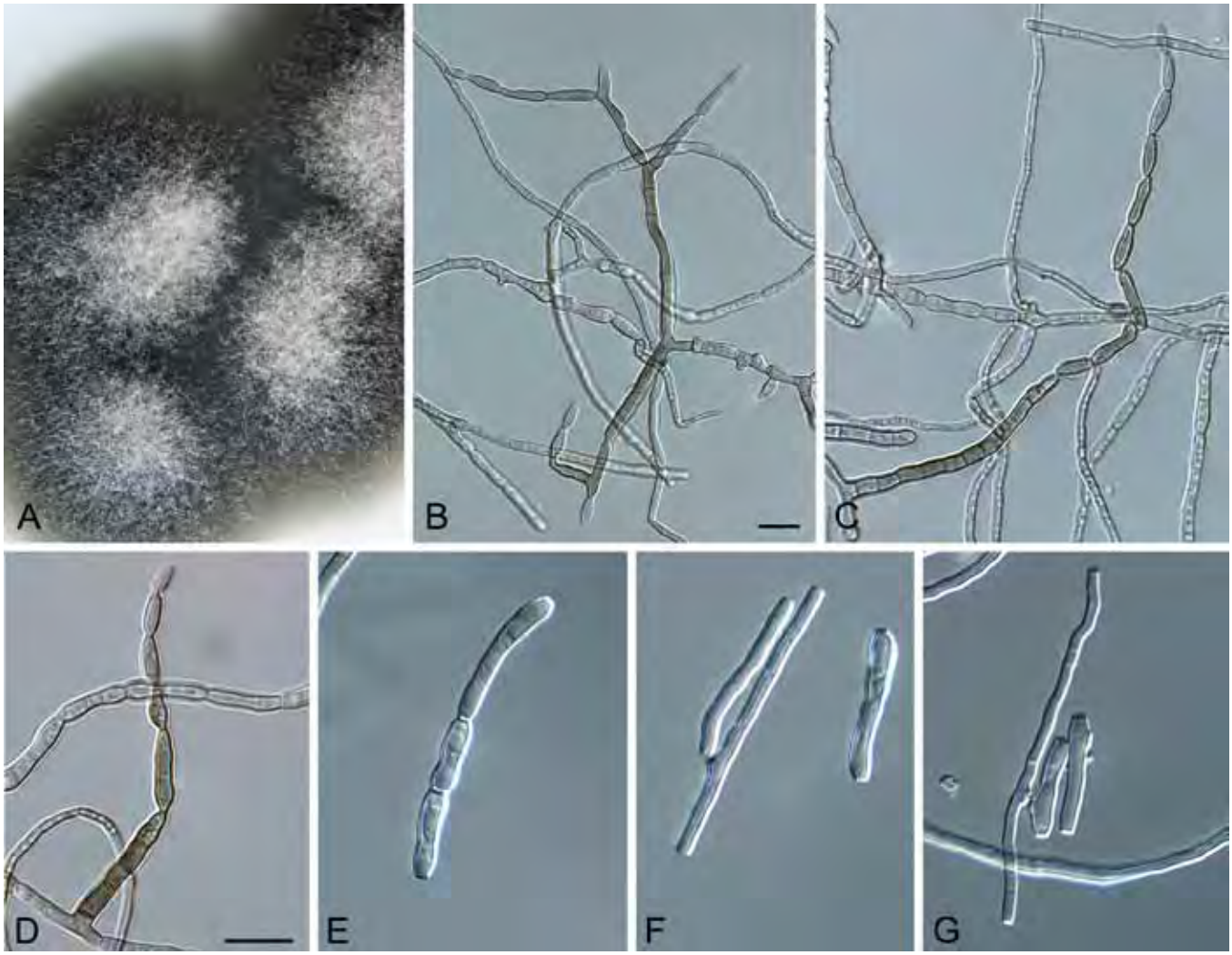



**Fig. 8.** *Devriesia hilliana*. A. Sporulating colony on OA. B–D. Conidiophores giving rise to catenulate conidia. E–G. Fragmenting conidial segments from aerial hyphae. Scale bars = 10 µm.

***Devriesia lagerstroemiae*** Crous & M.J. Wingf., **sp. nov.** MycoBank MB514701. Fig. 9.

*Etymology*: Named after the host on which it occurs, *Lagerstroemia*.

*Devriesiae strelitziae* similis, sed conidiis latioribus, (5–)7–10(–12) × (2–)2.5(–3) µm.

*Colonies* sporulating on OA. *Mycelium* consisting of smooth, branched, septate, 2–3 µm wide hyphae. *Conidiophores* rarely micronematous, predominantly macronematous, erect on creeping hyphae, brown, cylindrical with swollen basal cell, thick-walled, smooth, flexuous, 20–90 × 3–4 µm, 5–20-septate. *Conidiogenous cells* terminal, cylindrical to clavate, polyblastic, pale to medium brown, 5–10 × 2–3(–4) µm; scars somewhat thickened and darkened, not refractive. *Ramoconidia* medium brown, smooth, subcylindrical, 9–15 × 3–5 µm, (0–)1(–2)-septate, but with clavate apex and several flattened loci that are somewhat darkened and thickened, 1 µm diam. *Conidia* in branched chains of up to 10, pale brown, smooth, narrowly ellipsoid, 0–1-septate, (5–)8–12(–15) × 2–3(–4) µm; apical conidium with rounded apex, the rest with flattened loci that are somewhat darkened and thickened, not refractive, 0.5–1 µm diam.

*Culture characteristics*: On MEA erumpent, spreading, with sparse aerial mycelium and irregular margin; surface olivaceous-grey, with patches of iron-grey; reverse iron-grey, reaching 10 mm diam; on PDA similar, but on OA iron-grey, reaching 15 mm diam.

*Host range and geographic distribution*: *Lagerstroemia indica,* U.S.A., Louisiana.

*Specimen examined*: **U.S.A.**, Louisiana, Baton Rouge, Cod & Cook Centre, N30°24'50.3" W91°10'6.6", on *Lagerstroemia indica*, P.W. Crous & M.J. Wingfield, **holotype** CBS H-20341, culture ex-type CPC 14403 = CBS 125422.

*Notes*: *Devriesia lagerstroemiae* clusters close to *D. hilliana*. As far as we know, neither species is heat-resistant, nor forms chlamydospores, and hence the placement in *Devriesia* is more due to phylogenetic similarity than their ecology.

***Devriesia strelitziicola*** Arzanlou & Crous, **sp. nov.** MycoBank MB514702. Fig. 10.

*Etymology*: Named after its host plant, *Strelitzia*.

*Devriesiae strelitziae* similis, sed conidiis majoribus, (7–)25–45(–100) × (2–)2.5(–3) µm.

*Colonies* sporulating on OA. *Mycelium* consisting of medium brown, smooth, septate, branched, 2–3 µm wide hyphae; chlamydospores not observed. *Conidiophores* dimorphic. *Microconidiophores* reduced to conidiogenous cells on hyphae,

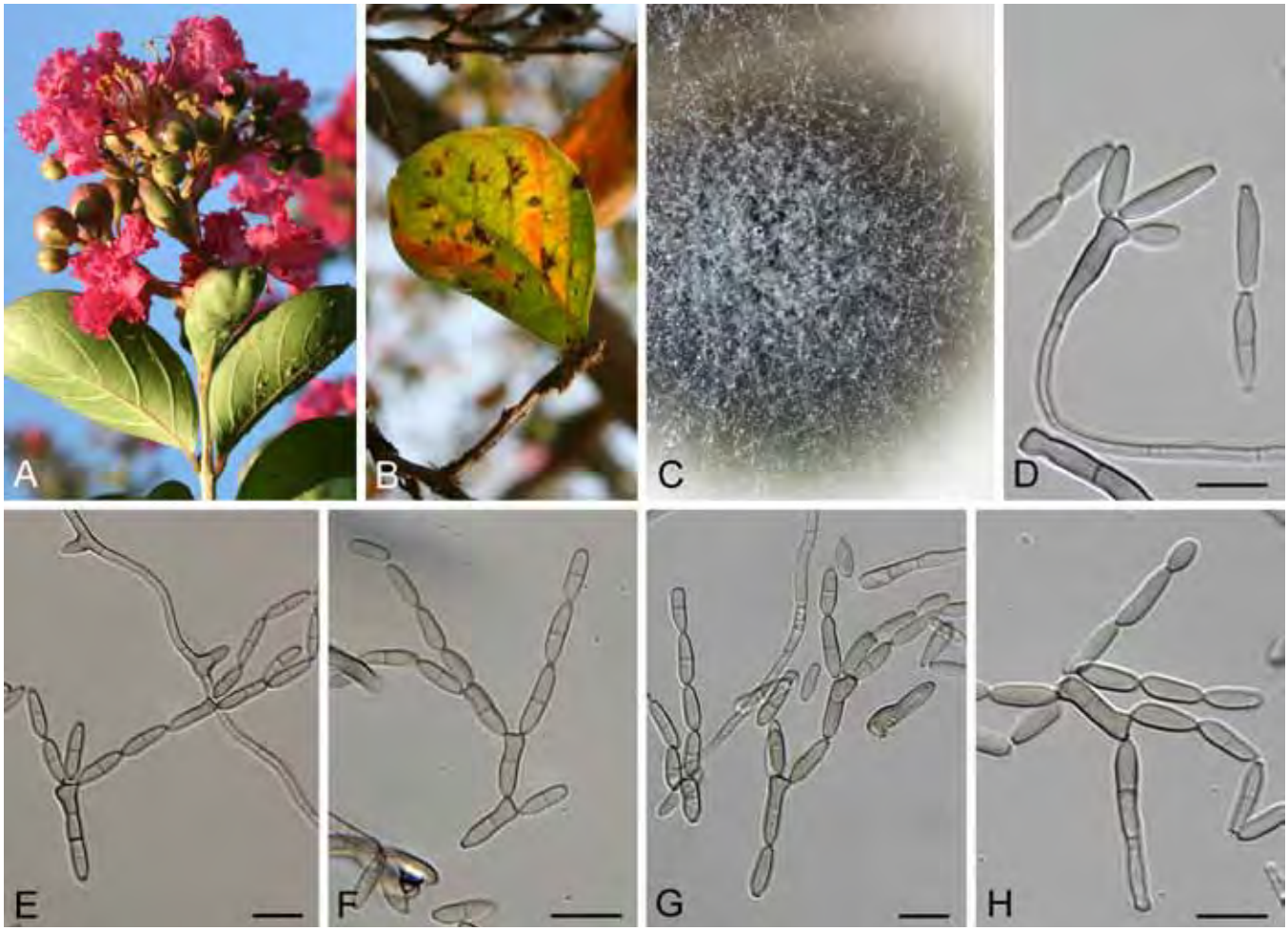



**Fig. 9.** *Devriesia lagerstroemiae*. A. Leaves and flowers of *Lagerstroemia indica*. B. Leaf spots. C. Colony on OA. D–H. Conidiophores giving rise to branched conidial chains. Scale bars = 10 µm.

erect, cylindrical, medium brown, smooth with truncate ends, proliferating sympodially, 4–7 × 2–3 µm. *Macroconidiophores* erect, cylindrical, straight to geniculate-sinuous, medium brown, smooth, unbranched or branched above, 30–100 × 2.5–3 µm, 3–10-septate. *Conidiogenous cells* terminal or lateral on branched conidiophores, medium brown, smooth, cylindrical, proliferating sympodially, 7–15 × 2.5–3 µm; loci truncate, inconspicuous, 1–1.5 µm wide. *Conidia* medium brown, smooth, guttulate, subcylindrical to narrowly obclavate, apex obtuse to truncate, base truncate, occurring in branched chains, widest at the basal septum, (7–)25–45(–100) × (2–)2.5(–3) µm, (0–)3–6(–13)-septate; hila inconspicuous to somewhat darkened and thickened, not refractive, 1–1.5 µm wide.

*Culture characteristics*: On MEA erumpent, slow growing, with moderate aerial mycelium and smooth margins; surface mouse-grey, reverse iron-grey, reaching 8 mm diam after 2 wk; similar on PDA and OA.

*Host range and geographic distribution*: *Strelitzia* sp., South Africa.

*Specimen examined*: **South Africa**, KwaZulu-Natal, Durban, Botanical Garden near Reunion, on leaves of *Strelitzia* sp., 5 Feb. 2005, W. Gams & H. Glen, CBS H-20342, holotype, culture ex-type X1045 = CBS 122480.

*Notes*: *Devriesia strelitziicola* is the second *Devriesia* species to be described from this host (Arzanlou *et al*. 2008a). The genus *Devriesia* was originally established to accommodate a group of heat-resistant, *Cladosporium*-like fungi (Seifert *et al*. 2004), and it appears that a different generic name will have to be introduced to accommodate those taxa occurring on plants. Further collections are required, however, to clarify the generic boundaries of *Devriesia* (Crous *et al*. 2007b).

***Hortaea thailandica*** Crous & K.D. Hyde, **sp. nov.** MycoBank MB514703. Fig. 11.

*Etymology*: Named after the country where it was collected, Thailand.

*Hortaeae werneckii* similis, sed conidiis brunneis, verruculosis, majoribus, (9–)10–13(–15) × (4–)5–6(–7) µm.

*Colonies* sporulating on MEA. *Mycelium* consisting of pale brown, smooth, septate, branched, 3–4 µm wide hyphae that become darker and thick-walled in the conidiogenous region. *Conidiogenous cells* integrated, intercalary on hyphae, reduced to short cylindrical loci, 2–2.5 µm wide, 1–4 µm tall; collarettes inconspicuous to minute; proliferating 1–2 times percurrently at apex. *Conidia* ellipsoid, aseptate, pale to medium brown, (4–)5–7(–9) × (2.5–)3 µm, verruculose, apex obtuse, base subtruncate with minute collarette; becoming swollen and elongate at maturity, with 1–4 transverse and 1–2 oblique septa; (9–)10–13(–15) × (4–)5–6(–9) µm; hila inconspicuous, up to 2 µm wide, frequently with visible marginal frill; microcyclic conidiation commonly observed on OA, MEA and PDA.


**Fig. 10.** *Devriesia strelitziicola*. A. *Strelitzia* sp. with dead leaves. B. Colony on OA. C–G. Conidiophores giving rise to conidia. H–M. Conidia. Scale bars = 10 µm.

*Culture characteristics*: On MEA erumpent, spreading; surface irregular, folded, greenish black, with sparse olivaceous-grey aerial mycelium and smooth, lobed, margins; reverse greenish black; reaching 12 mm diam after 2 wk; similar on OA and PDA.

*Host range and geographic distribution*: *Syzygium siamense*, Thailand.

*Specimen examined*: **Thailand**, Khao Yai National Park, N14°14'42.6" E101°22'15.7", on leaves of *Syzygium siamense*, in lesions with a cercosporoid fungus, 27 Mar. 2009, P.W. Crous & K.D. Hyde, **holotype** in BBH, **isotype** CBS H-20343, culture ex-type CPC 16652, 16651 = CBS 125423, also in BCC.

*Notes*: Similar to *Hortaea werneckii*, which is also frequently isolated from lesions in association with plant pathogenic fungi, *H. thailandica* occurred in leaf spots in association with a cercosporoid fungus. It is distinct from *H. werneckii* by forming larger conidia that turn medium brown and verruculose with age.

Several other taxa are newly placed in the *Teratosphaeriaceae* in the present study that require further evaluation. *Xenomeris juniperi*, a bitunicate ascomycete on *Jupinerus* with pseudothecia associated with a stroma, and pigmented, 1-septate ascospores, clusters close to *Teratosphaeria* species occurring on *Protea* and *Eucalyptus*, where the ascomata are also associated with stromatic tissue (Taylor & Crous 2000, Crous *et al.* 2006c). Fresh collections of this fungus would be required, however, to resolve its status. The occurrence of *Sporidesmium* species in the *Teratosphaeriaceae* should be interpreted with care, as the genus is polyphyletic, and further studies are required to resolve its status (Shenoy *et al*. 2006, Crous *et al.* 2008a, Yang *et al*., in prep.).


**Fig. 11.** *Hortaea thailandica*. A. Cercosporoid leaf spots on *Syzygium siamense*, in which *H. thailandica* occurred. B. Colonies on OA. C–E. Hyphae with conidiogenous loci (arrows). F–H. Conidia. Scale bars = 10 µm.

***Davidiellaceae*** C.L. Schoch, Spatafora, Crous & Shoemaker, Mycologia 98: 1048. 2006.

*Type species*: *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 3: 8. 2003.

*Notes*: The *Davidiellaceae* was introduced for the genus *Davidiella*, which has *Cladosporium* anamorphs. As shown in the present analysis, however, allied genera such as *Toxicocladosporium*, *Verrucocladosporium*, *Rachicladosporium* and *Graphiopsis* also belong in this family. Of interest is the position of *Melanodothis caricis* in *Cladosporium s. str*. This fungus, which infects florets of *Carex* and *Kobresia*, forms a stroma that gives rise to several immersed ascomata with bitunicate, oblong asci that are aparaphysate, and 0–(2)-septate, hyaline, 9–14.5 × 2–4 µm ascospores. In culture, a hyaline, *Ramularia*-like anamorph developed, with sympodial proliferation, catenulate conidia, with thickened, darkened loci (Arnold 1971). Although these characteristics are atypical of the *Davidiella*/*Cladosporium* species in this clade, the position of *Melanodothis caricis* in this family cannot simply be disregarded. However, the ex-type culture of this fungus (CBS 860.72) proved to be sterile.

A further unconfirmed sequence (CBS 354.29, culture sterile, but fast growing, grey-brown, *Cladosporium*-like), is that submitted as *Sphaerulina polyspora*. The culture was accessioned in 1929, deposited by A.E. Jenkins, and there is reason to believe that it was derived from BPI 623724!, which is authentic for the species, and collected by F.A. Wolf in May 1924. Wolf (1925) described this fungus from twigs of *Oxydendron arboretum* with die-back disease symptoms, collected in Raleigh, North Carolina. *Sphaerulina polyspora* (623723 = Type!) has pseudothecia with aparaphysate, bitunicate asci, and ascospores that are hyaline, 3–5-septate, 20–24 × 6–7 µm. On the host it was linked to a *Phoma*-like anamorph, which also grew similar in culture (yeast-like budding), and has hyaline conidia which are ellipsoidal, 7–8 × 3.8–4 µm.

Colonies were reported as slow-growing, grey, appressed, with germinating ascospores forming yeast-like budding cells, and rarely having hyphae that extended from the margin of the colonies. The link between *Sphaerulina*-like species, with *Selenophoma* and *Aureobasidium* synanamorphs was recently illustrated by Cheewangkoon *et al*. (2009). Although members of the *Dothideomycetes*, these taxa do not cluster in the *Davidiellaceae*, and hence it seems a fair assumption that CBS 354.29 is not representative of *Sphaerulina polyspora*.

***Rachicladosporium cboliae*** Crous, **sp. nov.** MycoBank MB514704. Fig. 12.

*Etymology*: Named after the Consortium for the Barcode of Life, CBOL, who organised a Fungal Barcoding Symposium, during which this fungus was collected.

*Rachicladosporio americano* similis, sed conidiophoris dense fasciculatis et conidiis minoribus.

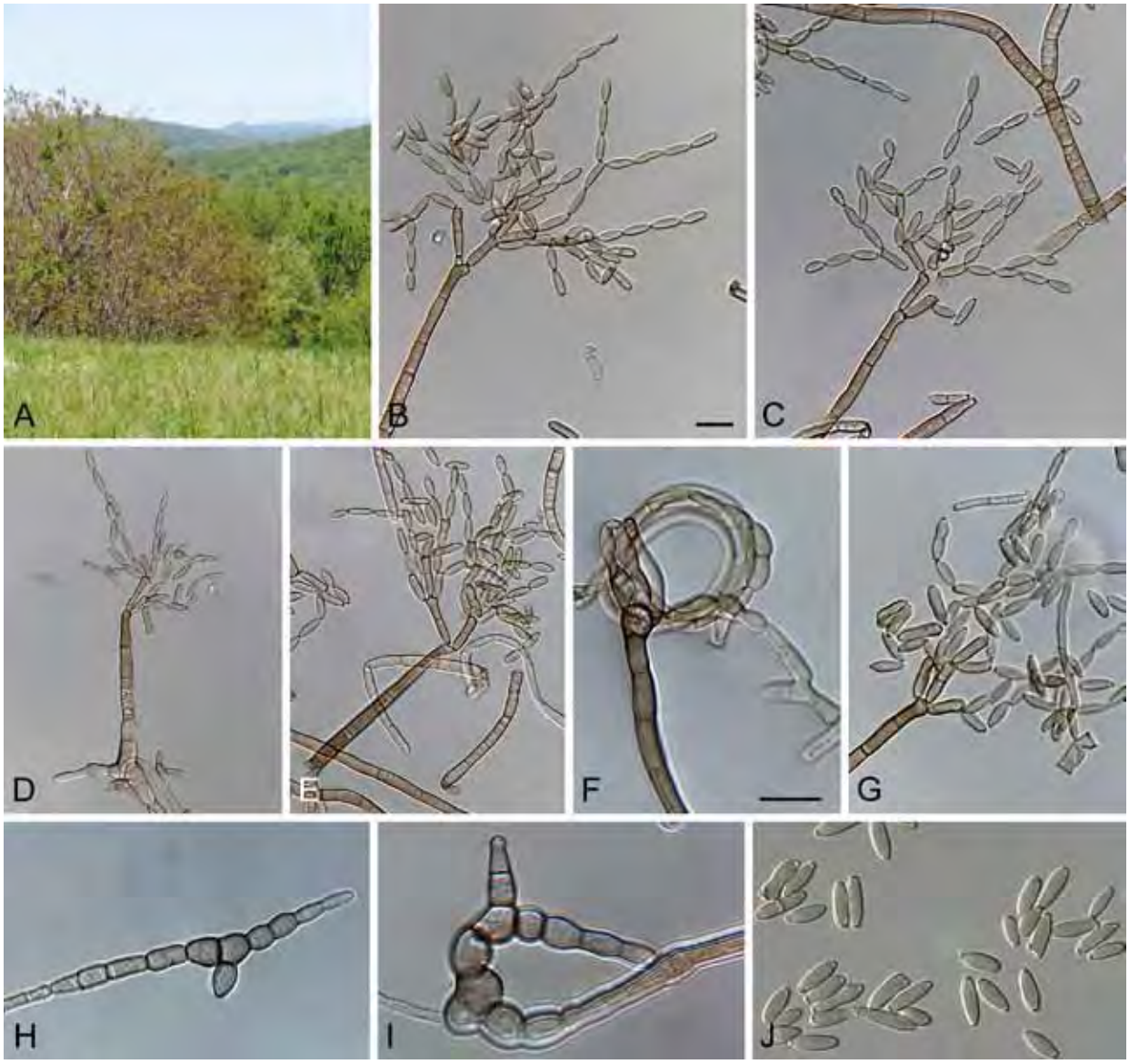



**Fig. 12.** *Rachicladosporium cboliae*. A. Front Royal collection site in Virginia. B–E, G. Conidiophores with branched conidial chains. F. Hyphal coil. H–I. Chlamydospores in chains. J. Conidia. Scale bars = 10 µm.

*Colonies* sporulating on OA. *Mycelium* consisting of branched, septate hyphae, pale brown, smooth, 1.5–3 µm wide, frequently constricted at septa, forming hyphal coils, but characteristically also forming intercalary and terminal clusters of chlamydospores that are brown, thick-walled, up to 6 µm diam. *Conidiophores* forming laterally on creeping hyphae, erect, visible as densely branched tufts on agar surface; conidiophores medium brown, smooth, thick-walled with bulbous base, lacking rhizoids, cylindrical, unbranched, flexuous, up to 250 µm long, 4–6 µm wide, 10–20-septate. *Conidiogenous cells* terminal, medium brown, smooth, polyblastic, subcylindrical, 10–20 × 3–4 µm; loci terminal, thickened, darkend, refractive, 1 µm diam. *Ramoconidia* 0(–1)-septate, subcylindrical, medium brown, smooth, 7–12 × 3–4 µm. *Conidia* 0(–1)-septate, in branched chains of up to 10, ellipsoid, pale brown, smooth, (6–)7–8(–10) × (2–)2.5(–3) µm; hila thickened, darkened and refractive, up to 1 µm diam.

*Culture characteristics*: On MEA spreading with sparse aerial mycelium and smooth margins; surface folded, centre pale mouse-grey to mouse-grey, margin iron-grey; reverse greenish black, reaching 15–20 mm diam after 2 wk; on PDA spreading with moderate aerial mycelium and smooth margins; surface olivaceous-grey, margin mouse-grey, reverse olivaceous-grey; reaching 30 mm diam; on OA spreading, folded with moderate aerial mycelium; surface pale mouse-grey (centre) to olivaceous-grey at margin, reaching 20 mm diam.

*Host range and geographic distribution*: Twig litter, Virginia, U.S.A.

*Specimen examined*: **U.S.A.**, Virginia, Front Royal, N38°53'35" W78°10'50", on twig debris, 14 May 2007, *P.W. Crous*, **holotype** CBS H-20344, cultures ex-type CPC 14034 = CBS 125424, CPC 14035, 14036.

*Notes*: *Rachicladosporium cboliae* is a cryptic species close to *R. americanum*, which was collected at the same site. They can be distinguished on the litter in that *R. cboliae* has conidiophores with densely branched tufts of conidia, in contrast to the more sparsely branched conidiophores of *R. americanum*. Furthermore, *R. cboliae* also forms prominent chains of chlamydospores in culture, which lacks in *R. americanum*. Finally, *R. cboliae* has smaller ramoconidia and conidia than those found in *R. americanum* (ramoconidia 13–23 × 3–4 µm; conidia 10–18 × 3–4 µm; Cheewangkoon *et al*. 2009).

## DISCUSSION

The class *Dothideomycetes* incorporates fungal taxa exhibiting a wide range of nutritional modes, and results in these fungi being found in many diverse niches (Fig. 13). The two largest orders *Pleosporales* (Zhang *et al.* 2009; this volume) and *Capnodiales* encapsulate this diversity. Here we continue to expand sampling within the *Capnodiales* in order to provide a well founded phylogenetic scaffold for taxonomic classification, informative genomic sampling, ecological studies and evolutionary evaluations.

### *Capnodiales*

The *Capnodiales* currently contain nine families (Lumbsch & Huhndorf 2007, Kirk *et al*. 2008), a selection of which are included in this study, namely *Capnodiaceae*, *Davidiellaceae*, *Mycosphaerellaceae*, *Piedraiaceae*, and *Teratosphaeriaceae*. Unfortunately, no cultures were available of the *Antennulariellaceae* and *Metacapnodiaceae*, while *Coccodiniaceae* was again shown to cluster outside the order, in *Chaetothyriales* (Crous *et al*. 2007a). Families supported within *Capnodiales* (Fig. 1) include *Capnodiaceae*, *Davidiellaceae*, *Teratosphaeriaceae*, *Dissoconiaceae*, *Schizothyriaceae* and *Mycosphaerellaceae*. No support was obtained for *Piedraiaceae*, which appeared to cluster within *Teratosphaeriaceae*.

One of the main aims of the present study was to resolve the status of the *Capnodiales* and *Mycosphaerellales*. Although we were able to distinguish a clear, well resolved node for the *Mycosphaerellales* (incl. *Mycosphaerellaceae*), this node was not well supported, and elevating it to ordinal level would mean that additional orders need to be introduced to accommodate several families outside the *Capnodiales s. str*. This finding led us to conclude that it is best to retain all families within a single, diverse order, namely the *Capnodiales*.

### Evolution of nutritional modes and ecological growth habits

The ancestral state of the present assemblage of taxa is likely to be saprobic, as *Phaeotheca* (Sigler *et al*. 1981, de Hoog *et al*. 1997, Tsuneda *et al.* 2004), and *Comminutispora* (Ramaley 1996) represent the earliest diverging lineages. This was similarly found for a majority of lineages in the larger context of *Ascomycota* (Schoch *et al*. 2009a, b). These taxa were not only all isolated from dead materials or substrates, but they also share the same unique mode of conidiogenesis, namely endoconidia, and a "black-yeast" appearance in culture. *Phaeotheca,* which is strongly halophilic (Zalar *et al*. 1999) is closely related to the lichen *Racodium rupestre*, which forms an association with *Trentepohlia* algae, in which the filamentous algae is enclosed by melanised hyphae of the fungus. This feature is also shared by another lichen, namely *Cystocoleus ebeneus* (*Teratosphaeriaceae*) (Muggia *et al*. 2008). The *Capnodiaceae* (sooty molds) that also cluster in a basal position in the tree are epiphytes, growing on insect exudates (honey dew). The *Capnodiaceae* are related to the *Davidiellaceae*, which represent *Cladosporium* and allied genera. This family contains a wide range of ecological adaptations, from primary plant pathogens, such as *Graphiopsis chlorocephala* on *Paeonia* (Schubert *et al*. 2007a, Braun *et al*. 2008), "*Mycosphaerella*" *iridis* on *Iris* (David 1997), to taxa opportunistic on humans, *Cladosporium bruhnei* (Schubert *et al*. 2007b), to halotolerant taxa, *Cladosporium sphaerospermum* (Zalar *et al*. 2007, Dugan *et al*. 2008), to saprobes, *C. herbarum, C. cladosporioides* (Schubert *et al*. 2007b).

The *Teratosphaeriaceae* contains several disjunct elements, many of which may still eventually be removed from the family as more taxa and additional sequence data are added, providing a better resolution to some of these clades. In its widest sense, the family contains lichens (*Anisomeridium, Cystocoleus*), saprobes (*Catenulostroma* spp.), and halophilic, hyperhydrotic or lipophilic species that have been reported from humans (*Piedraia, Hortaea, Penidiella, Stenella*) (de Hoog *et al*. 2000, Bonifaz *et al*. 2008, Plemenitaš *et al*. 2008), with the most derived clades tending to contain plant pathogens (*Readeriella, Teratosphaeria*).

*Dissoconiaceae* is an early diverging lineage to the *Mycosphaerellaceae* and *Schizothyriaceae*. Whereas most members of *Dissoconiaceae* appear to be commensalists, there is evidence that some species could be plant pathogenic (Jackson *et al*. 2004), while the *Schizothyriaceae* contains epiphytes (Batzer *et al*. 2007). The *Mycosphaerellaceae* contains species that are biotrophic (*Polythrincium*; Simon *et al.* 2009), necrotrophic plant pathogens (*Brunneosphaerella, Cercospora, Dothistroma, Pseudocercospora*, *Pseudocercosporella, Ramularia,* and *Septoria*), as well as some species that are saprobic (*Passalora, Pseudocercospora*, *Ramichloridium* and *Zasmidium*; Arzanlou *et al*. 2007), or endophytic (*Pseudocercosporella endophytica*; Crous 1998).

Within the *Capnodiales*, the positioning of saprobes such as *Phaeotheca* and *Comminutispora* and the sooty moulds (*Capnodiaceae*) may represent the more primitive state, from where transitions occurred to more lichenised, saprobic, biotrophic and nectrotrophic, plant pathogenic members of the order (Fig. 13). This appears to mirror the other large and diverse order in the class, the *Pleosporales* (Zhang *et al*. 2009; this volume). Lichenisation, as well as the ability to be saprobic or plant pathogenic evolved more than once, though the taxa in the later diverging clades of the tree tend to be strictly nectrotrophic plant pathogens. This should be interpreted with care, however, as *Polythrincium* is presently the only biotrophic member included in this analysis, and other biotrophic members of the *Capnodiales* may end up clustering here, among the presently dominant nectrotropic plant pathogens. One important and recent addition to *Capnodiales* diversity is the rock-inhabiting fungi (Ruibal *et al*. 2008, 2009; this volume). Although so far mainly isolated from sources in Antarctica and the Mediterranean area, it is clear that they are a ubiquitous group of fungi likely found throughout the globe. Their genetic diversity is underscored by the fact that rock inhabiting fungi of convergent morphology are also placed in other ascomycotan classes and orders (Gueidan *et al*. 2008). The fact that many of these species have reduced morphologies and are slow growers make their taxonomy challenging, but their phylogenetic placement within *Teratosphaeriaceae* and several other lineages within *Capnodiales* makes their inclusion in subsequent phylogenetic assessments of this order essential.

For this study, we designed novel primers to supplement primers presently available in literature. Although primers are usually designed for the genus or family of interest, they frequently tend to have a wider application. Therefore, we attempted to design our primers using a wide range of sequences from the GenBank sequence database, in the hope that these primers will eventually find application outside of the *Capnodiales* as well. Although this remains to be tested, we expect it to be the case. Our sequencing of the complete SSU and LSU for the selected members of the *Capnodiales* had a surprisingly large number of insertions present


**Fig. 13.** Members of *Capnodiales* exhibiting different ecological growth habits. A–C. *Mycosphaerella marksii* (plant pathogen). A. Leaf spot on *Eucalyptus*. B. Homothallic colony on MEA. C. Asci. D. Conidiophore of *Cladosporium sphaerospermum* (saprobe). E–G. Ascomata and asci of *Davidiella macrocarpa* (saprobe). H–J. *Dissoconium dekkeri* (plant pathogen, commensalist). H. Colony sporulating on MEA, with discharged conidia at the margin. I. Asci. J. Primary and secondary conidia attached to conidiophore. K–L. *Dissoconium proteae* (commensalist). K. Sporulation on MEA with microsclerotia. L. Two conidial types attached to conidiophore (arrow). M–Q. *Conidioxyphium gardeniorum* (sooty mold). M. Sporulation on MEA. N–P. Elongated, branched conidiomata with apical ostiolar hyphae. Q. Conidia. R–T. Leaf spot, ascus and verruculose ascospores of *Teratosphaeria fibrillosa* (plant pathogen). U–X. *Schizothyrium pomi* (epiphyte). U. Thyrothecia occurring on a *Rhus* stem. V. Ascomatal initials forming on OA. W. Asci. X. Conidiophore and conidia *in vitro*. Scale bars: E = 200 µm, M–O = 50 µm, all others = 10 µm.

for numerous strains. Although some of these insertions were anticipated based on data already present in GenBank's database, the insertions in the LSU were not expected based on the sequences used for primer design. However, this could be a result of the fewer complete LSU sequences available in the database rather than a deviation on the part of members of the *Capnodiales*. More complete LSU sequences are needed from diverse orders to test whether this is the case or not. Some of the taxa sequenced during this study had insertions present at almost all of the possible insertion positions, *e.g. Mycosphaerella latebrosa*, *Septoria quercicola* and *Teratosphaeria mexicana*. These taxa are distributed throughout the tree, and do not only cluster in a basal position, and therefore it is difficult to predict why so many insertions were present. If these insertions were all present in a basal position, it would have been possible to argue that the higher number of insertions represents the ancestral condition, and that these insertions are lost during evolution. However, this proved not to be the case, and it could be that these taxa accumulated these insertions.

Although the present study adds significantly to our knowledge of the *Capnodiales*, the *Capnodiaceae* are still underrepresented, and probably consist of numerous diverse lineages that can be elevated to family level once our phylogenies become more resolved. Regardless of this fact, the *Mycosphaerellaceae* clade appears to be quite robust. It seems likely that further sampling of the diverse *Teratosphaeriaceae* will necessitate further taxonomic changes. The fact that the saprobic and plant pathogenic and endophytic modes have evolved several times in different families, suggest that many taxa can still easily adapt to changing environments. A focus on adding more lichenicolous taxa, and taxa occurring on non-plant substrates is crucial to provide further insight into the ecological adaptations occurring in the *Capnodiales*.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Details of the isolates for which novel sequences were generated. Samples without an 18S rDNA accession number were only used in the 28S rDNA analysis; sequences of CBS 723.79 and CBS 123.26 were used in both analyses. The accession number for 5.8S nrDNA also includes the flanking spacer regions.

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| *Aulographina pinorum* | CBS 302.71; ETH 7129; UAMH 4037 | *Pinus maritima* | France | E. Müller | —, GU214622, GU214393 |
| *Batcheloromyces leucadendri* | CBS 110892; CPC 1837 | *Leucadendron* sp. | South Africa | L. Swart | GU214515, AY260100, EU019246 |
| *Batcheloromyces proteae* | CBS 110696; CPC 1518 | *Protea cynaroides* | South Africa | L. Viljoen | AY251102, AY260099, EU019247 |
| *Brunneosphaerella protearum* | CPC 13905 | *Protea* sp. | South Africa | P.W. Crous | —, GU214623, GU214394 |
| | CPC 13914 | *Protea* sp. | South Africa | P.W. Crous | —, GU214624, GU214395 |
| | CPC 15231 | *Protea nitida* | South Africa | L. Mostert | —, GU214625, GU214396 |
| | CPC 16338 | *Protea* sp. | South Africa | P.W. Crous | —, GU214626, GU214397 |
| *Capnobotryella renispora* | CBS 214.90; CBS 176.88; IAM 13014; JCM 6932; TNS F-198506 | *Capnobotrys neessii* | Japan | J. Sugiyama | AY220612, AY220612, GU214398 |
| | CBS 215.90; IAM 13015 | *Capnobotrys neessii* | Japan | J. Sugiyama | AY220613, AY220613, GU214399 |
| *Capnodium coffeae* | CBS 147.52 | *Coffea robusta* | Zaire | — | DQ247808, AJ244239, GU214400 |
| *Catenulostroma chromoblastomycosum* | CBS 597.97 | Man, chromoblastomycosis | Zaire | V. de Brouwere | GU214516, AJ244260, EU019251 |
| *Catenulostroma elginense* | CBS 111030; CPC 1958 | *Protea grandiceps* | South Africa | J.E. Taylor | GU214517, AY260093, EU019252 |
| *Catenulostroma germanicum* | CBS 539.88 | Stone | Germany | — | GU214518, EU019253, EU019253 |
| *Catenulostroma microsporum* | CBS 110890; CPC 1832 | *Protea cynaroides* | South Africa | L. Swart | GU214520, AY260097, EU019255 |
| *Catenulostroma protearum* | CBS 125421; CPC 15370 | *Leucadendron tinctum* | South Africa | F. Roets | —, GU214627, GU214401 |
| | CPC 15368 | *Hakea sericea* | South Africa | F. Roets | —, GU214628, GU214402 |
| | CPC 15369 | *Leucadendron tinctum* | South Africa | F. Roets | —, GU214629, GU214403 |
| *Cercospora apii* | CBS 118712 | — | Fiji | P. Tyler | GU214653, GU214653, GU214653 |
| *Cercospora beticola* | CBS 116456; CPC 11557 | *Beta vulgaris* | Italy | V. Rossi | AY840527, AY840527, GU214404 |
| *Cercospora capsici* | CPC 12307 | *Capsicum annuum* | South Korea | H.D. Shin | GU214654, GU214654, GU214654 |
| *Cercospora janseana* | CBS 145.37; CPC 4303; IMI 303642 | *Oryza sativa* | U.S.A. | E.C. Tullis | AY251103, AY260064, GU214405 |
| *Cercospora sojina* | CPC 12322 | *Glycine soja* | South Korea | H.D. Shin | GU214655, GU214655, GU214655 |
| *Cercospora zebrinae* | CBS 112893; CPC 3955 | *Trifolium protense* | Canada | K. Seifert | AY251104, AY260078, GU214406 |
| | CBS 118789; WAC 5106 | *Trifolium subterraneum* | Australia | M.J. Barbetti | GU214656, GU214656, GU214656 |
| | CBS 118790; IMI 262766; WAC 7973 | *Trifolium subterraneum* | Australia | M.J. Barbetti | GU214657, GU214657, GU214657 |
| *Cercosporella virgaureae* | CBS 113304 | *Erigeron annueus* | South Korea | H.D. Shin | GU214658, GU214658, GU214658 |
| *Cladosporium bruhnei* | CBS 115683; ATCC 66670; CPC 5101 | CCA-treated Douglas-fir pole | U.S.A. | — | AY251096, AY251078, GU214408 |
| | CBS 188.54; ATCC 11290; IMI 049638; CPC 3686 | — | — | G.A. de Vries | AY251098, AY251077, EU019263 |
| *Cladosporium cladosporioides* | CBS 109.21; ATCC 11277; ATCC 200940; CPC 3682; IFO 6368; IMI 049625 | *Hedera helix* | U.K. | G.A. de Vries | AY251093, AY251073, EU019262 |
| | CBS 401.80; CPC 3683 | *Triticum aestivum* | Netherlands | N.J. Fokkema | AY251091, AY251074, GU214409 |
| *Cladosporium herbarum* | CBS 723.79 | *Allium porrum* | New Zealand | A.C. Jamieson | EU167558, EU167558, GU214410 |
| *Cladosporium* sp. | CPC 15513 | *Rubus fruticosus* | Italy | P.W. Crous | —, GU214630, GU214411 |
| | CPC 15516 | *Pyrus communis* | Ukraine | A. Akulov | —, GU214631, GU214412 |
| *Cladosporium uredinicola* | ATCC 46649; CPC 5390 | *Quercus nigra* | U.S.A. | G. Morgan-Jones | AY251097, AY251071, EU019264 |
| *Davidiella rosigena* | CBS 330.51 | Leaf spot in *Rosa* sp. | Netherlands | — | —, GU214632, GU214413 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| *Devriesia hilliana* | CBS 123187; CPC 15382 | *Macrozamia communis* | New Zealand | C.F. Hill | —, GU214633, GU214414 |
| *Devriesia lagerstroemiae* | CBS 125422; CPC 14403 | *Lagerstroemia indica* | U.S.A. | P.W. Crous & M.J. Wingfield | —, GU214634, GU214415 |
| *Devriesia staurophora* | CBS 375.81; ATCC 200934; CPC 3687 | Páramo soil | Colombia | H. Valencia | EF137359, AF393723, GU214416 |
| *Devriesia strelitziicola* | CBS 122480; X1045 | *Strelitzia* sp. | South Africa | W. Gams & H. Glen | —, GU214635, GU214417 |
| *Dissoconium aciculare* | CBS 201.89 | *Brassica* sp. | Netherlands | T. Hijwegen | GU214522, AY725519, GU214418 |
| | CBS 204.89 | *Astragalus* sp. | Germany | T. Hijwegen | GU214523, AY725520, GU214419 |
| | CBS 342.82; CPC 1534 | *Erysiphe*, on *Medicago lupulina* | Germany | T. Hijwegen | GU214524, AF173308, EU019266 |
| *Dissoconium commune* | CBS 110747; CPC 831 | *Eucalyptus nitens* | South Africa | P.W. Crous | GU214525, AY725535, GU214420 |
| | CBS 114238; CPC 10440 | *Eucalyptus globulus* | Spain | J.P.M. Vazquez | GU214526, AY725541, EU019267 |
| | CBS 114239; CPC 10492 | *Eucalyptus globulus* | New Zealand | W. Gams | GU214527, AY725542, GU214421 |
| *Dissoconium dekkeri* | CBS 110748; CMW 14906; CPC 825 | *Eucalyptus grandis* | South Africa | G. Kemp | GU214528, AF309625, GU214422 |
| | CBS 111169; CMW 5164; CPC 1232 | *Eucalyptus globulus* | Zambia | — | GU214529, AY725550, GU214423 |
| | CBS 111272; CPC 1188 | *Eucalyptus nitens* | South Africa | M.J. Wingfield | GU214530, AY725551, GU214424 |
| | CBS 111282; CPC 1233 | *Eucalyptus globulus* | Zambia | — | GU214531, AF173305, GU214425 |
| | CBS 567.89; CPC 1535 | *Juniperus chinensis* | Netherlands | T. Hijwegen | AY251101, AF173309, EU019268 |
| *Dothistroma pini* | CBS 116487; CMW 10951 | *Pinus nigra* | U.S.A. | G. Adams | GU214532, AY808302, GU214426 |
| *Dothistroma septosporum* | CBS 112498; CPC 3779 | *Pinus radiata* | Ecuador | — | GU214533, AY293062, GU214427 |
| *Graphiopsis chlorocephala* | CBS 121523; CPC 11969 | *Paeonia officinalis* | Germany | K. Schubert | GU214534, EU009458, EU009458 |
| *Hortaea acidophila* | CBS 113389 | Lignite, pH 1 | Germany | U. Hölker | —, GU214636, GU214428 |
| *Hortaea thailandica* | CBS 125423; CPC 16651 | *Syzygium siamense* | Thailand | P.W. Crous & K.D. Hyde | —, GU214637, GU214429 |
| *Lecanosticta acicola* | CBS 871.95; MPFN 314 | *Pinus radiata* | France | M. Morelet | GU214663, GU214663, GU214663 |
| *Leptoxyphium fumago* | CBS 123.26; ATCC 11925; IMI 089363; LSHB X13 | *Hibiscus tiliaceus* | Indonesia | — | GU214535, —, GU214430 |
| *Melanodothis caricis* | CBS 860.72; ATCC 24309; DAOM 116433 | *Carex sitchensis* | Canada | — | —, GU214638, GU214431 |
| *Miuraea persicae* | CPC 10069 | *Prunus persica* | South Korea | H.D. Shin | GU214660, GU214660, GU214660 |
| *Mycosphaerella acaciigena* | CBS 112515; CPC 3837 | *Acacia mangium* | Venezuela | M.J. Wingfield | AY251116, AY752143, GU214432 |
| | CBS 112516; CPC 3838 | *Acacia mangium* | Venezuela | M.J. Wingfield | GU214661, GU214661, GU214661 |
| *Mycosphaerella africana* | CBS 116154; CMW 4945; CPC 794 | *Eucalyptus viminalis* | South Africa | P.W. Crous | GU214536, AF173314, GU214433 |
| *Mycosphaerella bixae* | CBS 111804; CPC 2554 | *Bixa orellana* | Brazil | P.W. Crous & R.L. Benchimol | GU214557, AF362056, GU214455 |
| *Mycosphaerella ellipsoidea* | CBS 110843; CPC 850 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | GU214537, AY725545, GU214434 |
| *Mycosphaerella endophytica* | CBS 114662; CPC 1193 | *Eucalyptus* sp. | South Africa | P.W. Crous | GU214538, DQ302953, GU214435 |
| *Mycosphaerella graminicola* | CBS 100335; IPO 69001.61 | *Triticum aestivum* | — | G.H.J. Kema | GU214539, EU019297, EU019297 |
| | CBS 110744; CPC 658 | *Triticum* sp. | South Africa | P.W. Crous | AY251117, AF362068, EU019298 |
| | CBS 115943; IPO323 | *Triticum aestivum* | Netherlands | R. Daamen | GU214540, AF181692, GU214436 |
| *Mycosphaerella handelii* | CBS 113302 | *Rhododendron* sp. | Netherlands | P.W. Crous & U. Braun | EU167581, EU167581, GU214437 |
| *Mycosphaerella heimii* | CBS 110682; CMW 4942; CPC 760 | *Eucalyptus* sp. | Madagascar | P.W. Crous | GU214541, AF309606, GU214438 |
| *Mycosphaerella heimioides* | CBS 111190; CMW 3046; CPC 1312 | *Eucalyptus* sp. | Indonesia | M.J. Wingfield | GU214542, AF309609, GU214439 |
| *Mycosphaerella holualoana* | CBS 110699; CPC 2155 | *Leucospermum* sp. | U.S.A.: Hawaii | P.W. Crous | GU214543, AY260084, GU214440 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| *Mycosphaerella irregulariramosa* | CBS 111211; CPC 1362 | *Eucalyptus saligna* | South Africa | M.J. Wingfield | GU214544, AF309608, GU214441 |
| *Mycosphaerella keniensis* | CBS 111001; CMW 5147; CPC 1084 | *Eucalyptus grandis* | Kenya | M.J. Wingfield | GU214545, AF173300, GU214442 |
| *Mycosphaerella latebrosa* | CBS 652.85 | *Acer pseudoplatanus* | Netherlands | H.A. van der Aa | AY251114, AF362067, GU214443 |
| | CBS 687.94 | *Acer pseudoplatanus* | Netherlands | G. Verkley | GU214546, AY152553, GU214444 |
| *Mycosphaerella lupini* | CPC 1661 | *Lupinus* sp. | U.S.A. | W. Kaiser | GU214547, AF362050, FJ839661 |
| *Mycosphaerella marasasii* | CBS 110790; CPC 348 | *Syzygium cordatum* | South Africa | M.J. Wingfield | GU214548, AF309591, GU214445 |
| *Mycosphaerella marksii* | CBS 110942; CPC 982 | *Eucalyptus botryoides* | Australia | A.J. Carnegie | GU214549, AF309589, GU214446 |
| | CPC 11222 | *Eucalyptus grandis* | Bolivia | M.J. Wingfield | GU214550, DQ302983, GU214447 |
| *Mycosphaerella parkii* | CBS 387.92; CMW 14775; CPC 353 | *Eucalyptus grandis* | Brazil | M.J. Wingfield | GU214551, AF309590, GU214448 |
| *Mycosphaerella* sp. | CBS 111166; CPC 1224 | *Eucalyptus cladocalyx* | South Africa | A.R. Wood | GU214552, AF173302, GU214449 |
| | CBS 111167; CPC 1225 | *Eucalyptus cladocalyx* | South Africa | A.R. Wood | GU214553, AF309593, GU214450 |
| *Mycosphaerella sphaerulinae* | CBS 112621; CPC 4314 | *Eucalyptus* sp. | Chile | — | GU214554, AY293066, GU214451 |
| *Mycosphaerella stromatosa* | CBS 101953; CPC 1731 | *Protea* sp. | South Africa | S. Denman | AY251115, EU167598, EU167598 |
| *Mycosphaerella tasmaniensis* | CBS 111687; CMW 14780; CPC 1555 | *Eucalyptus nitens* | Australia | — | GU214555, AF310107, GU214452 |
| *Passalora ageratinae* | CBS 125419; CPC 15365 | *Ageratina adenophora* | South Africa | A.R. Wood | —, GU214639, GU214453 |
| *Passalora bellynckii* | CBS 150.49; CPC 3635 | *Cynanchum vincetoxicum* | Switzerland | S. Blumer | GU214556, AF222831, GU214454 |
| *Passalora brachycarpa* | CBS 115124 | — | — | C.F. Hill | GU214664, GU214664, GU214664 |
| *Passalora armatae* | CBS 125420; CPC 15419 | *Dalbergia armata* | South Africa | A.R. Wood | —, GU214640, GU214456 |
| *Passalora dioscoreae* | CPC 10855 | *Dioscorea tokora* | South Korea | H.D. Shin | GU214665, GU214665, GU214665 |
| *Passalora dodonaea* | CPC 1223 | *Dodonaea* sp. | — | P.W. Crous | AY251108, GU214641, GU214457 |
| *Passalora eucalypti* | CBS 111318; CPC 1457 | *Eucalyptus saligna* | Brazil: Suzano | P.W. Crous | GU214558, AF309617, GU214458 |
| *Passalora fulva* | CBS 119.46; CPC 3688 | *Lycopersicon esculentum* | Netherlands | — | AY251109, AY251069, DQ008163 |
| *Passalora graminis* | CBS 113303 | *Alopecurus aequalis* var. *amurensis* | South Korea | H.D. Shin | GU214666, GU214666, GU214666 |
| *Passalora perplexa* | CBS 116364; CPC 11150 | *Acacia crassicarpa* | Indonesia | M.J. Wingfield | GU214559, AY752163, GU214459 |
| *Passalora sequoiae* | CPC 11258 | *Juniperus virginiana* | U.S.A. | C.S. Hodges | GU214667, GU214667, GU214667 |
| *Passalora* sp. | CBS 115525; CPC 3951 | *Tilia americana* | Canada | K. Seifert | GU214560, AY293064, GU214460 |
| | CPC 12319 | *Ambrosia artemisifolia* var. *elatior* | South Korea | H.D. Shin | GU214668, GU214668, GU214668 |
| *Passalora vaginae* | CBS 140.34; DSM 1148; IMI 303641 | *Saccharum officinarum* | Taiwan | — | GU214561, AF222832, GU214461 |
| *Passalora zambiae* | CBS 112970; CPC 1228 | *Eucalyptus globulus* | Zambia | T. Coutinho | GU214562, AY725522, EU019272 |
| | CBS 112971; CMW 14782; CPC 1227 | *Eucalyptus globulus* | Zambia | T. Coutinho | GU214563, AY725523, EU019273 |
| *Passalora*-like genus | CPC 11876 | *Avicermia* sp. | South Africa | W. Gams | GU214564, GU214642, GQ852622 |
| *Penidiella columbiana* | CBS 486.80 | *Paepalanthus columbianus* | Colombia | W. Gams | GU214565, AJ244261, EU019274 |
| *Phacellium paspali* | CBS 113093; RoKI 1144 | *Setaria palmicola* | Taiwan | R. Kirschner & C.-J. Chen | GU214669, GU214669, GU214669 |
| *Phaeophleospora atkinsonii* | CBS 124565; ICMP 17860 | Leaf of *Hebe* sp. | New Zealand | — | —, GU214643, GU214462 |
| | CBS 124566; ICMP 17862 | Leaf of *Hebe* sp. | New Zealand | — | —, GU214644, GU214463 |
| *Phaeophleospora eugeniicola* | CPC 2557 | *Eugenia* sp. | Brazil | — | GU214566, FJ493190, FJ493208 |
| | CPC 2558 | *Eugenia* sp. | Brazil | — | GU214567, FJ493191, FJ493209 |
| *Phloeospora maculans* | CBS 115123 | — | — | C.F. Hill | GU214670, GU214670, GU214670 |
| *Piedraia hortae* var. *hortae* | CBS 374.71 | Man | French Guiana | — | —, GU214645, GU214464 |
| | CBS 375.71 | Man | Brazil | — | —, GU214646, GU214465 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| | CBS 480.64; IHEM 3823; UAMH 4341 | Man, hair | Brazil | — | —, GU214647, GU214466 |
| *Piedraia hortae* var. *paraguayensis* | CBS 276.32; VKM F-393 | — | — | — | —, GU214648, GU214467 |
| *Piedraia quintanilhae* | CBS 327.63; IMI 101644 | *Genetta tigrina* | Central African Republic | — | —, —, GU214468 |
| *Polychaeton citri* | CBS 116435 | *Citrus aurantium*, leaf, with *Pseudococcus citri* | Iran | R. Zare & W. Gams | —, GU214649, GU214469 |
| *Pseudocercospora angolensis* | CBS 112933; CPC 4118 | *Citrus* sp. | Zimbabwe | — | GU214568, AY260063, GU214470 |
| | CBS 149.53; ATCC 11669 | *Citrus sinensis* | Angola | — | AY251106, AF222847, GU214471 |
| *Pseudocercospora atromarginalis* | CPC 11372 | *Solanum nigrum* | South Korea | H.D. Shin | GU214671, GU214671, GU214671 |
| *Pseudocercospora chengtuensis* | CPC 10785 | *Lycium chinense* | South Korea | H.D. Shin | GU214672, GU214672, GU214672 |
| *Pseudocercospora cordiana* | CBS 114685; CPC 2552 | *Cordia goeldiana* | Brazil | P.W. Crous & R.L. Benchimol | GU214569, AF362054, GU214472 |
| *Pseudocercospora cruenta* | CBS 462.75 | *Phaseolus* sp. | Fiji | W. IJzermans-Lutgerhorst | AY251105, AF362065, GU214473 |
| | CPC 10846 | *Vigna* sp. | Trinidad | H. Booker | GU214673, GU214673, GU214673 |
| *Pseudocercospora eucommiae* | CPC 10802 | *Eucommia ulmoides* | South Korea | H.D. Shin | GU214674, GU214674, GU214674 |
| *Pseudocercospora fijiensis* | X300 | *Musa* sp. | Tonga | — | GU214570, AY752150, GU214474 |
| *Pseudocercospora fuligena* | CPC 12296 | *Lycopersicum* sp. | Thailand | — | GU214675, GU214675, GU214675 |
| *Pseudocercospora griseola* f. *griseola* | CBS 194.47; ATCC 22393 | *Phaseolus vulgaris* | Portugal | — | DQ289861, DQ289801, GU214475 |
| | CBS 880.72 | *Phaseolus vulgaris* | Netherlands | H. A. v. Kesteren | DQ289862, DQ289802, GU214476 |
| *Pseudocercospora humuli* | CPC 11358 | *Humulus japonicus* | South Korea | H.D. Shin | GU214676, GU214676, GU214676 |
| *Pseudocercospora kaki* | CPC 10636 | *Diospyros lotus* | South Korea | H.D. Shin | GU214677, GU214677, GU214677 |
| *Pseudocercospora luzardii* | CPC 2556 | *Hancornia speciosa* | Brazil | A.C. Alfenas & P.W. Crous | GU214571, AF362057, GU214477 |
| *Pseudocercospora macrospora* | CBS 114696; CPC 2553 | *Bertholletia excelsa* | Brazil | P.W. Crous & R.L. Benchimol | GU214572, AF362055, GU214478 |
| *Pseudocercospora ocimicola* | CPC 10283 | *Ocimum basilicum* | Mexico | M.E. Palm | GU214678, GU214678, GU214678 |
| *Pseudocercospora opuntiae* | CBS 117708; CPC 11772 | *Opuntia* sp. | Mexico | M. De Jesus Yanez | GU214679, GU214679, GU214679 |
| *Pseudocercospora pallida* | CPC 10776 | *Campsis grandiflora* | South Korea | H.D. Shin | GU214680, GU214680, GU214680 |
| *Pseudocercospora paraguayensis* | CBS 111317; CPC 1458 | *Eucalyptus nitens* | Brazil: Suzano | P.W. Crous | GU214573, AF309596, GU214479 |
| *Pseudocercospora protearum* var. *leucadendri* | CPC 1869 | *Leucadendron* sp. | South Africa | S. Denman & P.W. Crous | AY251107, AY260089, GU214480 |
| *Pseudocercospora pseudoeucalyptorum* | CBS 114242; CMW 14908; CPC 10390 | *Eucalyptus globulus* | Spain | J.P.M. Vazquez | GU214574, AY725526, GU214481 |
| *Pseudocercospora punctata* | CBS 113315 | *Syzygium cordatum* | South Africa | M.J. Wingfield | EU167582, EU167582, GU214407 |
| | CPC 10532 | *Syzygium cordatum* | South Africa | M.J. Wingfield | GU214659, GU214659, GU214659 |
| *Pseudocercospora* sp. | CPC 11592 | *Zelkova serrata* | South Korea | H.D. Shin | GU214575, DQ303085, GU214482 |
| *Pseudocercospora vitis* | CPC 11595 | *Vitis vinifera* | South Korea | H.D. Shin | DQ073923, DQ073923, GU214483 |
| *Pseudocercospora*-like genus | CPC 10712 | *Quercus* sp. | Netherlands | G. Verkley | GU214681, GU214681, GU214681 |
| *Pseudocercosporella capsellae* | CPC 10301 | *Brassica* sp. | U.K. | R. Evans | GU214662, GU214662, GU214662 |
| *Pseudocercosporella fraxini* | CPC 11509 | *Fraxinus rhynchophylla* | South Korea | H.D. Shin | GU214682, GU214682, GU214682 |
| *Pseudocercosporella* sp. | CBS 112737; CPC 3959 | *Rhus typhina* | Canada | K. Seifert | GU214684, GU214684, GU214684 |
| | CPC 4008 | *Rhus typhina* | Canada | K. Seifert | GU214686, GU214686, GU214686 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| | CPC 10050 | *Rubus oldhamii* | South Korea | H.D. Shin | GU214685, GU214685, GU214685 |
| | CPC 11414 | *Vicia amurense* | South Korea | H.D. Shin | GU214683, GU214683, GU214683 |
| *Pseudotaeniolina globosa* | CBS 109889 | Rock | Italy | C. Urzi | GU214576, AY128700, EU019283 |
| *Rachicladosporium cboliae* | CBS 125424; CPC 14034 | Twig debris | U.S.A. | P.W. Crous | —, GU214650, GU214484 |
| *Ramichloridium apiculatum* | CPC 12310 | *Vicia amurensis* | South Korea | H.D. Shin | GU214687, GU214687, GU214687 |
| *Ramichloridium cerophilum* | CBS 103.59; MUCL 10034 | *Sasa* sp. | Japan | — | EU041798, EU041798, GU214485 |
| *Ramichloridium musae* | CBS 190.63; MUCL 9557 | *Musa sapientum* | — | — | GU214577, EU041800, EU041857 |
| *Ramichloridium*-like genus | CPC 10672 | *Phellodendron amurense* | South Korea | H.D. Shin | GU214688, GU214688, GU214688 |
| *Ramularia acroptili* | CBS 120252 | *Acroptilon repens* | Turkey | R. Sobhian | GU214689, GU214689, GU214689 |
| *Ramularia brunnea* | CPC 4903 | — | — | — | GU214691, GU214691, GU214691 |
| *Ramularia coleosporii* | CPC 11516 | *Plectranthus excisus* | South Korea | H.D. Shin | GU214692, GU214692, GU214692 |
| *Ramularia endophylla* | CBS 113265 | *Quercus robur* | Netherlands | G. Verkley | AY490775, AY490763, AY490776 |
| *Ramularia grevilleana* | CPC 656 | *Fragaria* sp. | South Africa | P.W. Crous | GU214578, AF173312, GU214486 |
| *Ramularia nagornyi* | CBS 120253 | *Centaurea solstitiales* | Greece | D. Berner | GU214579, EU019257, EU019257 |
| *Ramularia pratensis* var. *pratensis* | CPC 11294 | *Rumex crispus* | South Korea | H.D. Shin | GU214580, EU019284, EU019284 |
| *Ramularia* sp. | CBS 324.87 | leaf spot on *Brassica* sp., in *Mycosphaerella* sp. | Netherlands | — | GU214581, EU019285, EU019285 |
| | CPC 10066 | *Alangium plataniflium* | South Korea | H.D. Shin | GU214690, GU214690, GU214690 |
| | CPC 11297 | *Stellaria aquatica* | South Korea | H.D. Shin | GU214693, GU214693, GU214693 |
| *Ramularia uredinicola* | CPC 10813 | *Salix* sp. | South Korea | H.D. Shin | GU214694, GU214694, GU214694 |
| *Ramularia*-like genus | CPC 10852 | *Polygonum* sp. | South Korea | H.D. Shin | GU214695, GU214695, GU214695 |
| *Ramulispora sorghi* | CBS 110578; CPC 905 | *Sorghum* sp. | South Africa | D. Nowell | AY251110, AY259131, GU214487 |
| | CBS 110579; CPC 906 | *Sorghum* sp. | South Africa | D. Nowell | AY251111, AY259132, GU214488 |
| *Readeriella dimorphospora* | CBS 120034; CPC 12636 | *Eucalyptus nitens* | Australia | — | GU214521, EF394850, EU019258 |
| *Readeriella mirabilis* | CBS 116293; CPC 10506 | *Eucalyptus fastigata* | New Zealand | W. Gams | EU754110, AY725529, EU019291 |
| *Schizothyrium pomi* | CBS 228.57 | — | Italy | R. Ciferri | EF134947, EF134947, EF134947 |
| | CBS 406.61 | *Rubus idaeus* | Netherlands | — | EF134949, EF134949, EF134949 |
| | CBS 486.50 | *Polygonum sachalinense* | Netherlands | — | EF134948, EF134948, EF134948 |
| *Scorias spongiosa* | CBS 325.33 | Aphid | — | — | GU214696, GU214696, GU214696 |
| *Septoria apiicola* | CBS 400.54; IMI 092628 | *Apium graveolens* | Netherlands | J.A. von Arx | GU214584, AY152574, GU214490 |
| *Septoria convolvuli* | CBS 102325 | *Calystegia sepium* | Netherlands | G. Verkley | GU214697, GU214697, GU214697 |
| *Septoria cucubali* | CBS 102368 | *Cucubalus baccifer* | Netherlands | G. Verkley | GU214698, GU214698, GU214698 |
| *Septoria dysentericae* | CPC 12328 | *Daucus carota* | Brazil | N. Massola | GU214699, GU214699, GU214699 |
| *Septoria lactucae* | CBS 352.58 | *Lactuca sativa* | Germany | — | GU214585, AY489282, GU214491 |
| *Septoria leucanthemi* | CBS 109090 | *Chrysanthemum leucanthemum* | Austria | G. Verkley | GU214586, AY489277, GU214492 |
| *Septoria obesa* | CBS 354.58; BBA 8554; IMI 091324 | *Chrysanthemum indicum* | Germany | — | GU214587, AY489285, GU214493 |
| *Septoria protearum* | CPC 1470 | *Protea cynaroides* | South Africa | L. Viljoen | GU214588, AY260081, GU214494 |
| *Septoria pyricola* | CBS 222.31; CPC 3677 | *Pyrus communis* | — | — | GU214589, AY152591, GU214495 |
| *Septoria quercicola* | CBS 663.94 | *Quercus robur* | Netherlands | — | GU214590, AY490771, GU214496 |
| *Septoria rosae* | CBS 355.58; ATCC 24311; PD 341; CPC 4302 | *Rosa* sp. | — | — | AY251113, AY293065, GU214497 |
| *Septoria senecionis* | CBS 102366 | *Senecio fluviatilis* | Netherlands | G. Verkley | GU214591, AY489272, GU214498 |
| *Septoria*-like genus | CBS 102377 | *Castanea sativa* | Netherlands | G. Verkley | GU214592, AY152588, GU214499 |
| *Sonderhenia eucalypticola* | CPC 11252 | *Eucalyptus globulus* | Spain | M.J. Wingfield | GU214593, DQ303064, GU214500 |
| *Sphaerulina polyspora* | CBS 354.29 | — | — | — | —, GU214651, GU214501 |
| *Staninwardia suttonii* | CBS 120061; CPC 13055 | *Eucalyptus robusta* | Australia | B.A. Summerell | GU214594, DQ923535, DQ923535 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers 18S nrDNA, 5.8S nrDNA, 28S nrDNA |
|---|---|---|---|---|---|
| *Stenella araguata* | CBS 105.75; ATCC 24788; FMC 245 | Man | Venezuela | — | GU214596, EU019250, EU019250 |
| *Stigmina platani* | CBS 110755; IMI 136770; CPC 4299 | *Platanus orientalis* | India | — | GU214598, AY260090, FJ839663 |
| *Stigmina* synanamorph | CPC 11721 | *Platanus occidentalis* | South Korea | H.D. Shin | GU214700, GU214700, GU214700 |
| *Stomiopeltis betulae* | CBS 114420 | *Betula* sp. | Sweden | K. & L. Holm | GU214701, GU214701, GU214701 |
| *Teratosphaeria* aff. *nubilosa* | CBS 114419; CPC 10497 | *Eucalyptus globulus* | New Zealand | — | GU214599, AY725574, EU019303 |
| | CBS 116283; CPC 10495 | *Eucalyptus globulus* | Spain | W. Gams | GU214600, AY725573, GU214503 |
| *Teratosphaeria alcornii* | CBS 313.76; CPC 3632 | *Eucalyptus tessellaris* | Australia | J.L. Alcorn | GU214514, AF362061, EU019245 |
| *Teratosphaeria angophorae* | CBS 120493; DAR 77452 | *Angophora floribunda* | Australia | A.J. Carnegie | —, GU214652, GU214504 |
| *Teratosphaeria bellula* | CBS 111700; CPC 1821; JT 196 | *Protea eximia* | South Africa | J.E. Taylor | GU214601, EU019301, EU019301 |
| *Teratosphaeria cryptica* | CBS 110975; CMW 3279; CPC 936 | *Eucalyptus globulus* | Australia | A.J. Carnegie | GU214602, AF309623, GU214505 |
| *Teratosphaeria destructans* | CBS 111369; CPC 1366 | *Eucalyptus grandis* | Indonesia | M.J. Wingfield | GU214603, DQ267595, EU019287 |
| | CBS 111370; CPC 1368 | *Eucalyptus* sp. | Indonesia | P.W. Crous | GU214702, GU214702, GU214702 |
| *Teratosphaeria fibrillosa* | CPC 1876 | *Protea nitida* | South Africa | J.E. Taylor | EU019282, EU019282, GU214506 |
| *Teratosphaeria juvenalis* | CBS 110906; CMW 13347; CPC 40 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY720715, AY725513, FJ493217 |
| | CBS 111149; CPC 23 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY720714, AY725514 , EU019294 |
| *Teratosphaeria macowanii* | CBS 110756; CPC 1872 | *Protea nitida* | South Africa | J.E. Taylor | GU214519, AY260095, EU019254 |
| | CBS 111029; CPC 1488 | *Protea* sp. | South Africa | P.W. Crous | AY251118, AY260096, FJ493199 |
| *Teratosphaeria mexicana* | CBS 110502; CMW 14461 | *Eucalyptus globulus* | Australia | — | GU214604, AY725558, GU214507 |
| | CBS 120744; CPC 12349 | *Eucalyptus* sp. | U.S.A.: Hawaii | W. Gams | GU214605, EU019302, EU019302 |
| *Teratosphaeria molleriana* | CBS 111164; CMW 4940; CPC 1214 | *Eucalyptus globulus* | Portugal | M.J. Wingfield | GU214606, AF309620, EU019292 |
| | CBS 116370; CPC 10397 | *Eucalyptus globulus* | Spain | J.P.M. Vazquez | GU214607, AY725561, GU214508 |
| | CPC 4577 | *Eucalyptus* sp. | Australia | — | GU214582, AY725524, GU214489 |
| *Teratosphaeria nubilosa* | CBS 115669; CPC 933 | *Eucalyptus nitens* | South Africa | M.J. Wingfield | GU214608, AY725548, GU214509 |
| | CBS 116005; CMW 3282; CPC 937 | *Eucalyptus globulus* | Australia | A.J. Carnegie | GU214609, AY725572, GU214510 |
| *Teratosphaeria ohnowa* | CBS 112896; CMW 4937; CPC 1004 | *Eucalyptus grandis* | South Africa | M.J. Wingfield | AY251119, AF309604, EU019305 |
| | CBS 112973; CMW 4936; CPC 1005 | *Eucalyptus grandis* | South Africa | M.J. Wingfield | GU214610, AF309605, GU214511 |
| *Teratosphaeria pseudosuberosa* | CBS 118911; CPC 12085 | *Eucalyptus* sp. | Uruguay | M.J. Wingfield | GU214611, DQ303011, EU019256 |
| *Teratosphaeria secundaria* | CBS 115608; CPC 504 | *Eucalyptus grandis* | Brazil | A.C. Alfenas | GU214612, DQ303018, EU019306 |
| *Teratosphaeria* sp. | CBS 208.94; CPC 727 | *Eucalyptus grandis* | Indonesia | A.C. Alfenas | GU214613, AY626982, EU019307 |
| *Teratosphaeria stellenboschiana* | CBS 116428; CPC 10886 | *Eucalyptus* sp. | South Africa | P.W. Crous | GU214583, AY725518, EU019295 |
| *Teratosphaeria suberosa* | CPC 11032 | *Eucalyptus* sp. | Colombia | M.J. Wingfield | GU214614, DQ303044, GU214512 |
| *Teratosphaeria suttonii* | CPC 11279 | *Eucalyptus tereticornis* | Bolivia | M.J. Wingfield | GU214615, DQ303055, FJ493222 |
| | CPC 12352 | *Eucalyptus* sp. | U.S.A.: Hawaii | W. Gams | GU214616, EU019288, EU019288 |
| *Teratosphaeria toledana* | CBS 113313; CMW 14457 | *Eucalyptus* sp. | Spain | P.W. Crous & G. Bills | GU214617, AY725580, GU214513 |
| | CBS 115513; CPC 10840 | *Eucalyptus* sp. | Spain | P.W. Crous & G. Bills | GU214618, FJ493198, FJ493225 |
| *Teratosphaeria verrucosa* | CPC 18 | *Eucalyptus cladocalyx* | South Africa | P.W. Crous | AY720713, AY725517, EU019293 |
| *Thedgonia*-like genus | CPC 12304 | *Oplismenus undulatifolius* | South Korea | H.D. Shin | GU214703, GU214703, GU214703 |

**Table 1.** (Continued).

| Species | Accession number[1] | Host | Country | Collector | GenBank Accession numbers |
|---|---|---|---|---|---|
| | | | | | **18S nrDNA, 5.8S nrDNA, 28S nrDNA** |
| *Toxicocladosporium irritans* | CBS 185.58 | Mouldy paint | Suriname | M.B. Schol-Schwarz | GU214619, EU040243, EU040243 |
| *Verrucisporota daviesiae* | CBS 116002; VPRI 31767 | *Daviesia latifolia* | Australia | V. Beilharz | GU214620, FJ839633, FJ839669 |
| *Verrucisporota proteacearum* | CBS 116003; VPRI 31812 | *Grevillea* sp. | Australia | J.L. Alcorn | GU214621, FJ839635, FJ839671 |
| *Zasmidium anthuriicola* | CBS 118742 | *Anthurium* sp. | Thailand | C.F. Hill | GU214595, FJ839626, FJ839662 |
| *Zasmidium citri* | CBS 116366; CMW 11730; CPC 10522 | *Acacia mangium* | Thailand | K. Pongpanich | GU214597, AY752145, GU214502 |

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI) of the University of Pretoria, Pretoria, South Africa; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DAR: Plant Pathology Herbarium, Orange Agricultural Institute, Forest Road, Orange. NSW 2800, Australia; DSM: Deutsche Sammlung von Mikrorganismen und Zellkulturen GmbH, Braunschweig, Germany; ETH: Swiss Federal Institute of Technology Culture Collection, Zurich, Switzerland; FMC: Venezuelan School of Medicine; IAM: IAM Culture Collection, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IFO: Institute for Fermentation, Osaka, Japan; IHEM: Collection of the Laboratorium voor Microbiologie en Microbiele Genetica, Rijksuniversiteit, Ledeganckstraat 35, B-9000, Gent, Belgium; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; IPO: Culture collection of the Research Institute for Plant Protection, Wageningen, The Netherlands; JCM: Japan Collection of Microorganism, RIKEN BioResource Center, Japan; JT: Working collection of Joanne E. Taylor; LSHB: London School of Hygiene & Tropical Medicine, London, U.K.; MPFN: Culture collection at the Laboratoire de Pathologie Forestie`re, INRA, Centre de Recherches de Nancy, 54280 Champenoux, France; MUCL: Université Catholique de Louvain, Louvain-la-Neuve, Belgium; PD: Plant Protection Service, Wageningen, The Netherlands; RoKI: Private culture collection Roland Kirschner; TNS: Herbarium of the National Museum of Nature and Science of Japan, Tokyo, Japan; UAMH: University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada; VKM: All-Russian Collection of Microorganisms, Russian Academy of Sciences, Institute of Biochemistry and Physiology of Microorganisms, 142292 Pushchino, Moscow Region, Russia; VPRI: Victorian Department of Primary Industries, Knoxfield, Australia; WAC: Department of Agriculture Western Australia Plant Pathogen Collection, Perth, Australia; X: Working collection of Mahdi Arzanlou.

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# A molecular phylogenetic reappraisal of the *Hysteriaceae*, *Mytilinidiaceae* and *Gloniaceae* (*Pleosporomycetidae*, *Dothideomycetes*) with keys to world species

E.W.A. Boehm[1*], G.K. Mugambi[2], A.N. Miller[3], S.M. Huhndorf[4], S. Marincowitz[5], J.W. Spatafora[6] and C.L. Schoch[7]

[1]*Department of Biological Sciences, Kean University, 1000 Morris Ave., Union, New Jersey 07083, U.S.A.;* [2]*National Museum of Kenya, Botany Department, P.O. Box 40658, 00100, Nairobi, Kenya;* [3]*Illinois Natural History Survey, University of Illinois Urbana-Champaign, 1816 South Oak Street, Champaign, IL 6182, U.S.A.;* [4]*The Field Museum, 1400 S. Lake Shore Dr, Chicago, IL 60605, U.S.A.;* [5]*Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa;* [6]*Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 93133, U.S.A.;* [7]*National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, GenBank, 45 Center Drive, MSC 6510, Building 45, Room 6an.18, Bethesda, MD, 20892, U.S.A.*

**Correspondence*: E.W.A. Boehm, eboehm@kean.edu

**Abstract:** A reappraisal of the phylogenetic integrity of bitunicate ascomycete fungi belonging to or previously affiliated with the *Hysteriaceae*, *Mytilinidiaceae*, *Gloniaceae* and *Patellariaceae* is presented, based on an analysis of 121 isolates and four nuclear genes, the ribosomal large and small subunits, transcription elongation factor 1 and the second largest RNA polymerase II subunit. A geographically diverse and high density taxon sampling strategy was employed, including multiple isolates/species from the following genera: *Anteaglonium* (6/4), *Encephalographa* (1/1), *Farlowiella* (3/1), *Gloniopsis* (8/4), *Glonium* (4/2), *Hysterium* (12/5), *Hysterobrevium* (14/3), *Hysterographium* (2/1), *Hysteropatella* (2/2), *Lophium* (4/2), *Mytilinidion* (13/10), *Oedohysterium* (5/3), *Ostreichnion* (2/2), *Patellaria* (1/1), *Psiloglonium* (11/3), *Quasiconcha* (1/1), *Rhytidhysteron* (8/3), and 24 outgroup taxa. Sequence data indicate that although the *Hysteriales* are closely related to the *Pleosporales*, sufficient branch support exists for their separation into separate orders within the *Pleosporomycetidae*. The *Mytilinidiales* are more distantly related within the subclass and show a close association with the *Gloniaceae*. Although there are examples of concordance between morphological and molecular data, these are few. Molecular data instead support the premise of a large number of convergent evolutionary lineages, which do not correspond to previously held assumptions of synapomorphy relating to spore morphology. Thus, within the *Hysteriaceae*, the genera *Gloniopsis*, *Glonium*, *Hysterium* and *Hysterographium* are highly polyphyletic. This necessitated the transfer of two species of *Hysterium* to *Oedohysterium gen. nov.* (*Od. insidens comb. nov.* and *Od. sinense comb. nov.*), the description of a new species, *Hysterium barrianum sp. nov.*, and the transfer of two species of *Gloniopsis* to *Hysterobrevium gen. nov.* (*Hb. smilacis comb. nov.* and *Hb. constrictum comb. nov.*). While *Hysterographium*, with the type *Hg. fraxini*, is removed from the *Hysteriaceae*, some of its species remain within the family, transferred here to *Oedohysterium* (*Od. pulchrum comb. nov.*), *Hysterobrevium* (*Hb. mori comb. nov.*) and *Gloniopsis* (*Gp. subrugosa comb. nov.*); the latter genus, in addition to the type, *Gp. praelonga*, with two new species, *Gp. arciformis sp. nov.* and *Gp. kenyensis sp. nov*. The genus *Glonium* is now divided into *Anteaglonium* (*Pleosporales*), *Glonium* (*Gloniaceae*), and *Psiloglonium* (*Hysteriaceae*). The hysterothecium has evolved convergently no less than five times within the *Pleosporomycetidae* (e.g., *Anteaglonium*, *Farlowiella*, *Glonium*, *Hysterographium* and the *Hysteriaceae*). Similarly, thin-walled mytilinidioid (e.g., *Ostreichnion*) and patellarioid (e.g., *Rhytidhysteron*) genera, previously in the *Mytilinidiaceae* and *Patellariaceae*, respectively, transferred here to the *Hysteriaceae*, have also evolved at least twice within the subclass. As such, character states traditionally considered to represent synapomorphies among these fungi, whether they relate to spore septation or the ascomata, in fact, represent symplesiomorphies, and most likely have arisen multiple times through convergent evolutionary processes in response to common selective pressures.

**Key words:** Evolution, fungi, *Hysteriales*, *Mytilinidiales*, *Patellariales*, phylogeny, speciation, taxonomy.
**Taxonomic novelties: New species:** *Gloniopsis arciformis* E.W.A. Boehm, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch, *Gp. kenyensis* E.W.A. Boehm, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch, *Hysterium barrianum* E.W.A. Boehm, A.N. Miller, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch. **New genera:** *Hysterobrevium* E.W.A. Boehm & C.L. Schoch, *Oedohysterium* E.W.A. Boehm & C.L. Schoch. **New combinations:** *Gloniopsis subrugosa* (Cooke & Ellis) E.W.A. Boehm & C.L. Schoch, *Hysterobrevium constrictum* (N. Amano) E.W.A. Boehm & C.L. Schoch, *Hb. mori* (Schwein.) E.W.A. Boehm & C.L. Schoch, *Hb. smilacis* (Schwein.) E.W.A. Boehm & C.L. Schoch, *Oedohysterium insidens* (Schwein.) E.W.A. Boehm & C.L. Schoch, *Od. pulchrum* (Checa, Shoemaker & Umaña) E.W.A. Boehm & C.L. Schoch, *Od. sinense* (Teng) E.W.A. Boehm & C.L. Schoch, *Psiloglonium araucanum* (Speg.) E.W.A. Boehm, S. Marincowitz & C.L. Schoch, *P. chambianum* (Guyot) E.W.A. Boehm & C.L. Schoch, *P. colihuae* (Lorenzo & Messuti) E.W.A. Boehm & C.L. Schoch, *P. ephedrae* (Henn.) E.W.A. Boehm & C.L. Schoch, *P. hysterinum* (Rehm) E.W.A. Boehm & C.L. Schoch, *P. pusillum* (H. Zogg) E.W.A. Boehm & C.L. Schoch, *P. sasicola* (N. Amano) E.W.A. Boehm & C.L. Schoch, and *P. uspallatense* (Speg.) E.W.A. Boehm & C.L. Schoch.

## INTRODUCTION

Class *Dothideomycetes*, subphylum *Pezizomycotina* (*Ascomycota*), is currently classified into two subclasses, based on centrum type (Schoch *et al.* 2006, 2009b, Spatafora *et al.* 2006). The *Dothideomycetidae* is characterised by the absence of sterile centrum elements (*e.g.*, pseudoparaphyses). This subclass includes the *Dothideales*, *Capnodiales*, and *Myriangiales*. The *Microthyriales*, and *Trypetheliales*, while within the *Dothideomycetes*, lie outside of the *Dothideomycetidae* (Schoch *et al.* 2009a). The second subclass recognised within the *Dothideomycetes* is the *Pleosporomycetidae*, characterised by a hamathecium of wide to narrow cellular to trabeculate pseudoparaphyses, which may or may not persist at maturity. This subclass currently comprises the *Pleosporales*, *Hysteriales*, and *Mytilinidiales*, and tentatively the *Jahnulales*. The *Botryosphaeriales*, and *Patellariales*, possess pseudoparaphyses, and would be expected to fall into the *Pleosporomycetidae*, however, at present, statistical support is weak. A greater number of orders, families, and genera still await placement, and are currently designated as *incertae sedis* within the *Dothideomycetes* (Lumbsch & Huhndorf 2007).

Fungi classified in the *Hysteriaceae* (*Hysteriales*), *Mytilinidiaceae* (*Mytilinidiales*), and *Gloniaceae* (*Pleosporomycetidae fam. incertae sedis*), possess persistent, carbonaceous ascomata that characteristically dehisce by a longitudinal suture. Recent molecular data support the inclusion of all three families within

the *Pleosporomycetidae* (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugami & Huhndorf 2009). In the *Hysteriaceae* ascomata are thick-walled, navicular, characteristically dehiscing by an invaginated slit or sulcus (Zogg 1962). Fungi in the *Mytilinidiaceae*, on the other hand, possess strongly laterally compressed, connivent, thin-walled conchate ascomata, reminiscent of miniature bivalve molluscs. These mytilinidioid ascomata typically dehisce by an evaginated slit, in some species forming a longitudinal keel or cristate apex (Barr 1990a). Fungi belonging to the *Gloniaceae*, have dichotomously branched, laterally anastomosed pseudothecia, that form radiating pseudo-stellate composites and dehisce by an inconspicuous, longitudinal, but evaginated slit (Boehm *et al.* 2009).

We are broadly interested in the evolution of character states traditionally used to define higher taxa within each family. Essentially, we wish to address whether morphological features historically used in the classification of these fungi are phylogenetically informative in the context of sequence-based phylogenies. This would have bearing on which morphological features are phylogenetically significant, and therefore useful for a natural delineation of higher taxa. Morphological character states traditionally used to classify these fungi have related primarily to features associated with (1) the pseudothecium, (2) the peridium, (3) the hamathecium, and (4) differences in ascospore symmetry (Barr 1987, 1990a). Character states within each family relate primarily to ascospore septation and pigmentation (Zogg 1962).

Due to the seemingly transitional nature of the ascoma, neither fully open nor closed, hysteriaceous fungi have been placed in the discomycetes and pyrenomycetes about equally by various mycologists throughout the 19th Century (Bisby 1923). In his *Systema Mycologicum*, Fries (1823) initially considered hysteriaceous fungi to belong to the pyrenomycetes and placed them in the *Phacidiacei*, but later (Fries 1835) placed them in his new class *Discomycetes*, stating: "*Transitum sistunt ad Discomycetes, sed discum verum non monstrant*." Chevallier (1826) recognised the unique nature of the hysterothecium and established the *Hysteriineae*, which he considered as pyrenomycetes distinct from Fries' *Phacidiei*. Corda (1842), on the other hand, retained the *Phacidiei* within the *Hysteriaceae*, and divided the family into a number of subfamilies. De Notaris (1847) considered the *Hysteriaceae* to belong to the pyrenomycetes and used spore pigmentation to classify hysteriaceous fungi into the *Phaeosporii* and the *Hyalosporii*. Saccardo (1873) initially followed Fries, but later (1874) placed hysteriaceous fungi in the pyrenomycetes, and carried de Notaris' (1847) spore classification scheme further by dividing the *Hysteriaceae* into nine sections based on pigmentation and the morphology of spore septation (Saccardo 1883). Ellis & Everhart (1892), in their *North American Pyrenomycetes*, tentatively included the *Hysteriaceae*, but stated that they had not at first intended to do so due to the transitional nature of the hysterothecium. In Rabenhorst's *Kryptogamen-Flora, Die Pilze*, Rehm (1896) compromised and placed the *Hysteriales* as an order intermediate between the pyrenomycetes and the discomycetes.

Mytilinidioid fungi have also historically been classified within the family *Hysteriaceae*, due to perceived similarities in ascocarp morphology, specifically its means of longitudinal dehiscence (Fries 1823, De Notaris 1847, Saccardo 1875, 1883, Ellis & Everhart 1892, Massee 1895, Rehm 1896, von Höhnel 1918, Bisby 1923). Modern authors have likewise included mytilinidioid fungi within the *Hysteriaceae*, placing the family in the *Pseudosphaeriales* (Nannfeldt 1932, Gäumann 1949), the *Dothiorales* (Müller & von Arx 1950, von Arx & Müller 1954), the *Dothideales* (von Arx & Müller 1975), and in a separate order *Hysteriales*, closely related to the *Pleosporales* (Miller 1949, Luttrell 1955). The *Hysteriales* were placed in the subclass *Loculoascomycetes* by Luttrell (1955), due to the presence of bitunicate asci, corresponding to the *Ascoloculares* first proposed by Nannfeldt (1932).

Duby (1862) was the first to propose that hysteriaceous fungi be divided into two sections, the *Hystériées* and the *Lophiées*, the latter to accommodate mytilinidioid forms. One hundred years later, Zogg (1962) proposed two families: the *Hysteriaceae s. str.* to accommodate thick-walled hysteriaceous forms, and the *Lophiaceae* (Zogg 1962, von Arx & Müller 1975) to accommodate thin-walled, mytilinidioid fungi. Barr (1990a) made the argument for retention of the earlier name *Mytilinidiaceae* over the *Lophiaceae*, despite the proposal to conserve the latter (Hawksworth & Eriksson 1988). Luttrell (1953) studied ascomatal ontogeny and hamathecial development in *Glonium stellatum*. and concluded that the *Hysteriaceae* possess the pseudoparaphysate *Pleospora*-type centrum, in which cellular, septate pseudoparaphyses grow downwards from the cavity roof, initially anchored at both ends, and occupy the locule prior to the formation of asci (Luttrell 1951). Luttrell (1973) held a wide concept of the *Hysteriales*, but did not recognise the family *Lophiaceae*, instead proposing a subfamily, the *Lophioideae,* within the *Hysteriaceae* to accommodate mytilinidioid forms. Barr (1979) however maintained the two-family distinction. The *Mytilinidiaceae* was placed in the *Melanommatales* based on a thin-walled peridium of scleroparenchymatous cells enclosing a hamathecium of narrow trabeculate pseudoparaphyses, asci borne in a peripheral layer and with ascospores typically showing bipolar symmetry (Barr 1987, 1990a). Later, Barr & Huhndorf (2001) noted that the family was somewhat atypical of the *Melanommatales*, in that, as a consequence of reduced locule space attributed to lateral compression, they possess a basal, rather than peripheral, layer of asci and a reduced hamathecium at maturity. More recently, the *Melanommatales* have been included within the *Pleosporales* (Lumbsch & Huhndorf 2007). Barr (1983) eventually abandoned the *Hysteriales* and placed the *Hysteriaceae* within the *Pleosporales* due to the presence of cellular pseudoparaphyses, asci borne in a basal rather than peripheral layer and ascospores typically showing bipolar asymmetry. Eriksson (2006) removed the *Mytilinidiaceae* from the *Hysteriales* and considered it as *Dothideomycetes et Chaetothyriomycetes incertae sedis*, leaving the *Hysteriaceae* as the sole family in the *Hysteriales*.

More recently, Boehm *et al.* (2009) presented the first combined use of DNA and amino acid sequence data to reconstruct the phylogeny of hysteriaceous fungi. This study was based on a wide taxon sampling strategy, and employed four nuclear genes, namely the nuSSU and nuLSU, Transcription Elongation Factor 1 (*TEF1*) and the second largest RNA polymerase II subunit (*RPB2*). A number of specific conclusions were reached: (1) Multigene phylogenies provided strong support for the monophyly of the *Hysteriaceae* and of the *Mytilinidiaceae*, both within the *Pleosporomycetidae*. However, sequence data also indicated that both families were not closely related within the subclass. (2) Although core groups for many of the genera in the *Hysteriaceae* were defined, the genera *Hysterium*, *Gloniopsis*, and *Hysterographium* were demonstrated to be polyphyletic, with affinities not premised on spore septation and pigmentation. (3) The genus *Glonium* was also shown to be polyphyletic, but along two highly divergent lines. *Glonium* lies outside of the *Hysteriaceae*, and instead finds close affinities with the family *Mytilinidiaceae*, for which was proposed the *Gloniaceae* (Boehm *et al.* 2009), to accommodate the type, *G. stellatum*, and related forms. (4) The genus *Psiloglonium* was reinstated within the *Hysteriaceae*, with *P. lineare* as type, to accommodate

didymospored species segregated from *Glonium*. (5) The genera *Mytilinidion* and *Lophium* formed a strongly supported clade within the *Pleosporomycetidae*, thus defining the monophyletic *Mytilinidiaceae*, adjacent to the *Gloniaceae*, for which was proposed the *Mytilinidiales* (Boehm *et al.* 2009). (6) The genus *Farlowiella*, previously in the *Hysteriaceae*, was placed as *Pleosporomycetidae gen. incertae sedis.* (7) The genus *Ostreichnion*, previously in the *Mytilinidiaceae*, was transferred to the *Hysteriaceae*. (8) The genus *Rhytidhysteron*, previously in the *Patellariaceae*, was transferred to the *Hysteriaceae*.

These taxonomic changes present a number of challenges for understanding evolution within this group of fungi. The lack of agreement between morphological character states, previously considered synapomorphic (*e.g.*, Zogg 1962), and recent molecular data based on the nuSSU, nuLSU, *TEF1* and *RPB2* (Boehm *et al.* 2009), had resulted in a highly polyphyletic core set of genera for the *Hysteriaceae* (*e.g.*, *Hysterium*, *Hysterographium*, *Gloniopsis*, and *Glonium*). This presented us with a complicated picture of past speciation events for the family, and necessitated the current reappraisal. Essentially, the challenge was to reconcile discrepancies between morphological and molecular data, in order to more accurately reflect natural phylogenetic relationships within the family. As a result, the revised *Hysteriaceae* bears little resemblance to the original concept of the family (Zogg 1962).

In an effort to facilitate species identification, a number of dichotomous keys are presented in the current study. These keys take into consideration taxonomic changes brought about by DNA and amino acid sequencing studies (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugambi & Huhndorf 2009), and attempt to provide a morphological basis for the many new relationships revealed by molecular data. Although the keys are based on those first presented by Zogg (1962), they considerably expand upon them to include a number of new species and genera described since the original publication (*e.g.*, Darker 1963, Tilak & Kale 1968, Barr 1975, 1990a, Barr & Blackwell 1980, Amano 1983, Speer 1986, Pande & Rao 1991, van der Linde 1992, Kantvilas & Coppins 1997, Lorenzo & Messuti 1998, Messuti & Lorenzo 1997, 2003, 2007, Vasilyeva 2000, 2001, Chlebicki & Knudsen 2001, Checa *et al.* 2007). In addition to incorporating new species and genera, the revised keys also take into consideration variation in ascospore measurements as presented by different authors, and include widened distribution reports as well. Additional information can be found at www.eboehm.com/.

## MATERIALS AND METHODS

### Taxon sampling

Fungal cultures, collection data and DNA GenBank accession numbers are listed in Table 1 - see online Supplementary Information. Fungal cultures initiated for this study were based on the isolation of individual ascospores, employing a method whereby individual ascomata were affixed to Petri plate lids suspended over potato-dextrose agar. Every 12 h the lids were rotated 45 degrees, such that after 96 h, confirmation of spore deposits could be made under a stereomicroscope using transmitted light. Discharged spores were observed microscopically to confirm identity, transferring a single ascospore to initiate an axenic culture (*e.g.*, EB cultures and deposits with the CBS; Centraalbureau voor Schimmelcultures). In some cases, spore discharge was not obtained, necessitating DNA extraction from individual fruitbodies (*e.g.*, all GKM, SMH, ANM and some EB accessions). Lastly, a number of original cultures, from the CBS were employed in this study, the provenance of which could not be ascertained beforehand. Confirmation of taxonomic identity was based on whether different isolates of the same species co-segregated in the final tree.

An attempt was made to include a broad range of fungal isolates, belonging to or previously affiliated with the *Hysteriaceae*, *Mytilinidiaceae*, *Gloniaceae* and *Patellariaceae* (Table 1). A geographically diverse (Cuba, Europe, Ghana, Kenya, New Zealand, South Africa, Tasmania, North and South America) and high density taxon sampling strategy was employed. This included multiple isolates/species from the genera: *Anteaglonium* (6/4), *Encephalographa* (1/1), *Farlowiella* (3/1), *Gloniopsis* (8/4), *Glonium* (4/2), *Hysterium* (12/5), *Hysterobrevium* (14/3), *Hysterographium* (2/1), *Hysteropatella* (2/2), *Lophium* (4/2), *Mytilinidion* (13/10), *Oedohysterium* (5/3), *Ostreichnion* (2/2), *Patellaria* (1/1), *Psiloglonium* (11/3), *Quasiconcha* (1/1), *Rhytidhysteron* (8/3), and 24 outgroup taxa, for a total of 121 taxa. All cultures and the herbarium specimens from which they were derived, have been deposited and are permanently conserved in the certified public institutions given in Table 1.

Within the *Pleosporales*, we sampled *Anteaglonium abbreviatum*, *A. globosum*, *A. latirostrum* and *A. parvulum*, *Byssothecium circinans*, *Cochliobolus heterostrophus*, *Delitschia winteri*, *Herpotrichia diffusa*, *Leptosphaeria maculans*, *Phoma herbarum*, and *Pleospora herbarum*. Eight representatives from the *Dothideomycetidae* were included as outgroups to the *Pleosporomycetidae*, namely *Elsinoë veneta* and *Myriangium duriaei* (*Myriangiales*), *Dothidea sambuci* and *D. insculpta* (*Dothideales*), *Mycosphaerella punctiformis* and *Scorias spongiosa* (*Capnodiales*). *Botryosphaeria dothidea*, and *Guignardia gaultheriae* (*Botryosphaeriales*). *Jahnula aquatica* and *Aliquandostipite khaoyaiensis* (*Jahnulales*), were also included. Four taxa in the *Arthoniomycetes*, were used as outgroups to the *Dothideomycetes*, namely *Opegrapha dolomitica*, *Simonyella variegata*, *Roccella fuciformis*, and *Arthonia caesia*. These are not presented in Fig. 1, due to space limitations, but are presented as a full tree available on TreeBASE, as well as in Table 1.

### DNA extraction, amplification and sequencing

Genomic DNA was recovered using the DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, CA, U.S.A.), following the instructions of the manufacturer, but using sterile white quartz sand and a Kontes® battery-powered pestle grinder in 1.5 mL microfuge tubes. The nuSSU was amplified and double-strand sequenced using the primers NS1 and NS4 (White *et al.* 1990), while amplification of the nuLSU utilised the primers LROR (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990), in addition to the internal sequencing primers LR3R and LR16 (Moncalvo *et al.* 1993). Final concentrations for 50 µL PCR amplification reactions were as follows: 1 µM of each forward and reverse primer, 2 mM $MgCl_2$, 200 µM dNTP, 1X Promega GoTaq® Flexi Reaction Buffer, 1.25 U of Promega GoTaq® Polymerase, and 2 µL template DNA diluted tenfold. For the nuSSU and nuLSU, PCR reaction parameters were as follows: a 95 °C pre-melt for 3 min, and 35 cycles of 95 °C for 20 s, 54 °C for 30 s and 72 °C for 60 s, followed by a final extension at 72 °C for 10 min. For *TEF1* and *RPB2*, PCR amplification conditions followed those in Schoch *et al.* (2006). Primers used for the amplifications and sequencing of these protein coding genes were for *TEF1*: 983 & 2218R; and for *RPB2*: fRPB2-5F & fRPB2-7cR. PCR reactions were performed using PCR Master Mix Polymerase from Promega

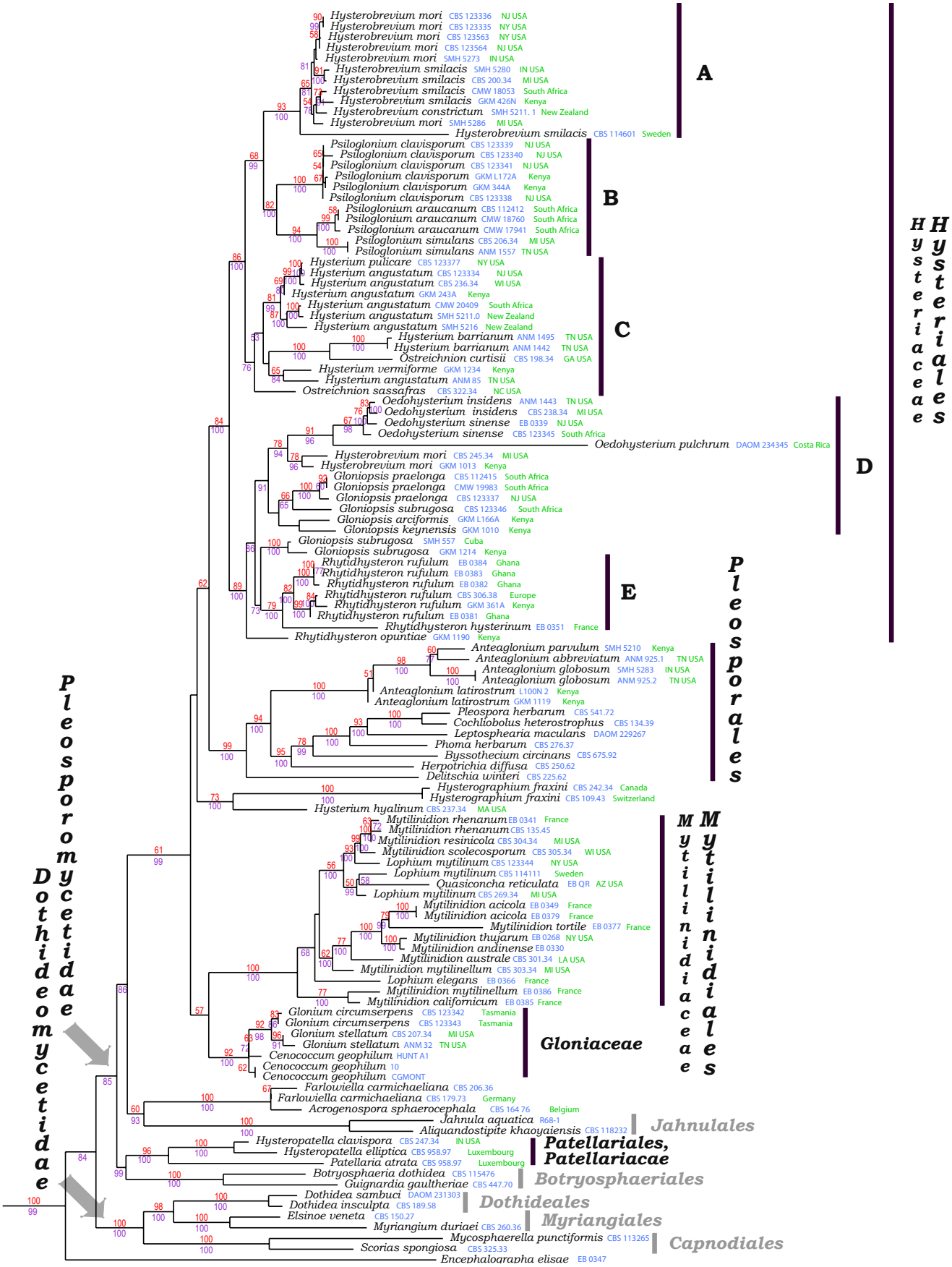


**Fig. 1.** Combined ribosomal (nuSSU & nuLSU) and protein coding gene (*TEF1* & *RPB2*) DNA phylogeny for bitunicate ascomycetes belonging to or previously affiliated with the *Hysteriaceae*, *Mytilinidiaceae*, *Gloniaceae* and *Patellariaceae*. Also included are representatives from allied groups such as the *Pleosporales*, *Jahnulales*, *Patellariales*, and *Botryosphaeriales*, as well as representatives from the *Dothideales*, *Myriangiales* and *Capnodiales* in the *Dothideomycetidae*. The *Arthoniomycetes*, chosen as outgroup, are not presented here due to space limitations, but are available in the full tree on TreeBASE. The tree is the highest scoring tree obtained by maximum likelihood in RAxML. Nodal values, given as percentages, are as follows: Bayesian posterior probability / maximum likelihood bootstrap. Only values above 50 % are shown.

Corporation (Fitchburg, Wisconsin, U.S.A.) and run on an iCycler® from Biorad (Hercules, California, U.S.A.). For the amplification of DNA fragments used to infer the *TEF1* amino acid sequence, the following conditions were used: (1) 94 °C for 2 min; (2) five cycles of 94 °C for 40 s, 55 °C for 45 s lowering by 0.8 °C per cycle and 72 °C for 90 s; (3) 30 cycles of 94 °C for 30 s, 52 °C for 45 s and 72 °C for 120 s and (4) a cycle for 10 min at 72 °C. Amplifications of DNA fragments used to infer the *RPB2* amino acid sequence utilised the same cycle parameters, except for changes in steps (2) and (3) where the annealing temperatures of 55 °C and 52 °C were changed to 50 °C and 45 °C, respectively. Amplified PCR products were cleaned using the QIAquick® PCR Purification Kit (Qiagen Inc.) and resuspended in water prior to outsourcing for sequencing (Macrogen U.S.A., Inc.).

## Phylogenetic analysis

DNA sequences were derived from previous studies (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugambi & Huhndorf 2009), as well as from a number of new accessions generated in this study (Table 1). Sequences were aligned using default options for a simultaneous method of estimating alignments and tree phylogenies, SATé (Liu *et al.* 2009). Protein coding fragments were translated using BioEdit v. 7.0.1 (Hall 2004), and aligned within SATé as amino acid sequence data. These were then aligned with their respective DNA sequences using the RevTrans v. 1.4 Server (Wernersson & Pedersen 2003). Newly generated sequences were subsequently added to the core alignment with MAFFT v. 6.713 (Katoh *et al.* 2009). A supermatrix of four genes (nuLSU, nuSSU *TEF1*, *RPB2*) consisting of 56 % gaps and undetermined characters, across 121 taxa was obtained.

The matrix was analysed using maximum likelihood in RAxML v. 7.0.4 (Stamatakis 2006). Data was partitioned by individual gene and, where applicable, by codon, as in Schoch *et al.* (2009). A most likely tree was obtained after 100 successive searches in RAxML under the GTR model with gamma rate distribution across 11 partitions and starting each search from a randomised tree with a rapid hill climbing option and joint branch length optimisation. Five hundred fast bootstrap pseudoreplicates (Stamatakis *et al.* 2008) were run under the same conditions and these values are given above each node. The matrix analysed in this study produced 4174 distinct alignment patterns and the most likely tree had a log likelihood of -72114.22899. The average log likelihood over 100 trees was -72117.730727. Three independent Bayesian phylogenetic analyses were performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) using a uniform [GTR+I+G] model. The Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP). For each Bayesian run four Markov chains were run from a random starting tree for 5 000 000 generations and trees sampled every 100 generations. The first 50 000 generation trees were discarded as burn-in prior to convergence of four of the chains. All three runs reached a plateau that converged. One run was chosen to construct a 50 % majority rule consensus tree of all trees remaining after the burn in was discarded. Bayesian Posterior Probabilities with those equal or greater than 50 % are given below each node (Fig. 1).

# RESULTS AND DISCUSSION

## Phylogenetic analysis – ordinal level

At the ordinal level in the *Pleosporomycetidae*, molecular data indicate that the *Hysteriales* are closely related to the *Pleosporales* (Fig. 1), as was indicated in earlier studies (Schoch *et al.* 2006, Boehm *et al.* 2009). This is also confirmed by morphological evidence related to the centrum. Thus, the *Hysteriales* share a very similar centrum with the *Pleosporales*, that is, one defined by the *Pleospora*-type, in which cellular, septate pseudoparaphyses grow downwards from the cavity roof, initially anchored at both ends, and occupy the locule prior to the formation of asci (Luttrell 1951). However, there is also strong branch support for its separation from the *Pleosporales* (Boehm *et al.* 2009). The *Hysteriales* are therefore retained as defined by Luttrell (1955), to emphasise the elongated hysteriaceous locule, capable of relatively indeterminate linear growth, as distinct from the strict *Pleospora*-type centrum, defined as it is by constrained concentric growth. In contrast to the close association between the *Hysteriales* and the *Pleosporales*, the *Mytilinidiales* forms a more distant clade within the *Pleosporomycetidae* (Boehm *et al.* 2009).

## Phylogenetic analysis – family level

### *Hysteriaceae*

Although the *Hysteriales* receives high branch support as a monophyletic entity, distinct from the closely related *Pleosporales*, two major groups can be defined within the family. The first supports Clades A–C, whereas the second supports Clades D and E (Fig. 1).

**Clade A:** This first clade is characterised by *Hysterographium mori*, with short pigmented dictyospores, *Gloniopsis constricta*, and *Gp. smilacis*, the latter two with short hyaline dictyospores. The *Gp. smilacis* isolates originate from highly diverse geographical sources (*e.g.*, Sweden, South Africa, North America; Table 1), thus strongly supporting its phylogenetic placement. As these taxa are far removed from the types for their respective genera, we propose here to unite them in *Hysterobrevium gen. nov.*, as *Hb. mori comb. nov.*, *Hb. constrictum comb. nov.*, and *Hb. smilacis comb. nov.*

**Clade B:** This clade (Fig. 1) appears monophyletic for the newly reinstated genus *Psiloglonium* (Boehm *et al.* 2009), with hyaline didymospores. It includes the following species: *P. simulans*, *P. clavisporum,* and *P. araucanum comb. nov.* In this study, we propose a number of new combinations for the genus *Psiloglonium*, with *P. lineare* as the type (Boehm *et al.* 2009), to accommodate species previously classified under the genus *Glonium*, now in the *Gloniaceae*.

**Clade C:** This clade is characterised by pigmented phragmospores belonging to four species of the genus *Hysterium*, namely *H. pulicare*, *H. angustatum*, *H. vermiforme*, which have 3-septate spores, and *H. barrianum sp. nov.*, which has 9-septate spores. Again, a geographically diverse set of isolates were surveyed (Table 1). For instance, taxon sampling for *H. angustatum* included isolates originating from Kenya, New Zealand, South Africa, and North America (Fig. 1). Also within this clade, but with weak bootstrap support, is *Ostreichnion sassafras*, and *O. curtisii*, previously transferred from the *Mytilinidiaceae* to the *Hysteriaceae* (Boehm *et al.* 2009).

**Clade D:** This clade is heterogeneous, but can be divided into two sub-clades. The first sub-clade includes two species formerly in the genus *Hysterium*, namely *H. insidens* and *H. sinense*. Molecular data indicate that these species are not related to the type species, *H. pulicare*, nor to related species within Clade C. Morphology also supports this separation, as *H. insidens* and *H. sinense* both possess phragmospores with a swollen supra-median cell. We therefore propose *Oedohysterium gen. nov.*, to accommodate *Od. insidens comb. nov.* and *Od. sinense comb. nov.* Also grouping in Clade D is *Hysterographium pulchrum*. Despite the fact that *Hg. pulchrum* possesses dictyospores, we propose to unite it within *Oedohysterium*, as *Od. pulchrum comb. nov.*, on account that it too possesses a swollen supra-median cell. Also present in this subclade are two isolates of *Hb. mori*, distant from the other *Hb. mori* accessions in Clade A; this anomaly will be discussed later. A separate subclade is evident in Clade D, and defines the type species for the genus *Gloniopsis*, namely *Gp. praelonga*. Closely associated with *Gp. praelonga* is one representative of *Hg. subrugosum*. Dictyospores of both species are of similar shape, size and degree of septation, differing only in the lack of pigmentation and a gelatinous sheath. We thus propose that *Gp. praelonga* and *Hg. subrugosum* be united within the same genus, proposing *Gloniopsis subrugosa comb. nov*. The other two representatives of *Gp. subrugosa* do not fall into Clade D, but lie adjacent. Lastly, an additional two species are described in Clade D, namely *Gloniopsis arciformis sp. nov.* and *Gp. kenyensis sp. nov.*, both from East Africa (Table 1).

**Clade E:** This clade is well-supported and defines two species in the genus *Rhytidhysteron*, namely *R. rufulum*, and *R. hysterinum*. Taxon sampling included isolates originating from France, Ghana, Kenya and North America. This clade therefore supports the transference of the genus *Rhytidhysteron* from the *Patellariaceae* to the *Hysteriaceae*, as initially proposed by Boehm *et al.* (2009). The third species of *Rhytidhysteron*, *R. opuntiae*, is distant to the first two species, but remains adjacent to Clade E.

### *Mytilinidiaceae*

In contrast to the *Hysteriales*, the family *Mytilinidiaceae* represents a highly monophyletic entity, defining the order *Mytilinidiales* (Boehm *et al.* 2009). The conchate nature of the fruitbody and the thin-walled peridium, seem to unite what at first may seem a disparate group of fungi into a single family (Fig. 1). In this study, we have sampled 10 of the 15 species of *Mytilinidion*, characterised by phragmospores and scolecospores, two of the four species of *Lophium*, with filiform spores, as well as the monotypic *Quasiconcha*, with reticulated 1-septate spores (Table 1). Although monophyletic, sequence data also indicate a complex pattern of speciation within the family, one that is not premised on past assumptions based on spore morphology (Fig. 1).

### *Gloniaceae*

As for the monotypic family *Gloniaceae* (Boehm *et al.* 2009), based on the genus *Glonium*, previously classified within the *Hysteriaceae* (Zogg 1962), surprisingly, sequence data indicate that it finds close affinity with the *Mytilinidiaceae* (Fig. 1). This is based on four isolates, representing two species, *Glonium stellatum* and *G. circumserpens*. However, the *Gloniaceae* is not included within the *Mytilinidiales*, due to the highly divergent morphology associated with the genus *Glonium*. These include character states related to the hamathecium (persistent cellular pseudoparaphyses *versus* narrow trabeculate pseudoparaphyses) and to the fruitbody (dichotomously branched *versus* conchate), for the *Gloniaceae* and *Mytilinidiaceae*, respectively. Thus, for the present, we propose that the family *Gloniaceae* be considered *Pleosporomycetidae fam. incertae sedis*.

## TAXONOMY

***Hysteriaceae*** Chevall. 1826, ***Hysteriales*** Lindau 1897.

Fungi classified in the *Hysteriaceae* (Chevallier 1826) have been traditionally defined by a specialised ascocarp termed the hysterothecium (Clements 1909). Hysterothecia are dense, persistent carbonaceous structures, distinctly navicular in outline, and bear a pronounced longitudinal slit running the length of the long axis of the fruitbody. They can be immersed to erumpent to entirely superficial, solitary to gregarious, ellipsoid to greatly elongated, sometimes branched or triradiate. In vertical section, hysterothecia are globose to obovoid, typically with a thick three-layered peridium, composed of small pseudoparenchymatous cells, the outer layer heavily encrusted with pigment and often longitudinally striated on the surface, the middle layer lighter in pigmentation and the inner layer distinctly thin-walled, pallid and compressed (Barr 1987). The hamathecium is composed of persistent, narrow cellular pseudoparaphyses, often borne in a gel matrix, with tips darkened or branched at maturity above the asci. Bitunicate asci are borne in a basal layer and at maturity are typically clavate to cylindric, bearing 8 ascospores, overlapping biseriate, ranging from hyaline to dark brown, obovoid, clavate, ellipsoid or fusoid. Ascospores are highly diverse in septation and range from didymospores to phragmospores to dictyospores, at times surrounded by a gel coating, and often show bipolar asymmetry (Barr 1987). Zogg (1962) accepted the following seven genera within the *Hysteriaceae*: *Farlowiella*, *Gloniella*, *Gloniopsis*, *Glonium*, *Hysterium*, *Hysterocarina*, and *Hysterographium*.

The traditional circumspection of the *Hysteriaceae* was based on character states related to the hysterothecium and spore morphology (*e.g.*, septation and pigmentation), character states previously considered synapomorphic (Zogg 1962). However, recent molecular data underscore the potential for morphology to be difficult to interpret, and even unhelpful in phylogenetic inference and reconstruction for this group of fungi (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugambi & Huhndorf 2009). Thus, a number of examples of convergent evolution are presented in the current study, which relate not only to the fruitbody, but to spore morphology as well. As a result, three genera have been removed from the family (*Glonium*, *Hysterographium* and *Farlowiella*), based on convergence associated with the fruitbody. Additionally, within the family, several genera have their members spanning different clades (Fig. 1). This necessitated the description of two new genera (*Oedohysterium* and *Hysterobrevium*), as well as three new species, one in *Hysterium* and two in *Gloniopsis*, in addition to a number of new combinations involving *Psiloglonium*, *Oedohysterium*, *Hysterobrevium* and *Gloniopsis*. These taxonomic changes have de-emphasised both spore septation and spore pigmentation as reliable character states for deducing phylogenetic relationships within the family. Nevertheless, in the keys that follow, we have endeavoured to provide a morphological basis for the new phylogenies revealed by molecular data.

Data have also necessitated that we expand the concept of the *Hysteriaceae* to include thin-walled mytilinidioid forms previously in the *Mytilinidiaceae* (*e.g.*, *Ostreichnion*), as well as patellarioid forms previously in the *Patellariaceae* (*e.g.*, *Rhytidhysteron*). The inclusion of *Ostreichnion* within the *Hysteriaceae* was unexpected. Unlike most members of the family, the peridium in *Ostreichnion* is sclerenchymatoid and thin-walled, defining a fragile mytilinidioid ascoma, and with a hamathecium typified by trabeculate pseudoparaphyses (Barr 1975, 1990a). Including the genus *Ostreichnion* in the *Hysteriaceae* implies that, either morphological features within the genus need to be re-evaluated, or that the family *Hysteriaceae* must also encompass mytilinidioid forms. More difficult to understand perhaps is the inclusion of the genus *Rhytidhysteron* within the *Hysteriaceae*. Although included within the *Patellariaceae* (Kutorga & Hawksworth 1997), phylogenetic data presented here and elsewhere (Boehm e*t al.* 2009), clearly indicate that this genus lies quite distant from other members of the *Patellariaceae*.

Some authors have included a number of additional genera within the *Hysteriaceae*. For instance, the genera *Hysteropatella*, *Hysteroglonium*, and *Pseudoscypha* were included in the *Hysteriaceae* by Eriksson (2006). In addition, the genera *Hemigrapha*, *Graphyllium*, and *Encephalographa* were included in the family by Kirk *et al.* (2001). In Boehm *et al.* (2009), two species belonging to *Hysteropatella*, namely *Hp. clavispora* (CBS 247.34) and *Hp. elliptica* (CBS 935.97), did not cluster with any of the hysteriaceous taxa surveyed. Instead, they formed a distant clade within the *Pleosporomycetidae*, postulated to represent the emergence of the *Patellariales*. In the present study, these two species of *Hysteropatella* continue to be distant from the *Hysteriaceae*, and also cluster now with *Patellaria atrata* (CBS 958.97). Therefore, we do not include the genus *Hysteropatella* within the *Hysteriaceae*.

Reid & Pirozynski (1966) in describing *Pseudoscypha abietis* on the needles of *Abies balsamea* did not mention the *Hysteriaceae*, and in fact stated that the fungus cannot be assigned to any presently known order. In their illustrations, no sterile tissue or excipulum is presented, and the bitunicate asci and pseudoparaphyses arise directly from an erumpent orange basal stromatic cushion. As such, we do not include *Pseudoscypha* as a member of the *Hysteriaceae*. As for the genus *Hemigrapha*, Diederich & Wedin (2000) make the argument for the inclusion of the genus in the *Microthyriaceae*, not the *Hysteriaceae*. The genus *Graphyllium* possesses applanate, clathrate ascospores borne in thin-walled membranous hysterothecia, at first subcuticular, later erumpent, often associated with aquatic poaceous hosts. The genus was included in the *Hysteriaceae* by Shoemaker & Babcock (1992) and Kirk *et al.* (2001), but was earlier classified in the *Phaeosphaeriaceae* (Barr 1987). A new species was recently described from Costa Rica (Checa *et al.* 2007). The unique ascospore and the lack of carbonisation or peridial wall thickness argue against the inclusion in the *Hysteriaceae*, but molecular data are lacking.

The genus *Encephalographa* was originally placed in the *Hysteriaceae* by Renobales & Aguirre (1990) who thought it to be lichenicolous. Tretiach & Modenesi (1999) demonstrated it to be lichenised, and maintained its placement within the *Hysteriaceae*. The latter authors illustrate endolithic, saxicolous, dichotomously branched, laterally anastomosed, lirelliform pseudothecia with a longitudinal sulcus, and clavate bitunicate asci bearing pigmented didymospores, highly reminiscent of the saxicolous forms of *Glonium circumserpens*, in the *Gloniaceae*. We recently were able to obtain fresh material of *Encephalographa elisae* from Mauro Tretiach (Dipartimento di Biologia, Università di Trieste, Trieste, Italy), and, although cultures failed, we were able to isolate DNA directly from the ascomata (EB 0347 / BPI 879773). Sequence data presented here indicate that *E. elisae* does not reside within the *Hysteriaceae*, nor within the *Gloniaceae*. Instead, *E. elisae* lies outside of the *Pleosporomycetidae* and *Dothideomycetidae* (Fig. 1).

To summarise, we accept the following genera in the *Hysteriaceae*: *Actidiographium*, *Gloniella*, *Gloniopsis, Hysterium*, *Hysterobrevium*, *Hysterocarina*, *Oedohysterium*, *Ostreichnion*, *Psiloglonium*, and *Rhytidhysteron*. Dichotomous keys are presented here for hysteriaceous fungi, with the caveat that phylogenetically unrelated taxa share the same key. Thus, despite their transference from the *Hysteriaceae* (Boehm *et al.* 2009), the genera *Hysterographium*, *Farlowiella*, *Glonium* and *Anteaglonium* (Mugambi & Huhndorf 2009), are nevertheless included in the key. This is because they typically possess ascomata that have traditionally been referred to as hysterothecia.

## Key to the genera and allied genera of the *Hysteriaceae*

1. Ascomata apothecioid, opening widely when hydrated, fully exposing the hymenium, which may be red or black (*i.e.*, patellarioid) ..... ***Rhytidhysteron***
1. Hysterothecia usually remaining closed, or only opening slightly through a longitudinal fissure or sulcus to reveal a lenticular, disk-like hymenium when hydrated and mature ..... 2

2. Ascospores pedicellate amerospores, the upper cell pigmented and much larger than the lower, which remains un- or less-pigmented; anamorph *Acrogenospora* ..... ***Farlowiella***
   *Note*: The genus *Farlowiella* has been removed from the *Hysteriaceae* and is currently listed as *Pleosporomycetidae gen. incertae sedis* (Boehm *et al.* 2009).
2. Ascospores not as above, didymospores, phragmospores or dictyospores, sometimes pigmented ..... 3

3. Didymospores small, the two cells more or less equal in size ..... 4
3. Ascospores not as above, phragmospores, dictyospores, +/- pigmentation, or very large didymospores (*O. curtisii*) ..... 7

4. Ascospores hyaline ..... 5
4. Ascospores pigmented ..... ***Actidiographium***

5. Didymospores less than 8 µm long ..... ***Anteaglonium***
   *Note*: The genus *Anteaglonium* lies within the *Pleosporales* (Mugambi & Huhndorf 2009), but is keyed out here with *Psiloglonium*.
5. Didymospores longer than 8 µm ..... 6

6. Didymospores hyaline, borne in solitary or gregarious hysterothecia, rarely associated with a subiculum, not laterally anastomosed to form radiating stellate composites ..... ***Psiloglonium***
   *Note*: One species of *Anteaglonium*, *A. latirostrum*, will key out here, but belongs in the *Pleosporales* (Mugambi & Huhndorf 2009) and is also keyed out in the *Psiloglonium* key.
6. Didymospores hyaline, borne in modified hysterothecia, usually associated with a subiculum, strongly laterally anastomosed along their length to form radiating stellate composites ..... ***Glonium***
   *Note*: The genus *Glonium* has been transferred from the *Hysteriaceae* to the *Gloniaceae*, currently listed as *fam. incertae sedis* within the *Pleosporomycetidae* (Boehm *et al.* 2009).

7. Ascospores transversely septate phragmospores, or if with dictyospores then also with red pigmentation ..... 8
7. Ascospores transversely and longitudinally septate dictyospores, or very large didymospores (*O. curtisii*) ..... 10

8. Ascospores hyaline phragmospores ..... ***Gloniella***
8. Ascospores pigmented phragmospores or in one case (*Od. pulchrum*) with pigmented dictyospores and red pigmentation in the hamathecium ..... 9

9. Phragmospores 3-septate or rarely more, but without swollen supra-median cell(s) ..... ***Hysterium***
9. Phragmospores with swollen supra-median cell, usually more than 3-septate, in one case with pigmented dictyospores and red centrum pigmentation (*Od. pulchrum*) ..... ***Oedohysterium***

10. Dictyospores hyaline, +/- gelatinous sheath, or pigmented, but short, less than 25 µm in length ..... ***Hysterobrevium***
10. Dictyospores hyaline, +/- gelatinous sheath, or pigmented, but longer than 25 µm, or very large didymospores (*O. curtisii*) ..... 11

11. Dictyospores, if hyaline, then longer than 25 µm, or if pigmented, then measuring (22–)25–34(–45) x (6–)8–12(–17) µm, with 7–11 transverse and 1–2 vertical septa, and no red pigment associated with the hamathecium (*Gp. subrugosa*) ..... ***Gloniopsis***
11. Dictyospores pigmented, of different length, or if similar in length to *Gp. subrugosa*, then tropical with red pigment associated with the hamathecium, or very large didymospores (*O. curtisii*) ..... 12

12. Dictyospores or large didymospores borne in conchate, mytilinidioid, thin-walled, slerenchymatous, fragile fruitbodies ..... ***Ostreichnion***
    *Note*: The genus *Ostreichnion*, previously in the *Mytilinidiaceae*, was transferred to the *Hysteriaceae* (Boehm *et al.* 2009).
12. Dictyospores borne in thick-walled, navicular hysterothecia ..... **13**

13. Dictyospores pigmented, borne in typical hysterothecia, that are erumpent or sessile on the substrate ..... ***Hysterographium***
    *Note*: The genus *Hysterographium*, with the type species *Hg. fraxini*, has been transferred out of the *Hysteriaceae* as *Pleosporomycetidae gen. incertae sedis* (Boehm *et al.* 2009). Residual species classified as *Hysterographium*, for which sequence data are lacking, are provisionally retained within the genus.
13. Hysterothecia borne within the substrate, hardly erumpent, with cristate longitudinal apex instead of a sulcus; neotropical ..... ***Hysterocarina***

***Hysterium*** Tode, Schriften Berlin. Ges. Naturf. Freunde 5: 53 (1784).

The genus *Hysterium* is characterised by pigmented versicolorous or concolorous asymmetric phragmospores, three- or more transversely-septate, borne in hysterothecia. A historical overview of the nomenclature of the genus was presented in Boehm *et al.* (2009). Zogg (1962) recognised two morphological types within the genus. Type I is characterised by 3-septate phragmospores, and includes the versicolorous type species *H. pulicare* (Fig. 2A–B), and its closely related concolorous counterpart, *H. angustatum* (Fig. 2C–F), both extremely common in the temperate zones of both hemispheres. These are followed by *H. vermiforme* (Fig. 2G–K), from Africa, and the much larger-spored *H. macrosporum*, reported from North America and China (Teng 1933). Although Zogg (1962) did not accept *H. hyalinum*, Lohman (1934) provided legitimacy to the epithet, noting that pigmentation is delayed in the maturation of the 3-septate ascospores (Boehm *et al.* 2009).

Type II corresponds to a different phragmospore, one in which, typically, there are five or more septa, and in which there exists a swollen cell, either just above the median septum (*i.e.*, supramedian) or, rarely, some distance up from the median septum. Type II includes, by increasing spore length, the cosmopolitan *H. insidens* (Fig. 3A–D), the larger-spored counterpart *H. sinense* (Fig. 3E–H), and the unusual *H. magnisporum*, 7-septate, with three of the septa crowded to each end, the two central cells much larger. The latter two species are reported from China (Teng 1933) and North America (Boehm, unpubl. data). *Hysterium velloziae*, provisionally included by Zogg (1962), with up to 21 septa at maturity, has only been reported from Africa (van der Linde 1992).

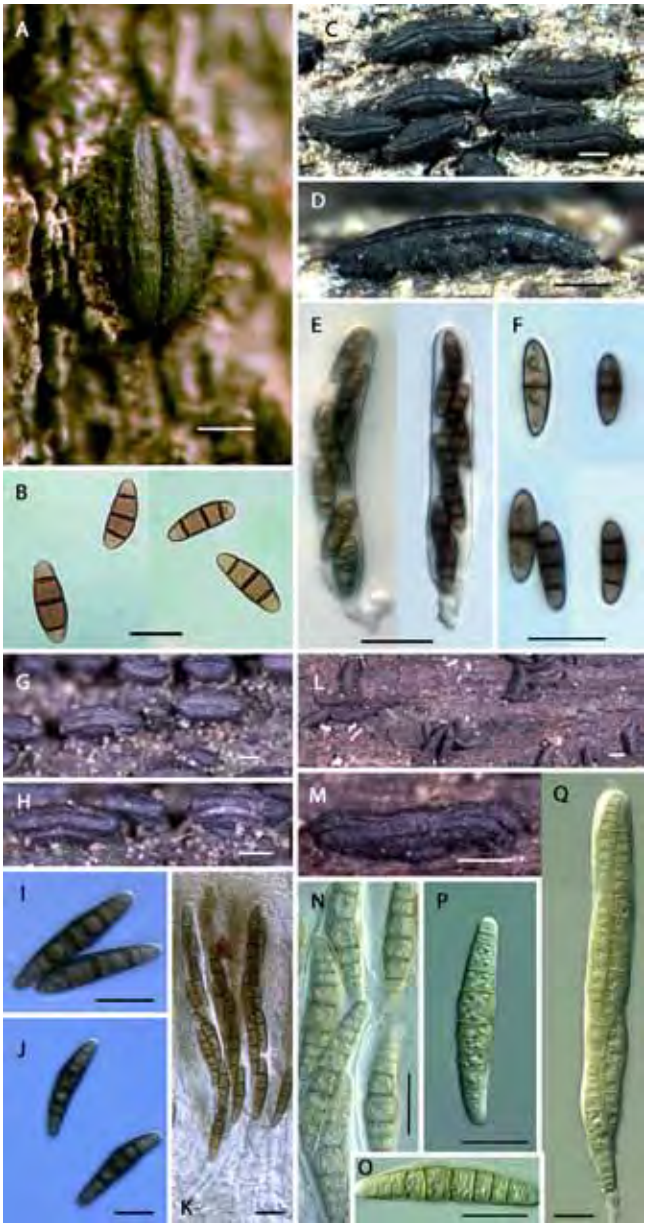


**Fig. 2.** The genus *Hysterium* (Clade C). A–B. *Hysterium pulicare* [CBS 123377 (BPI 878723), U.S.A.]; C–F. *Hysterium angustatum* [ANM 120 (ILLS), U.S.A.; not incl.]; G–K. *Hysterium vermiforme* [GKM 1234 (BPI 879785), Kenya]; L–Q. *Hysterium barrianum sp. nov.* [ANM 1495 (ILLS 59908 = holotype), U.S.A.]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 20 µm.

An additional two species have been recently described. *Hysterium asymmetricum* (Checa *et al.* 2007) from Costa Rica, has outer centrum tissues pigmented red, and 3-septate phragmospores, showing an extended basal cell. *Hysterium andinense* has been recently described from the conifer *Austrocedrus chilensis* in Argentina (Messuti & Lorenzo 1997). However, molecular and morphological data (Boehm *et al.* 2009) has placed this taxon in the *Mytilinidiaceae*, as *Mytilinidion andinense*, based on CBS 123562 (BPI 878737). This brings the total number of species within the genus *Hysterium* to 10. An additional new species is described here.

***Hysterium barrianum*** E.W.A. Boehm, A.N. Miller, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch, **sp. nov.** MycoBank MB515330. Fig. 2L–Q.

*Etymology*: Named after the late Dr Margaret E. Barr, preeminent American mycologist.

Ascomata inconspicue hysterothecioidea, modice compressa e latere in parte superiore, paulo conniventia, sulco inconspicuo angusto, latera paucis striis profundis praedita; ascomata recta vel flexuosa, sessilia, raro furcata, matura altiora quam lata, 1–2.5 mm longa, 250–450 µm alta, 200–300 µm lata. Pseudoparaphyses hyalinae, cellulares, 1–2 µm latae, supra ascos ramosae epithecium formantes. Asci bitunicati, cylindrici, breviter stipitati, (110–)125–135 x 15–20 µm. Phragmosporae fusiformes, angustae, rectae vel paulo curvatae, primum hyalinae, maturae pallide luteae, quaque cellula guttulis magnis refringentibus repleta, (7–)9(–11)-septatae, (35–)40–45(–55) x (7–)9–10(–12) µm.

*Ascomata* atypically hysterithecioid, somewhat laterally compressed in the upper region, slightly connivent, sulcus very shallow, existing as a narrow rim, sides laterally striate, striae few and deep, straight to flexuous, sessile on the substrate, rarely bifurcating, taller than wide at maturity: 1–2.5 mm long x 250–450 µm high, 200–300 µm wide. *Pseudoparaphyses* hyaline, cellular, 1–2 µm wide, branched above the ascal layer to form an epithecium. *Asci* bitunicate, cylindrical, short-stipitate, (110–)125–135 x 15–20 µm (n = 9). *Phragmospores* fusiform, narrow, hyaline and straight when young, becoming pale-yellow to lightly clear-brown, and curved when mature, highly guttulate, with guttulae large, highly refractive, present in every cell, with (7–)9(–11) septa, measuring (35–)40–45(–55) x (7–)9–10(–12) µm when mature (n = 27).

*Specimens examined*: **U.S.A.**, Tennessee, Sevier Co., Great Smoky Mountains National Park, Elkmont, Little River Trail, 35° 39' 13.4" N, 83° 34' 44.7" W, 686 m elev., 5 Nov. 2007, A.N. Miller, S.M. Huhndorf, J.L. Crane, T.J. Atkinson, I. Promputtha, M. Grief, G.K. Mugambi, & P. Chaudhary, deposited as ILLS 59908 (ANM 1495) = **holotype**; BPI 879783 = **paratype**; Tennessee, Sevier Co., Great Smoky Mountains National Park, Chimney Tops Picnic Area, Cove Hardwood Loop Trail, 35° 38' 10.7" N, 83° 29' 32.1" W, 4 Nov. 2007, A.N. Miller, S.M. Huhndorf, J.L. Crane, T.J. Atkinson, I. Promputtha, M. Grief, G.K. Mugambi & P. Chaudhary, deposited as ILLS 59907 (ANM 1442), and BPI 879784.

*Notes*: A superficial resemblance exists between *Hysterium barrianum* in Clade C, with *H. sinense* in Clade D. The phragmospores of *H. barrianum* (Fig. 2N–Q) have a similar number of septa, (7–)9(–11), as those of *H. sinense* (Fig. 3H), the latter with (3–)5–9(–11) septa. The two species also have spores of similar length. However, the width measurements of *H. barrianum*, (35–)40–45(–55) x (7–)9–10(–12) µm, serve to separate it from *H. sinense*, (34–)38–50 x 11–15 µm. Most importantly, *H. barrianum* does not possess a swollen or tumid supra-median cell, as does *H. sinense* and the closely related *H. insidens*. Furthermore, *H. barrianum* is highly guttulate, and lightly pigmented at maturity, whereas *H. sinense* and *H. insidens* possess few if any guttulae, and are much darker in pigmentation at maturity. Lastly, molecular data place the species in different groups within the *Hysteriaceae*.

In this study, we were able to secure a wide taxon sampling strategy for the genus *Hysterium* (Table 1), including multiple isolates for seven of the eleven currently recognised species, namely: *H. pulicare* (1), *H. angustatum* (7), *H. vermiforme* (1), *H. insidens* (2), *H. sinense* (2), *H. barrianum* (2) and *H. hyalinum* (1). Multiple gene phylogenies indicate that the genus *Hysterium* is polyphyletic, along three separate lines, two within the *Hysteriaceae* and one, *H. hyalinum*, outside of the family (Fig. 1). This implies that the evolution of pigmented phragmospores borne in hysterothecia has occurred at least three times within the *Pleosporomycetidae*.

Sequence data indicate that Clade C contains the type species, *Hysterium pulicare*, as well as the closely related *H. angustatum*, and *H. vermiforme* (Fig. 1). All three taxa have 3-septate, pigmented phragmospores, corresponding to Type I. Also, within Clade C resides the newly described *H. barrianum*, with 9-sepate spores. None of these species has a swollen supra-median cell. Accessions of *H. angustatum*, originating from South Africa (CMW

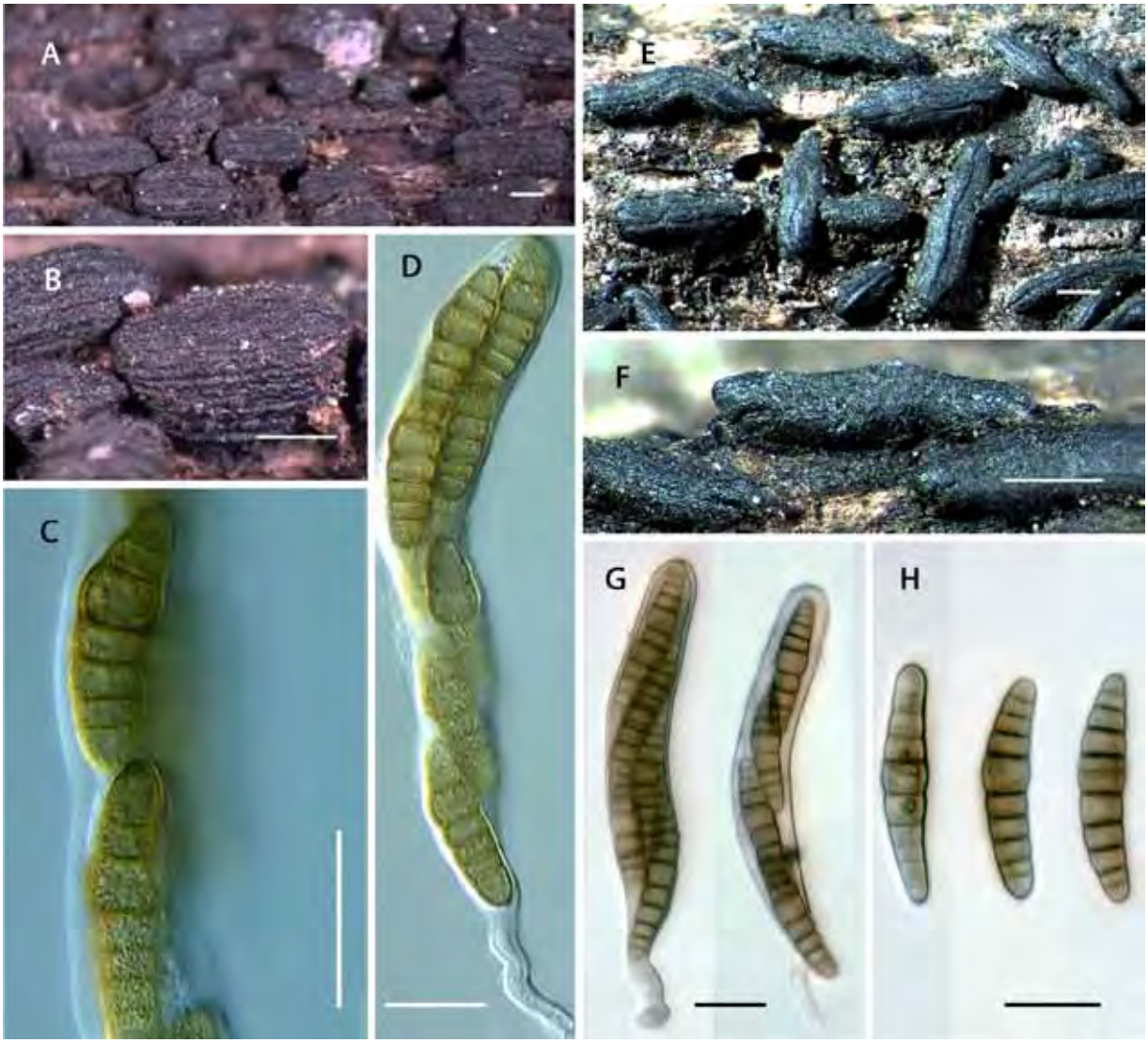


**Fig. 3.** The genus *Oedohysterium* (Clade D). A–D. *Oedohysterium insidens* [ANM 1443 (BPI 879799), U.S.A.]; E–H. *Oedohysterium sinense* [ANM 119 (ILLS), U.S.A.; not incl.]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 20 µm.

20409), Kenya (GKM 243A), New Zealand (SMH 5211.0, SMH 5216) and the United States, New Jersey (CBS 123334) and Wisconsin (CBS 236.34), form a highly supported monophyletic clade with *H. pulicare*, collected from the United States, New York (CBS 123377). Both species possess similar pigmented 3-septate phragmospores, versicolorous in *H. pulicare* and concolorous in *H. angustatum*. Interestingly, ~10 % of the ascospores within a given hysterothecium of *H. pulicare* are typically found to be concolorous (Bisby 1941). Likewise, versicolorous ascospores have also been observed in *H. angustatum*, stated at less than ~5 % for a given hysterothecium (Lee & Crous 2003). Although ascospore size in *H. pulicare* may be twice that found in *H. angustatum* (Zogg 1962), a certain degree of overlap in spore length measurements exists between the two, and molecular data presented here and elsewhere (Boehm *et al.* 2009) indicate that they are closely related.

In this study, one of the *H. angustatum* accessions from Tennessee (ANM 85), did not cluster with the other surveyed *H. angustatum* in Clade C. Instead, ANM 85 clustered with *H. vermiforme* from Kenya (GKM 1234). Spore measurements of ANM 85 (ILLS) were compared to the other *H. angustatum* accessions from the United States (CBS 123334 / BPI 878724), Kenya (GKM 243A, EA), and New Zealand (SMH 5211.0, F) which formed the other sub-clade within Clade C. All of these specimens showed remarkably little variability in their spore morphology. Additionally, no obvious differences were noted in their fruitbody morphology. This may indicate early stages of speciation within the taxon, with sequence variation preceding morphologic change.

Grouping with the anomalous *H. angustatum* ANM 85, was *H. vermiforme*, a taxon known only from the original description by Massee in 1901 from West Africa (Ghana). The isolate included here (GKM 1234 / BPI 879785; Fig. G–K) originated from Mt. Kenya, Kenya, and possesses smaller spore measurements, (20–)25–28 x (4–)5–6 µm, than those given by Massee (1901), and reiterated by Zogg (1962), as (30–)35–40 x 12–14 µm. In other respects, however, BPI 879785 matches closely Massee's (1901) original description, and we choose here to simply expand the spore measurements for *H. vermiforme* to (20–)25–40 x (4–)5–14 µm, rather than describe a new species.

The 3-septate *H. hyalinum* (CBS 237.34) lies outside of the *Hysteriaceae* altogether. It falls in a small, isolated, but well-supported clade along with the type species of *Hysterographium*, namely *Hg. fraxini*. Since only one isolate is represented, it is premature to draw conclusions. Molecular data indicate that the remaining two species of *Hysterium* in our survey, namely *H. sinense* and *H. insidens*, are not related to the type *H. pulicare* and associated species within Clade C. Rather, data indicate that they belong to Clade D. As such, we propose the following new genus to accommodate these taxa.

***Oedohysterium*** E.W.A. Boehm & C.L. Schoch, **gen. nov.** MycoBank MB515421.

*Etymology*: Greek, *Oedo-* meaning swollen, referring to the swollen supra-median cell of the ascospores and *Hys-* from *Hysterium.*

Hysterothecia solitaria vel gregaria, iuvenia erumpentia, deinde superficialia, navicularia, nonnumquam linearia, plus minusve parallela, neque confluentia, nonnumquam angulo inserta, raro flexuosa vel furcata, plerumque utrinque obtuse, et fissura longitudinali prominente praedita. Latitudo altitudine minor vel major. Peridium crassum, carbonaceum, maturum fragile, per longitudinem striatum, basim versus incrassatum, sursum attenuatum, bistratosum. Pseudoparaphyses cellulares, 1–2.5 µm latae, hyalinae, septatae, sursum ramosae, vulgo epithecium pigmentatum ascos obtegens formantes. Asci cylindrici vel clavati, bitunicati. Ascosporae irregulariter biseriatae, phragmoseptatae (dictyoseptatae), fusiformes, curvatae, utrinque angustatae, ad septum medium constrictae, (4–)6–8(–11) septis divisae, primum pallide luteae, deinde brunnescentes. Cellula (raro duo cellulae) ascosporarum supramediana conspicue inflata. Anamorphe ad *Septonema* pertinens.

*Hysterothecia* isolated to gregarious, erumpent when young, superficial when mature, navicular, sometimes linear in more or less parallel rows, but non confluent laterally, or sometimes situated at angles, rarely flexuous or bifurcating, usually with obtuse ends, and with a prominent longitudinal slit. Sometimes, taller than wide, other times wider than tall. *Peridium* thick, carbonaceous, brittle with age, longitudinally striated on the margins, thickened towards base, less thick apically, composed of two to three distinct layers, the inner compressed and pallid, the outer thickened and pigmented. *Pseudoparaphyses* cellular, 1–2.5 µm wide, hyaline, septate, branched above, forming a usually pigmented epithecium above the asci. *Asci* cylindrical to clavate, usually short stipitate, and bitunicate. *Ascospores* irregularly biseriate in ascus, typically phragmospores, in one case dictyospores, curved, fusiform, with tapering apices, constricted at the median septum, with (4–)6–8(–11) septa, at first hyaline-yellow, then pigmented sepia to brown at maturity. Genus characterised by a swollen or tumid supra-median cell, rarely with two cells swollen. *Anamorph*: *Septonema*.

*Type species*: *Oedohysterium insidens* (Schwein.) E.W.A. Boehm & C.L. Schoch, comb. nov.

***Oedohysterium insidens*** (Schwein.) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515422. Fig. 3A–D.

*Basionym*: *Hysterium insidens* Schwein., Trans. Amer. Philos. Soc., New Series 4(2): 244. 1832.

≡ *Hysterographium insidens* (Schwein.) Sacc., Syll. Fung. 2: 778. 1883.
= *Hysterium complanatum* Duby, Mém. Soc. Phys. Genève 16(1): 38. 1862.
= *Hysterium depressum* Berk. & M.A. Curtis, Grevillea 4(29): 10. 1875.
= *Hysterium fusigerum* Berk. & M.A. Curtis, Grevillea 4(29): 11. 1875 (as '*fusiger*').
= *Hysterium berengeri* Sacc., Syll. Fung. 2: 751. 1883.
= *Hysterium janusiae* Rehm, Hedwigia 37: 299. 1898.
= *Hysterium apiculatum* Starbäck, Bidrag Kungl. Svenska Vetensk.-Akad. Hist. 25(1): 19. 1899.
= *Hysterium batucense* Speg., Revista Fac. Agron. Univ. Nac. La Plata 6(1): 116. 1910.
= *Hysterium andicola* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 23: 85. 1912.
= *Hysterium atlantis* Maire, Mém. Soc. Sci. Nat. Maroc. 45: 35. 1937.
= *Hysterium lavandulae* Urries, Ann. Jard. Bot. Madrid 1: 64. 1941.

*Hysterothecia* isolated to gregarious, variably erumpent to sessile, 0.5–2.5 mm long, 0.2–0.5 mm high, lying parallel, but not confluent laterally, generally in line with the grain of the wood, and striated laterally with age. *Pseudoparaphyses* hyaline, cellular, 1–2.0 µm wide, walls thickened at apices, forming an epithecium, borne in mucilage, above the ascal layer, often encrusted with dark, pigmented crystals. *Asci* bitunicate, cylindrical, 8-spored, irregularly biseriate, 130–150 x 15–24 µm, short stipitate, and with a prominent apical nasse, especially when young. *Ascospores* phragmospores transversely (4–)6–8(–11)-septate, constricted at the median septum, inequilateral, slightly curved, at first hyaline-yellow, then brown at maturity, with a prominent swollen supra-median cell. If 5-septate, then swollen cell located at the second position; if 6-septate, then often the third from the top, measuring (20–)23–28(–38) x (5–)7–10(–13) µm. Principally North- and South-America, and Europe (Italy), from bark and old wood of *Pinus, Larix, Castanea, Quercus, Eucalyptus, Fraxinus, Aspidosperma,* and *Lavandula* (Zogg 1962). Also reported from South Africa (van der Linde, 1992). Anamorph: *Septonema spilomeum*.

***Oedohysterium sinense*** (Teng) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515423. Fig. 3E–H.

*Basionym*: *Hysterium sinense* Teng, Sinensia 4: 134. 1933.

= *Hysterium macrosporum* Teng, Sinensia 4: 134. 1933, non Peck, Rep. (Annual) New York State Mus. Nat. Hist. 26: 83. 1874 (1873).

*Hysterothecia* scattered to subgregarious, linear, sometimes parallel but non-confluent laterally, more often lying at irregular angles, depending on the grain of the substrate, striated in age, usually of a similar size (2–3.5 mm in length), that is, maturing synchronously in a given colony. *Pseudoparaphyses* hyaline to pale-yellow, cellular, 2–2.5 µm wide, apically branched, walls of even thickness along length, forming a darkened gelatinous epithecium above the ascal layer, +/- encrusted with pigmented crystals. *Asci* bitunicate, cylindrical, 8-spored, irregularly biseriate, 140–170 x 26–30 µm, short-stipitate, ascospores biseriate to subseriate in ascus, with a prominent apical nasse, especially when young, but sometimes persisting through maturity. *Ascospores* large, fusiform, asymmetric, curved phragmospores, at first hyaline, then pale-yellow to -brown, finally deep brown at maturity, with (3–)5–9(–11) septa, with a medial septal constriction, measuring (34–)38–50 x 11–15 µm, and, like *Od. insidens*, with a prominent swollen or tumid supra-median cell, usually located just above the median septum. From North America (Boehm, unpubl. data), Europe (Zogg 1962), China (Teng 1933), and South Africa (van der Linde 1992), on decorticated hardwood trees and structures (*e.g.*, aged fence posts).

*Notes*: Species of *Oedohysterium* belonging to Clade D are characterised by elongate asymmetric spores with more than 3 septa, typically showing a swollen or tumid supra-median cell. In this study, two single-ascospore isolates of *Od. sinense*, one from South Africa (CBS 123345 / BPI 878730), and one from the United States, New Jersey (EB 0339 / BPI 879800), cluster with two isolates of *Od. insidens*, both from the United States, Massachusetts (CBS 238.34) and Tennessee (ANM 1443 / BPI 879799). Both species have remarkably similar phragmospores (*e.g.*, Fig. 3D *versus* Fig. 3H). As these two taxa belong to Clade D and are far removed from the type species, *H. pulicare*, in Clade C, we propose that they be accommodated in the new genus *Oedohysterium*. An additional new combination is proposed below.

***Oedohysterium pulchrum*** (Checa, Shoemaker & Umaña) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515424.

*Basionym*: *Hysterographium pulchrum* Checa, Shoemaker & Umaña, *Mycologia* 99: 289. 2007.

*Notes*: The newly described *Hg. pulchrum* from Costa Rica (Checa *et al.* 2007) also falls within Clade D (Fig. 1) and is here transferred to *Oedohysterium*, as *Od. pulchrum* (DQ 402184 / DAOM 234345). This is because molecular data indicate a close association with the two species of *Oedohysterium*, *Od. insidens* and *Od. sinense*. At first surprising, on further consideration, this sub-clade forms a natural assemblage premised on morphological features. The spores of all three taxa show a remarkable degree of similarity in morphology, which includes being similarly pigmented, slightly curved and fusiform, with a common number of transverse septa. The sole difference is the presence of one or two vertical septa in *Od. pulchrum*, a feature noted by the authors to be absent in some spores (Checa *et al.* 2007). Most importantly, like *Od. insidens* and *Od. sinense*, *Od. pulchrum* also possesses a swollen supra-median cell. Interestingly, a striking resemblance to the phragmospores of *Od. insidens* can be seen for those spores of *Od. pulchrum* that do not possess vertical septa (Checa *et al.* 2007). This is based on similarities in shape (*e.g.*, curved and fusiform), size [(20–)23–28(–38) x (5–)7–10(–13) µm *versus* 22–25(–27) x 5–6 µm], and in the number of transverse septa (4–)6–8(–11) *versus* (5–)6, for *Od. insidens* and *Od. pulchrum*, respectively. As molecular data indicate that the presence or absence of vertical septa should be considered a sympleisiomorphic character state within the *Hysteriaceae* (Boehm *et al.* 2009), we feel justified in including both phragmospores and dictyospores within the genus *Oedohysterium*.

We choose to provide the following dichotomous key whereby all hysteriaceous fungi, bearing transversely septate pigmented phragmospores (including *Od. pulchrum* with dictyospores) are identified together, with the caveat that unrelated taxa appear in the same key.

## Key to the species of *Hysterium* and *Oedohysterium*

1. Phragmospores mainly 3-septate ..... 2
1. Phragmospores concolorous, more than 3-septate, in one instance pigmented dictyospores with 1-2 vertical septa (*Od. pulchrum*) ..... 7

2. Phragmospores either versicolorous or delayed concolorous ..... 3
2. Phragmospores truly concolorous (sepia to dark brown in colour) ..... 4

3. Terminal cell mainly remaining hyaline with inner spore cells pigmented brown (versicolorous); ascospores 20–40 x 6–12 µm; cosmopolitan ..... ***H. pulicare***
3. Phragmospores tardily pigmented, often remaining hyaline for quite some time after discharge, but eventually becoming uniformly concolorous; 20–26(–28) x 6–8.5 µm; North America ..... ***H. hyalinum***
*Note*: Currently recognised as *Pleosporomycetidae sp. incertae sedis* (Boehm *et al.* 2009).

4. Phragmospores 3-septate, 28 µm or less in length ..... 5
4. Phragmospores 3-septate, longer than 28 µm ..... 6

5. Phragmospores (12–)14–21(–28) x (3–)4–8(–10) µm, firmly 3-septate, no septal constrictions; end-cells obtuse; cosmopolitan ..... ***H. angustatum***
5. Phragmospores (14–)15–18(–20) x 5–7 µm; 3- (rarely 2- or 4)-septate; prominently constricted at first-formed septum; basal cell extended; red hamathecial pigment; neotropical ..... ***H. asymmetricum***

6. Phragmospores fusoid, slightly curved, guttulate; (20–)25–40 x (4–)5–14 µm; West and East Africa ..... ***H. vermiforme***
6. Phragmospores fusoid, curved, highly guttulate; 40–57 x 11–15 µm; on *Pinus*, North America and China ..... ***H. macrosporum* Peck**

7. Phragmospores or dictyospores (4-) 6- to 8- (11-) celled, fusiform in outline, with +/- swollen supra-median cell(s) ..... 8
7. Phragmospores with more than 11 septa, fusiform, pale brown, (13–)14–15(–21)-septate, (35–)45–50(–60) x (10–)12–13(–14) µm; Africa ..... ***H. velloziae***

8. Swollen supra-median cell(s) present, either phragmospores or dictyospores (*Oedohysterium*) ..... 9
8. Phragmospores only, no swollen supra-median cells(s) present ..... 11

9. Dictyospores lightly pigmented, 22–25(–27) x 5–6 µm, with (5–)6 transverse and 1 vertical septum in either cell or both cells adjacent to the primary septum, absent in some spores, with a swollen supra-median cell; typically with red pigment in the hamathecium; neotropical (Costa Rica) ..... ***Od. pulchrum***
9. With no red pigment present ..... 10

10. Phragmospores with (4–)6–8(–11) septa, slightly curved, fusiform, at first hyaline-yellow then reddish brown at maturity, if 5-septate, showing a swollen cell at the second position, if 6-septate, often the third from the top, +/- median septal constriction, (20–)23–28(–38) x (5–)7–10(–13) µm; cosmopolitan ..... ***Od. insidens***
10. Phragmospores larger, fusiform, straight to curved, at first hyaline, then yellow or pale brown, finally deep brown; swollen supra-median cell(s) present, (3–)5–9(–11) septa, with median septal constriction; (34–)38–50 x 11–15 µm; cosmopolitan ..... ***Od. sinense***

11. Phragmospores fusiform, narrow, straight to very slightly curved, pale hyaline at first, then pale-yellow at maturity, with highly refractive guttules, in every cell, with (7–)9(–11) septa, no supra-median swollen cell(s), (35–)40–45(–55) x (7–)9–10(–12) µm; North America ..... ***H. barrianum***
11. Phragmospores oblong, wide, slightly curved, bulging on one side, nearly hyaline and 1-septate at first, becoming clear brown and 7-septate, septa highly asymmetric, (2–)3 of the septa close to each end, the two central cells much larger; 48–67 x 15–20 µm; China and North America ..... ***H. magnisporum***

***Gloniella*** Sacc., Syll. Fung. 2: 765 (1883).

The genus *Gloniella* was established by Saccardo (1883) to accommodate hysteriaceous fungi that possess hyaline phragmospores, from 3- to 9-septate. Zogg (1962) recognised six species: three collected on ferns from Europe and the Mediterranean, namely *Gl. adianti* on *Adiantum*, and *Gl. graphidioidea* and *Gl. normandina*, both on *Pteridium*. Zogg also accepted *Gl. sardoa* from *Populus* in Europe, *Gl. typhae* on *Typha*, the latter described from Europe (Zogg 1962) and Chile (Lorenzo & Messuti 1998), and *Gl. bambusae* on *Bambusa* from Brazil. Since then, an additional three species have been described: *Gl. gracilis* from Costa Rica (Checa *et al.* 2007), *Gl. corticola* from India (Pande & Rao 1991), and *Gl. clavatispora* from South Africa (Steinke & Hyde 1997). More recently, Barr (2009) recognised *Gl. abietina* on *Abies* from Idaho, and *Gl. lapponica* on *Arctostaphylos* from Washington. A number of species in the key may be conspecific, since reported spore measurements are identical or nearly so.

## Key to the species of *Gloniella*

1. Ascospores 3-septate, shorter than 15 µm ..... 2
1. Ascospores 3- or more-septate, and longer ..... 3

2. Ascospores 10–15 x 5–6 µm; India ..... ***Gl. corticola***
2. Ascospores 12–14 x 4–5 µm; on *Typha*, Europe ..... ***Gl. typhae***

3. On ferns in Europe ..... 4
3. Not on ferns ..... 6

4. Ascospores (2–)3(–4)-septate, (11–)15–20(–23) x 3–5 µm; on *Adiantum*, Europe ..... ***Gl. adianti***
4. Ascospores (3–)5(–7)-septate, slightly longer ..... 5

5. Ascospores (3–)5-septate, (15–)18–20(–22) x 4–5 µm; on *Pteridium*, Europe ..... ***Gl. graphidoidea***
5. Ascospores 5–7-septate, (22–)25–27(–30) x 3–4 µm; on *Pteridium*, Europe ..... ***Gl. normandina***

6. Ascospores 1–3-septate, 36–39 x 10 µm; on *Arctostaphylos*, Western North America ..... ***Gl. lapponica***
6. Ascospores with more septa ..... 7

7. Ascospores 3(–5) septate, 20–27 um x 7–8 µm; on *Abies grandis*, Western North America ..... ***Gl. abietina***
7. Ascospores with more septa ..... 8

8. Ascospores (6–)7(–8)-septate, (16–)18–21(–26) x 6–7(–8) µm; on *Populus*, Europe ..... ***Gl. sardoa***
8. Ascospores larger ..... 9

9. Ascospores (5–)6(–8)-septate, (18–)37(–41) x 10–11.5 µm, hyaline, smooth; on *Avicennia marina*, South Africa ..... ***Gl. clavatispora***
9. Ascospores smaller, neotropical ..... 10

10. Ascospores 6–7-septate, 32–37(–40) x 4–6 µm; Costa Rica ..... ***Gl. gracilis***
10. Ascospores (5–)6–7-septate, (28–)32–38(–44) x (3–)4–8(–9) µm; on *Bambusa*, Brazil ..... ***Gl. bambusae***

***Hysterographium*** Corda, Icon. Fung. 5: 34. 1842.

= *Hysteriopsis* Speg., Revista Fac. Agron. Univ. Nac. La Plata 2: 308. 1907.
= *Polhysterium* Speg., Anales Mus. Nac. Buenos Aires 23: 87. 1912.
= *Fragosoa* Cif., in Ciferri & Fragoso, Bol. Real Soc. Esp. Hist. Nat., Secc. Biol. 26(3-4): 194. 1926.

Although the genus *Hysterographium* has been removed from the *Hysteriaceae* (Boehm *et al.* 2009), and is currently recognised as *Pleosporomycetidae gen. incertae sedis*, it is included here. This is because it forms the basis for a number of new combinations within the family. The genus is characterised by pigmented dictyospores, with one to several longitudinal septa, ovoid to ellipsoid-fusoid, relatively broad, usually constricted at the first-formed septum. Zogg (1962) extensively revised the synonymy of the genus and accepted four species: *Hysterographium flexuosum* (Fig. 4A–B) and *Hg. fraxini* (Fig. 4C–D), the type, both with large dictyospores, prominently constricted at the median septum, the former with slightly longer, narrower spores. Zogg (1962) also accepted *Hg. mori* and *Hg. subrugosum*, with smaller, fewer-celled dictyospores, short and squat in the former, longer and more slender in the latter, both also constricted at the median septum.

Since then, an additional three species have been described: *Hysterographium minus* from Japan (Amano 1983), *Hg. spinicola*

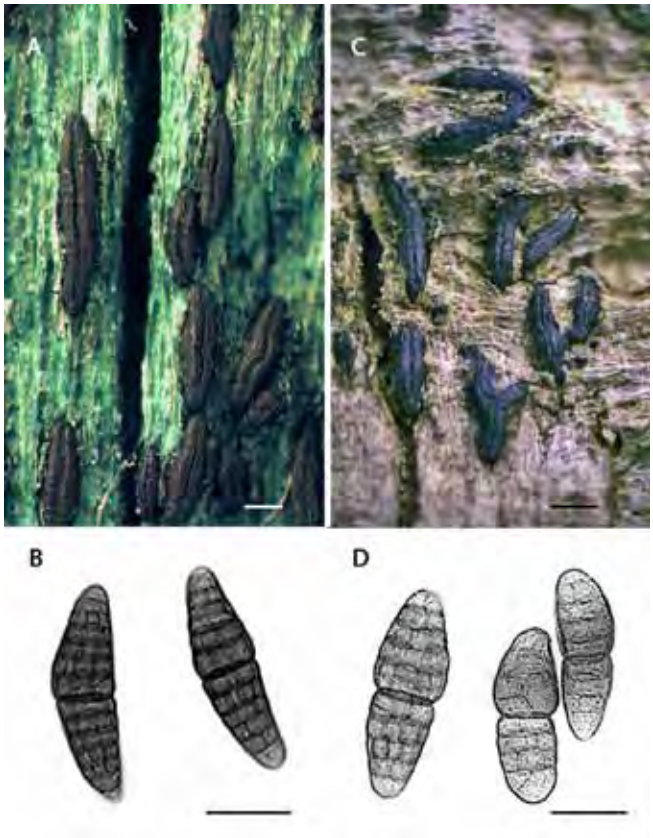


**Fig. 4.** The genus *Hysterographium*. A–B. *Hysterographium flexuosum* (EB 0098, U.S.A.; not incl.); C–D. *Hysterographium fraxini* (EB 0100, U.S.A.; not incl.). Scale bar (habitat) = 1 mm; Scale bar (spores) = 20 µm.

from South Africa, recollected from the thorns of *Acacia* and validated by van der Linde (1992), with a brick-red epithecium and spores only slightly longer than those of *Hg. mori*, and, lastly, *Hg. pulchrum* from Costa Rica, also with a red pigment in the hamathecium (Checa *et al.* 2007), here transferred to *Oedohysterium*, as *Od. pulchrum*.

Four of the seven species were surveyed in the present study, with multiple isolates (Table 1): *Hysterographium mori* (8), *Hg. subrugosum* (3), *Hg. fraxini* (2) and *Od. pulchrum* (1), falling into no fewer than three separate clades, two within the *Hysteriaceae* (Clades A and D) and one far removed from the family (Fig. 1). The latter clade includes the type species for the genus *Hysterographium*, namely *Hg. fraxini*, represented by isolates from Switzerland (CBS 109.43), deposited by Zogg in 1943, and from Canada (CBS 242.34), deposited by Lohman in 1934. *Hysterographium fraxini* forms a well-supported clade distant from the *Hysteriaceae,* but remains within the *Pleosporomycetidae* (Fig. 1). As this is substantiated by two geographically disparate isolates from two different continents, deposited by two reputable workers, it is significant. The implication is that the genus *Hysterographium* must follow the type species and be removed from the *Hysteriaceae* (Boehm *et al.* 2009). Species with pigmented dictyospores remaining within the *Hysteriaceae*, previously classified in *Hysterographium*, must therefore be accommodated in other genera. In this study, these would include the following species, for which we have sequence data: *Hysterographium mori*, *Hg. subrugosum*, and *Hg. pulchrum* (= *Od. pulchrum*). The remaining species for which we do not have sequence data, namely *Hg. minus*, *Hg. spinicola* and *Hg. flexuosum*, must remain as species of *Hysterographium*, until such time that sequence data are available. We therefore propose the following new genus.

***Hysterobrevium*** E.W.A. Boehm & C.L. Schoch, **gen. nov.** MycoBank MB515329.

*Etymology*: *Hystero-* from *Hysterographium*, Latin *brevis*, short, referring to the spores of the type, *Hb. mori*.

Hysterothecia navicularia, fissura longitudinali prominente praedita, utrinque acuminata vel obtusa, linearia vel flexuosa, solitaria vel gregaria, vulgo per longitudinem striata, nonnumquam erecta, quasi stipitata, superficialia vel partim in substrato immersa. Asci bitunicati, cyindrici vel clavati. Dictyosporae pigmentatae vel hyalinae, plerumque breviores quam 25 µm, ad septum medium constrictae; ascosporae hyalinae vel luteae iuvenes vulgo strato mucido circumdatae; pigmentatae pallide brunneae, pariete levi; ascosporae ovoideae vel obovoideae, apice obtuso vel acuminato, 3–4(–6) septis transversalibus et 1–2 longitudinalibus divisae.

*Hysterothecia* navicular, with a prominent longitudinal slit, variable with acuminate to obtuse ends, linear to flexuous, solitary to densely gregarious, surface usually longitudinally striate, sometimes erect, superficial, almost stipitate, to erumpent and partially embedded in substrate, the latter especially when gregarious. *Asci* bitunicate, cylindrical to clavate. *Ascospores* pigmented or hyaline dictyospores, usually less than 25 µm long, constricted at least at the median septum. If hyaline to pale-yellow, then typically associated with a gelatinous sheath when young, dissipating with age. If pigmented then lightly so, transparent clear brown, walls smooth; ascospores generally ovoid to obovoid, with either obtuse or acuminate ends, 3–4(–6) transverse septa, and 1–2 longitudinal septa, these mostly associated with the two central cells, but highly variable and sometimes at oblique angles in the end cells.

*Type species*: *Hysterobrevium mori* (Schwein.) E.W.A. Boehm & C.L. Schoch, comb. nov.

***Hysterobrevium mori*** (Schwein.) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515335. Fig. 5J–R.

*Basionym*: *Hysterium mori* Schwein., Trans. Amer. Philos. Soc. 4(2): 244. 1832.

≡ *Hysterographium mori* (Schwein.) Rehm, Ascomyceten No. 363. 1876.
= *Hysterium grammodes* De Not., Giorn. Bot. Ital. 2 (7–8): 55. 1847.
≡ *Hysterographium grammodes* (De Not.) Sacc., Syll. Fung. 2: 782. 1883.
= *Hysterium rousselii* De Not., Piren. Ister. 2(7–8): 19. 1847.
≡ *Hysterographium rousselii* (De Not.) Sacc., Syll. Fung. 2: 779. 1883.
= *Hysterium vulgare* De Not., Piren. Ister. 2(7–8): 18. 1847.
= *Hysterium australe* Duby, Mém. Soc. Phys. Genève 16(1): 44. 1862.
= *Hysterium lesquereuxii* Duby, Mém. Soc. Phys. Genève 16(1): 41. 1862.
≡ *Hysterographium lesquereuxii* (Duby) Sacc., Syll. Fung. 2: 779. 1883.
= *Hysterium gerardi* Cooke & Peck, Bull. Buffalo Soc. Nat. Sci. 3: 33. 1875.
≡ *Hysterographium gerardi* (Cooke & Peck) Sacc., Syll. Fung. 2: 783. 1883.
= *Hysterium viticolum* Cooke & Peck, Bull. Buffalo Soc. Nat. Sci. 3: 33. 1875.
≡ *Hysterographium viticola* (Cooke & Peck) Rehm, Ascomyc. No. 316, in Sacc., Syll. Fung. 2: 782. 1883.
= *Hysterium variabile* Cooke & Peck, Bull. Buffalo Soc. Nat. Sci. 3: 33. 1875.
≡ *Hysterographium variabile* (Cooke & Peck) Sacc., Syll. Fung. 2: 780. 1883.
= *Hysterium formosum* Cooke, in Harkness & Cooke, Grevillea 7: 3. 1878.
≡ *Hysterographium formosum* (Cooke) Sacc., Syll. Fung. 2: 783. 1883.
= *Hysterium putaminum* Cooke, Grevillea 7: 48. 1878.
≡ *Hysterographium putaminum* (Cooke) Sacc., Syll. Fung. 2: 783. 1883.
= *Hysterographium portenum* Speg., Anales Soc. Ci. Argent., Secc. Santa Fe. 9(4): 185. 1880.
= *Hysterographium grammodes* var. *minus* Sacc., Syll. Fung. 2: 783. 1883.
= *Hysterographium pumilionis* Rehm, Discom. 1(3): 21. 1887.
= *Hysterographium guaraniticum* Speg., Anales Soc. Ci. Argent., Secc. Santa Fe. 26(1): 56. 1888.
= *Hysterographium punctiforme* Pat., Bull. Soc. Mycol. France 4: 120. 1888.
= *Hysterographium ruborum* Cooke, in Rehm, Ascom., No. 918. 1888.
= *Hysterium insulare* P. Karst. & Har., Rev. Mycol. Toulouse No. 47: 1890.
= *Hysterographium incisum* Ellis & Everh., Bull. Torrey Bot. Club 24: 462. 1897.

= *Hysterographium ziziphi* Pat., Cat. Rais. Pl. Cell. Tunisie: 112. 1897 (as *"zizyphi"*).
= *Hysterographium rousselii* (De Not.) Sacc. var. *piri* Feltgen, Vorst. Pilz. Luxemb. Nachtr. 3: 111. 1903.

*Hysterothecia* erumpent-superficial, ellipsoidal, oblong, linear or cylindrical, 1–2(–3.5) mm long, 220–275(–440) µm wide, by 190–330 µm high, mostly straight and lying parallel, but not confluent laterally, often gregarious and crowded so as to cover the substrate, longitudinally striate in age, navicular with tapering ends. Two types of hysterothecial aggregations regularly observed, depending on substrate: (1) Colonies on weathered, whitened decorticated hardwood often forming large oval colonies, with acuminate ends, measuring 5–15 cm in length, with hysterothecia gregarious in the center, densely packed in longitudinal formations, showing multiple stages of development, and darkening the adjacent substrate; when young, prior to emergence of hysterothecia, smaller colonies are seen, but still presenting darkened oval patches, often with coelomycetous anamorph present. (2) Colonies on bark (*i.e.*, corticolous) less gregarious, not darkening the substrate, hysterothecia often situated at angles, rather than in parallel orientation. *Peridium* 30–60 µm thick medially, to 100+ µm at the base, distinctly three-layered in cross-section, the outer layer darkly pigmented, the middle less so, and the inner layer, thin-walled, pallid and compressed. *Pseudoparaphyses* cellular, septate, persistent, 1–2 µm wide, hyaline, thickened apically, branched and forming an epithecium in a gelatinous matrix above the ascal layer. *Asci* cylindrical to clavate, bitunicate, short-stipitate, (50–)80–110 x 10–18 µm. *Ascospores* pigmented, thin-walled dictyospores, obovoid, ends obtuse, 3–(5–7)-septate, with 1–2(–3) vertical septa usually associated with mid-cells, but on occasion also present obliquely in end cells, constricted at the median septum, sometimes, when fully hydrated, at additional, more distal septa, measuring (12–)14–22(–26) x (5–)7–10(–11) µm. *Anamorph* coelomycetous, *Aposphaeria*-like in nature, in culture conidiomata as irregular locules, with conidiogenous cells 8–10 x 1.5–2 µm; *conidia* (2–)2.5–3.5(–4) x 1–2 µm (Lohman 1932). Cosmopolitan, on aged, usually decorticated, weathered wood or bark of *Pinus, Juniperus, Salix, Ostrya, Castanea, Quercus, Ulmus, Morus, Pyrus, Amelanchier, Crataegus, Rubus, Cercocarpus, Prunus, Gleditsia,* various *Fabaceae, Melia, Pistacia, Cotinus, Rhus, Acer, Ziziphus, Vitis, Fraxinus, Olea,* and *Aspidosperma* (Zogg 1962).

**Fig. 5.** The genus *Hysterobrevium* (Clade A). A–E. *Hysterobrevium constrictum* [SMH 5211.1 (F), New Zealand]; F–I. *Hysterobrevium smilacis* [GKM 426N (EA), Kenya]; L–N. *Hysterobrevium mori* [SMH 5273 (BPI 879787), U.S.A.]; O–R. *Hysterobrevium mori* [ANM 43 (ILLS), U.S.A.; not incl]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 10 µm.

***Hysterobrevium smilacis*** (Schwein.) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515336. Fig. 5F–I.

*Basionym*: *Hysterium smilacis* Schwein., Schriften Naturf. Ges. Leipzig 1: 49. 1822.

≡ *Gloniopsis smilacis* (Schwein.) Underw. & Earle, Bull. Alabama Agric. Exp. Sta. 80: 196. 1897.
≡ *Hysterographium smilacis* (Schwein.) Ellis & Everh., N. Amer. Pyrenomyc. 709. 1892.
= *Hysterium biforme* Fr., Observ. Mycol. (Havniae) 2: 354. 1818.
≡ *Gloniopsis biformis* (Fr.) Sacc., Syll. Fung. 2: 773. 1883.
= *Hysterium elongatum β curvatum* Fr., Elench. Fung. (Greifswald) 2: 138. 1828.
= *Hysterium curvatum* Fr., Elench. Fung. 2: 139. 1828.
≡ *Gloniopsis curvata* (Fr.) Sacc., Syll. Fung. 2: 775. 1883.
= *Hysterium rocheanum* Duby, Mém. Soc. Phys. Genève 16: 51. 1862.
≡ *Gloniopsis rocheana* (Duby) Sacc., Syll. Fung. 2: 773. 1883.
= *Hysterographium naviculare* P. Karst., Symb. Mycol. Fenn. 6: 37. 1877.
= *Hysterium gloniopsis* W.R. Gerard in Peck, Rep. New York State Mus. 32: 49. 1877 (1879).
≡ *Hysterographium gloniopsis* (W.R. Gerard) Ellis & Everh., N. Amer. Pyrenomyc. 708. 1892.
≡ *Gloniopsis gloniopsis* (W.R. Gerard) House, Bull. New York State Mus. 219-220: 235. 1920.
= *Gloniella scortechiniana* Sacc. & Roum., Rev. Mycol. Toulouse 5: tab. 41, fig. 17. 1883.
= *Gloniopsis gerardiana* Sacc., Syll. Fung. 2: 774. 1883.
= *Gloniopsis decipiens* var. *cisti* Rehm, Hedwigia 25: 13. 1886.
= *Gloniopsis cisti* Rehm, Hedwigia 25: 13. 1896.
= *Gloniopsis ambigua* Sacc., Ann. Mycol. 10(3): 317. 1912.
= *Gloniopsis ellisii* Cash, Mycologia 31: 294. 1939.

*Hysterothecia* erumpent, many times surrounded at the base by ruptured epidermis or periderm, especially when borne in herbaceous stems, much less so on wood, then completely superficial, 0.5–1.5 mm long, 300–400 µm wide, 200–250 µm high, longitudinally striated. *Peridium* 25–50 µm wide, narrower at base within the substrate, widest at mid-point, carbonaceous and brittle when dry. *Pseudoparaphyses* cellular, septate, persistent, 1–1.5 µm wide, hyaline to pale yellow in mass, branched above, forming an epithecium, but not darkly pigmented, exposed surface yellow-brown. *Asci* cylindrical to clavate, bitunicate, short-stipitate, 70–120 x 15–25 µm at maturity. *Ascospores* asymmetric, hyaline to pale yellow dictyospores, with acuminate ends, and a gelatinous sheath that usually dissipates at maturity, measuring (13–)15–26(–

31) x (4–)5–9(–10) µm. Spore septation highly variable, usually 3–5(–9)-septate and with 1(–3) vertical septa, passing through multiple mid-cells, and usually prominently constricted at the median septum, when fresh and hydrated, sometimes constriced along multiple transverse septa. *Anamorph* coelomycetous, *Aposphaeria*-like. Cosmopolitan on *Pinus, Chamaerops, Smilax, Populus, Salix, Juglans, Betula, Fagus, Quercus, Ficus, Pyrus, Crataegus, Rubus*, *Rosa, Prunus, Robinia, Butea, Pistacia, Cotinus, Acer, Cistus, Erica,* and *Lavandula* (Zogg 1962).

*Notes*: *Hysterobrevium mori*, while falling within the *Hysteriaceae*, finds itself in two separate clades (Fig. 1). In Clade A, one set of North American *Hb. mori* isolates associates with six highly geographically diverse isolates of *Hb. smilacis*. The *Hb. mori* isolates originate from the United States, from New Jersey (CBS 123336, CBS 123564), New York (CBS 123335, CBS 123563), Indiana (SMH 5273) and Michigan (SMH 5286). The *Hb. smilacis* isolates originate from the United States, from Indiana (SMH 5280) and Michigan (CBS 200.34), as well as from South Africa (CMW 18053), Sweden (CBS 114601) and Kenya (GKM 426N). Dictyospores of both species are of similar shape, size and degree of septation: (12–)14–22(–26) x (5–)7–10(–11) µm, 3–(5–7)-septate, with 1–2(–3) vertical septa, for *Hb. mori versus* (13–)15–26(–31) x (4–)5–9(–10) µm, 3–5(–9)-septate, with 1(–3) vertical septa, for *Hb. smilacis*. They differ in the absence of pigmentation and the presence of a gelatinous sheath in the latter. Thus, these two species, previously classified in two separate genera, *Hysterographium* and *Gloniopsis*, are in fact closely related, with each species far removed from the type species of their respective genera. Further support for this argument, can be found in Lohman (1933a), who found a similar *Aposphaeria* anamorph for both *Hb. mori* (as *Hg. mori*) and *Hb. smilacis* (as *Gp. gerardiana*) and stated that they were indistinguishable in culture. The implication is that both taxa should be united within the same genus, for which we propose *Hysterobrevium*.

In addition to the association with *Hb. smilacis* in Clade A, *Hb. mori* also finds itself in Clade D. As this is validated by two geographically diverse isolates, one from the United States, Michigan (CBS 245.34) and one from Kenya (GKM 1013 / BPI 879788), it is significant. Spore measurements of the Kenyan accession GKM 1013 (BPI 879788) in Clade D *versus* those of other *Hb. mori* accessions in Clade A, represented by SMH 5273 / BPI 879787, CBS 123335 / BPI 878734, and CBS 123336 / BPI 878733, failed to detect any significant morphological differences; nor were there any appreciable differences detected in their hysterothecia. The association of *Hb. mori* with unrelated taxa within the *Hysteriaceae* in Clade A and D may be significant in that *Hb. mori* has long been regarded as a highly variable taxon (Ellis & Everhart 1892, Lohman 1933a), resulting in the synonymy of no fewer than 30 names since its inception by Schweinitz in 1832 (Zogg 1962). Future studies may well reveal that *Hb. mori* contains a number of cryptic species, morphologically similar, but genetically unrelated. We propose an additional new combination below.

***Hysterobrevium constrictum*** (N. Amano) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515337. Fig. 5A–E.
*Basionym*: *Gloniopsis constricta* N. Amano, Trans. Mycol. Soc. Japan 24: 289. 1983.

*Notes*: Amano (1983) described a small-spored species of *Gloniopsis* from Japan, *Gp. constricta*, noting a prominent median septal constriction. The measurements of the dictyospores were given as 10.4–13.2 x 4.4–5.8 µm, usually with 3–4 transverse and one vertical septum that passes through one to three cells. Although not mentioned (Amano 1983), the illustrations depict a very thick wall and dictyospores highly symmetric in outline and septation. Amano (1983) stated of the spores "...hyaline, later becoming brown...", but did not mention the presence of a gelatinous sheath. He also noted that the closest resemblance is with *Hb. smilacis* (as *Gp. curvata*), the latter however with slightly larger spores. In this study, we were fortunate to obtain a specimen from New Zealand (SMH 5211.1, deposited in F; Fig. 5A–E) that corresponds to the published description given by Amano (1983), but differs on several counts. Like *Gp. constricta*, the hyaline dictyospores in SMH 5211.1, are highly symmetric and thick-walled, (1–)3(–4)-septate, with 1(–2) vertical septa, but the constriction at the median septum in SMH 5211.1, while present, is not prominent. Also unlike *Gp. constricta*, the spores in SMH 5211.1 have an obvious gelatinous sheath when young, but this quickly dissipates with age, and may be completely absent in mature specimens. In SMH 5211.1, the spores measure (18–)20(–23) x 10–12 µm, which is considerably larger than those of *Gp. constricta*. Nevertheless, these differences, in our opinion, are not sufficient to warrant a new species, and we choose here to simply expand the spore measurements to (11–)13–20(–23) x 5–12 µm, rather than describe a new species, proposing instead the new combination *Hb. constrictum*.

***Gloniopsis*** De Not., Giorn. Bot. Ital. 2(2): 23. 1847.

A review of the nomenclatural history of the genus *Gloniopsis* was given in Boehm *et al.* (2009). The genus is characterised by hyaline to yellow dictyospores, often inequilateral, curved, in outline obovoid, ends obtuse to sub- to acuminate, multi-septate, with one or more longitudinal septa, constricted at the first-formed septum, sometimes constricted at additional septa, and usually surrounded by a gelatinous sheath, which may dissipate with age. Zogg (1962) synonymised a number of names under the type species, *Gp. praelonga* (Fig. 6A–B), and accepted only one additional species, namely *Gp. curvata* with smaller ascospores. Barr (1990a) proposed to include this latter species under the earlier name *Gp. smilacis*, following Cash (1939). In this study, we have transferred *Gp. smilacis* to *Hysterobrevium*, closely related to *Hb. mori* in Clade A. Recently, *Gp. argentinensis*, previously considered by Zogg (1962) as a doubtful species, was reinstated by Lorenzo & Messuti (1998). The authors state that the ascospores are 7-septate, with 1–3(–4) longitudinal septa, some passing through multiple cells, in outline widely ellipsoid, measuring 20–26 x 9–12 µm. The septation and spore measurements are nearly identical to those of *Gp. praelonga*, the latter 5–7(–10)-septate, with 2–3 longitudinal septa, (16–)20–32(–34) x (6–)9–12(–15) µm. We therefore synonymise *Gp. argentinensis* under *Gp. praelonga*. Lastly, Amano (1983) described an additional two species of *Gloniopsis* from Japan, namely *Gp. macrospora* and *Gp. constricta*, the latter transferred here to *Hysterobrevium* (Clade A).

Molecular data indicate that the genus *Gloniopsis* is polyphyletic, with the type, *Gp. praelonga*, belonging to Clade D (Fig. 1). Closely associated with the type, are a number of species possessing pigmented dictyospores, which would previously have been classified in the genus *Hysterographium* (*e.g.*, *Hysterographium subrugosum*). Based on molecular data presented here, we therefore propose to emend the genus *Gloniopsis*, to include both hyaline and pigmented dictyospores. The following new combination is proposed, as well as two new species from Africa.

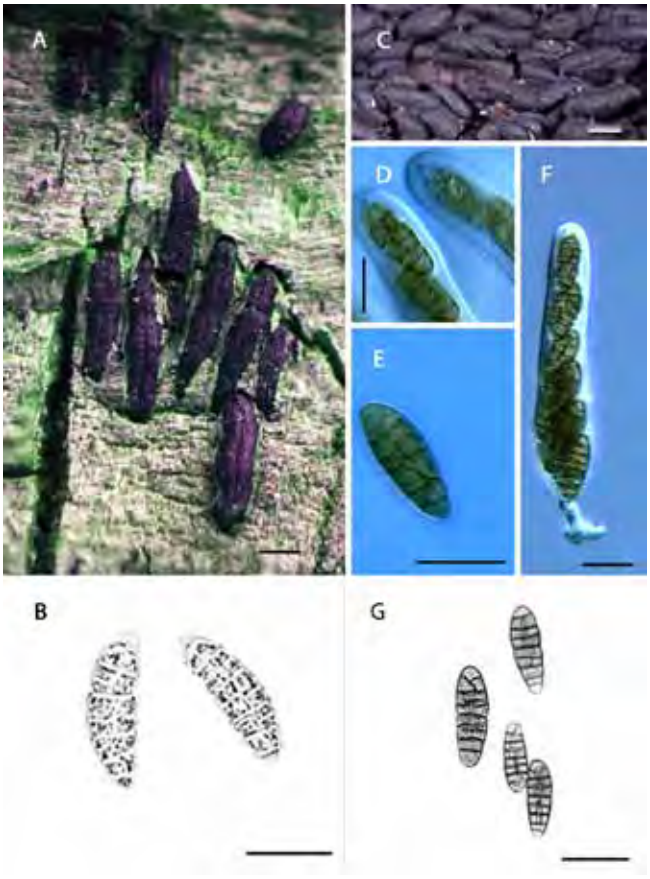


**Fig. 6.** The genus *Gloniopsis* (Clade D). A–B. *Gloniopsis praelonga* [CBS 123337 (BPI 878725), U.S.A.]; C–F. *Gloniopsis subrugosa* [GKM 1214 (BPI 879776), Kenya]; G. *Gloniopsis subrugosa* (CBS 123346, BPI 878735; South Africa). Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 20 µm.

***Gloniopsis subrugosa*** (Cooke & Ellis) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515338. Fig. 6C–G. *Basionym*: *Hysterium subrugosum* Cooke & Ellis, Grevillea 5: 54. 1876.

≡ *Hysterographium subrugosum* (Cooke & Ellis) Sacc., Syll. Fung. 2: 780. 1883.

= *Hysterographium hiascens* Rehm, Ber. Naturhist. Vereins. Augsburg 26: 780. 1881.

= *Hysterographium kansense* Ellis & Everh., Erythea 2: 22. 1894.

= *Hysterographium cylindrosporum* Rehm, Bih. Kongl. Svenska Vetensk.-Akad. Handl. 25(6): 11. 1899.

= *Hysterographium minutum* M.L. Lohman, Pap. Michigan Acad. Sci. 17: 267. 1933.

*Hysterothecia* erumpent to superficial, scattered to densely crowded, navicular, straight to flexuous, with tapered ends, surface not striated in age, but smooth to sub-rugose in texture, 1–2 mm long, 250–350 µm diam. *Peridium* composed of small pseudoparenchymatous cells, heavily pigmented at the surface, not showing a distinct number of layers, relatively smooth on outer surface. *Pseudoparaphyses* narrowly cellular, septate, 1–1.5 µm in diam., hyaline, branched above the asci, borne in a gelatinous matrix. *Asci* cylindrical to clavate, bitunicate, short-stipitate, 80–150 x 18–25 µm, with a prominent apical nasse, especially when young. *Ascospores* pigmented thin-walled, dictyospores (22–)25–34(–45) x (6–)8–12(–17) µm, mostly with 7–11 transverse and 1–2 vertical septa, hardly constricted at septa, clear brown, ends paler at times, slightly asymmetric in outline. *Anamorph* coelomycetous, *Aposphaeria*-like (Lohman 1933a). Less frequently collected, but reported from North America (Barr 1990b), Europe (Zogg 1962), Argentina (Messuti & Lorenzo 2003) and from South Africa (van der Linde 1992) as well. Old wood and bark of *Populus, Quercus, Celtis, Crataegus, Rosa,* and *Cotinus* (Zogg 1962), as well as on weathered fence posts and old planks (Boehm, unpubl. data).

*Notes*: In the current study, we were able to include three geographically diverse isolates of *Gp. praelonga* (Table 1), two from South Africa (CBS 112415 and CMW 19983 / PREM 57539), and one from the United States, New Jersey (CBS 123337 / BPI 878725). These isolates cluster together in Clade D and associate with one isolate of *Gp. subrugosa* from South Africa (CBS 123346 / BPI 878735). Both *Gp. praelonga* and *Gp. subrugosa* are somewhat similar in the shape, size and septation of their dictyospores, hyaline in the former (Fig. 6B), pigmented in the latter (Fig. 6G). The spores of *Gp. praelonga* are (16–)20–32(–34) x (6–)9–12(–15) µm, and those of *Gp. subrugosa* are (22–)25–34(–45) x (6–)8–12(–17) µm. Septation is also similar in both species, with 5–7(–10) transverse and 2–3 vertical septa in *Gp. praelonga* and 7–11 transverse and 1–2 vertical septa in *Gp. subrugosa*. They differ in pigmentation and the presence of a gelatinous sheath in the type. Molecular data indicate that they are closely related.

An additional two isolates of *Gp. subrugosa*, from Kenya (GKM 1214 / BPI 879776) and Cuba (SMH 557 / BPI 879777), are more distantly related and do not fall in Clade D. Moreover, no morphological differences were noted between these two more distantly associated isolates of *Gp. subrugosa* and CBS 123346 (BPI 878735) from South Africa in Clade D. Although spore morphology dictates that all three specimens of *Gp. subrugosa* should be classified as the same species, molecular data point to genetic heterogeneity within the taxon. This is similar to the situation in *Hb. mori*, mentioned earlier, which, despite identical morphologies, finds affinities in both Clades A and D. *Hysterobrevium mori* and, to a lesser extent, *Gp. subrugosa*, may represent ancestral lineages that have maintained stable morphologies, while simultaneously incurring sufficient genetic change to, in the case of *Hb. mori*, fall into different clades within the family. Alternatively, these isolates may represent examples of convergent evolution among genetically unrelated lineages, which produce remarkably similar ascospores and hysterothecia. Also associating with *Gp. praelonga* and *Gp. subrugosa* in Clade D are two new species from East Africa, described below.

***Gloniopsis arciformis*** E.W.A. Boehm, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch, **sp. nov.** MycoBank MB515331. Fig. 7A–H.

*Etymology*: Latin *arcus*, a bow or arch, referring to the arcuate or arciform dictyospores.

Hysterothecia solitaria vel pauca aggregata, recta vel flexuosa, carbonacea, plerumque erecta, conspicue applanata et altiora quam lata, (0.5–)1–2.5 mm longa, 250–350 µm lata, 400–600 µm alta, per longitudinem striata, sulco inconspicuo maturitate clauso. Peridium 40–75 µm crassum in medio, basim versus crassius, sursum tenuius, bistratosum. Pseudoparaphyses cellulares 1–1.5 µm latae, ramosae, sursum magis crassitunicatae, epithecium pigmentatum ascos obtegens formantes. Asci cylindrici vel clavati, stipite sinuoso, bitunicati, 50–75 × 14–18 µm; ascosporae irregulariter biseriatae, dictyosporae, pigmentatae, tenuitunicatae, fragiles, facile dilabentes, conspicue arcuatae, 3–5(–7)-septatae, 1–2(–3) septis verticalibus divisae; cellulis centralibus multo maioribus quam distales, ad septa haud constrictae, (10–)12–18(–22) x 6–10 µm.

*Hysterothecia* solitary to sparsely aggregated, straight to flexuous, carbonaceous, mainly erect, distinctly flattened and taller than wide, (0.5–)1–2.5 mm long, 250–350 µm wide, by 400–600

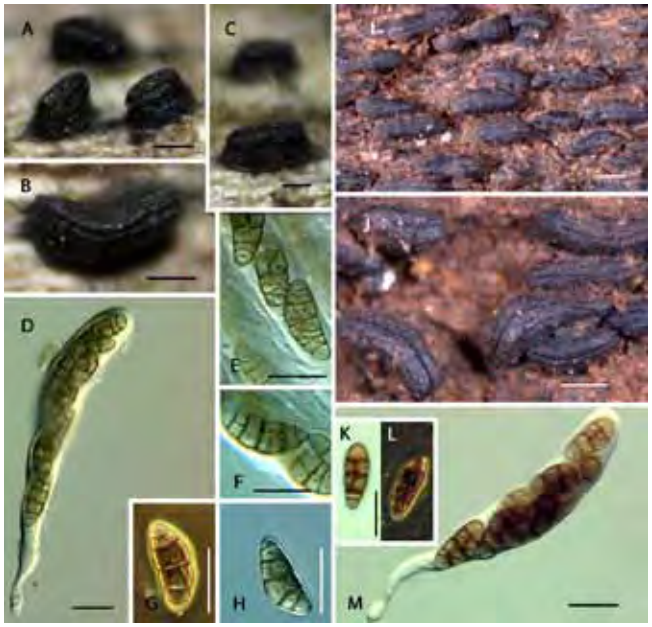


**Fig. 7.** The genus *Gloniopsis* (Clade D). A–H. *Gloniopsis arciformis sp. nov.* [GKM L166A (BPI 879774 = holotype), Kenya]; I–M. *Gloniopsis kenyensis sp. nov.* [GKM 1010 (BPI 879775 = holotype), Kenya]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 10 µm.

µm high, longitudinally striated, with an inconspicuous sulcus remaining closed at maturity. *Peridium* 40–75 µm thick medially, thicker towards the base, thinner towards the sulcus, composed of two layers, the inner thin, compressed and hyaline, the outer denser, and darkly pigmented. *Pseudoparaphyses* cellular 1–1.5 µm wide, branched and thicker-walled distally towards the top, forming a pigmented epithecium above the asci. *Asci* cylindrical to clavate, with a sinuous stalk, bitunicate, 50–75 x 14–18 µm (n = 7), ascospores irregularly biseriate. *Ascospores* pigmented, thin-walled, dictyospores, fragile, easily breaking under the slightest pressure, pronouncedly arcuate or bent (arciform), and thus highly asymmetric, 3–5(–7)-septate, with 1–2(–3) vertical septa, these mostly associated with the mid cells, which are much larger and swollen than the end-cells, no septal constrictions, measuring (10–) 12–18(–22) x 6–10 µm (n = 17).

*Specimen examined*: **Kenya**, Coast Province, Malindi District, Arabuko-Sokoke National Park, 6 Nov. 2006, G.K. Mugambi. Deposited as BPI 879774, **holotype** [formerly, GKM L166A (EA)].

*Notes*: *Gloniopsis arciformis* is represented by a single specimen (BPI 879774) of only ~30 fruitbodies in the protected crevice of a small piece of decorticated hardwood, collected in Arabuko-Sokoke National Park, Malindi District, Kenya. Although the material is sparse, it does permit the description of a new species on account of the highly unusual arcuate dictyospores. *Gloniopsis arciformis* resides in Clade D, and is phylogenetically closely associated with two other species of *Gloniopsis* (*Gp. praelonga* and *Gp. subrugosa*), as well as with an additional new species described below.

***Gloniopsis kenyensis*** E.W.A. Boehm, G.K. Mugambi, S.M. Huhndorf & C.L. Schoch, **sp. nov.** MycoBank MB515359. Fig. 7I–M.

*Etymology*: From the Latin *-ensis* to denote origin, from Kenya.

Hysterothecia navicularia, carbonacea, recta vel flexuosa, utrinque obtusa, dense aggregata, erumpentia, ad latera inconspicue striata vel levia, (0.5–)1–3 mm longa, 250–350 µm lata, 250–350 µm alta. Peridium prope basim ad 100 µm crassum, bi- vel tristratosum, stratum internum compressum, hyalinum, strata exteriora densiora et fusca. Pseudoparaphyses cellulares, septatae, 1–1.5 µm latae, sursum ramosae et anastomosantes, epithecium pigmentatum ascos obtegens formantes. Asci cylindrici vel clavati, bitunicati, 60–80 x 12–16 µm, ascosporas irregulariter biseriatas continentes. Ascosporae dictyoseptatae, pigmentatae, obovoideae, tenuitunicatae, fragiles, polis asymmetricis: apice obtuso, ad basim acuminatae vel nonnumquam protrudentes, 3(–4)-septatae, 1–2 septis verticalibus, utrinque saepe septis obliquis divisae, ad septa vix constrictae, iuvenes guttulis repletae, (12–)15–18(–19) x 5–7(–8) µm.

*Hysterothecia* navicular, carbonaceous, straight to flexuous, with obtuse ends, densely aggregated, erumpent, slightly striated laterally to smooth, (0.5–)1–3 mm long, 250–350 µm wide, by 250–350 µm high. *Peridium* up to 100 µm thick at base, composed of two to three layers, the inner thin, compressed and hyaline, the outer two progressively denser, and darkly pigmented. *Pseudoparaphyses* cellular, septate, 1–1.5 µm wide, branched, anastomosed distally, forming a pigmented epithecium above the asci. *Asci* cylindrical to clavate, bitunicate, 60–80 x 12–16 µm (n = 5), ascospores irregularly biseriate. *Ascospores* pigmented dictyospores, in outline obovoid, thin-walled, very fragile, spore apices asymmetric, the upper obtuse, the lower acuminate and sometimes drawn out, 3(–4[rarely])-septate, with 1–2 vertical septa, often with oblique septa in end cell, hardly constricted at the septa, highly gutulate when young, (12–)15–18(–19) x 5–7(–8) µm (n = 14). Known from only one collection, from Kenya, East Africa.

*Specimen examined*: **Kenya**, Coast Province, Malindi District, Arabuko-Sokoke National Park, 6 Apr. 2005, G.K. Mugambi. Deposited as BPI 879775, **holotype**; GKM 1010 (EA), **paratype**.

*Notes*: Molecular data indicate that both *Gp. kenyensis* and *Gp. arciformis* are closely associated, adjacent to *Gp. praelonga* and *Gp. subrugosa* in Clade D. The spores of all four taxa, however, are different, and thus their association would not have been predicted based on traditional morphology. The spores of *Gp. kenyensis* do bear a close resemblance, however, to those of *Hb. mori*. Both have predominantly 3-septate, thin-walled, pigmented dictyospores, with 1–2 vertical septa, often with oblique septa in the end cell. They can be differentiated on spore size: (12–)14–22(–26) x (5–)7–10(–11) µm for *Hb. mori, versus* (12–)15–18(–19) x 5–7(–8) µm for *Gp. kenyensis*. The spores of *Hb. mori* are usually longer and wider, and also show prominent septal constrictions, especially when fresh and hydrated. Additionally, *Gp. kenyensis* is highly guttulate when young, where this is rarely observed in *Hb. mori*. Molecular data indicate that they are not related.

To summarise, molecular data have necessitated the break up of the genus *Hysterographium*, because the type, *Hg. fraxini*, no longer resides within the *Hysteriaceae* (Boehm *et al.* 2009). This break up has resulted in: (1) the new genus *Hysterobrevium*, which includes both species with hyaline dictyospores, previously classified as *Gloniopsis* (*Hb. constrictum* and *Hb. smilacis*), and species with pigmented dictyospores, previously classified as *Hysterographium* (*Hb. mori*) in Clade A; (2) the inclusion in *Gloniopsis* of both hyaline (*Gp. praelonga*) and pigmented (*Gp. subrugosa*, *Gp. arciformis*, *Gp. kenyensis*) dictyospores in Clade

D; (3) the inclusion in *Oedohysterium* of pigmented dictyospored species previously classified in *Hysterographium* (*Od. pulchrum*), also in Clade D; and, lastly, (4) the removal of *Hysterographium*, with the type *Hg. fraxini*, from the *Hysteriaceae*, currently placed as *Pleosporomycetidae gen. incertae sedis*. As the taxonomy of *Hysterographium*, *Hysterobrevium* and *Gloniopsis* is currently in flux, we chose to provide the following dichotomous key, whereby all hysteriaceous fungi, bearing transversely and longitudinally septate dictyospores, whether pigmented or hyaline, are identified together, with the caveat that unrelated taxa share the same key.

## Key to the species of *Hysterographium, Hysterobrevium* and *Gloniopsis*

1. Dictyospores, usually shorter than 25 µm ..... 2
1. Dictyospores mostly longer than 25 µm ..... 6

2. Dictyospores pigmented, thin-walled, fragile, pronouncedly arcuate or bent, 3–5(–7)-septate, with 1–2(–3) vertical septa, which are mostly associated with the mid-cells, these much larger and swollen than the end-cells, no septal constrictions, (10–)12–18(–22) x 6–10 µm; Kenya ..... ***Gp. arciformis***
2. Not with the above combination of characters ..... 3

3. Dictyospores hyaline at maturity ..... 4
3. Dictyospores pigmented at maturity ..... 5

4. Dictyospores highly symmetric in outline and septation, with thickened walls, gelatinous sheath present when young, absent at maturity, (1–)3(–4)-septate, with 1(–2) vertical septa, that may pass through one to two cells; (11–)13–20(–23) x 5–12 µm; Japan, New Zealand ..... ***Hb. constrictum***
4. Dictyospores asymmetric, with acuminate ends, with a gelatinous sheath when young, mostly 3–5(–9)-septate and with 1(–3) vertical septa, passing through multiple mid-cells, prominently constricted at the median septum, sometimes constriced at multiple septa, (13–)15–26(–31) x (4–)5–9(–10) µm; cosmopolitan ..... ***Hb. smilacis***

5. Dictyospores thin-walled, obovoid, with obtuse ends, 3–(5–7)-septate, with 1–2(–3) vertical septa, usually associated with mid-cells, but occasionally present obliquely in end-cells, constricted at the median septum, sometimes at additional septa, (12–)14–22(–26) x (5–)7–10(–11) µm; cosmopolitan ..... ***Hb. mori***
5. Dictyospores thin-walled, very fragile, obovoid, 3[–4(rarely)]-septate, with 1–2 vertical septa, highly gutulate when young, spore apices asymmetric, the upper obtuse, the lower acuminate and sometimes drawn out, often with oblique septa in end cell(s), hardly constricted at the septa, measuring (12–)15–18(–19) x 5–7(–8) µm; Kenya ..... ***Gp. kenyensis***

6. Red pigment present in hamathecium and/or centrum; dictyospores pigmented ..... 7
6. No red pigment present, spores pigmented or hyaline ..... 8

7. Dictyospores, 22–25(–27) x 5–6 µm, with (5–)6 transverse and 1 vertical septum in either cell or both cells adjacent to the primary septum; typically with red pigment in the hamathecium; neotropical (Costa Rica) ..... ***Od. pulchrum***
*Note*: *Od. pulchrum* is accommodated in the genus *Oedohysterium* and is present in both keys.
7. Dictyospores 25–28 x 11–13 µm, with 5–6 transverse and mostly one longitudinal septum; hamathecium brick-red; on *Acacia* thorns, South Africa ..... ***Hg. spinicola***

8. Dictyospores hyaline or turning brown tardily ..... 9
8. Dictyospores pigmented in the ascus ..... 10

9. Dictyospores hyaline turning yellow in age, obovoid, ends usually obtuse, 5–7(–10)-septate, with 2–3 longitudinal septa, constricted at the median and often other septa, gelatinous sheath when young, (16–)20–32(–34) x (6–)9–12(–15) µm; cosmopolitan ..... ***Gp. praelonga***
9. Ascospores irregularly biseriate, ellipsoid, hyaline but becoming brown tardily, with the upper half generally wider than the lower half, sometimes surrounded by a gelatinous sheath, with 7–13 transverse and 1–3 longitudinal septa, constricted at the median transverse septum; 25–49 x 8–17 µm; Japan ..... ***Gp. macrospora***

10. Dictyospores usually less than 38 µm long ..... 11
10. Dictyospores 30–80 µm long ..... 12

11. Dictyospores (22–)25–34(–45) x (6–)8–12(–17) µm, mostly with 7–11 transverse and 1–2 vertical septa; cosmopolitan ..... ***Gp. subrugosa***
11. Dictyospores 26–38 x 10–15 µm, with 6–13 transverse and 1–3 vertical septa, obovoid, ends obtuse; Japan ..... ***Hg. minus***

12. Dictyospores (25–)30–45(–51) x (10–)12–15(–22) µm, with 7–9 transverse and 2–3 vertical septa, obovoid, ends obtuse; cosmopolitan ..... ***Hg. fraxini***
*Note*: *Hysterographium fraxini*, the type species for the genus *Hysterographium*, lies outside of the *Hysteriaceae*, as *Pleosporomycetidae incertae sedis* (Boehm *et al.* 2009).
12. Ascospore outline ellipsoid, fusoid, ends slightly acuminate, (30–)40–65(–80) x (8–)10–18(–19) µm, with 7–15 transverse and 1–3 vertical septa; cosmopolitan ..... ***Hg. flexuosum***

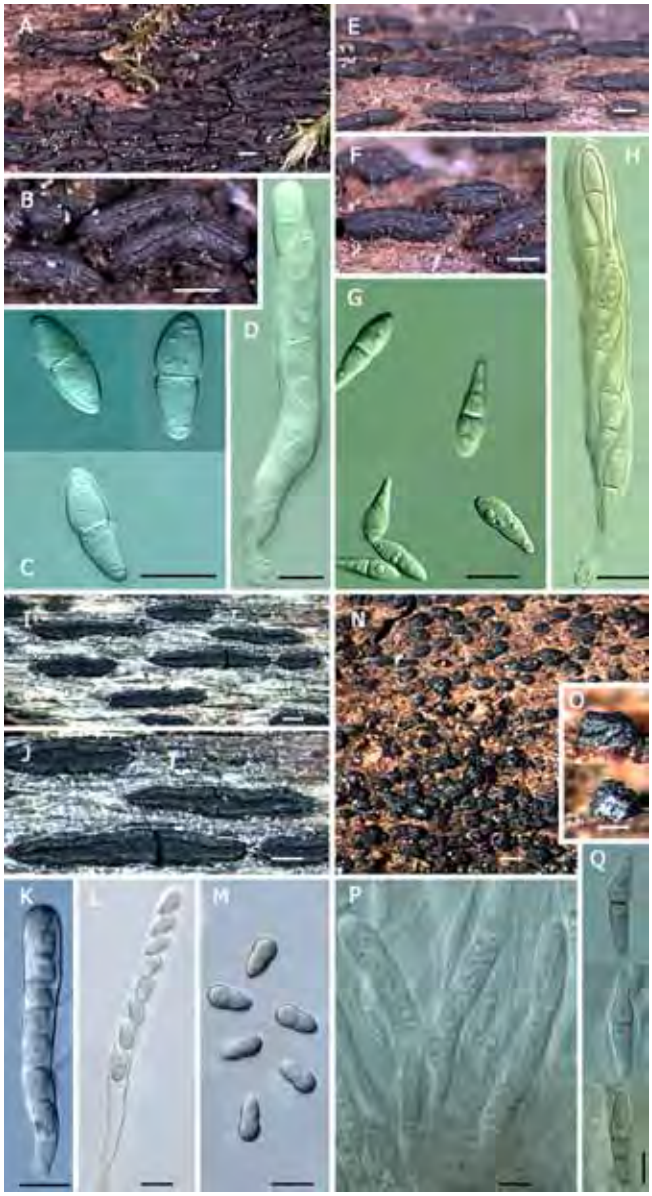


**Fig. 8.** The genus *Psiloglonium* (Clade B). A–D. *Psiloglonium simulans* [ANM 1557 (BPI 879803), U.S.A.]; E–H. *Psiloglonium clavisporum* [GKM 344A (BPI 879801), Kenya]; I–M. *Psiloglonium lineare* [ANM 117 (ILLS), U.S.A.; not incl.]; N–Q. *Psiloglonium araucanum* [ANM 42 (ILLS), U.S.A.; not incl.]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 10 µm.

## ***Psiloglonium*** Höhn.**,** Ann. Mycol. 16: 145. 1918.

A discussion of the genus *Psiloglonium* (von Höhnel 1918; Petrak 1923a, b) by necessity must begin with the genus *Glonium*. This is because Zogg (1962) synonymised a number of species under the genus *Glonium* that were originally classified in *Psiloglonium* by von Höhnel (1918) and Petrak (1923a, b). Both *Psiloglonium* and *Glonium* possess hyaline to yellow didymospores, somewhat constricted at the septum, with obtuse or acuminate ends, typically with cells unequal in size, borne in hysterothecia.

Von Höhnel (1918) was the first to view the genus *Glonium* as comprised of two distinct morphological types, and stressed the importance of subicula, using it to divide the genus, at first, into two subgenera, *Glonium* and *Psiloglonium*, and, further in the same article, into two separate genera, with or without subicula, respectively. Petrak (1923a) recognised that von Höhnel (1918) had established the genus *Psiloglonium*, both at sub-generic and generic rank, but it was Petrak (1923a) who explicitly designated the type species for *Psiloglonium* as *P. lineare* (Fig. 8I–M), retaining *G. stellatum* as the type species for the genus *Glonium sensu* von Höhnel (1918). Petrak (1923a, b) eventually placed a number of species in *Psiloglonium*, all subsequently transferred to *Glonium* by Zogg (1962). Müller & von Arx (1950) originally accepted the genus *Psiloglonium*, but later reduced it to a synonym of *Glonium* (von Arx & Müller 1975). Lohman (1933a, 1937) also did not support *Psiloglonium*, based on the observation that similar anamorphs were shared between species of the two subgenera. Barr (1987), was the only modern author to retain the genus *Psiloglonium*, as distinct from the subiculate *Glonium*.

Although von Höhnel (1918) and Petrak (1923a, b) both stressed the importance of subicula as a major morphological distinction between *Psiloglonium* and *Glonium*, Zogg (1962) noted that some species previously classified as *Psiloglonium* by Petrak (1923a) do in fact possess subicula on occasion (*e.g.*, *P. lineare*). Zogg (1962) further noted an additional two species that were occasionally associated with subicula, namely *G. pusillum* and *G. graphicum*, stating: *"…ohne Subiculum oder auf ziemlich deutlichem Subiculum sitzend..."* Hence, Zogg (1962) considered subicula not to be a synapomorphic character state, and transferred those species previously classified by Petrak (1923a, b) in *Psiloglonium* (*e.g.*, *P. lineare*, *P. microspermum*, *P. ruthenicum*, and *P. finkii*) to the genus *Glonium*.

Although Zogg (1962) did not support *Psiloglonium*, he did in fact recognise three distinct morphological forms within his concept of *Glonium*, two of which (Types I and II) we incorporate in *Psiloglonium*, the third (Type III) forming the basis for the *Gloniaceae* (Boehm *et al.* 2009). Zogg (1962) arranged the species of *Glonium* based on (1) didymospore shape: spore apices obovoid to rounded (Type I) *versus* spores fusiform with acuminate apices (Type II and III); and (2) the degree of complexity surrounding the architecture of the hysterothecia, simple, linear, solitary to gregarious (Types I, II) *versus* complex bifurcating, laterally anastomosing to form flabelliform pseudostellate composites, sometimes associated with a thin stromal crust (Type III). Thus, the genus *Glonium sensu* Zogg (1962) was comprised of two groups of species, one with obovoid to rounded spores apices borne in regular hysterothecia (Type I) *versus* those with acuminate spore apices borne in complex bifurcating or modified hysterothecia (Type III). Species belonging to Type II possess fruitbodies of Type I, but spores of Type III; the assumption was that they constituted an intermediate, perhaps transitional, morphological group. This, then, de-emphasised the presence or absence of subicula *per se*, as stressed by von Höhnel (1918) and Petrak (1923a, b). Nevertheless, Zogg (1962) maintained all three types within the genus *Glonium*. Molecular data

presented here (see below), indicate that Types I & II are closely related, with Type III forming a distant clade in the *Gloniaceae* (Boehm *et al.* 2009).

**Type I:** This type is characterised by hysterothecia that may be solitary to gregarious, erumpent to entirely superficial, navicular to linear to highly flexuous, even triradiate, sometimes arranged in parallel orientation and confluent linearly to some degree, but never dichotomously branched, or associated with a stromal crust, as found in the *Gloniaceae* (Type III). These species correspond to *Psiloglonium sensu* von Höhnel (1918). Here, the didymospores are relatively small, hyaline, and have at least one, if not both ends, obovoid to obtuse (Type I), rather than acuminate (Types II and III). Zogg (1962) recognised five species, listed here by increasing ascospore length: *Glonium abbreviatum*, *G. pusillum*, *G. lineare*, *G. chambianum*, and *G. curtisii.* Barr (1975) transferred the last species to *Ostreichnion*, as *O. curtisii* in the *Mytilinidiaceae*, since transferred to the *Hysteriaceae* (Boehm *et al.* 2009). A sixth species, *G. finkii*, was included by Zogg (1962), based on ascospore shape, but placed apart in the key due to the unusual arrangement of the ascospores within the upper part of the ascus (Lohman 1937).

*Psiloglonium lineare* was previously reinstated within the *Hysteriaceae*, listing *G. lineare* as a synonym (Boehm *et al.* 2009). Here we also reinstate *Psiloglonium finkii*. An additional two species are included in Type I, namely *G. simulans* and *G. clavisporum*, synonymised by Zogg (1962) under *G. lineare*, but earlier recognised by Lohman (1932a, 1937) to be distinct from *G. lineare*. Boehm *et al.* (2009) proposed new combinations for these taxa, based on morphological as well as molecular data, as *P. simulans* (Fig. 8A–D) and *P. clavisporum* (Fig. 8E–H). To these species can also be added *G. sasicola* from Japan, the first report of a gelatinous sheath in the genus (Amano 1983). In this same publication Amano (1983) proposed an additional new species, *G. macrosporum*, also from Japan. The spore measurements were given as 13.1–16.8 x 4–5.6 µm, nearly identical to those of *P. simulans* at (10–)14–16(–18) x (4.5–)5–6 µm (Lohman 1937). Moreover, the illustrations given by Amano (1983) match closely those given by Lohman (1932a) for *P. simulans*. We therefore synonymise *G. macrosporum* under *P. simulans*.

More recently, Lorenzo & Messuti (1998), in a reappraisal of the type specimens collected by Spegazzini and Hennings from Argentina and Chile, have reinstated *Glonium costesii*. In a later publication, Messuti & Lorenzo (2007) synonymised *G. costesii* under the earlier epithet *G. ephedrae*. With spore measurements of 26–35 x 8–15 µm, *G. ephedrae* possesses the largest spores in Type I. In the same publication, Messuti & Lorenzo (2007) also accepted two additional species, *G. chilense* and *G. uspallatense*, previously considered by Zogg (1962) to be doubtful species. The spores of *G. chilense* measure 15–16 x (5–)7–8 µm, which places it very close to *P. lineare*, the latter with slightly smaller spores, (10–)12–14(–18) x (4–)5–7(–8) µm (Zogg 1962). However, *G. chilense* has almost identical ascomatal and spore measurements as *P. simulans*, given above. We therefore synonymise *G. chilense* with the earlier name *G. simulans*, as *P. simulans*. For *G. uspallatense*, Messuti & Lorenzo (2007) gave spore measurements of 18–24 x 10–12 µm, intermediate between *G. chambianum*, (14–)16–18(–21) x (6–)8–9(–10) µm (Zogg 1962), and *G. sasicola*, 25–32 x 5–8 µm (Amano 1983).

Recently, Mugambi & Huhndorf (2009) proposed a new genus, *Anteaglonium*, outside of the *Hysteriales* but within the *Pleosporales*, to accommodate *A. abbreviatum* (Fig. 9A–E), *A. globosum* (Fig. 9F–I), *A. parvulum* (Fig. 9J–M), and *A. latirostrum* (Fig. 9N–R). The


**Fig. 9.** The genus *Anteaglonium* (*Pleosporales*). A–E. *Anteaglonium abbreviatum* [ANM 37 (ILLS), U.S.A.; not incl.]; F–I. *Anteaglonium globosum* [ANM 925.2 (ILLS), U.S.A.]; J–M. *Anteaglonium parvulum* [GKM 219N (EA), Kenya; not incl.]; N–R. *Anteaglonium latirostrum* [GKM L100N.2 (EA), Kenya]. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 5 µm.

first three species are characterised by hyaline didymospores that belong to Type I, as defined by Zogg (1962), and are less than 8 µm in length. The fourth species, *A. latirostrum*, belongs to Type II (see below), with longer spores. Although phylogenetically unrelated to *Psiloglonium*, these species share a similar morphology and thus are included in the key below.

**Type II:** This type is characterised by relatively large didymospores, distinctly fusoid in outline, prominently constricted at the septum, and with acuminate apices. Zogg (1962) recognised two species, namely *Glonium caucasicum* and the much larger-spored, neotropical *G. hysterinum*, to which can be added the newly described *G. colihuae*, on *Chusquea culeou* from Argentina (Lorenzo & Messuti 1998). *Glonium caucasicum* has recently been synonymised under the earlier name *G. araucanum* by Messuti & Lorenzo (2007), based on a comparison of the type specimen of *G. caucasicum* to Spegazini's earlier type of *G. araucanum* from Chile.

**Type III:** This type corresponds to von Höhnel's (1918) and Petrak's (1923a, b) circumscription of the genus *Glonium*, and includes species with fusiform spores, with acuminate apices, typically producing complex laterally anastomosing hysterothecia, forming stellate composites, usually with prominent subicula, with or without stroma. Zogg (1962) included the type, *G. stellatum* (Fig. 12A–E), *G. compactum*, and *G. graphicum*, the later sometimes variably associated with subicula. Zogg (1962) also stated that *G. compactum* possesses a subiculum, much like *G. stellatum*, and with similar spore size, but whereas hysterothecia in *G. stellatum* are merely seated on the subiculum, in *G. compactum* the hysterothecia are embedded in and arise from a thin stromal crust, which is itself seated on subicula. Recently, a fourth species was added, based on molecular evidence (Boehm *et al.* 2009), namely *G. circumserpens* (Fig. 12F–H), from Tasmania (Kantvilas & Coppins 1997).

Sequence data presented here (Fig. 1) and elsewhere (Boehm *et al.* 2009, Mugambi & Huhndorf 2009), clearly indicate that the genus *Glonium sensu* Zogg (1962) actually comprises three entirely unrelated lineages within the *Pleosporomycetidae*, one within the *Hysteriaceae* and two forming clades outside of the family. The first lineage corresponds to *Psiloglonium sensu* von Höhnel (1918), and forms a highly supported monophyletic clade in this study (Clade B in Fig. 1). This clade includes: *Psiloglonium clavisporum*, with four single-ascospore isolates from New Jersey, the United States (CBS 123338 / BPI 878726, CBS 123339 / BPI 878727, CBS 123340 / BPI 878728 and CBS 123341 / BPI 878729), and two from Kenya (GKM 344A / BPI 879801, GKM L172A in EA), *P. simulans*, with two isolates from the United States, one from Michigan (CBS 206.34), deposited in 1934 by Lohman, and a more recent collection from Tennessee (ANM 1557 / BPI 879803), and, lastly, *P. araucanum*, with three isolates from South Africa, two from Kirstenbosch (CBS 112412 / PREM 57570, CMW 18760/ PREM 57569) and one from Jonkershoek (CMW 17941 / PREM 575566). *Psiloglonium clavisporum* and *P. simulans* belong to Type I, whereas *P. araucanum* belongs to Type II. Both are phylogenetically related and reside in Clade B (Fig. 1). Recently, a second lineage has been shown to be associated with the *Pleosporales*, now accommodated in the new genus *Anteaglonium* (Mugambi & Huhndorf 2009), for which we include six accessions representing four species (Table 1). The third lineage corresponds to *Glonium* (Type III), in the *Gloniaceae* (Boehm *et al.* 2009), for which we have included four isolates, representing two species (Table 1). We treat here all species of *Glonium sensu* Zogg (1962), belonging to Types I and II, outside of *Anteaglonium*, as belonging to *Psiloglonium*. Since the generic name *Glonium* is reserved for species in the *Gloniaceae* (Boehm *et al.* 2009), we propose eight new combinations for the genus *Psiloglonium*.

***Psiloglonium pusillum*** (H. Zogg) E.W.A. Boehm & C.L. Schoch, ***comb. nov.*** MycoBank MB515327.
*Basionym*: *Glonium pusillum* H. Zogg, Beitr. Kryptogamenfl. Schweiz. 11(3): 62. 1962.

*Notes*: Zogg (1962) described this species as *G. pusillum* from *Juniperus phoenicea* and *Pinus sylvestris* from Southern France, noting that it was quite rare. Zogg (1962) stated that this species may or may not be associated with a subiculum, and hence was one of the factors behind his transfer of Petrak's (1923a, b) *Psiloglonium* species to *Glonium*. *Psiloglonium pusillum* has ascospores only slightly larger than those of *P. abbreviatum*, measuring (9–)10–12(–13) x 4–5(–6) µm. Lee & Crous (2003) also identified this fungus from *Proteaceae* and *Restionaceae* in South Africa, and Sivanesan & Hsieh (1989) reported it from Taiwan. It has also been found in North America (Boehm, unpubl. data).

***Psiloglonium chambianum*** (Guyot) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515320.
*Basionym*: *Glonium chambianum* Guyot, Ann. Serv. Bot. Tunisie 28: 90. 1955.

*Notes*: Originally from North Africa, on *Lonicera implexa* (*Caprifoliaceae*), the fungus has since been reported from the *Proteaceae* in South Africa (Lee & Crous 2003) and Europe. Zogg (1962) gave the spore measurements for *G. chambianum* as (14–)16–18(–21) x (6–)8–9(–10) µm, whereas Lee & Crous (2003) gave slightly larger measurements, (18–)20–21(–23) x (4–)5–6(–7) µm. Spores ellipsoid to oblong, with upper cell broader than the lower, and with an obovoid, obtuse apex. *Psiloglonium chambianum* possesses larger spores than *P. lineare*, *P. simulans*, and *P. clavisporum*, but smaller than *P. uspallatense*.

***Psiloglonium uspallatense*** (Speg.) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515321.
*Basionym*: *Glonium uspallatense* Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires. 19: 436. 1909.

*Notes*: Zogg (1962) listed the species a "doubtful", but Messuti & Lorenzo (2007) reinstated *G. uspallatense* after locating the original holotype material. They gave the spore measurements as 18–24 x 10–12 µm, placing it intermediate between *P. chambianum* and *P. sasicola*.

***Psiloglonium sasicola*** (N. Amano) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515322.
*Basionym*: *Glonium sasicola* N. Amano, Trans. Mycol. Soc. Japan 24: 287. 1983.

*Notes*: Amano (1983) described this species from dead culms of *Sasa* sp. (*Bambusaceae*) in Japan. The ascospore measurements were given as 25–32 x 5–8 µm, with a rounded apical cell, placing it between *P. uspallatense* and *P. ephedrae*. Amano (1983) further reported that ascospores of this species are associated with a gelatinous sheath, previously not known among these didymospored fungi.

***Psiloglonium ephedrae*** (Henn.) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515323.
*Basionym*: *Glonium ephedrae* Henn., Öfvers. K. Vet. Akad. Förhandl. 2: 328. 1900.
= *Glonium costesi* Speg., Bol., Acad. Ci., Córdoba 25: 78. 1921.

*Notes*: Messuti & Lorenzo (2007) reinstated *G. ephedrae* with the synonym *G. costesi*, after locating and comparing original type materials. *Psiloglonium ephedrae* possesses very large didymospores, measuring 26–35 x 8–15 µm, the upper cells broadly ovate. It has been collected from *Ephedra andicola*, and, as *G. costesi*, from *Proustia pyrifolia* in Chile.

***Psiloglonium hysterinum*** (Rehm) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515324.
*Basionym*: *Glonium hysterinum* Rehm, Hedwigia 37: 298. 1898.

*Notes*: Rehm (1898) originally described a species of *Glonium* from Southern Brazil with large fusiform didymospores, prominently

constricted at the septum, and with acuminate spore apices ("*Enden zugespitzt*"). The spore measurements were given as 45 x 9 µm.

***Psiloglonium colihuae*** (Lorenzo & Messuti) E.W.A. Boehm & C.L. Schoch, **comb. nov.** MycoBank MB515325.
*Basionym*: *Glonium colihuae* Lorenzo & Messuti, Mycol. Res. 102: 1104. 1998.

*Notes*: Lorenzo & Messuti (1998) described a new species on culms of *Chusquea culeou* from the Argentine *Nothofagus* rainforests. The spore measurements were given as 30–43 x 4–9.8 µm, and, although the spores are fusiform in outline, they possess moderately acuminate apices. In comparing this species to other acuminate-spored species of *Glonium*, the authors noted that the greatest degree of similarity was with the slightly smaller-spored *G. caucasicum*.

***Psiloglonium araucanum*** (Speg.) E.W.A. Boehm, S. Marincowitz & C.L. Schoch, **comb. nov.** MycoBank MB515326. Fig. 8N–Q.
*Basionym*: *Glonium araucanum* Speg., Revista Fac. Agron. Univ. Nac. La Plata 6: 110. 1910.
= *Gloniella caucasica* Rehm, Vestn. Tiflissk. Bot. Sada 25:12. 1912.
≡ *Glonium caucasicum* (Rehm) H. Zogg, Beitr. Kryptogamenfl. Schweiz. 11(3): 67. 1962.

*Notes*: Messuti & Lorenzo (2007) transferred *Glonium caucasicum* to *G. araucanum*, after examining the types for both species. Previously, Zogg (1962) had transferred *Gloniella caucasica* to *Glonium*. Here we transfer *G. araucanum* to *Psiloglonium*. This taxon possesses fusiform spores with highly acuminate apices. Messuti & Lorenzo gave the spore measurements as 22–28 x 8–10 µm, whereas Zogg (1962) gives them as (19–)22–25(–27) x (6–)7–9(–10) µm. Although originally European in distribution (Zogg 1962), the taxon has subsequently been collected from South (Messuti & Lorenzo 2007) and North America (Boehm unpubl. data), and from South Africa (Lee & Crous 2003).

Lee & Crous (2003) identified a series of isolates from South Africa on the *Restionaceae* as *Glonium compactum* (CBS 112412, CMW 18760, CMW 17941). However, in their study they did not note the presence of subicula, nor a stromal crust. These features were stressed for this taxon by Zogg (1962). These same isolates were used in Boehm *et al.* (2009), and were shown to associate, with high branch support, with two species of *Psiloglonium*, *P. clavisporum* and *P. simulans*, distant from the other species of *Glonium* surveyed (*e.g.*, *G. stellatum* and *G. circumserpens*). Thus, a new combination was proposed, *Psiloglonium compactum*. However, it is now realised that this new combination was made in error and is hereby retracted. It must be concluded that the South African isolates (Lee & Crous 2003) were not *G. compactum*, due to the absence of subicula and stroma, but rather, we suspect, the cosmopolitan *P. araucanum*, which has similar, but slightly smaller, fusiform acuminate didymospores. Lee & Crous (2003) give the ascospore measurements for the South African "*G. compactum"* as (24–)26–27(–30) x (4–)5–6(–7) µm, which matches closely those given above for *P. araucanum*. Furthermore, the illustrations in Lee & Crous (2003) closely match *P. araucanum*, and not those of *G. compactum*, as given by Zogg (1962). If we are correct in assuming that the South African isolates used in Boehm *et al.* (2009) are in fact *P. araucanum* (Type II) and not *G. compactum* (Type III), then this would provide a high degree of support for the inclusion of species with acuminate spore apices, belonging to Type II, in the genus *Psiloglonium*, along with species with obtuse spore apices, belonging to Type I (*e.g.*, *P. simulans* and *P. clavisporum*). A reanalysis of the original South African herbarium specimens from which the sequences were derived (PREM 57570, PREM 57569, PREM 57566), by S. Marincowitz, has confirmed that they do indeed correspond to *P. araucanum* and not to *G. compactum*. Molecular data thus supports the association of Types I and II within the genus *Psiloglonium*.

In addition to the 12 currently recognised species in *Psiloglonium*, the following key also includes entries for the unrelated *Gloniaceae*, *Anteaglonium* and *Ostreichnion curtisii*.

## Key to the species of *Psiloglonium* and *Anteaglonium*

1. Asci ovoid, +/- cylindrical; ascospores borne in the upper portion of the ascus, not evenly distributed; ascospores (12–)13–15 x 6–7 µm; Puerto Rico ..... ***P. finkii***
1. Asci typically cylindrical to club-shaped; ascospores in one row or distichous in the asci, but always regularly arranged for its full length ..... 2

2. Ascospores obovoid, with at least one, often both, ends obtuse, typically with upper cell larger, +/- constricted at the septum (Type I) ..... 3
2. Ascospores fusiform (*i.e.*, spindle-shaped), with both ends acuminate, usually constricted at the septum (Types II and III) ..... 14

3. Ascospores small, 8 µm or less in length (*Anteaglonium*, in part) ..... 4
3. Ascospores longer than 8 µm (*Psiloglonium* Type I) ..... 6

4. Ascospores 6–8 x 2.5–3 µm; hysterothecia with apices acuminate, but not associated with a darkened crust; no KOH-soluble pigments; New Zealand, East Africa, North America ..... ***A. parvulum***
*Note*: *A. parvulum* lies within the *Pleosporales* (Mugambi & Huhndorf 2009).
4. Not with the above combination of characters ..... 5

5. Ascospores (5–)6–7(–8) x 2–3(–3.5) µm (as in *A. parvulum*); but hysterothecia with apices truncated, and associated with a darkened crust (tending to darken the substratum); minute amounts of soluble pigment in KOH (easily missed); Europe, East Africa, North America ..... ***A. abbreviatum***
*Note*: *A. abbreviatum* lies within the *Pleosporales* (Mugambi & Huhndorf 2009)

5. Ascospores 6–7 x 2–3 µm (as in *A. parvulum* and *A. abbreviatum*); but hysterothecia globose with roughened walls, an indistinct slit, and associated with sparse, short subicula, and also with short tomentum on the walls of the ascomata; like *A. abbreviatum* also associated with a darkened crust on substrate; producing a strong green soluble pigment in KOH; eastern and mid-western North America ..... ***A. globosum***
*Note*: *A. globosum* lies within the *Pleosporales* (Mugambi & Huhndorf 2009).

6. Ascospores (9–)10–12(–13) x 4–5(–6) µm; cosmopolitan ..... ***P. pusillum***
6. Ascospores slightly larger ..... 7

7. Ascospores (10–)12–14(–18) x (4–)5–7(–8) µm; ascomata +/- confluent laterally, in parallel rows, semi-immersed to erumpent; cosmopolitan ..... ***P. lineare***
7. Ascospores similar in length; ascomata not confluent laterally, usually entirely superficial ..... 8

8. Ascospores (10–)14–16(–18) x (4.5–)5–6 µm; cosmopolitan ..... ***P. simulans***
8. Ascospores slightly larger ..... 9

9. Ascospores (15–)16–18(–20) x 5–6(–7) um; *Sporidesmium stygium* anamorph usually present; North and South America, Africa ..... ***P. clavisporum***
9. Ascospores slightly larger in length and breadth ..... 10

10. Ascospores (14–)16–18(–21) x (6–)8–9(–10) µm; Europe, North Africa ..... ***P. chambianum***
10. Ascospores slightly larger ..... 10

11. Ascospores 18–24 x 10–12 µm; Argentina ..... ***P. uspallatense***
11. Ascospores slightly larger ..... 12

12. Ascospores 25–32 x 5–8 µm, with a gelatinous sheath; Japan ..... ***P. sasicola***
12. Ascospores slightly larger ..... 13

13. Ascospores 26–35 x 8–15 µm; Chile ..... ***P. ephedrae***
13. Ascospores (59–)62–68(–76) x 13–15 µm; North and South America ..... ***O. curtisii***
*Note*: The genus *Ostreichnion*, previously placed in the *Mytilinidiaceae*, has been transferred to the *Hysteriaceae* (Boehm *et al*. 2009).

14. Hysterothecia usually borne in/on subicula, typically bifurcated, forming radiating flabelliform or pseudo-stellate composites, with or without a stroma (Type III) ..... ***Gloniaceae***
*Note*: In this study, a key to the species of the *Gloniaceae* is provided under that family.
14. Hysterothecia not bifurcated, forming radiating flabelliform or pseudo-stellate composites, nor with a stroma ..... 15

15. Ascospores less than 30 µm long ..... 16
15. Ascospores more than 30 µm long ..... 17

16. Ascospores (19–)22–25(–27) x (6–)7–9(–10) µm, both ends acuminate, with a prominent septal constriction; cosmopolitan (Type II) ..... ***P. araucanum***
16. Ascospores 22–28 x 4–6 µm, acuminate, 1-septate, hyaline and with a mucilaginous sheath when young, but acquiring additional septa and pigmentation with age, to become 3–5-septate and pale brown at maturity; Kenya ..... ***A. latirostrum***
*Note*: *A. latirostrum* lies within the *Pleosporales* (Mugambi & Huhndorf 2009).

17. Ascospores 30–43 x 4–9.8 µm; Argentina (Type II) ..... ***P. colihuae***
17. Ascospores about 45 x 9 µm; Brazil (Type II) ..... ***P. hysterinum***

***Actidiographium*** Lar.N. Vassiljeva**,** Mikol. Fitopatol. 34 (6): 4. 2000.

Vasilyeva (2000) established the monotypic genus *Actidiographium* to accommodate a hysteriaceous fungus with pigmented one-septate ascospores, reminiscent of those found in *Actidium* in the *Mytilinidiaceae*. However, in *Actidiographium orientale*, the two-celled spores are borne in a typical thick-walled hysterothecium. The pigmented didymospores measure 13.2–16.5 x 3–4 µm. Molecular data are lacking for this taxon.

***Hysterocarina*** H. Zogg, Ber. Schweiz. Bot. Ges. 59: 39. 1949.

Zogg (1949) erected this monotypic genus for *Hysterocarina paulistae*, with pigmented dictyospores as in *Hysterographium*, but the hysterothecia are borne within the substrate, barely erumpent at maturity, and with a cristate, slightly evaginated longitudinal keel, instead of the invaginated sulcus typical of most members of the *Hysteriaceae*. Described from old wood of *Eucalyptus* sp. in Brazil, the pigmented dictyospores measure 20–25 x 8–10 µm.

The presence of an evaginated keel-like fissure in *Hysterocarina* is intriguing, as it seems to belong to an evolutionary trend that culminates in the *Mytilinidiaceae* and *Gloniaceae*. Clearly, molecular data are needed to resolve these issues.

***Ostreichnion*** Duby, Mém. Soc. Phys. Genève 16: 22. 1862.
= *Ostreion* Sacc., Syll. Fung. 2: 765. 1883.

Since its reappraisal (Barr 1975), the genus *Ostreichnion* has been heterogeneous, due to the inclusion of *O. curtisii* an unusual taxon, from the southeastern United States (Lohman 1937) and Brazil (Zogg 1962). It is very different from the other two species of this genus, namely the type *O. sassafras* and *O. nova-caesariense*. Whereas the latter two species possess pigmented dictyospores, in *O. curtisii* the ascospores are 1-septate below the middle, with walls greatly thickened towards the spore apices. When mounted under different stains, the spore cytoplasm appears subdivided into numerous compartments, giving the impression of a potentially muriform structure. Lohman (1937) provided details as to the highly unusual spore germination process in this fungus, which involves a distended apical plug and numerous median germ tubes, differing from that found in species of *Psiloglonium* and *Glonium*, which send out apical germ tubes (Lohman 1931, 1932a). *Ostreichnion sassafras* occurs on both sides of the Atlantic, as well as in China, and has been recovered from *Sassafras*, *Quercus*, *Liriodendron*, and *Liquidambar* (Bisby 1932, Teng 1933, Barr 1975). It is unusual in having very large dictyospores, measuring (65–)76–100(–135) x 20–32 µm, with up to 27 septa, borne four to an ascus. *Ostreichnion nova-caesariense* is known only from the type locality in New Jersey on *Pinus*, and has similar, but smaller, ascospores (Barr 1975).

Based on a recent four-gene analysis (Boehm *et al.* 2009), the genus *Ostreichnion*, previously in the *Mytilinidiaceae* (Barr 1975, 1990a), was transferred to the *Hysteriaceae*. This was based on sequence data derived from two of the three species (Table 1), namely *O. curtisii* (CBS 198.34) and *O. sassafras* (CBS 322.34), deposited by Lohman in 1934. Although both species find residency within Clade C (Fig. 1), their association with the genus *Hysterium* could not have been predicted. Given the unique nature of the ascospore in *O. curtisii*, considered potentially muriform, one would assume affinities with the genus *Hysterographium sensu* Zogg (1962), or, given its 1-septate ascospores at maturity, with *Psiloglonium*, where it was originally treated by Lohman (1937) as *Glonium curtisii*. However, molecular data suggest neither. Instead, *O. curtisii* shares a subclade with *Hysterium barrianum*, with 9-septate phragmospores (Fig. 1). *Ostreichnion sassafras* is more distant within Clade C. Although we recognise the genus as artificial, we present the following key, adapted from Barr (1975), to facilitate species identification.

## Key to the species of *Ostreichnion*

1. Ascospores mostly 1-septate, ends greatly thickened, (45–)62–80 x (10–)12–15 µm; North & South America ..... ***O. curtisii***
1. Ascospores with both transverse and longitudinal septa ..... 2

2. Ascospores measuring 35–45(–50) x 11–13 µm, with 7–13 septa, borne eight to an ascus; North America ..... ***O. nova-caesariense***
2. Ascospores measuring (65–)76–100(–135) x 20–32 µm, with up to 27 septa, borne four to an ascus; cosmopolitan ..... ***O. sassafras***

***Rhytidhysteron*** Speg., Anales Soc. Ci. Argent. 12: 188. 1881.

The genus *Rhytidhysteron* is characterised by ascomata that are at first closed and navicular (*e.g.*, Fig. 10K), somewhat resembling those found in the *Hysteriaceae*, but then later opening by a longitudinal sulcus to become irregularly apothecioid at maturity, often with incurved margins (*e.g.*, Fig. 10M) – a feature never observed in the *Hysteriaceae*. The peridium in *Rhytidhysteron* is somewhat gelatinous when wet, as compared to the hard, carbonaceous peridium found in the *Hysteriaceae*. Although ascomata may possess striations, in *Rhytidhysteron* these are perpendicular to the long axis (Fig. 10K), rather than parallel, as in the *Hysteriaceae* (*e.g.*, Figs 1A, 2B, and 6A). The ascospores in *Rhytidhysteron* tend to be heavily pigmented and thick-walled, as opposed to lightly pigmented and thin-walled in the *Hysteriaceae.* These features, among others, have been used to place *Rhytidhysteron* within the *Patellariaceae* (*e.g.*, Kutorga & Hawksworth 1997). Samuels & Müller (1979) revised the genus, providing a number of synonyms, and accepted only two species, namely the type, *R. rufulum* (Fig. 10E–K), with 3-septate phragmospores, and *R. hysterinum* (Fig. 10M), with 1-septate spores, both darkly pigmented and thick-walled. Anamorphs have been characterised as *Diplodia*- and *Aposphaeria*-like (Samuels & Müller 1979). Subsequently, another two species have been accepted in the genus, namely *R. dissimile* (Magnes 1997), with 5-septate phragmospores, and *R. opuntiae* (1990b), from the American South West, with short pigmented dictyospores (Fig. 10A–D), reminiscent of those found in *Hb. mori*.

Dictyospores of both *R. opuntiae* and *Hb. mori* are similar in shape, obovoid, with obtuse ends, and are also similar in size and septation. In both, the longitudinal septum is usually associated with the mid-cells, but on occasion it can be found obliquely in the end cells. However, unlike *Hb. mori*, the spores of *R. opuntiae* are thick-walled, verruculose and darkly pigmented. The most surprising morphological feature of *R. opuntiae* is that the spores are not borne within patellarioid ascomata, as in other members of the genus. Rather, the ascomata are hysterithecioid, that is, carbonaceous, navicular, with an invaginated longitudinal sulcus (Fig. 10A–B). In hindsight, it is remarkable that Barr (1990) recognised *R. opuntiae* as a member of *Rhytidhysteron*, transferring it from *Hysterographium opuntiae,* despite the presence of hysterithecioid ascomata. In this study we were fortunate to acquire an isolate of *R. opuntiae* from Kenya (GKM 1190 / BPI 879805). *Rhytidhysteron opuntiae* falls distant from *R. rufulum* and *R. hysterinum,* lying outside of Clade E altogether (Fig. 1). Although both morphological and molecular data suggest that *R. opuntiae* should be removed from the genus *Rhytidhysteron*, this is based only on a single specimen, and clearly needs to be substantiated with other isolates.

The six isolates of *R. rufulum* included one from Kenya (GKM 361A / BPI 879806; Fig. 10E–J), four from Ghana (EB 0381 / BPI 879807, Fig. 10L; EB 0382 / BPI 879808, Fig. 10K; EB 0383 / 879809; EB 0384 / BPI 879810), and one from Europe (CBS 306.38). Also included was one isolate of *R. hysterinum* from

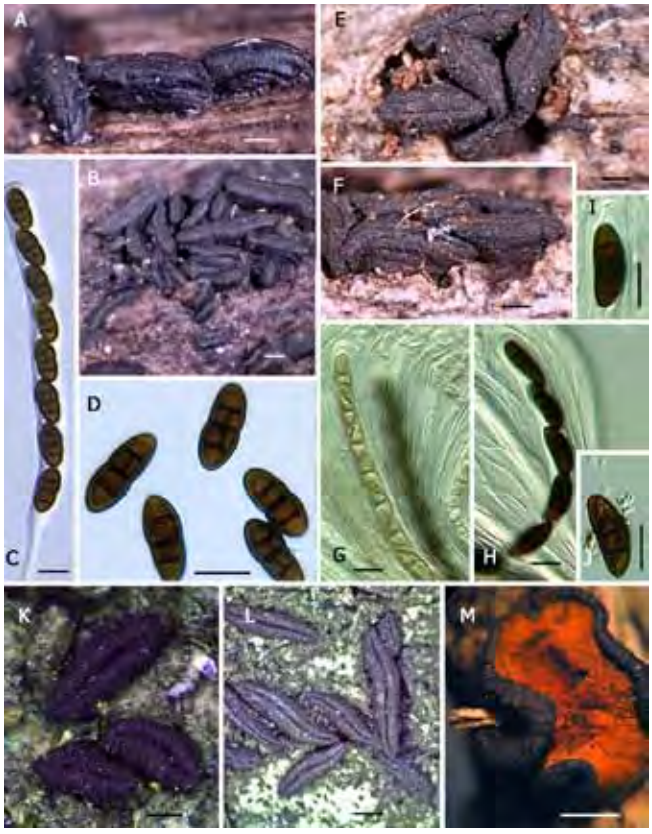


**Fig. 10.** The genus *Rhytidhysteron* (Clade E). A–D. *Rhytidhysteron opuntiae* [GKM 1190 (BPI 879805), Kenya]; E–J. *Rhytidhysteron rufulum* [GKM 361A (BPI 879806), Kenya]; K. *Rhytidhysteron rufulum* [EB 0382 (BPI 879808), Ghana]; L. *Rhytidhysteron rufulum* [EB 0381 (BPI 879807), Ghana]; M. *Rhytidhysteron hysterinum* [EB 0351 (BPI 879804) France, photo by Alain Gardiennet]. Scale bar (habitat) = 1 mm; Scale bar (spores and asci) = 10 µm.

France (EB 0351 / BPI 879804). Three of the Ghanian isolates clustered together in Clade E (Fig. 1), but one (EB 0381 / BPI 879807) associated in another subclade, along with the Kenyan (GKM 361A) and European (CBS 306.38) accessions of *R. rufulum*. The morphology of the ascomata (Fig. 10L) of *R. rufulum* EB 0381 (BPI 879807) differs from other more typical specimens of *R. rufulum* (*e.g.*, Fig. 10 K), although the 3-septate spores in both are identical. Finally, molecular data indicate that *R. hysterinum*, with 1-septate spores, falls outside of the *R. rufulum* subclades, while still within Clade E (Fig. 1).

Boehm *et al.* (2009) were the first to provide sequence data indicating that *Rhytidhysteron* does not lie within the *Patellariaceae*. Although initially based on only a single isolate of *R. rufulum* (CBS 306.38), the genus was tentatively noted to be associated with the *Hysteriaceae*. In the current study, a total of eight isolates, representing three species, clearly indicates that that the genus *Rhytidhysteron* belongs to the family *Hysteriaceae*, and not to the *Patellariaceae*, the latter defined in this study to include *Hysteropatella clavispora* (CBS 247.34), *Hp. elliptica* (CBS 935.97), and *Patellaria atrata* (CBS 958.97).

Earlier, Barr (1987) had noted the differences between *Rhytidhysteron* and other members of the *Patellariaceae*, stating: "*Rhytidhysteron rufulum* illustrates the problem: paraphysoids and a well-developed pseudoepithecium are conspicuous, but the structure of the peridium, thickened base of ascoma, cylindric asci, are all features attributed to members of the *Hysteriaceae*. When the heterogeneous family *Patellariaceae* is revised, *Rhytidhysteron* should be segregated in its own family". Samuels & Müller (1979) also noted that "The genus does not have any close relatives in the heterogeneous *Patellariaceae*". However, other authors (Bezerra & Kimbrough 1982) presented arguments against the inclusion of *Rhytidhysteron* within the *Hysteriaceae*, based on patterns of centrum development. Nevertheless, molecular data presented here, necessitate a radical reappraisal of the *Hysteriaceae* to include patellarioid forms.

## Key to the species of *Rhytidhysteron*

1. Ascospores mainly 1-septate; Europe ..... ***R. hysterinum***
1. Ascospores with more than one septum ..... 2

2. Ascospores mainly 3-septate ..... 3
2. Ascospores with five or more septa; Europe ..... ***R. dissimile***

3. Ascospores with three transverse, but also one or more longitudinal septa; Southwestern United States, East Africa ..... ***R. opuntiae***
3. Ascospores transversely 3-septate, with no longitudinal septa; cosmopolitan ..... ***R. rufulum***

***Mytilinidiaceae*** Kirschst. 1924, ***Mytilinidiales*** E.W.A. Boehm, C.L. Schoch & J.W. Spatafora 2009.

= *Lophiaceae* H. Zogg *ex* Arx & E. Müll., Stud. Mycol. 9: 60. 1975.

≡ *Lophiaceae* H. Zogg, Beitr. Kryptogamenfl. Schweiz. 11(3): 90. 1962, *nom. inval.* ICBN Art. 36.

Fungi classified in the *Mytilinidiaceae* (Kirschstein 1924) are characterised by fragile yet persistent carbonaceous ascomata, which range from globoid to obovoid to strongly laterally compressed erect, bivalve shell-shaped (*i.e.*, conchate) structures, standing on edge, with lateral walls more or less connivent, and extended vertically, in some species, to a prominent longitudinal keel or cristate apex. Mytilinidioid fungi possess a thin-walled, scleroparenchymatous peridium enclosing a hamathecium of narrow trabeculate pseudoparaphyses, borne in a gel matrix, which are often sparse to lacking at maturity. Bitunicate asci are borne in a basal, rarely lateral orientation within the centrum, and contain eight, rarely four, ascospores, overlapping uniseriate, biseriate or in one or two fascicles. Ascospores are diverse, ranging from scolecospores to didymospores to phragmospores or dictyospores, hyaline, soon yellow to dark brown, and generally showing bipolar symmetry (Zogg 1962, Barr 1987, 1990a). Anamorphs in the *Mytilinidiaceae* are primarily coelomycetous (*e.g.*, *Aposphaeria*, *Pyrenochaeta*, *Camaroglobulus*, *Dothiorella*-like, and *Sclerochaeta*) and less frequently hyphomycetous (*e.g.*, *Chalara*-like, *Papulaspora*, and *Septonema*) (Lohman 1932b, 1933a, b, Blackwell & Gilbertson 1985, Speer 1986). Typically temperate in distribution, mytilinidioid fungi are found in association with the wood, bark, resin, cones, scales, needles, seeds, and roots of gymnosperms.

Currently accepted genera in the *Mytilinidiaceae* include: *Actidium*, *Lophium*, *Mytilinidion*, *Ostreola*, and *Quasiconcha*, to

which has recently been added *Zoggium* (Lohman 1932b, Zogg 1962, Darker 1963, Barr 1975, 1990a, Barr & Blackwell 1980, Vasilyeva 2001). The genus *Ostreichnion*, previously classified within the *Mytilinidiaceae*, has been removed to the *Hysteriaceae* (Boehm *et al.* 2009). The genus *Glyphium*, originally classified within the *Mytilinidiaceae*, has recently been transferred to the *Chaetothyriales* in the *Eurotiomycetes* (Lindemuth *et al.* 2001, Lumbsch *et al.* 2005). This has been restated in a number of subsequent publications (Lücking *et al.* 2004, Schmitt *et al.* 2005, Geiser *et al.* 2006, Kodsueb *et al.* 2006), including the Assembling the Fungal Tree of Life (AFTOL) Project (Lutzoni *et al.* 2004). A study currently in preparation (Boehm *et al*.) will address issues related to the phylogenetic placement of the genus *Glyphium*. Despite their transference out of the *Mytilinidiaceae*, both *Ostreichnion* and *Glyphium* are included in the current key to effectuate identification of morphologically similar fungi, regardless of whether close phylogeny is implied or not.

## Key to the genera of the *Mytilinidiaceae*

1. Ascospores 1-septate, small, shorter than 30 µm ..... 2
1. Ascospores not didymospores, or if 1-septate, then longer than 30 µm ..... 3

2. Didymospores brown, ellipsoid, symmetric, with coarsely reticulate wall; 6–8 x 5–5.5 µm ..... ***Quasiconcha***
2. Didymospores olive- to reddish brown, walls thin, smooth or delicately longitudinally striate, but not reticulated; longer than 10 µm ..... ***Actidium***

3. Ascospores filiform, multi-septate, about equal in length to the ascus, in some case, at maturity longer than the ascus, often spirally arranged ..... 4
3. Ascospores ellipsoid, fusoid, cylindrical, if scolecospores, then shorter than the ascus and not spirally arranged ..... 6

4. Ascomata conchate, solitary to gregarious, but never forming fused, ridge-like assemblages ..... ***Lophium***
4. Ascomata either forming rigid, fused band- or ridge-like structures or solitary, erect, dolabrate to ligulate ..... 5

5. Ascomata densely gregarious, forming band- or ridge-like assemblages ..... ***Zoggium***
5. Ascomata erect, dolabrate to ligulate in outline; often with subtending hyphal strands; cosmopolitan ..... ***Glyphium***
*Note*: A key to the species is not presented here.

6. Ascospores transversely septate phragmospores, or scolecospores ..... ***Mytilinidion***
6. Ascospores dictyospores, or large and remaining 1-septate ..... 7

7. Ascospores ellipsoid, less than 30 µm long, with a single longitudinal septum, usually passing through the mid-cells, or spanning the entire length of the ascospore ..... ***Ostreola***
7. Ascospores ellipsoid or cylindric, longer than 30 µm, with several longitudinal septa in cells or large and remaining 1-septate ..... ***Ostreichnion***
*Note*: The genus *Ostreichnion* previously classified within the *Mytilinidiaceae* (Barr 1990a) has been transferred to the *Hysteriaceae* (Boehm *et al.* 2009).

***Actidium*** Fr., Observ. Mycol. 1: 190. 1815.
= *Mytilinidion* subgen. *Bulliardella* Sacc., Syll. Fung. 2: 764. 1883.
= *Bulliardella* (Sacc.) Paoli, Nuovo Giorn. Bot. Ital. 12:101. 1905.
= *Ostreionella* Seaver, Sci. Surv. Porto Rico & Virgin Islands 8(1): 77. 1926.

The genus *Actidium* was established by Fries (1823) to accommodate *A. hysterioides*, a stellate mytilinidioid fungus found on *Pinus* and *Picea* in Europe, with two-celled, symmetric ascospores, light olive- to reddish-brown, later noted to be faintly longitudinally striate (Barr 1990a). Fries (1823) noted its similarity with the genus *Glonium*. Zogg (1962) recognised a total of four species, namely *A. hysterioides*, *A. baccarinii*, both from Europe, *A. pulchra*, from China, and *A. nitidum*, from Europe and North America, on *Pinus*, *Picea, Juniperus*, and *Thuja* (Zogg 1962, Barr 1990a). Due to similarities in ascospore morphology, the genus *Actidium* may have affinities with other didymospored hysteriaceous genera (*e.g.*, *Actidiographium, Glonium* and *Psiloglonium*), although molecular data are presently lacking.

## Key to the species of *Actidium*

1. Ascomata stellate; spores 11–14 x (1.5–)2–3 µm; on *Pinus*, *Picea*, Europe ..... ***A. hysterioides***
1. Ascomata shell-shaped (conchate), not star-shaped ..... 2

2. Ascospores (9–)11–14(–16) x (1.5–)2–3 µm; on *Pinus*, *Picea, Juniperus*, Europe, North America ..... ***A. nitidum***
2. Ascospores larger ..... 3

3. Ascospores (16–)18–22(–24) x (3–)4–5(–6) µm; on *Pinus*, *Picea*, *Thuja,* Europe ..... ***A. baccarinii***
3. Ascospores 23–28 x 6–7.5 µm; China ..... ***A. pulchra***

***Quasiconcha*** M.E. Barr & M. Blackw., Mycologia 72: 1224. 1980.

The genus *Quasiconcha* was established by Barr & Blackwell (1980) to accommodate *Q. reticulata*, an unusual mytilinidioid fungus, with 1-septate, highly reticulated ascospores, borne in conchate, thin-walled ascomata, found in association with *Juniperus* seeds excreted in dung and the roots of two conifers from the southwestern United States (Barr & Blackwell 1980, Blackwell & Gilbertson 1985). In the present study, we were fortunate to obtain original material (RLG 141189) of *Q. reticulata* (Table 1) from Meredith Blackwell (Louisiana State University, Baton Rouge, LA), from which we isolated DNA (EB QR). Sequence data (Fig. 1) clearly indicate that the genus *Quasiconcha* belongs to the *Mytilinidiaceae*, in close association with *Lophium*, to which its fruitbodies most closely resemble.

***Mytilinidion*** Duby, Mém. Soc. Phys. Genève 16: 34. 1862.
= *Mytilidion* Sacc., Atti Soc. Veneto-Trentino Sci. Nat. Padova 4: 99. 1875.
= *Hypodermopsis* Earle, Bull. New York Bot. Gard. 2: 345. 1902.
= *Murashkinskija* Petr., Hedwigia 68: 203. 1928.

The genus *Mytilinidion*, the type for the family *Mytilinidiaceae*, was established by Duby (1862) with an etymology from *Mytilus*, a genus of mussels. Saccardo (1883, p. 760) considered the name *Mytilinidion* to be linguistically incorrect and replaced it with *Mytilidion*. It remained for Barr (1975) to note that the name *Mytilinidion* had historical precedence (Rogers 1953), and therefore should replace the later name *Mytilidion*. Species of *Mytilinidion* are characterised by yellow- to reddish-brown, ellipsoid, fusoid, obovoid to elongate, transversely septate, usually symmetric, ascospores, or scolecospores, borne in thin-walled globoid to conchate pseudothecia, with lateral walls more or less connivent and extended vertically to a cristate apex. There are currently 15 recognised species, occurring on the *Pinaceae*, *Cupressaceae*, and *Taxodiaceae* (Lohman 1932b, Zogg 1962, Speer 1986, Barr 1990a).

Ascospore morphology can be used to discern four morphological types within the genus, listed here by increasing ascospore length: (1) Short squat phragmospores: *M. acicola*, *M. resinae*, *M. decipiens*, *M. tortile* (Fig. 11A–B), and *M. resinicola*; (2) Elongate phragmospores, with a spore length to width ratio of 10 : 1 or less: *M. californicum*, *M. mytilinellum* (Fig. 11C–D), *M. rhenanum*, and *M. gemmigenum*; (3) Fusoid or spindle-shaped spores: *M. thujarum*, *M. oblongisporum*, and *M. andinense*; and (4) Highly elongated phragmospores, termed scolecospores, with a length to width ratio of 20 : 1: *M. scolecosporum*, *M. parvulum* and *M. australe* (Fig. 11E–I). These last three scolecosporous species were postulated to form a transitional series to connect *Mytilinidion* with the heretofore somewhat isolated genus *Lophium* (Fig. 11J–K), and formed the basis for subgenus *Lophiopsis*, distinct from subgenus *Eu-Mytilinidion sensu* Lohman (Lohman 1932b), a concept accepted by Zogg (1962).

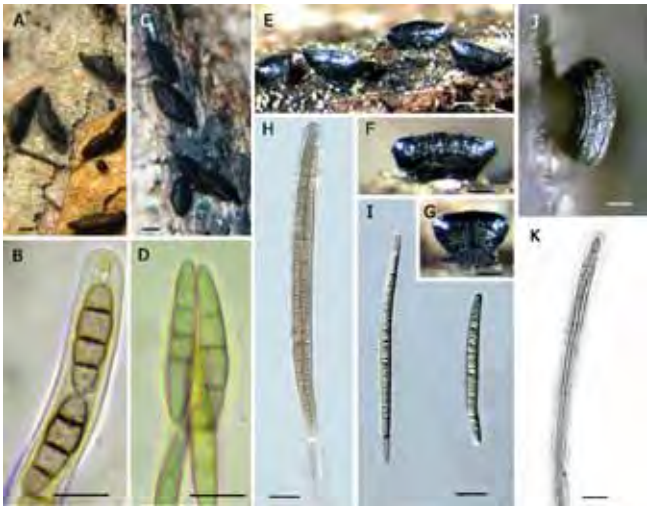


**Fig. 11.** The *Mytilinidiaceae*. A–B. *Mytilinidion tortile* [EB 0377 (BPI 879798), France]; C–D. *Mytilinidion mytilinellum* [EB 0386 (BPI 879796), France]; E–I. *Mytilinidion australe* [ANM 1524 (ILLS), U.S.A.; not incl.]; J–K. *Lophium mytilinum* [CBS 123344 (BPI 878736), U.S.A.]. Photo credits Alain Gardiennet, Figs. A–D. Scale bar (habitat) = 500 µm; Scale bar (spores and asci) = 10 µm.

Sequence data presented here (Fig. 1), based on an analysis of 10 of the 15 currently recognised species (Table 1), do not support subgenus *Lophiopsis sensu* Lohman (1932b): *Mytilinidion scolecosporum* (CBS 305.34) does not belong to the same clade as *M. australe* (CBS 301.34) (Fig. 1). This implies that the scolecospore has independently evolved at least twice within the family. Data do however support the association of fusoid or spindle-shaped spores belonging to *M. thujarum* (EB 0268 / BPI 879797) and to *M. andinense* (CBS 123562 / BPI 878737), thus defining a lineage for this type of spore within the genus. On the other hand, species possessing short, squat phragmospores, namely *M. acicola* (EB 0349 / BPI 879794, EB 0379 / BPI 879793), *M. tortile* (EB 0377 / BPI 879798), and *M. resinicola* (CBS 304.34) display complex relationships with species possessing elongate phragmospores, such as *M. californicum* (EB 0385 / BPI 879795), *M. mytilinellum* (EB 0386 / BPI 879796, CBS 303.34) and *M. rhenanum* (EB 0341, CBS 135.45). This indicates that phragmospores with different length to width ratios have also evolved multiple times within the genus (Fig. 1). A manuscript currently in preparation (Boehm *et al.*) will address speciation events within the *Mytilinidiaceae*. Despite the lack of molecular support for the subgenus *Lophiopsis*, it is included in the key below to facilitate species identification.

## Key to the species of *Mytilinidion*

1. Spore length to width ratio = 10 : 1 or less (phragmospores): Subgenus *Eu-Mytilinidion sensu* Lohman (1932b) ..... 2
1. Spore length to width ratio = approx. 20 : 1 (scolecospores): Subgenus *Lophiopsis sensu* Lohman (1932b) ..... 13

2. Ascomata not conchate, but erect, low and spreading at the base (scutate), seated on a shield-like process fused to the substrate, apical portion slightly connivent; ascospores 3–5(–6)-septate ..... 3
2. Ascomata conchate, standing on edge, usually with a clearly defined longitudinal cristate apex ..... 4

3. Ascospores 23–25 x 4–4.5(–5) µm, 3-septate; California on *Sequoia* ..... ***M. californicum***
3. Ascospores 14–22(–28) x (4.5–)6–8(–10) µm, 3–4–5–(–6) septate; on *Juniperus*, *Thuja*, Europe and North America ..... ***M. acicola***

4. Ascospores elongate phragmospores, usually not constricted at the septa ..... 5
4. Ascospores shorter, squat, or longer, but not narrowly elongated, usually constricted at median septum ..... 7

5. Ascospores (2–)3(–5)-septate, measuring (14–)16–22(–24) x (2.5–)3–4(–5) µm; cosmopolitan ..... ***M. mytilinellum***
5. Ascospores longer, with more septa ..... 6

6. Ascospores 3–5(–7)-septate, measuring (24–)30–42(–50) x 3–5 µm; Europe ..... ***M. rhenanum***
6. Ascospores slightly curved, asymmetric, (3–)7–9(–11)-septate, measuring (27–)32–38(–48) x (4–)5–6(–8) µm; cosmopolitan ..... ***M. gemmigenum***

7. Ascospores (2–)3-septate, small, 10–13 x 4–6 µm; resinicolous on *Araucaria*, Brazil ..... ***M. resinae***
7. Ascospores 3(–5)-septate, longer ..... 8

8. Ascospores 3-septate, slightly curved, oblong-elliptic, with obtuse ends, unconstricted, measuring (11–)13–15(–21) x 3–4(–6) µm; on *Larix*, *Juniperus*, Europe ..... ***M. decipiens***
8. Ascospores longer, or similar in length but then slightly wider ..... 9

9. Ascospores 3-septate, slightly curved, but oblong, fusiform, with slight constrictions, measuring (11–)14–17(–21) x 5–7(–8) µm; cosmopolitan ..... ***M. tortile***
9. Ascospores longer ..... 10

10. Ascospores 3-septate, elliptic-oblong, deeply constricted at the septa, measuring 24–26 x 8–9 µm; North America ..... ***M. resinicola***
10. Ascospores longer, fusoid ..... 11

11. Ascospores 3-septate, constricted at the median septum, measuring 27–33 x 7–8.5 µm; China and northwestern North America ..... ***M. oblongisporum***
11. Ascospores longer ..... 12

12. Ascospores 3-(4–5)-septate, measuring (26–)30–34(–40) x (10–)12–13(–15) µm; on *Thuja*, cosmopolitan ..... ***M. thujarum***
12. Ascospores wider, 3–7(–9)-septate, with swollen middle cells, 32–44 x 10–15 µm; on *Austrocedrus chilensis*, Argentina ..... ***M. andinense***

13. Ascospores 5–7-septate, measuring 40–50 x 2–2.5 µm, slightly constricted at central septa; North America and Europe ..... ***M. scolecosporum***
13. Ascospores longer, with more septa, less constricted ..... 14

14. Ascospores 7–9(–11)-septate, measuring (48–)54–62(–65) x 2.7–3 µm; North America ..... ***M. parvulum***
14. Ascospores (10–)11–14-septate, measuring (54–)58–70(–75) x 3–4 µm; North America ..... ***M. australe***

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***Lophium*** Fr., Syst. Mycol. 2: 534. 1823.

= *Lophidium* P. Karst., Bidrag. Kännedom Finlands Natur Folk. 23: 33, 247. 1873.

The genus *Lophium* is characterised by fragile, conchate ascocarps, sometimes seated on a foot-like base or sessile directly on the substrate. The thin-walled scleroparenchymatous peridium encloses a basal hamathecium of narrow trabeculate pseudoparaphyses, with very elongate asci, each bearing one fascicle of transversely septate filiform ascospores, often spirally arranged. The type species, *Lophium mytilinum* (Fig. 11J–K), is cosmopolitan in the temperate zones and has been recorded from both sides of the Atlantic (Zogg 1962, Barr 1990a). Zogg (1962) described two additional species, namely *L. elegans* on *Juniperus* from alpine regions of France, Italy and Switzerland, and *L. mayorii* on *Pinus* and *Larix* from the European Alps. Like *Mytilinidion*, most species of *Lophium* have only been recovered from coniferous substrates. The exception being the recently described *L. igoschinae*, recovered on *Dryas octopetala* and *D. crenulata* (*Rosaceae*) from Russia and Greenland (Chlebicki & Knudsen 2001).

Three isolates of the type species, *L. mytilinum*, were surveyed (Table 1), two from the United States, one from Michigan (CBS 269.34) and one from New York (CBS 123344 / BPI 878736), and one from Sweden (CBS 114111). An additional species of *Lophium*, namely a single-spored isolate of *L. elegans* from France (EB 0366 / BPI 879792), was included as well (Table 1). Both species are morphologically similar, with *L. elegans* having spirally arranged spores in the ascus and *L. mytilinum* having them in parallel orientation (Zogg 1962). Molecular data indicate that the two species are not closely related within the family. *Lophium mytilinum*, with filiform ascospores, shows a close phylogenetic relationship however to the genus *Quasiconcha* (EB QR), with reticulated didymospores (Fig. 1). Although having dissimilar spores, the fruitbodies of both taxa are remarkably similar in their shape, size and fragility.

## Key to the species of *Lophium*

1. Fruitbody erect, conchate, with thin-walled sclerenchymatoid peridium ..... 2
1. Fruitbody conchate, but crowded, band- or ridge-like, horizontal to recumbent and elongated; ascospores arranged parallel in the ascus, measuring (60–)80–100(–110) x 3–4(–5) µm; Europe, Russian Far East ..... ***L. mayorii***
   *Note*: Transferred to the genus *Zoggium* (Vasilyeva 2001).

2. Ascospores filiform, 12–15-septate, measuring 78–86 x 2.6–3 µm; on *Dryas*, Greenland, Russia ..... ***L. igoschinae***
2. Ascospores filiform, but longer; on conifers ..... 3

3. Ascospores arranged parallel in the ascus; measuring (130–)170–250(–300) x 1–2(–2.5) µm; cosmopolitan ..... ***L. mytilinum***
3. Ascospores spirally arranged in the ascus; measuring (200–)260–280(–300) x 2 µm; Europe ..... ***L. elegans***

***Zoggium*** Lar.N. Vassiljeva, Mikol. Fitopatol. 35: 17. 2001.

Zogg described *Lophium mayorii* on *Pinus* and *Larix* from the Swiss and French Alps, but noted that it differed from other species of *Lophium* in having rigid, band-forming ascomata, with a less fragile peridium as compared to *Lophium* and *Mytilinidion*. Vasilyeva (2001) found the same fungus in the Russian Far East and stated that it differed sufficiently from other species of *Lophium* in having gross, erumpent crowded ascomata, band- or ridge-like in appearance, as compared to the smaller, fragile, and entirely superficial fruitbodies typical of species of *Lophium* and made the transfer to *Zoggium mayorii*. Molecular data are presently lacking.

***Ostreola*** Darker, Canad. J. Bot. 41: 1383. 1963.

Barr (1975, 1990a) recognised two genera with muriform ascospores in the *Mytilinidiaceae*, namely *Ostreichnion* and *Ostreola*. Darker (1963) originally established the genus *Ostreola* for dictyospored forms that otherwise resembled species of *Mytilinidion* – that is, mytilinidioid counterparts to *Hysterographium sensu* Zogg (1962). Barr (1990a) differentiated *Ostreola* from *Ostreichnion* by smaller ascospores in the former, and recognised two species from North America, *Ot. consociata* from northeastern North America, and *Ot. formosa*, the latter common on conifers in western North America and Europe, with spores similar to those of *Hysterobrevium mori*. Tilak & Kale (1968) added another two species from India, namely *Ot. indica* and *Ot. ziziphi*, surprisingly both from non-coniferous substrates. Molecular data are presently lacking for this genus.

## Key to the species of *Ostreola*

1. Ascomata on coniferous hosts; North America, Europe ..... 2
1. Ascomata on non-coniferous hosts; India ..... 3

2. Base of ascoma foot-like, immersed in substrate; ascospores 3–5(–7)-septate, with a longitudinal septum in the mid-cells, 14–18(–22) x 5–7 µm; on *Picea*, Northeastern North America ..... ***Ot. consociata***
2. Base of ascoma tapered or applanate on surface of substrate; ascospores (3–)5(–6)-septate, wider than in *O. consociata*, 15–21 x 6.5–9.5 µm; alpine, on *Abies*, Europe and Western North America ..... ***Ot. formosa***

3. Ascospores transversely 3–7-septate, with 2–3 longitudinal septa, slightly constricted in the middle; 24–30 x 8–9.6 µm; on culms of *Maduca*, India ..... ***Ot. indica***
3. Ascospores as above but smaller, 19–23 x 6–7.5 µm; on culms of *Ziziphus*, India ..... ***Ot. ziziphi***

***Gloniaceae*** (Corda) E.W.A. Boehm, C.L. Schoch & J.W. Spatafora 2009, *Pleosporomycetidae fam. incertae sedis.*
= *Gloniaceae* Corda, Icon. Fung. (Abellini) 5: 34. 1842.

Corda (1842) originally proposed the *Gloniaceae* as an intrafamilial taxonomic rank under the family *Hysteriaceae*, to comprise *Hysterographium* and *Glonium*. Boehm *et al.* (2009) emended and restricted the circumscription and elevated the taxon to family rank. The genus *Glonium* was retained as circumscribed first by von Höhnel (1918) and then by Petrak (1923a). We feel justified in reinstating the *Gloniaceae* and, more importantly, recognising it at family rank for a single genus, because of the high support the group receives in a recent four-gene analysis (Boehm *et al.* 2009), and corroborated here.

***Glonium*** Muhl., Cont. Lab. Plant Disease Sci. Fac. Agric. Gifu Univ. 101. 1813.
= *Solenarium* Spreng., Syst. Veg. 4(1): 376, 414. 1827.
= *Psiloglonium* Höhn., Ann. Mycol. 16(1): 149. 1918.

The genus *Glonium* is characterised by modified hysterothecia, progressively dichotomously branched, laterally anastomosed along their length to form radiating flabelliform or pseudo-stellate composites, usually seated upon a conspicuous brown felt-like subiculum, sometimes borne in a stroma (Zogg 1962). Hysterothecia in vertical section globose to obovoid, typically with a thick, three-layered peridium, but fragile, unlike the robust peridium of the *Hysteriaceae*. Luttrell (1953) described the development of the ascocarp in the type species, *G. stellatum* as composed of small pseudoparenchymatous cells, the outer layer heavily encrusted with pigment and often longitudinally striate on the surface, the middle

layer lighter in pigmentation and the inner layer distinctly thin-walled, pallid and compressed. The hamathecium consisted of persistent narrow cellular pseudoparaphyses, often borne in a gel matrix, with tips darkened or branched at maturity. Bitunicate asci are borne in a basal layer and at maturity are typically clavate to cylindric, bearing eight ascospores, overlapping biseriate; ascospores ranging from hyaline to pale yellow, 1-septate, conspicuously constricted at the septum, fusoid in outline, with at least one end, often both, acuminate, and showing bipolar asymmetry.

Zogg (1962) listed three species that he grouped together in his key, that later formed the basis for the *Gloniaceae* (Boehm *et al.* 2009). These are the type species, *G. stellatum* (Fig. 12A–E), *G. graphicum*, and *G. compactum*, the latter associated with both subicula and stroma. To these three species, we can add the recently described saxicolous, terricolous and lignicolous *G. circumserpens* (Fig. 12F–H) from Tasmania (Kantvilas & Coppins 1997). Although von Höhnel (1918) and Petrak (1923a) stressed the importance of subiculum as a synapomorphic character state, Zogg (1962) noted that *G. graphicum* may or may not be associated with a subiculum. This, combined with the observation that *P. lineare* may also on occasion be associated with subiculum, led Zogg not to accept the genus *Psiloglonium*. Data presented here and elsewhere (Boehm *et al.* 2009), however, indicate that the synapomorphic character state is not subicula *per se*, but the ascomata, which are modified hysterothecia that are progressively dichotomously branched, to form radiating pseudostellate composites. This is most pronounced in *G. stellatum* and *G. circumserpens*, but may also be found to a lesser extent in *G. graphicum* (Zogg 1962). One distinguishing feature that separates *G. stellatum* from *G. circumserpens* is that, although both are associated with subicula, in the former this extends as a wide margin in front of the developing hysterothecia (Fig. 12A–C), whereas in *G. circumserpens* (Fig. 12F–G) the subicula is only associated with the under surface of the hysterothecia, closely appressed to the substrate, with only minor deviations from the long axis of the fruitbody.

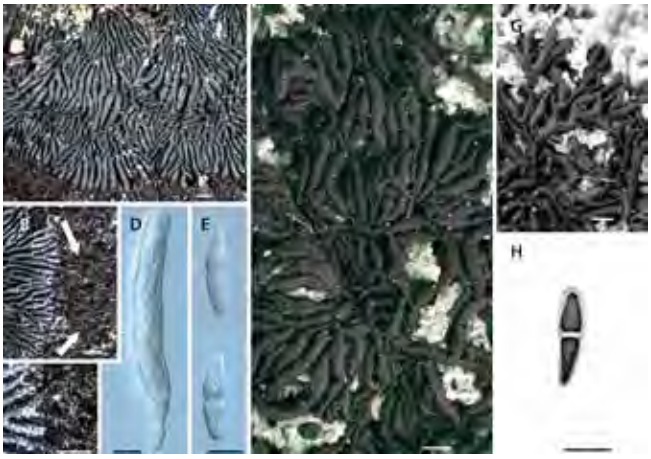


**Fig. 12.** The *Gloniaceae*. A–E. *Glonium stellatum* [ANM 41 (ILLS), U.S.A.; not incl.], arrows in 12B, subiculum. F–H. *Glonium circumserpens* [CBS 123343 (BPI 878739), Tasmania]. Scale bar (habitat) = 1 mm; Scale bar (spores and asci) = 10 µm.

Four isolates were surveyed for the *Gloniaceae*. Two of *G. stellatum*, from Michigan (CBS 207.34) and Tennessee (ANM 32), the United States, and two of *G. circumserpens*, recently isolated from wood (CBS 123342 / BPI 878738) and dolerite stone (CBS 123343 / BPI 878739) from Tasmania (Table 1). Molecular data indicate that all four isolates are closely related. Surprisingly, this clade also includes multiple isolates of *Cenococcum geophilum*, an ecologically important ectomycorrhizal fungus with a global distribution and a wide host range, but with no known teleomorph (LoBuglio *et al.* 1996).

## Key to the species of *Glonium*

1. Hysterothecia associated and seated upon a thin crust-like stroma, or arising from within a stromal crust; stroma itself seated on subiculum; didymospores spindle-shaped with the upper cell slightly swollen and larger than the lower cell, measuring 24–28 x 5–6 µm; Africa ..... ***G. compactum***
1. Hysterothecia not associated with stroma ..... 2

2. Hysterothecia somewhat branched, irregular, "graphoid"; without well-developed subiculum; didymospores oblong to spindle-shaped; upper cell pear-shaped, constricted at septum; both ends acuminate, measuring (13–)15–18(–21) x (3–)5–6 µm; on *Pinus*, *Juniperus*, Europe ..... ***G. graphicum***
2. Hysterothecia in mature specimens highly bifurcated, closely appressed to the substrate, dichotomously branched to form irregular creeping masses; usually seated upon or sitting behind a front of well-developed brown to black subiculum ..... 3

3. Didymospores hyaline, constricted at the septum, apices pointed, measuring (15–)16–17 x 6–7 µm; on soil (terricolous) or rock (saxicolous), or lignicolous; Tasmania ..... ***G. circumserpens***
3. Didymospores oblong to spindle-shaped; upper cell pear-shaped, constricted at septum; both ends acuminate, measuring (18–)21–26(–28) x (4–)5–6(–7) µm; cosmopolitan ..... ***G. stellatum***

***Farlowiella*** Sacc., Syll. Fung. 9: 1101. 1891.
= *Farlowia* Sacc., Syll. Fung. 2: 727. 1883.

Recent molecular data (Schoch *et al.* 2006; Boehm *et al.* 2009) support the transference of the genus *Farlowiella* from the *Hysteriaceae*, and its current placement as *Pleosporomycetidae gen. incertae sedis*. The genus is characterised by 1-celled pedicellate slightly laterally compressed amerospores, the upper cell pigmented and much larger than the lower, which remains hyaline or moderately pigmented, and can be considered as an associated papilla. The hysterothecia are somewhat laterally compressed, but nonetheless thick-walled and with a prominent sunken slit. They can be solitary to gregarious, but remain erect, and elevated, presenting an almost stipitate appearance. Anamorphs have been described in the genus *Acrogenospora* (Goh *et al.* 1998). Two species are recognised, namely *Farlowiella carmichaeliana* from Europe (Belgium, England, Germany, Switzerland), from the bark and wood of *Fagus, Quercus, Sorbus* and *Prunus*, and *F. australis*

known only from the original collection on *Phylica arborea* from Tristan da Cunha in the South Atlantic (Dennis 1955). Sequence data from two isolates of *F. carmichaeliana* (CBS 206.36 and CBS 179.73) indicate that this taxon lies quite distant from the *Hysteriaceae* (Fig. 1). An additional isolate of the anamorph, *Acrogenospora sphaerocephala* (CBS 164.76), further supports the current placement of the genus *Farlowiella*.

## Key to the species of *Farlowiella*

1. Ascospores unequally 2-celled; upper cell pigmented, much larger than the lower cell, which is smaller and hyaline, together measuring 18–21 x 7–12 µm; Europe ..... ***F. carmichaeliana***
1. Ascospores as above, but smaller, 13–15 x 6–7.5 µm; Tristan da Cunha ..... ***F. australis***

## CONCLUSIONS

Hysteriaceous fungi are an ancient and ecologically successful group of organisms, as attested by their wide geographic distribution on a multitude of gymnosperm and angiosperm host species. Whereas the *Mytilinidiaceae* are found almost exclusively on conifers, the *Hysteriaceae* occur primarily on angiosperms (Zogg 1962). Presumably, the *Hysteriaceae* underwent rapid speciation in response to the angiosperm radiation of the mid- to late-Cretaceous, 65–100 mya (Palmer *et al.* 2004). However, this must have occurred prior to the complete loss of continental contiguity, which occurred during the same time period. This is because we see today a remarkable degree of intraspecific stability, in both morphology and sequence data, among geographically disparate accessions (Fig. 1). For example, little morphological or sequence variation was detected in *Hysterium angustatum*, from North America (CBS 123334), Kenya (GKM 243A), New Zealand (SMH 5211.0), and South Africa (CMW 20409; Lee & Crous 2003). Similarly, little variation was detected in *Psiloglonium clavisporum*, from Kenya (GKM L172A, GKM 344A) and North America (*e.g.*, CBS 123338), or in *Oedohysterium sinense*, from South Africa (CBS 123345) and North America (EB 0339). As we are presumably sampling remnants of once contiguous sexual populations, their similarity today must imply that speciation occurred prior to complete genetic isolation. The break-up of Pangea during the Triassic 200 mya, and the formation of the nascent central Atlantic Ocean, separating Gondwana from Laurasia, during the Jurassic, 150 mya, must have effectively disrupted once contiguous populations. Although most flowering plant families were established by the end of the Cretaceous, 65–70 mya, it is now believed that they diversified into their present lineages (*e.g.*, eudicots, Magnoliids and monocots) much earlier, around 140 mya (Davies *et al.* 2004, Palmer *et al.* 2004, Moore *et al.* 2007). This may have allowed for remnants of once contiguous populations to colonise early angiosperm lineages, prior to the complete dissolution of continental integrity during the mid- to late-Cretaceous. Recent studies (Lücking *et al.* 2009), based on a recalibration of published molecular clock trees, using internally unconstrained, uniform calibration points, have suggested an origin for the fungi between 760 mya to 1.0 bya, with the origin of the *Ascomycota* set at 500-650 mya. Whatever the timing, hysteriaceous fungi incurred little appreciable intraspecific morphological or genetic (*e.g.*, nuLSU, nuSSU, *TEF1* and *RPB2*) change over significant periods of geologic time, on different continents. Thus, with the exception of *Hb. mori*, and perhaps, *Gp. subrugosa* (see below), most members of the *Hysteriaceae* appear to be stable species.

Sequence data indicate that *Hb. mori* occurs in both Clades A and D. However, analysis of *Hb. mori* specimens originating from each clade (*e.g.*, CBS 123563 / BPI 878731, and others, in Clade A *versus* GKM 1013 / BPI 879788 in Clade D), failed to find any appreciable difference in either spore morphology (*e.g.*, septation, pigmentation, symmetry, or measurement), substrate-choice, or features associated with the hysterothecium. Likewise, no morphological difference could be detected among genetically unrelated accessions of *Gp. subrugosa*, from South Africa (CBS 123346 / BPI 878735), in Clade D, *versus* those from Kenya (GKM 1214 / BPI 879776) and Cuba (SMH 557 / BPI 879777), outside of Clade D. These two examples illustrate a lack of correspondence between the morphospecies concept (Burnett 2003) and the genealogical concordance phylogenetic species recognition concept (Taylor *et al.* 2000), the latter indicating here the presence of cryptic species within the two morphospecies. *Hysterobrevium mori* and, to a lesser extent, *Gp. subrugosa*, may represent examples of convergent evolution, whereby similar ascospores borne in hysterothecia have evolved multiple times within the family. This is supported by the polyphyly inherent in the circumspection of the classical genera of the *Hysteriaceae* (*e.g.*, *Gloniopsis*, *Glonium*, *Hysterium*, and *Hysterographium*), revealed by recent studies (Schoch *et al.* 2006, Boehm *et al.* 2009, Mugambi & Huhndorf 2009). Alternatively, *Hb. mori* and *Gp. subrugosa* may have retained ancestral character states, and thus may represent evolutionary lineages that did not incur appreciable morphological change, while at the same time accumulating sufficient genetic change to fall, in the case of *Hb. mori*, into distant clades within the family. If this is the case, then these two taxa may represent examples of speciation in progress, with genetic change preceding morphological change, thus differing from independent convergent character states. Whatever the mechanism, it is difficult to see how *Hb. mori*, for example, may be classified into different species, in different genera (*e.g.*, *Hysterobrevium* and *Oedohysterium*), without a sound morphological basis. We conclude that both *Hb. mori* and *Gp. subrugosa* contain genetically unrelated, cryptic, and potentially different biological species, that can not at present be morphologically differentiated.

Although there are examples of concordance between morphological and molecular data in this study (see below), these are few. For the most part, molecular data support the premise of a large number of convergent evolutionary lineages, sharing similar spore morphologies, but that are not closely related. This resulted in a polyphyletic core set of genera for the *Hysteriaceae*, and presented us with a complicated picture of past speciation events within the family (Boehm *et al.* 2009). To achieve a natural phylogeny, that is, one based on the concordance of morphological and molecular data, required that we break-up what were once thought to be stable genera. Thus, two species of *Hysterium* were transferred to *Oedohysterium* (*Od. insidens* and *Od. sinense*), and two species of *Gloniopsis* to *Hysterobrevium* (*Hb. smilacis* and *Hb. constrictum*). While *Hysterographium*, with the type *Hg. fraxini*, was removed from the *Hysteriaceae* (Boehm *et al.* 2009), some of its species remained within the family, transferred here

to *Oedohysterium* (*Od. pulchrum*), *Hysterobrevium* (*Hb. mori*) and *Gloniopsis* (*Gp. subrugosa*). New species were described (*e.g.*, *Gp. arciformis* and *Gp. kenyensis*) which would previously have been classified in *Hysterographium*, but are now accommodated in *Gloniopsis*. Molecular data necessitated that both *Gloniopsis* and *Hysterobrevium* include hyaline and pigmented dictyospores, and the genus *Oedohysterium*, both phragmospores and dictyospores. This, then, de-emphasised spore morphology as a synapomorphic character state. Likewise, the genus *Glonium sensu* Zogg (1962) was divided into *Psiloglonium* in the *Hysteriaceae* and *Glonium* in the *Gloniaceae* (Boehm *et al.* 2009), and, more recently, *Anteaglonium* in the *Pleosporales* (Mugambi & Huhndorf 2009). These taxonomic changes were unexpected, as they were not premised on past assumptions of synapomorphy related to spore morphology (Zogg 1962). Although we have included here a total of 59 accessions, representing 22 species in seven genera, for the *Hysteriaceae*, and another 62 outside of the family (Table 1), taxon sampling may still be insufficient. Clearly, additional species and genera need to be sampled before a complete picture emerges for the family.

The quest for synapomorphic character states that correlate with molecular data was one of the goals of this study. If traditional character states associated with spore septation/pigmentation or the fruitbody (Zogg 1962) can not be relied upon to deduce phylogeny, are there other character states that can be emphasised instead? Two examples are discussed below, the first relating to spore morphology, the second to characters associated with the fruitbody. Although both *Oedohysterium* and *Hysterium* possess similar pigmented asymmetric phragmospores, species of *Oedohysterium* can be morphologically differentiated by the possession of an enlarged supra-median cell. Molecular data also revealed that a species previously classified as *Hysterographium*, namely *Hg. pulchrum*, belonged to *Oedohysterium*, despite the presence of dictyospores. Closer inspection, however, reveals that the dictyospores of *Od. pulchrum* also possess a swollen supra-median cell. Additionally, a certain number of spores remain as transversely septate phragmospores (Checa *et al.* 2007), thus reinforcing its placement within *Oedohysterium*, and perhaps underscoring the plasticity of spore septation configurations for this group of fungi.

The second example relates to character states associated with the fruitbody. Fruitbody morphology clearly supports the transfer of the genus *Glonium* out of the *Hysteriaceae* to its own family, the *Gloniaceae*, closely allied with the *Mytilinidiales*. The *Gloniaceae* possess a modified hysterothecium, one in which the frutibodies frequently bifurcate to a greater (*e.g.*, *G. stellatum* and *G. circumserpens*) or lesser (*e.g.*, *G. graphicum*) degree, the former two species with radiating stellate composites, usually seated on subicula. This is in contrast to hysterothecia found in the *Hysteriaceae* which may be gregarious, but are never laterally anastomosed to form radiating composites. Additionally, the morphology of the dehiscence slit found in the *Gloniaceae* is unlike that found in the *Hysteriaceae*. In the *Gloniaceae*, the aperture is in most cases evaginated, forming a miniscule crest, similar to the more extended version found in some species in the *Mytilinidiaceae*; whereas, in the *Hysteriaceae*, hysterothecia have deeply invaginated slits. Also, hysterothecia found in the *Gloniaceae*, like those in the *Mytilinidiaceae*, are considerably more fragile, as compared to those found within the *Hysteriaceae*. These character states were either not noted before (*e.g.*, swollen supra-median cell in *Oedohysterium* and evaginated slit in *Glonium*), or were noticed, but given less taxonomic weight (*e.g.*, modified hysterothecium in *Glonium*; Zogg 1962). These examples illustrate that morphological features can be found that correlate with molecular data, despite the anomalies presented by *Hb. mori* and *Gp. subrugosa*, mentioned earlier.

The hysterothecium, thick-walled, navicular, and with a prominent longitudinal slit, has long been considered synapomorphic, defining the *Hysteriales*. However, this type of fruitbody has evolved convergently no less than five times within the *Pleosporomycetidae* (*e.g.*, *Farlowiella*, *Glonium*, *Anteaglonium*, *Hysterographium* and the *Hysteriaceae*). Similarly, thin-walled mytilinidioid (*e.g.*, *Ostreichnion*) and patellarioid (*e.g.*, *Rhytidhysteron*) ascomata have also evolved at least twice within the subclass, the genera having been transferred from the *Mytilinidiaceae* and *Patellariaceae*, respectively, to the *Hysteriaceae*. As such, character states relating not only to the external features of the ascoma, but to the centrum as well (*e.g.*, cellular pseudoparaphyses *versus* trabeculae, etc.), previously considered to represent synapomorphies among these fungi, in fact, represent symplesiomorphies, and most likely have arisen multiple times through convergent evolutionary processes in response to common selective pressures. Similar findings have emerged for a number of other ascomycete lineages within the *Pezizomycotina* (*e.g.*, Schoch *et al.* 2009b). One selective advantage of the hysterothecium may be spore discharge over prolonged periods of time, since some, if not most, species may be perennial (Lohman 1931, 1933a). The thick-walled peridium further contributes to xerotolerance, as many of these fungi persist on decorticated, weathered woody substrates prone to prolonged periods of desiccation. Thus, the ability to perennate, and time spore discharge with environmental conditions suitable for germination, spanning multiple seasons, may be the driving force behind the repeated evolution of the hysterothecium.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Taxon sampling, provenance and GenBank accession numbers.

| Species | Accession | Provenance | Genbank No. | | | |
|---|---|---|---|---|---|---|
| | | | nuSSU | nuLSU | *TEF1* | *RPB2* |
| *Acrogenospora sphaerocephala* | **CBS 164.76** | W. Gams, Grande Tinémont, BELGIUM | **GU296129** | **GU301791** | **GU349059** | **GU371748** |
| *Aliquandostipite khaoyaiensis* | **CBS 118232** | P. Inderbitzin (AFTOL1364), Khao Yai NP, THAILAND | AF201453 | **GU301796** | **GU349048** | FJ238360 |
| *Anteaglonium abbreviatum* | **ANM 925.1** | A.N. Miller (ILLS), Smoky Mts. TN, U.S.A. | – | GQ221877 | GQ221924 | – |
| *A. globosum* | **SMH 5283** | S.M. Huhndorf (F), Indiana Dunes, IN, U.S.A. | – | GQ221911 | GQ221919 | – |
| | **ANM 925.2** | A.N. Miller (ILLS), Smoky Mts. TN, U.S.A. | – | GQ221879 | GQ221925 | – |
| *A. latirostrum* | **GKM L100N.2** | G.K. Mugambi (EA), Taita Hills, KENYA | – | GQ221876 | GQ221938 | – |
| | **GKM 1119** | G.K. Mugambi (EA), Taita Hills, KENYA | – | GQ221874 | GQ221937 | – |
| *A. parvulum* | **SMH 5210** | S.M. Huhndorf (F), NEW ZEALAND | – | GQ221907 | GQ221917 | – |
| *Arthonia caesia* | **AFTOL 775** | A. Amtoft, NC, U.S.A. | – | FJ469668 | FJ469669 | FJ469670 |
| *Botryosphaeria dothidea* | **CBS 115476** | B. Slippers (AFTOL 946), Crocifisso, SWITZERLAND | DQ677998 | DQ678051 | DQ767637 | DQ677944 |
| *Byssothecium circinans* | **CBS 675.92** | G. Semeniuk (AFTOL 1735), SD, U.S.A. | AY016339 | AY016357 | GU349061 | DQ767646 |
| *Cenococcum geophilum* | **HUNT A1** | K.F. LoBuglio, GenBank | L76616 | – | – | – |
| *C. geophilum* | **CGMONT** | K.F. LoBuglio, GenBank | L76617 | – | – | – |
| | **010** | K.F. LoBuglio, GenBank | L76618 | – | – | – |
| *Cochliobolus heterostrophus* | **CBS 134.39** | K. Böning (AFTOL 54) | AY544727 | AY544645 | DQ497603 | DQ247790 |
| *Delitschia winteri* | **CBS 225.62** | J.L. Bezerra (AFTOL1599), Baarn, NETHERLANDS | DQ678026 | DQ678077 | DQ677922 | DQ677975 |
| *Dothidea insculpta* | **CBS 189.58** | E. Müller (AFTOL921), Maupas, FRANCE | DQ247810 | DQ247802 | DQ471081 | AF107800 |
| *D. sambuci* | **DAOM 231303** | S. Hambleton & B. Shoemaker (AFTOL 274) | AY544722 | AY544681 | DQ497606 | DQ522854 |
| *Elsinoë veneta* | **CBS 150.27** | E.M. Wakefield (AFTOL 1853) | DQ767651 | DQ767658 | DQ767641 | – |
| *Encephalographa elisae* | **EB 0347** | M. Tretiach, (BPI 879773), Prov. Trieste, Karst, ITALY | **GU397358** | **GU397343** | – | – |
| *Farlowiella carmichaeliana* | **CBS 206.36** | E.W. Mason (AFTOL1787). EUROPE | AY541482 | AY541492 | DQ677931 | DQ677989 |
| *F. carmichaeliana* | **CBS 179.73** | W. Gams,Teutoburger Wald, Neuenheerse, GERMANY | GU296148 | – | – | – |
| *Gloniopsis arciformis* | **GKM L166A** | G.K. Mugambi (BPI 879774 = Holotype), Malindi, KENYA | **GU323180** | **GU323211** | – | – |
| *Gp. kenyensis* | **GKM 1010** | G.K. Mugambi (BPI 879775 = Holotype), EA, Malindi, KENYA | – | GQ221891 | – | – |
| *Gp. praelonga* | **CBS 112415** | S. Marincowitz (PREM), Kogelberg NR, SOUTH AFRICA | FJ161134 | FJ161173 | FJ161090 | FJ161113 |
| | **CBS 123337** | E.W.A. Boehm (BPI 878725), NJ, U.S.A. | FJ161154 | FJ161195 | FJ161103 | – |
| | **CMW 19983** | S. Marincowitz (PREM 57539), Jonkershoek, SOUTH AFRICA | FJ161152 | FJ161193 | – | – |
| *Gp. subrugosa* | **CBS 123346** | S. Marincowitz (BPI 878735), Gauteng, SOUTH AFRICA | FJ161170 | FJ161210 | – | FJ161131 |
| | **GKM 1214** | G.K. Mugambi (BPI 879776, EA), Mt. Kenya, KENYA | – | GQ221895 | **GU397336** | – |
| | **SMH 557** | S.M. Huhndorf (BPI 879777, F), Sancti Spiritus, CUBA | – | GQ221896 | **GU397337** | – |
| *Glonium circumserpens* | **CBS 123342** | G. Kantvilas (BPI 878738), Warra SST, TASMANIA | FJ161168 | FJ161208 | – | – |
| *G. circumserpens* | **CBS 123343** | G. Kantvilas (BPI 878739), Warra SST, TASMANIA | FJ161160 | FJ161200 | FJ161108 | FJ161126 |
| *G. stellatum* | **CBS 207.34** | M.L. Lohman (No. 265), MI, U.S.A. | FJ161140 | FJ161179 | FJ161095 | |
| | **ANM 32** | A.N. Miller (ILLS), Smoky Mts., TN, U.S.A. | – | GQ221887 | | |
| *Guignardia gaultheriae* | **CBS 447.70** | H.A. van der Aa (AFTOL 1784), Seattle, WA, U.S.A. | – | DQ678089 | | DQ677987 |
| *Herpotrichia diffusa* | **CBS 250.62** | M.C. Pande (AFTOL1588), Uttar Pradesh, INDIA | DQ678019 | DQ678071 | DQ677915 | DQ677968 |
| *Hysterium angustatum* | **CBS 236.34** | M.L. Lohman (No. 309), WI, U.S.A. | **GU397359** | FJ161180 | FJ161096 | FJ161117 |
| | **CBS 123334** | E.W.A. Boehm (BPI 878724), Sussex Co., NJ, U.S.A. | FJ161167 | FJ161207 | FJ161111 | FJ161129 |
| | **CMW 20409** | S. Marincowitz (PREM 57585), Kleinmond, SOUTH AFRICA | FJ161153 | FJ161194 | – | – |
| | **SMH 5211.0** | S.M. Huhndorf (F), NEW ZEALAND | **GU397360** | GQ221905 | – | GQ221923 |
| | **GKM 243A** | G.K. Mugambi (EA), Malindi, KENYA | – | GQ221899 | – | – |

**Table 1.** (Continued).

| Species | Accession | Provenance | Genbank No. | | | |
|---|---|---|---|---|---|---|
| | | | nuSSU | nuLSU | *TEF1* | *RPB2* |
| | **SMH 5216** | S.M. Huhndorf (F), NEW ZEALAND | – | – | – | GQ221933 |
| | **ANM 85** | A.N. Miller (ILLS), Smoky Mts., TN, U.S.A. | – | GQ221898 | – | – |
| *H. barrianum* | **ANM 1495** | A.N. Miller (ILLS59908 = Holotype, BPI879783 = Paratype), TN, U.S.A. | **GU323182** | GQ221885 | – | – |
| | **ANM 1442** | A.N. Miller (ILLS 59907, BPI 879784), Smoky Mts., TN, U.S.A. | **GU323181** | GQ221884 | – | – |
| *H. hyalinum* | **CBS 237.34** | M.L. Lohman (No. 425), MA, U.S.A. | FJ161141 | FJ161181 | – | – |
| *H. pulicare* | **CBS 123377** | E.W.A. Boehm (BPI 878723), NY, U.S.A. | FJ161161 | FJ161201 | FJ161109 | FJ161127 |
| *H. vermiforme* | **GKM 1234** | G.K. Mugambi (BPI 879785, EA), Mt. Kenya, KENYA | – | GQ221897 | – | – |
| *Hysterobrevium constrictum* | **SMH 5211.1** | S.M. Huhndorf (F), NEW ZEALAND | **GU397361** | GQ221905 | – | – |
| *Hb. mori* | **CBS 245.34** | M.L. Lohman (No. 6), MI, U.S.A. | FJ161143 | – | FJ161098 | – |
| | **CBS 123563** | E.W.A. Boehm (BPI 878731), NY, U.S.A. | FJ161155 | FJ161196 | FJ161104 | – |
| | **CBS 123564** | E.W.A. Boehm (BPI 878732), NJ, U.S.A. | FJ161158 | FJ161198 | FJ161106 | – |
| | **CBS 123336** | E.W.A. Boehm (BPI 878733), NJ, U.S.A. | FJ161164 | FJ161204 | – | – |
| | **CBS 123335** | E.W.A. Boehm (BPI 878734), NY, U.S.A. | FJ161162 | FJ161202 | – | – |
| | **SMH 5273** | S.M. Huhndorf (BPI 879787, F), IN, U.S.A. | – | GQ221910 | GQ221936 | – |
| | **GKM 1013** | G.K. Mugambi (BPI 879788, EA), Malindi, KENYA | – | **GU397344** | **GU397338** | – |
| | **SMH 5286** | G.K. Mugambi (BPI 879789, EA), MI, U.S.A. | – | **GU397345** | – | – |
| *Hb. smilacis* | **CBS 114601** | O. Constantinescu, as *Gp. curvata* (Fr.) Sacc., SWEDEN | FJ161135 | FJ161174 | FJ161091 | FJ161114 |
| | **CBS 200.34** | M.L. Lohman (No. 29), as Gp. gerardiana Sacc., MI, U.S.A. | FJ161138 | FJ161177 | – | – |
| | **CMW 18053** | S. Marincowitz (PREM 57546), Kirstenbosch, SOUTH AFRICA | FJ161150 | FJ161191 | – | – |
| | **SMH 5280** | G.K. Mugambi (EA), IN, U.S.A. | **GU323183** | GQ221912 | – | **GU371784** |
| | **GKM 426N** | G.K. Mugambi (EA), Taita Hills, KENYA | – | GQ221901 | – | – |
| *Hysterographium fraxini* | **CBS 109.43** | H. Zogg, SWITZERLAND | FJ161132 | FJ161171 | FJ161088 | – |
| *Hg. fraxini* | **CBS 242.34** | M.L. Lohman (No. 300), Manitoba, CANADA | – | FJ161189 | | – |
| *Hysteropatella clavispora* | **CBS 247.34** | M.L. Lohman (No. 143), IN, U.S.A. | DQ678006 | AY541493 | DQ677901 | DQ677955 |
| *Hp. elliptica* | **CBS 935.97** | G. Marson (AFTOL 1790), Fentange, LUXEMBOURG | EF495114 | DQ767657 | DQ767640 | DQ767647 |
| *Jahnula aquatica* | **R68-1** | Campbell *et al.* 2007 | EF175633 | EF175655 | – | – |
| *Leptosphaeria maculans* | **DAOM 229267** | S. Hambleton & B. Shoemaker (AFTOL 277), CANADA | DQ470993 | DQ470946 | DQ471062 | DQ470894 |
| *Lophium elegans* | **EB 0366** | A. Gardiennet (BPI 879792), Til-Chatel, FRANCE | **GU323184** | **GU323210** | – | – |
| *L. mytilinum* | **CBS 269.34** | M.L. Lohman (AFTOL 1609), MI, U.S.A. | DQ678030 | DQ678081 | DQ677926 | DQ677979 |
| | **CBS 114111** | K. & L. Holm & O Constantinescu, Uppland, SWEDEN | EF596819 | EF596819 | – | – |
| | **CBS 123344** | E.W.A. Boehm (BPI 878736), NY, U.S.A. | FJ161163 | FJ161203 | FJ161110 | FJ161128 |
| *Mycosphaerella punctiformis* | **CBS 113265** | G. Verkley (AFTOL 942), Utrecht, NETHERLANDS | DQ471017 | DQ470968 | DQ471092 | DQ470920 |
| *Myriangium duriaei* | **AFTOL 1304** | L. Grodsinsky (CBS 260.36), Delta del Parana, ARGENTINA | AY016347 | DQ678059 | DQ677900 | DQ677954 |
| *Mytilinidion acicola* | **EB 0379** | A. Gardiennet (BPI 879793), Veronnes, FRANCE | **GU397362** | **GU397346** | – | **GU397355** |
| *M. acicola* | **EB 0349** | A. Gardiennet (BPI 879794), Fixey, Combe Laveau, FRANCE | **GU323185** | **GU323209** | – | **GU371757** |
| *M. andinense* | **CBS 123562** | M.I. Messuti (BPI 878737), Barrio Don Orione, ARGENTINA. | FJ161159 | FJ161199 | FJ161107 | FJ161125 |
| *M. australe* | **CBS 301.34** | A.H. Smith & M.L. Lohman, (type culture), LA, U.S.A. | – | FJ161183 | – | – |
| *M. californicum* | **EB 0385** | A. Gardiennet (BPI 879795), Bois de la Chamage, FRANCE | **GU323186** | **GU323208** | – | – |
| *M. mytilinellum* | **CBS 303.34** | M.L. Lohman (No. 281), as M. laeviusculum, MI, U.S.A. | FJ161144 | FJ161184 | FJ161100 | FJ161119 |
| | **EB 0386** | A. Gardiennet (BPI 879796), Boissenois, FRANCE | **GU397363** | **GU397347** | – | **GU397356** |
| *M. resinicola* | **CBS 304.34** | M.L. Lohman, No. 260, MI, U.S.A. | FJ161145 | FJ161185 | FJ161101 | FJ161120 |
| *M. rhenanum* | **CBS 135.45** | NCTC 6434 (1945), as *M. karstenii* | FJ161136 | FJ161175 | FJ161092 | FJ161115 |

**Table 1.** (Continued).

| Species | Accession | Provenance | Genbank No. | | | |
|---|---|---|---|---|---|---|
| | | | nuSSU | nuLSU | *TEF1* | *RPB2* |
| | **EB 0341** | A. Brissard, Guesnes, FRANCE | **GU323187** | **GU323207** | – | – |
| *M. scolecosporum* | **CBS 305.34** | A.H. Smith & M.L. Lohman, WI, U.S.A. | FJ161146 | FJ161186 | FJ161102 | FJ161121 |
| *M. thujarum* | **EB 0268** | E.W.A. Boehm (BPI 879797), NY, U.S.A. | **GU323188** | **GU323206** | – | – |
| *M. tortile* | **EB 0377** | A. Gardiennet (BPI 879798), Veronnes, FRANCE | **GU323189** | **GU323205** | – | – |
| *Oedohysterium insidens* | **CBS 238.34** | M.L. Lohman (No. 308) MI, U.S.A. | FJ161142 | FJ161182 | FJ161097 | FJ161118 |
| *Od. insidens* | **ANM 1443** | A.N. Miller (BPI 879799, ILLS), Smoky Mts., TN, U.S.A. | **GU323190** | GQ221882 | – | **GU371785** |
| *Od. pulchrum* | **DQ 402184** | J. Checa (DAOM 234345), Guanacaste, COSTA RICA | DQ402184 | – | – | – |
| *Od. sinense* | **CBS 123345** | M. Gryzenhout (BPI 878730), Limpopo, SOUTH AFRICA | FJ161169 | FJ161209 | – | FJ161130 |
| | **EB 0339** | E.W.A. Boehm (BPI 879800), NJ, U.S.A. | **GU397364** | **GU397348** | **GU397339** | **GU397357** |
| *Opegrapha dolomitica* | **DUKE 0047528** | C. Gueidan (AFTOL 993), CROATIA | DQ883706 | – | DQ883732 | DQ883714 |
| *Ostreichnion curtisii* | **CBS 198.34** | M.L. Lohman (No. 464), GA, U.S.A. | FJ161137 | FJ161176 | FJ161093 | – |
| *O. sassafras* | **CBS 322.34** | M.L. Lohman (No. 530), NC, U.S.A. | FJ161148 | FJ161188 | – | FJ161122 |
| *Patellaria atrata* | **CBS 958.97** | G. Marson, Wasserbillig, Bahnhof, LUXEMBOURG | **GU296181** | **GU301855** | **GU349038** | **GU371726** |
| *Phoma herbarum* | **CBS 276.37** | AFTOL 1575 | DQ678014 | DQ678066 | DQ677909 | DQ677962 |
| *Pleospora herbarum* | **CBS 191.86** | E.G. Simmons, AFTOL_940, Uttar Pradesh, INDIA | DQ247812 | DQ247804 | DQ471090 | DQ247794 |
| *Psiloglonium araucanum* | **CBS 112412** | S. Marincowitz (PREM 57570), Kirstenbosch, SOUTH AFRICA | FJ161133 | FJ161172 | FJ161089 | FJ161112 |
| | **CMW 18760** | S. Marincowitz (PREM 57569), Kirstenbosch, SOUTH AFRICA | FJ161151 | FJ161192 | – | – |
| | **CMW 17941** | S. Marincowitz (PREM 57566), Jonkershoek, SOUTH AFRICA | FJ161149 | FJ161190 | – | – |
| *P. clavisporum* | **CBS 123338** | E.W.A. Boehm (BPI 878726), NJ, U.S.A. | FJ161156 | FJ161197 | – | FJ161123 |
| | **CBS 123339** | E.W.A. Boehm (BPI 878727), NJ, U.S.A. | FJ161157 | FJ167526 | FJ161105 | FJ161124 |
| | **CBS 123340** | E.W.A. Boehm (BPI 878728), NJ, U.S.A. | FJ161165 | FJ161205 | – | – |
| | **CBS 123341** | E.W.A. Boehm (BPI 878729), NJ, U.S.A. | FJ161166 | FJ161206 | – | – |
| | **GKM 344A** | G.K. Mugambi (BPI 879801, EA), Malindi, KENYA | **GU397365** | GQ221889 | – | – |
| | **GKM L172A** | G.K. Mugambi (EA), Malindi, KENYA | **GU323192** | **GU323204** | – | – |
| *P. simulans* | **CBS 206.34** | M.L. Lohman, MI, U.S.A. | FJ161139 | FJ161178 | FJ161094 | FJ161116 |
| | **ANM 1557** | A.N. Miller (BPI 879803, ILLS), Smoky Mts., TN, U.S.A. | – | GQ221873 | GQ221920 | – |
| *Quasiconcha reticulata* | **EB QR** | M. Blackwell (RLG 14189), AZ, U.S.A. | – | **GU397349** | – | – |
| *Roccella fuciformis* | **AFTOL 126** | Diederich 15572 | AY584678 | AY584654 | – | DQ782866 |
| *Rhytidhysteron hysterinum* | **EB 0351** | A. Gardiennet (BPI 879804), Gevrey-Chambertin, FRANCE | – | **GU397350** | **GU397340** | – |
| *R. opuntiae* | **GKM 1190** | G.K. Mugambi (BPI 879805, EA), Malindi, KENYA | – | GQ221892 | **GU397341** | – |
| *R. rufulum* | **CBS 306.38** | R.K. Voorhees (AFTOL 2109), EUROPE | AF164375 | FJ469672 | **GU349031** | – |
| | **GKM 361A** | G.K. Mugambi (BPI 879806, EA), Malindi, KENYA | **GU296192** | GQ221893 | **GU349031** | – |
| | **EB 0381** | E. Nkansah (BPI 879807), Kwame Nkrumah, GHANA | **GU397366** | **GU397351** | – | – |
| | **EB 0382** | E. Nkansah (BPI 879808), Kwame Nkrumah, GHANA | – | **GU397352** | – | – |
| | **EB 0383** | E. Nkansah (BPI 879809), Kwame Nkrumah, GHANA | **GU397353** | **GU397367** | – | – |
| | **EB 0384** | E. Nkansah (BPI 879810), Kwame Nkrumah, GHANA | **GU397368** | **GU397354** | – | – |
| *Scorias spongiosa* | **CBS 325.33** | L.H. Leonian (AFTOL 1594) | DQ678024 | DQ678075 | DQ677920 | DQ677973 |
| *Simonyella variegata* | **AFTOL 80** | DUKE Printzen14310a | AY584669 | – | DQ782891 | DQ782861 |

**AFTOL:** Assembling the Fungal Tree of Life; **BPI:** United States USDA ARS National Fungus Collections, Beltsville, MD; **CBS:** Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CMW:** Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, Republic of South Africa; **DAOM:** National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada; **DUKE:** Duke University Herbarium, Durham, North Carolina; **EA:** National Museums of Kenya East African Herbarium, Nairobi, Kenya; **F:** Field Museum of Natural History, Chicago, IL; **ILLS:** Illinois Natural History Survey Herbarium, Champaign, IL; **PREM:** The South African National Collection of Fungi, National Mycological Herbarium, Pretoria, South Africa; **RLG:** The Robert L. Gilbertson Mycological Herbarium at the University of Arizona. Culture and specimen abbreviations: ANM: A.N. Miller; EB: E.W.A. Boehm; GKM: G.K. Mugambi, SMH: S.M. Huhndorf. GenBank accessions marked in bold represent new sequences generated in the current study.

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# Multi-locus phylogeny of *Pleosporales*: a taxonomic, ecological and evolutionary re-evaluation

Y. Zhang[1], C.L. Schoch[2], J. Fournier[3], P.W. Crous[4], J. de Gruyter[4, 5], J.H.C. Woudenberg[4], K. Hirayama[6], K. Tanaka[6], S.B. Pointing[1], J.W. Spatafora[7] and K.D. Hyde[8, 9*]

[1]*Division of Microbiology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China;* [2]*National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, U.S.A.;* [3]*Las Muros, Rimont, Ariège, F 09420, France;* [4]*CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands;* [5]*Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands;* [6]*Faculty of Agriculture & Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan;* [7]*Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 93133, U.S.A.;* [8]*School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand;* [9]*International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming, Yunnan, P.R. China 650034*

*Correspondence: Kevin D. Hyde, kdhyde2@gmail.com

**Abstract:** Five loci, nucSSU, nucLSU rDNA, *TEF1*, *RPB1* and *RPB2*, are used for analysing 129 pleosporalean taxa representing 59 genera and 15 families in the current classification of *Pleosporales*. The suborder *Pleosporineae* is emended to include four families, *viz. Didymellaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae* and *Pleosporaceae*. In addition, two new families are introduced, i.e. *Amniculicolaceae* and *Lentitheciaceae*. *Pleomassariaceae* is treated as a synonym of *Melanommataceae*, and new circumscriptions of *Lophiostomataceae s. str.*, *Massarinaceae* and *Lophiotrema* are proposed. Familial positions of *Entodesmium* and *Setomelanomma* in *Phaeosphaeriaceae*, *Neophaeosphaeria* in *Leptosphaeriaceae*, *Leptosphaerulina*, *Macroventuria* and *Platychora* in *Didymellaceae*, *Pleomassaria* in *Melanommataceae* and *Bimuria*, *Didymocrea*, *Karstenula* and *Paraphaeosphaeria* in *Montagnulaceae* are clarified. Both ecological and morphological characters show varying degrees of phylogenetic significance. *Pleosporales* is most likely derived from a saprobic ancestor with fissitunicate asci containing conspicuous ocular chambers and apical rings. Nutritional shifts in *Pleosporales* likely occured from saprotrophic to hemibiotrophic or biotrophic.

**Key words:** Environmental habit, evolution, molecular phylogeny, nutritional mode, taxonomy.
**Taxonomic novelties:** *Amniculicolaceae* Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, fam. nov., *Kalmusia brevispora* (Nagas. & Y. Otani) Yin. Zhang, Kaz. Tanaka & C.L. Schoch, comb. nov., *Lentitheciaceae* Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, fam. nov., *Lophiotrema neoarundinaria* (Ellis & Everh.) Yin. Zhang, Kaz. Tanaka & K.D. Hyde, comb. nov., *Lophiotrema rubi* (Fuckel) Yin. Zhang, C.L. Schoch & K.D. Hyde, comb. nov., *Murispora rubicunda* (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov., *Murispora* Yin. Zhang, J. Fourn. & K.D. Hyde, gen. nov., *Neomassariosphaeria grandispora* (Sacc.) Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov., *Neomassariosphaeria typhicola* (P. Karst.) Yin. Zhang, J. Fourn. & K.D. Hyde, comb. nov., *Neomassariosphaeria* Yin. Zhang, J. Fourn. & K.D. Hyde, gen. nov.

## INTRODUCTION

*Pleosporales* is the largest order in the class *Dothideomycetes*, with a reported 23 families, 332 genera and more than 4 700 species (Kirk *et al*. 2008), or 19 families and 174 genera in Lumbsch & Huhndorf (2007)*. Members of *Pleosporales* can be endophytes or epiphytes (Huang *et al*. 2008, Sánchez Márquez *et al*. 2008, Tao *et al*. 2008), parasitic on green leaves or stems (Wetzel *et al*. 1999, Solomon *et al*. 2006), lichenicolous (Calatayud *et al*. 2001), saprobic on dead leaves or stems in terrestrial or aquatic environments (Câmara *et al*. 2002, Ramesh 2003, Kodsueb *et al*. 2008, Zhang *et al*. 2008b, 2009a), or occur on animal dung (Kruys *et al*. 2006, Kruys & Wedin 2009).

The circumscription of *Pleosporales* has undergone great changes in the last half century. The name *Pleosporales* was first proposed in 1955 by Luttrell to accommodate members of *Dothideomycetes* having perithecioid ascomata with pseudoparaphyses amongst the asci, and seven families, *i.e. Botryosphaeriaceae*, *Didymosphaeriaceae*, *Herpotrichiellaceae*, *Lophiostomataceae*, *Mesnieraceae*, *Pleosporaceae* and *Venturiaceae* were included. Luttrell (1973) redefined the concept of *Pleosporales* based on ascomatal morphology, ascal arrangement in locules, presence or absence of hamathecial tissue, shape of papilla or ostioles, ascospore features and type of habitats, and added three more families, *i.e. Dimeriaceae*, *Mycoporaceae* and *Sporormiaceae*. The morphology of the pseudoparaphyses was given much importance at the ordinal level classification when Barr (1983) introduced *Melanommatales* to accommodate pleosporalean taxa with trabeculate pseudoparaphyses (*Sporormia*-type centrum development) as compared to cellular pseudoparaphyses (*Pleospora*-type centrum development) possessed by other members of *Pleosporales*. Due to the lack of a Latin description in the original publication, *Pleosporales* was formally established in 1987 (Barr 1987b), and was characterised by perithecioid ascomata, usually with a papillate apex, an ostiole with or without periphyses, cellular pseudoparaphyses, fissitunicate asci, and ascospores with various shapes, pigmentation and septation. Barr's concept included previous families, *i.e. Botryosphaeriaceae*, *Dimeriaceae*, *Lophiostomataceae*, *Mesnieraceae*, *Pleosporaceae, Venturiaceae,* plus 15 additional families, *i.e. Arthopyreniaceae*,

*Note*: Recent phylogenetic studies indicated that *Mytilinidiaceae* (Boehm *et al*. 2009), *Phaeotrichaceae* (unpubl. data) and *Venturiaceae* (Schoch *et al*. 2009a; this volume) should be excluded from *Pleosporales*. Thus 23 families (including five newly introduced families: *Aigialaceae*, *Amniculicolaceae*, *Lentitheciaceae*, *Tetraplosphaeriaceae* and *Trematosphaeriaceae*), about 200 genera and 3 000 species are accepted in the current concept of *Pleosporales* in the present study.

*Coccoideaceae*, *Cucurbitariaceae*, *Dacampiaceae*, *Hysteriaceae*, *Leptosphaeriaceae*, *Micropeltidaceae*, *Parodiellaceae*, *Phaeosphaeriaceae*, *Phaeotrichaceae*, *Pleomassariaceae*, *Polystomellaceae*, *Pyrenophoraceae*, *Tubeufiaceae* and *Vizellaceae*. Recent phylogenetic analysis based on DNA sequence data however, have indicated that the *Pleospora*-type and *Sporormia*-type of centrum development (cellular *versus* trabeculate pseudoparaphyses) are not natural groupings, as taxa with these centrum types are dispersed in phylogenetic trees (Liew *et al*. 2000, Lumbsch & Lindemuth 2001). Thus members of *Melanommatales* were assigned to *Pleosporales*, and consequently, *Melanommatales* was treated as a synonym of *Pleosporales* (Eriksson 2006). Nineteen families have been assigned to *Pleosporales* in Kirk *et al*. (2001), 13 in Eriksson (2006), and 19 in Lumbsch & Huhndorf (2007).

One important reason for the unstable circumscriptions in the traditional classification of the *Pleosporales* is that the value given to the various morpho-characters, even those used at high-level classification, has proven to be overstated. For instance, fruiting-body shapes, *i.e.* cleistothecioid, perithecioid and apothecioid, previously considered sanctum at class level classification, were found to have undergone convergent evolution (Hawksworth & Lagreca 2007), as can be seen across *Ascomycota* (Schoch *et al*. 2009a). Another important distinguishing character, ascus type, has been reported to be phylogenetically misleading in numerous natural groups (Schmitt & Lumbsch 2004, Wedin *et al*. 2005, Lumbsch *et al*. 2007). Indeed, several DNA sequence based phylogenetic reconstructions have shown that ascospore morphology has little phylogenetic significance at familial or generic level classification (Crous *et al*. 2003, Schmitt & Lumbsch 2004, Kodsueb *et al*. 2006, Wang *et al*. 2007, Zhang *et al*. 2009b). Consequently, an increasing number of taxa designated only by morphological characterisations in *Pleosporales* have been reported to be polyphyletic, such as the families *Pleosporaceae* (Kodsueb *et al*. 2006), *Melanommataceae* (Liew *et al*. 2000, Wang *et al*. 2007) and genera *Massariosphaeria* (Wang *et al*. 2007), *Melanomma* (Wang *et al*. 2007), *Massarina* and *Lophiostoma* (Liew *et al*. 2002, Zhang *et al*. 2009b).

Various anamorph genera have been recorded in *Pleosporales* and include both hyphomycetes and coelomycetes. Anamorph genera are often associated with multiple teleomorph genera, and in many cases anamorph relationships described in older literature have not yet been tested with DNA sequence data (Farr *et al*. 1989, de Gruyter *et al*. 2009). In the few cases where this was done, anamorph genera such as *Ampelomyces, Ascochyta, Coniothyrium* and *Phoma* proved to be polyphyletic and associated with multiple teleomorphic genera (Aveskamp *et al.* 2008, de Gruyter *et al*. 2009).

Besides the morphological characters used in traditional taxonomy, several other biological characters have been used to define families. For instance, metabolite production and substrate staining reactions have been shown to be phylogenetically informative in xylariaceous and pleosporalean taxa (Stadler *et al*. 2001, 2004, 2007, Stadler & Fournier 2006, Bitzer *et al*. 2008, Zhang *et al*. 2009a). Host spectrum has been used to distinguish between *Phaeosphaeria* and *Leptosphaeria* (Holm 1957, Shoemaker & Babcock 1989), and anamorphic stages have been used to distinguish *Pleospora* and *Lewia* (Simmons 1986, 2007).

Since the first attempts at a classification of the order *Pleosporales* it has been a challenge to address the enormous diversity in biology, morphology and ecology within a stable classification. Thus, in molecular studies comprehensive taxon sampling is essential in order to avoid biased conclusions. To counteract this, a large number of taxa from various families and habitats, in particular generic types were included in the present phylogenetic analysis. The aims of the present investigation are: 1) to build up an overall molecular phylogenetic framework based on a multi-gene analysis showing the interfamilial relationships in the *Pleosporales*; 2) to re-evaluate the significance of morphological or ecological characters used in phylogeny and taxonomy of the order; and 3) to redefine hypotheses for evolutionary trends in the *Pleosporales*.

## MATERIALS AND METHODS

### Collection and examination of specimens

Twenty-eight fresh specimens were collected in Europe (the majority from France) during 2004 to 2008 by J. Fournier, and returned to the laboratory for examination. In most cases ascomata were collected directly on natural wood without incubation. The samples were processed and examined following the method described in Tsui *et al*. (2000). Colonies were sub-cultured onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), 2 % malt extract agar (MEA), and oatmeal agar (OA) (Crous *et al*. 2009b), and incubated under continuous near-UV light at 25 °C to promote sporulation. Observations and photographs were prepared from material mounted in water, congo red, cotton blue, chlorazol black, aqueous nigrosin, lactic acid or Indian ink. Additional cultures used in this study were obtained from the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands. Nomenclatural novelties and descriptions were deposited in MycoBank (Crous *et al*. 2004).

### Fungal isolates and DNA extraction

Total genomic DNA was extracted from mycelia following the protocols as outlined by Cai *et al*. (2006) and Shenoy *et al*. (2007). A second set of DNA samples were obtained following DNA extraction protocols outlined in Schoch *et al*. (2007). In cases where no cultures could be obtained, a Forensic Kit (UltraClean™ Forensic Kit, Cambio) was used to extract DNA from specimens directly.

### DNA amplification and sequencing

DNA amplification was performed by PCR. For partial large subunit (28S, LSU) nuclear rDNA amplification (nu-rDNA), LROR and LR5 primers (Vilgalys & Hester 1990) were used. Primer pairs NS1 and NS4 were used to amplify a region from the small subunit (18S, SSU) of the nu-rDNA (White *et al.* 1990). The f*RPB2*-5F and f*RPB2*-7cR primers were used for the amplification of the partial RNA polymerase second largest subunit (*RPB2*) (Liu *et al.* 1999). The *EF1*-Fα and *EF1*-Rα primers were used to amplify a region from the translation elongation factor 1-alpha gene (*TEF1*) (Schoch *et al*. 2006) and the *RPB1*-Ac and *RPB1*-Cr primers were used for *RPB1* region (Schoch *et al*. 2009; this volume). The amplification reaction for partial LSU, SSU and *TEF1* nu-rDNA genes was performed in a 50 µL reaction volume as outlined by Jeewon *et al*. (2004) and Shenoy *et al*. (2007): 1 × PCR buffer, 0.2 mM dNTPs, 0.3 µM of each primer; 1.5 mM $MgCl_2$, 0.8 units *Taq* polymerase and 5–10 ng gDNA. The PCR thermal cycle programme for partial LSU nu-rDNA amplication was as follows: 95 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 30 s and elongation at 72 °C for 1 min,

with a final extension step of 72 °C for 10 min (Vilgalys & Hester 1990). The PCR thermal cycle programme for the partial *RPB2* gene amplification consisted of 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 2 min and elongation at 72 °C for 90 s, with a final extension step of 72 °C for 10 min (Liu *et al*. 1999). The PCR products, spanning approximately 700 bp (*TEF1*), 900 bp (partial LSU) and 1200 bp (partial SSU and *RPB2*), were checked on 1 % agarose electrophoresis gels stained with ethidium bromide. The PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (GFX PCR DNA and Gel Band Purification Kit, Amersham Biosciences, Buckinghamshire, U.K.). DNA sequencing was performed using the above-mentioned primers in an Applied Biosystem 3730 DNA analyser at the Genome Research Centre, the University of Hong Kong.

## Sequence alignment and phylogenetic analyses

Sequences were obtained from WASABI (Kauff *et al*. 2007) as well as from previous publications (*e.g.* Lutzoni *et al*. 2004, Schoch *et al*. 2009b). Taxa was aligned by using default options for a simultaneous method of estimating alignments and tree phylogenies, SATé (Liu *et al*. 2009). Protein coding fragments were translated in BioEdit v. 7.0.1 (Hall 2004) and aligned within SATé as amino acids. These were aligned with their respective DNA sequences using the RevTrans 1.4 Server (Wernersson & Pedersen 2003). Subsequently, newly generated sequences were added to this initial alignment with MAFFT v. 6.713 (Katoh *et al*. 2009).

A supermatrix of five genes (LSU, SSU, *TEF1*, *RPB1*, *RPB2*) consisting of 47 % gaps and undetermined characters across 171 taxa was obtained. Most taxa had at least two genes present – except for a set of nine taxa with closely related species needed to confirm their identity (Table 1 - see online Supplementary Information).

### Conflict tests

Conflict tests were conducted by selecting single gene data sets and doing comparisons on a gene-by-gene basis applying the bootstrapping criterion in RAxML v. 7.0.4 (Stamatakis *et al*. 2008), using the CIPRES 2.1 webportal (Miller *et al*. 2009) to produce trees of comparative gene sets where all taxa have the gene present. Comparisons between two sets of gene trees were done using a script (compat.py; Kauff & Lutzoni 2002) obtained through the Lutzoni lab website (www.lutzonilab.net/downloads/index.shtml) to detect taxa within clades with a cut-off value of 70 %. This is also performed as in Schoch *et al*. (2009).

A phylogenetic analysis was performed using RAxML v. 7.2.2 (Stamatakis 2006) applying unique model parameters for each gene and codon. The data set was thus partitioned in 11 partitions as previously done in Schoch *et al.* (2009b). In addition a general time reversible model (GTR) was applied with a discrete gamma distribution and four rate classes. One hundred successive most likely tree searches were done in RAxML under the same model, each one starting from a randomised tree with joint branch length optimisation and a rapid hill climbing option. Bootstrap pseudoreplicates were performed 145 times using the fast bootstrapping option and a frequency-based bootstrapping criterion (Stamatakis *et al*. 2008). These were plotted above the nodes in the most likely tree obtained earlier. The values below the nodes are percentages of 500 jacknife resamplings performed in TNT for MS windows with a new technology search set to 20 (Goloboff *et al*. 2008).

# RESULTS AND DISCUSSION

## DNA phylogeny

The tree presented in Fig. 1 represents the most complete phylogeny of *Pleosporales* produced to date. In addition it contains the members of other potential orders in *Pleosporomycetidae* and *Dothideomycetes* for outgroup comparisons. The tree was rooted with two *Arthoniomycetes* as outgroups, *Opegrapha varia* and *O. dolomitica* (not shown). The supermatrix analysed in this study produced 4 290 distinct alignment patterns distributed as follows across the various partitions: SSU – 563, LSU – 807, *RPB1* codon1 – 232, *RPB1* codon2 – 198, *RPB1* codon3 – 333, *RPB2* codon1 – 467, *RPB2* codon2 – 404, *RPB2* codon3 – 614, *TEF1* codon1 – 185, *TEF1* codon2 – 176 and *TEF1* codon3 – 311. The highest scoring likely tree had a log likelihood of -107754.307532.

## Families of *Pleosporales*

In total, 151 taxa (171 strains) of *Ascomycota* (including the outgroups *Opegrapha dolomitica* and *O. varia*) were included in the analysis. It comprises 149 taxa (169 strains) of *Dothideomycetes*, of which 129 taxa (148 strains) were *Pleosporales*. The *Pleosporales* formed a well-supported clade (Fig. 1). The pleosporalean taxa comprised of representatives from 59 pleosporalean genera out of about 200 known genera (*ca*. 30 %), with 39 generic types of *Pleosporales* included in the analysis. As shown in Fig. 1, *Pleosporales* can be subdivided into 17 clades with more than 70 % ML bootstrap (MLB) or 65 % Jacknife (JK); 15 representing familial ranks, *i.e. Aigialaceae*, *Delitschiaceae*, *Didymellaceae*, *Leptosphaeriaceae*, *Lophiostomataceae s. str.*, *Massarinaceae*, *Melanommataceae*, *Montagnulaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Sporormiaceae*, *Trematosphaeriaceae* and *Massariaceae* (Lumbsch & Huhndorf 2007, Kirk *et al*. 2008), as well as *Amniculicolaceae* and *Lentitheciaceae*, which are newly introduced in this paper. Based on the multi-gene phylogenetic data generated here, a new circumscription of *Pleosporales* is given as follows:

***Pleosporales*** Luttr. ex M.E. Barr, *Prodromus to class Loculoascomycetes*: 67. 1987. **emend.**

Hemibiotrophic, saprobic, hypersaprobic, or lichenised. Habitats in freshwater, marine or terrestrial environment. *Ascomata* perithecioid, rarely cleistothecioid, immersed, erumpent to superficial, globose to subglobose, or lenticular to irregular, with or without conspicuous papilla or ostioles. *Ostioles* with or without periphyses. *Peridium* usually composed of a few layers of cells with various shapes and structures. *Hamathecium* persistent, filamentous, very rarely decomposing. *Asci* bitunicate, fissitunicate, cylindrical, clavate to obclavate, with or without pedicel. *Ascospores* hyaline or pigmented, ellipsoidal, broadly to narrowly fusoid or filiform, mostly septate.

*Anamorphs*: *Acroconidiellina*, *Alternaria*, *Aposphaeria*, *Ascochyta*, *Ascochytella*, *Bipolaris*, *Ceratophoma*, *Coniothyrium*, *Corynespora*, *Curvularia*, *Cytoplea*, *Drechslera*, *Exserohilum*, *Hendersonia*, *Leptophoma*, *Metabotryon*, *Microsphaeropsis*, *Myxocyclus*, *Nigrolentilocus*, *Nimbya*, *Phoma*, *Pithomyces*, *Pleurophomopsis*, *Prosthemium*, *Pseudospiropes*, *Pyrenochaeta*, *Scolecosporiella*, *Scolicosporium*, *Shearia*, *Sphaerellopsis*, *Stagonospora*, *Steganosporium*, *Stemphylium* and *Tiarospora* (www.cbs.knaw.

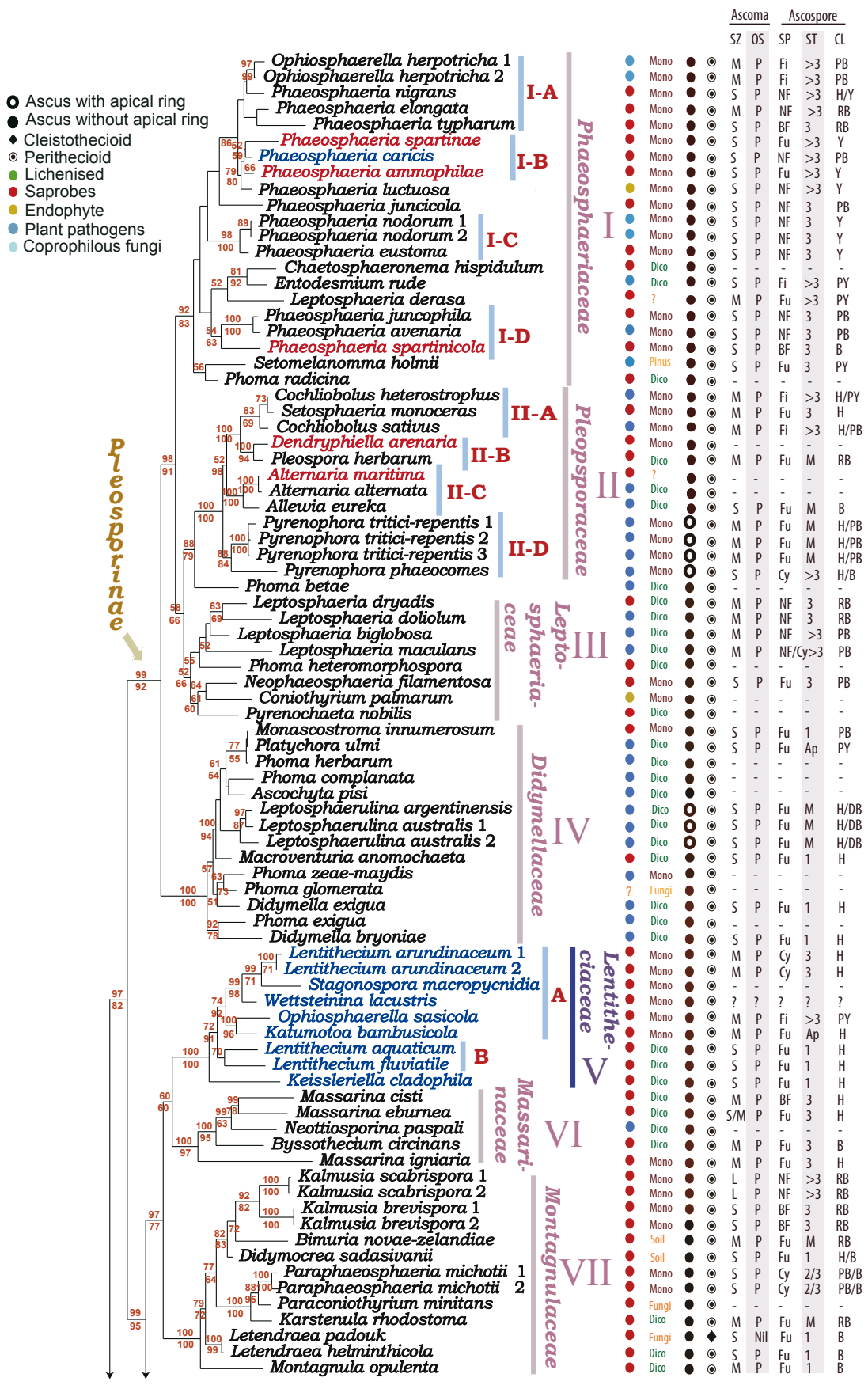


**Fig 1.** RAxML tree with bootstrap values after 1000 pseudo repetitions on the nodes. The values below the nodes are percentages of 500 jacknife resamplings. Pleosporalean leaves highlighted in red and bold are marine or maritime taxa, in blue and bold are freshwater taxa, and others are terrestrial ones. Relevant biological or morphological characters plotted on the leaves are abbreviated as follows: Biology: Mono – monocotyledons; Dico – dicotyledons; Gy – Gymnosperm; SF – Stream foam; ? – unknown; X – no information. Morphology: SZ – size, OS – ostiole, SP – shape, ST – septum, CL – colour; Ascoma size: S – small (diam < 300 µm), M –medial (300 µm < diam < 600 µm), L – large (diam > 600 µm); ostiole: P – pore-like ostiole, Sl – slite-like ostiole, Nil – no opening. Ascospore shape: Fi – filiform, Fu – fusiform, NF – narrowly fusiform, BF – broadly fusiform, Cy – cylindrical; ascospore septum: 1 – one transverse septum, 2 – two transverse septa, 3 – three transverse septa, >3 – more than three transverse septa, M– muriform, Ap – apiosporous; ascospore colour: H – hyaline, B – brown, PB – pale brown, RB – reddish brown, DB – dark brown, Y – yellow, PY – pale yellow. ? – characters unknown. - – anamorph strain.

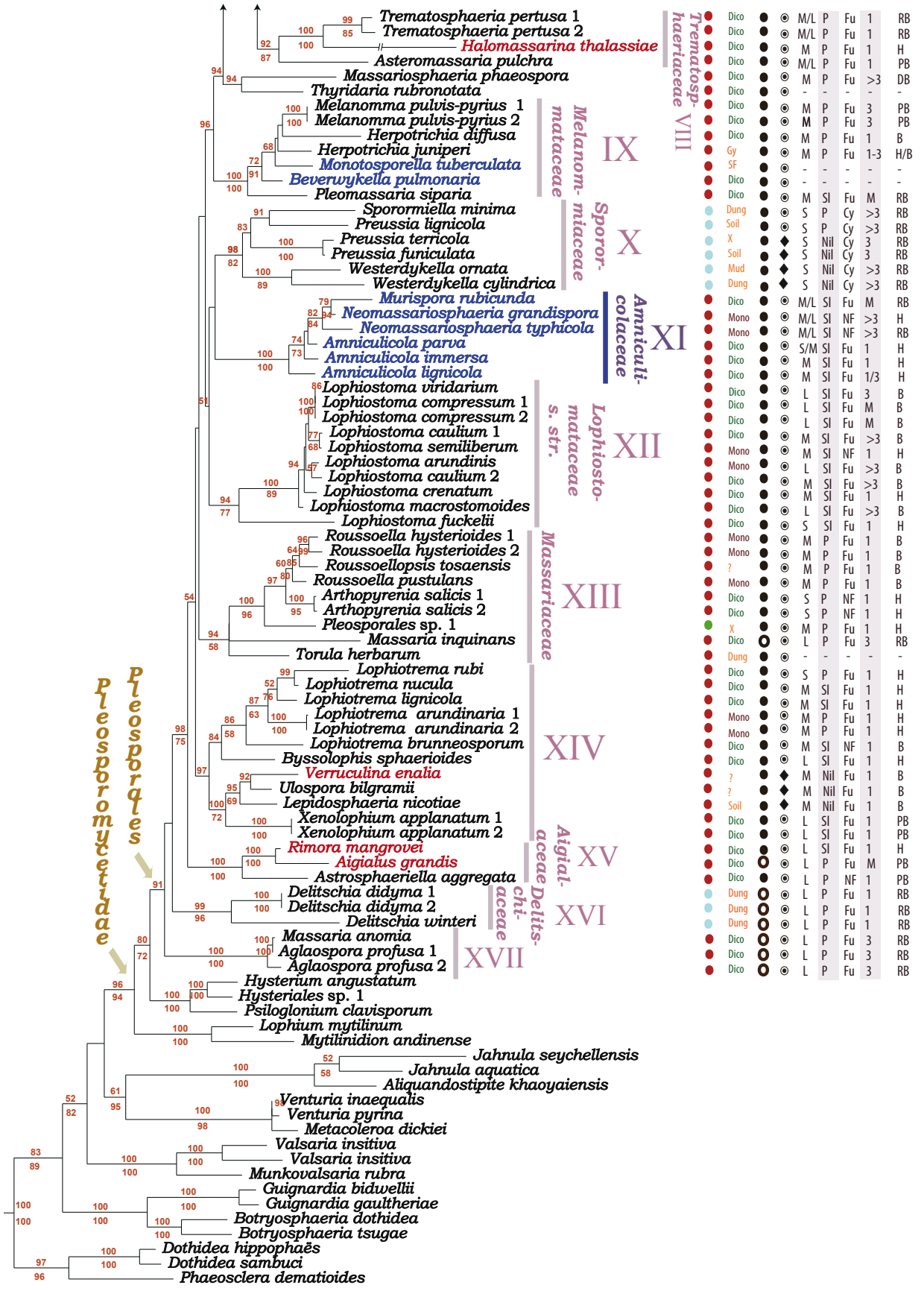


**Fig 1.** (Continued).

nl/databases/anateleo.htm 04-2009, www.indexfungorum.org/ 12-2009, www.mycobank.org/DefaultPage.aspx 12-2009). It should be noted that these anamorphs are based on literature data, and the anamorph-teleomorph relations based on *in vitro* studies or molecular data are provided in the following families.

## *Pleosporineae*

*Pleosporales* contains many notorious plant pathogens, most belonging to one of four families, *viz. Didymellaceae*, *Leptosphaeriaceae*, *Phaeosphaeriaceae* and *Pleosporaceae*. These four families cluster together with high support (MLB = 99 %, JK = 92 %) (Fig. 1). Most taxa in these families are associated with living plants and many are serious plant pathogens (Shoemaker & Babcock 1989, Ueng *et al*. 2003, Rouxel & Balesdent 2005). Examples of important plant pathogens representing the different families are *Cochliobolus heterostrophus* (*Pleosporaceae*), the cause of southern corn leaf blight on maize (White 1999), *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*) the cause of wheat glume blotch (Vergnes *et al*. 2006), *Didymella pisi* (*Didymellaceae*), the cause of Ascochyta blight of pea (Chilvers *et al.* 2009) and *Leptosphaeria maculans* (*Leptosphaeriaceae*) the cause of stem canker on *Brassica* crops (Rouxel & Balesdent 2005). Because of their economic importance, members of *Pleosporineae* have already been subject to extensive molecular phylogenetic and pathogenic investigations over several decades (Wehmeyer 1961, Shoemaker 1976, 1984a, Shoemaker & Babcock 1985, Simmons 1986, Barr 1992). This includes studies on taxonomy, fungus-host interactions, biochemistry and genomics. Recently, the production of full genome data sets have spurred renewed interest in species such as *Stagonospora nodorum* (Solomon *et al*. 2006, Hane *et al.* 2007), *Leptosphaeria maculans* (Rouxel & Balesdent 2005), and *Alternaria brassicicola* (Pedras *et al*. 2009). The designation of *Pleosporineae* was first proposed by Barr (1979) to accommodate fungi having "globose, depressed, conic or vertically elongated ascomata, with a peridium equal in thickness or thickened at the lower sides". Six families were included, *viz*. *Mesnieraceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Pyrenophoraceae*, *Tubeufiaceae* and *Venturiaceae* (Barr 1979). The findings here support previous phylogenetic studies in concluding that the ordinal type, *Pleosporaceae,* and the families *Phaeosphaeriaceae*, *Leptosphaeriaceae* and *Didymellaceae* form a robust clade, and consistently occupy the terminal branches of pleosporalean dendrograms (Liew *et al*. 2000, Kodsueb *et al*. 2006, Kruys *et al*. 2006, Schoch *et al*. 2006, de Gruyter *et al*. 2009). Thus *Pleosporineae* is emended here to accommodate these four families. Many anamorphic stages of the *Pleosporineae* are coelomyceteous genera, which includes *Ascochyta*, *Chaetosphaeronema, Coniothyrium, Microsphaeropsis, Pleurophoma, Phoma,* and *Stagonospora* (de Gruyter *et al*. 2009). However, hyphomyceteous anamorphs such as *Bipolaris*, *Alternaria* or *Stemphylium* are also included (Simmons 1986).

***Pleosporineae*** Barr, Mycologia 71: 947. 1979. **emend.**

Mostly hemibiotrophic or saprobic, rarely symbiotic. *Ascomata* perithecioid, immersed, erumpent to superficial; globose to subglobose, ovoid or obpyriform. *Hamathecium* broadly to narrowly trabeculate or cellular pseudoparaphyses, rarely deliquescing at maturity. *Asci* bitunicate, fissitunicate, usually basal, rarely extending laterally, cylindrical, clavate to oblong. *Ascospores* mostly pigmented, rarely hyaline, one- to multi-septate or muriform, symmetrical or rarely assymmetrical.

*Anamorphs*: *Acroconidiellina*, *Alternaria*, *Ascochyta*, *Ascochytella*, *Bipolaris*, *Coniothyrium*, *Curvularia*, *Drechslera*, *Exserohilum*, *Leptophoma*, *Metabotryon*, *Nimbya*, *Phoma*, *Pithomyces*, *Scolecosporiella*, *Stagonospora*, *Stemphylium* and *Tiarospora* (www.cbs.knaw.nl/databases/anateleo.htm 04-2009, www.indexfungorum.org/ 12-2009, www.mycobank.org/DefaultPage.aspx 12-2009).

### Clade I *Phaeosphaeriaceae*

The clade of *Phaeosphaeriaceae* (MLB = 92 %, JK = 83 %) comprises 19 taxa including the generic types of *Amarenomyces* (*A. ammophilae*), *Entodesmium* (*E. rude*) and *Setomelanomma* (*S. holmii*), as well as the species *Leptosphaeria derasa*, *Ophiosphaerella herpotricha* and some other *Phaeosphaeria* species, such as *P. avenaria*, *P. eustoma* and *P. nodorum* (Fig. 1). This clade could be further subdivided into four subclades, *i.e.* I-A–D. Of these, I-A comprises species of *Ophiosphaerella* and *Phaeosphaeria*; and I-B–D *Phaeosphaeria* species.

*Phaeosphaeriaceae* is an important family in the *Pleosporales*, comprising 19 genera and 394 species (Kirk *et al*. 2008), with many plant pathogens or forming associations with plants (Shoemaker & Babcock 1989, Carson 2005, Stukenbrock *et al*. 2006). *Phaeosphaeriaceae* was introduced by Barr (1979) based on a pseudoparenchymatous peridium almost equal in thickness, and narrowly fusiform or filiform, hyaline, pale brown or rarely dark brown ascospores, and was assigned under *Pleosporales sensu* Barr. The anamorphs are coelomycetes. Fourteen genera were included, *viz*. *Comoclathris*, *Didymella*, *Eudarluca*, *Heptameria*, *Leptosphaeria*, *Loculohypoxylon*, *Metameris*, *Microthelia*, *Nodulosphaeria*, *Ophiobolus*, *Paraphaeosphaeria*, *Rhopographus*, *Scirrhodothis* and *Teichospora* (Barr 1979). Subsequent phylogenetic studies indicated that the *Phaeosphaeriaceae* is heterogeneous, and *Leptosphaeriaceae* was introduced to accommodate species related to *Leptosphaeria* (Barr 1987a), which is supported by subsequent phylogenetic results (Fig. 1; Khashnobish & Shearer 1996, Câmara *et al*. 2002, de Gruyter *et al*. 2009).

*Phaeosphaeria*, as the familial type of *Phaeosphaeriaceae*, was first introduced by Miyake (1909), but was regarded as a synonym of *Leptosphaeria* for a long time. Holm (1957) noticed the presence of pseudoparaphyses in the generic type of *Phaeosphaeria* (*P. oryzae*), reinstated *Phaeosphaeria*, assigned some *Leptosphaeria* (*s. l.*) species with relatively small ascomata which occurred on monocotyledons to *Phaeosphaeria*, and treated 17 species. Subsequently, more species and information were added (Hedjaroude 1968, Leuchtmann 1984, Shoemaker & Babcock 1989). In a world monograph, 114 species of *Phaeosphaeria* were treated, and they were further divided into 6 subgenera, *viz. Ovispora*, *Fusispora*, *Phaeosphaeria*, *Spathispora*, *Vagispora* and *Sicispora*, based on differences in ascospore shape and the number of septa (Shoemaker & Babcock 1989). Many species of *Phaeosphaeria* have characteristic gelatinous sheaths on spores, and some are dictyosporous (Eriksson 1967). Currently, *ca.* 80 species are accepted under *Phaeosphaeria*, and many of them have *Stagonospora* anamorphs (Kirk *et al*. 2008).

Two of the three strains in subclade I-B are isolated from maritime environments; *e.g. P. ammophilae* from beach grass *Ammophila arenaria* and *Phaeosphaeria spartinae* from stems of *Spartina alterniflora* in estuarine salt marshes. A strain of *Phaeosphaeria caricis* (CBS 120249) used here was isolated from *Typha latifolia* occurring in or near freshwater. All species in the other three subclades (I-A, C–D, Fig. 1) are associated

with terrestrial or near freshwater grasses such as *P. elongata* with *Miscanthus sinensis*, *P. juncophila* with *Juncus articulatus* and *Ophiosphaerella herpotricha* with *Bromus erectus*. The only exception is *Phaeosphaeria spartinicola*, which was isolated from salt marsh grass (*Spartina alterniflora*).

*Amarenomyces* was separated from *Phaeosphaeria* (as *Amarenomyces ammophilae*) based on its multilayered endotunica and large and thick-walled, sheathed ascospores (Eriksson 1981). However, its relationship with other *Phaeosphaeria* species is supported in this study. Thus *Amarenomyces* is treated as a synonym of *Phaeosphaeria*. *Entodesmium* is exclusively associated with legumes, and is traditionally assigned to *Lophiostomataceae* based on its periphysate papilla (Eriksson & Hawksworth 1990, Barr 1992). But its immersed ascomata, non-compressed papilla and thin peridium, plus the multiseptate, lightly pigmented ascospores, which break up into part-spores support its inclusion in *Phaeosphaeriaceae*. In particular, *Entodesmium multiseptatum* and *E. niessleanum* were originally described as a *Leptosphaeria* species (Shoemaker 1984b), indicating their similarity with *Phaeosphaeria* which is commonly confused with *Leptosphaeria* (Shoemaker 1984a, Shoemaker & Babcock 1989).

*Notes*: Although members of the *Phaeosphaeriaceae* are usually known as saprobes or parasites of plants or other fungi, the strain of *Phaeosphaeria luctuosa* (CBS 308.79) in this clade is recorded as an endophyte in *Zea mays*. In addition, the inclusion of *Entodesmium rude* in this clade indicates the ascospores of this family can be filiform.

*Currently accepted genera:* ?* *Ophiosphaerella*, ? *Phaeosphaeria*, *Entodesmium* and *Setomelanomma*.

*Anamorphs*: *Ampelomyces*, *Chaetosphaeronema*, *Coniothyrium*, *Phoma*, *Plenodomus*, *Stagonospora* and *Wojnowicia* (Leuchtmann 1984, de Gruyter *et al*. 2009).

The genera *Ampelomyces, Coniothyrium, Phoma* and *Plenodomus* are polyphyletic (de Gruyter *et al*. 2009). The generic type species *Ampelomyces quisqualis* clustered in the *Phaeosphaeriaceae*, whereas *A. quercinus* grouped in the *Didymellaceae*. The type species of the genera *Phoma*, *Coniothyrium* and *Pleonodomus* clustered in the *Didymellaceae* and *Leptosphaeriaceae* respectively. Although *Chaetosphaeronema* was associated with *Ophiobolus* (Petrak 1944), this teleomorph-anamorph relation has not been confirmed. An isolate preserved as *Trematophoma* sp. was found in the *Phaeosphaeriaceae* (de Gruyter *et al*. 2009); however, its identity needs to be studied in more detail.

## Clade II *Pleosporaceae*

*Pleosporaceae* (Clade II), including the generic type of *Pleospora* — *P. herbarum*, forms a robust clade (MLB = 100 %, JK = 100 %), and comprises four subclades as well, *i.e.* II-A–D. Clade II-A, including the generic type – *Cochliobolus heterostrophus* represents *Cochliobolus*, II-B comprises two taxa, *i.e. Pleospora herbarum* and the anamorphic *Dendryphiella arenaria* (*Scolecobasidium arenarium*), which represents *Pleospora*, II-C represents anamorphic fungi – *Alternaria*, and II-D contains the generic type – *Pyrenophora phaeocomes*, represents *Pyrenophora*.

*Pleosporaceae* comprises 36 genera and 769 species (Kirk *et al*. 2008) and is the largest family in *Pleosporales.* Members have been reported as plant parasites or saprobes occurring on herbaceous or woody plant leaves or stems (Sivanesan 1984). *Pleosporaceae* was introduced by Nitschke (1869), which had been assigned to *Sphaeriales* based on the immersed ascomata and presence of pseudoparaphyses, then to *Pseudosphaeriales* (Theissen & Sydow 1917, Wehmeyer 1975), and the name of *Pseudosphaeriales* subsequently was replaced by *Pleosporales* (Luttrell 1955). Morphology of ascospores, *i.e.* shape, colour, septation and presence or absence of sheaths has been emphasised in defining the circumscriptions of genera under *Pleosporaceae* (Luttrell 1955, 1973, Wehmeyer 1961, 1975, von Arx & Müller 1975, Sivanesan 1984, Barr 1987b, Abler 2003). The polyphyletic nature of *Pleosporaceae* has been indicated in previous investigations, and some genera have been assigned to other families, such as *Leptosphaerulina* to *Leptosphaeriaceae*, and *Macroventuria* to *Phaeosphaeriaceae* (Kodsueb *et al*. 2006). In this study however, the generic types of both *Macroventuria* (*M. anomochaeta*) and *Leptosphaerulina* (*L. australis*) cluster within the *Didymellaceae*, as previously recorded (de Gruyter *et al*. 2009).

The current clade of *Pleosporaceae*, comprising the generic types of *Cochliobolus* (*C. heterostrophus*), *Pleospora* (*P. herbarum*) and *Pyrenophora* (*P. phaeocomes*), represents the core members of *Pleosporaceae*, and are mostly plant pathogens (Fig. 1). Species in subclades II-A and II-D are exclusively associated with monocotyledons, such as *Pyrenophora tritici-repentis* with wheat and *P. phaeocomes* with *Festuca rubra*. *Pleospora herbarum* (Clade II-B) has been recorded as associates of numerous monocotyledons and dicotyledons, while the strain of *Dendryphiella arenaria* is from the root zone soil of beachgrass (*Ammophila arenaria*). Subclade II-C comprises two *Alternaria* species and one *Allewia* species, of which *Alternaria maritima* was isolated from submerged wood in seawater, *A. alternata* is generally occurring on all kinds of substrates, and *Allewia eureka* is associated with terrestrial dicotyledons.

*Notes*: Members of this clade mostly have middle-sized ascomata, and the hyaline and filiform ascospores possessed by *Setosphaeria monoceras* expanded the familial concept from “brown” by Cannon & Kirk (2007) to “hyaline or brown”.

*Currently accepted genera*: ? *Allewia,* ? *Lewia, Cochliobolus*, *Pleospora*, *Pyrenophora* and ? *Setosphaeria*.

*Anamorphs*: *Alternaria*, *Ascochyta*, *Bipolaris, Curvularia*, *Drechslera, Embellisia, Exserohilum*, *Phoma* and *Stemphylium* (Simmons 1986, 1989, 1990, Cannon & Kirk 2007, Aveskamp *et al*. 2008, de Gruyter *et al*. 2009).

Most of the anamorphs in the *Pleosporaceae* are hyphomycetes. Both *Ascochyta* and *Phoma* species have been described in the *Pleosporaceae*. However, the generic type species, *Ascochyta pisi* and *Phoma herbarum*, belong to the *Didymellaceae* (de Gruyter *et al*. 2009).

*Note*: Genera lack generic types or other representative species in the clades are marked as “?” to indicate their uncertain status.

### Clade III *Leptosphaeriaceae*

The clade containing members of *Leptosphaeriaceae* is sister to the *Pleosporaceae*, but receives poor statistical support (Fig. 1), indicating the need for more thorough analysis. It comprises the generic types of *Leptosphaeria* (*L. doliolum*) and *Neophaeosphaeria* (*N. filamentosa*), as well as other taxa from numerous groups, such as *Coniothyrium palmarum*, *L. maculans* (*Leptosphaeriaceae*) and *Pyrenochaeta nobilis* (*Herpotrichia*, *Melanommataceae*).

The *Leptosphaeriaceae* is likely paraphyletic (Schoch *et al*. 2009a; this volume). This taxon was separated from the *Pleosporaceae* and formally introduced by Barr (1987a) based on its "coelomycetous anamorphs" and "narrower and thinner-walled asci" (Barr 1987b), and supported by phylogenetic data (Dong *et al*. 1998). Initially, five genera, *i.e. Curreya*, *Didymolepta*, *Heptameria*, *Leptosphaeria* and *Ophiobolus*, were accepted under *Leptosphaeriaceae* (Barr 1987b), while Eriksson & Hawksworth (1990) only accepted *Leptosphaeria* and *Ophiobolus* under this family. The *Leptosphaeriaceae* only comprises some species of *Leptosphaeria* and *Neophaeosphaeria filamentosa*, as well as the anamorph *Coniothyrium palmarium*. *Pyrenochaeta nobilis* also clustered in the *Leptosphaeriaceae.* However, this species probably represents a closely related subclade (de Gruyter *et al.* 2009).

Morphologically, *Leptosphaeriaceae* is mostly comparable with *Phaeosphaeriaceae*, and numerous characters have been used to distinguish them at generic or family level. For instance, anamorphic states (Câmara *et al*. 2002), peridium structure (Khashnobish & Shearer 1996, Câmara *et al*. 2002) and host spectrum (Câmara *et al*. 2002) have all been proposed in distinguishing *Leptosphaeria s. str*. and *Phaeosphaeria*. Of these characters, the host preference of *Leptosphaeria* on dicotyledons in contrast to *Phaeosphaeria* on monocotyledons has been widely reported (Eriksson 1967, Hedjaroude 1968, Eriksson 1981, Shoemaker & Babcock 1989). Currently, six of the eight species included in *Leptosphaeriaceae* (Fig. 1) have dicotyledonous hosts, while *Coniothyrium palmarum* is associated with palms. Thus present results further support the fact that the host spectrum has phylogenetic significance to some degree (Câmara *et al*. 2002, Voigt *et al*. 2005).

*Currently accepted genera*: *Leptosphaeria* and *Neophaeosphaeria*.

*Anamorphs*: *Chaetodiplodia*, *Coniothyrium*, *Phoma*, *Plectophomella* and *Pyrenochaeta* (Wehmeyer 1975, de Gruyter *et al*. 2009).

The genus *Chaetodiplodia* has been recorded as an anamorph of *Leptosphaeria* (Wehmeyer 1975), but not confirmed. A *Chaetodiplodia* sp. isolate clustered in the *Leptosphaeriaceae* (de Gruyter *et al*. 2009); however the identity of this strain is uncertain.

### Clade IV *Didymellaceae*

The *Didymellaceae* (Clade IV) receives high bootstrap support, and includes the generic types of *Didymella* (*D. exigua*), *Macroventuria* (*M. anomochaeta*), *Monascostroma* (*M. innumerosum*), *Leptosphaerulina* (*L. australis*) and *Platychora* (*P. ulmi*), as well as some species of *Phoma* and *Ascochyta* (Fig. 1).

This family was introduced to accommodate some species of *Phoma* and their phylogenetically closely related anamorphic taxa, as well as teleomorphs such as *Didymella* and *Leptosphaerulina* (de Gruyter *et al*. 2009, Woudenberg *et al*. 2009). The generic types of *Platychora*, *Monascostroma* and *Macroventuria* are also located in Clade IV. In particular, both *Platychora ulmi* and *Monascostroma innumerosum* have immersed ascomata and clavate asci with lightly pigmented, 1-septate ascospores, and they form a robust subclade (Fig. 1), which most likely represents a single genus. When compared with *M. innumerosum*, the apiosporous ascospores are the most striking character of *Platychora ulmi*. Thus the symmetry of ascospores might have no phylogenetic significance at the generic level.

What is most interesting is that *Leptosphaerulina argentinensis* forms a robust clade with two strains of *L. australis*. Although *L. argentinensis* can be distinguished from *L. australis* by its larger ascospores, their morphological similarity can not be ignored (Graham & Luttrell 1961). Thus this subclade most likely represents a species complex for *L. australis*.

Most species in this clade are associated with dicotyledons, such as *Macroventuria anomochaeta* with *Medicago sativa*, *Phoma cucurbitacearum* with *Cucurbita* spp., *Didymella exigua* with *Rumex arifolius*, *Leptosphaerulina argentinensis* with *Lonicera periclymenum* and *Ascochyta pisi* with *Pisum sativum*, while *Leptosphaerulina australis* and *Phoma herbarum* are associated with a wide range of hosts including dicotyledons and monocotyledons.

*Notes*: Besides the characters described by de Gruyter *et al*. (2009), members of *Didymellaceae* are also mostly hemibiotrophic or saprobic, and have sometimes setose ascomata, persistent or deliquescing pseudoparaphyses and fusiform, symmetric or apiosporous ascospores.

*Currently accepted genera*: *Didymella*, *Leptosphaerulina*, *Macroventuria*, *Monascostroma* and *Platychora*.

*Anamorphs*: *Chaetasbolisia*, *Diplodina*, *Microsphaeropsis* and *Phoma* (Aveskamp *et al*. 2008, de Gruyter *et al*. 2009).

The genus *Phoma* is subdivided in nine sections with teleomorphs in the genera *Didymella, Leptosphaeria, Mycosphaerella* and *Pleospora* (Boerema 1997). Molecular studies confirmed the polyphyletic character of *Phoma* in the *Pleosporineae* (de Gruyter *et al.* 2009). The generic type, *Phoma herbarum*, grouped in the *Didymellaceae*, and therefore, *Phoma* species in the *Didymellaceae* are considered as *Phoma s. str.* (de Gruyter *et al.* 2009). The taxonomy of *Phoma* species in the *Leptosphaeriaceae*, *Phaesphaeriaceae* and *Pleosporaceae* needs further study.

### Clade V *Lentitheciaceae*

The clade of *Lentitheciaceae* comprises the generic type *Lentithecium fluviatile,* as well as *L. arundinaceum*, *Stagonospora macropycnidia*, *Wettsteinina lacustris*, *Keissleriella cladophila*, and the bambusicolous species *Katumotoa bambusicola* and *Ophiosphaerella sasicola*, which receives high bootstrap support (MLB = 100 %, JK = 100 %). The teleomorphs have lenticular ascomata, trabeculate to broadly cellular pseudoparaphyses, cylindrical to clavate asci with short pedicels, uni-, 3- to multiseptate, fusiform or filiform ascospores. Based on morphological characters and current molecular phylogenetic results, a new family — *Lentitheciaceae* is introduced to accommodate them.

This clade is further subdivided into two groups. One subclade comprises *Lentithecium arundinaceum*, *Katumotoa bambusicola*, *W. lacustris*, *Ophiosphaerella sasicola* and *Stagonospora macropycnidia* (Clade V-A), while the other subclade (Clade

V-B) comprises *L. fluviatile* and *L. aquaticum* with *Keissleriella cladophila* basal to both. Species of Clade V-A exclusively occur on monocotyledons, such as *Lentithecium arundinaceum* and *Stagonospora macropycnidia* which are isolated from *Phragmites* sp., and *Wettsteinina lacustris* which is recorded on *Schoenoplectus* sp. The strain of *W. lacustris* (CBS 618.86) used here was isolated from *Schoenoplectus lacustris*, and both *Ophiosphaerella sasicola* (from *Sasa senanensis*) and *Katumotoa bambusicola* (from *Sasa kurilensis*) are bambusicolous. In contrast, species of Clade V-B seem to be exclusively associated with dicotyledonous woody substrates in freshwater environments, *i.e. L. aquaticum* and *L. fluviatile* are from submerged wood of *Fraxinus* sp. and *Populus* sp. from France, respectively. The habit details of the *Keissleriella cladophila* strain (CBS 104.55) used here are unknown, but it was isolated from dicotyledonous woody plants (*Smilax parvifolia*) in Pakistan.

The relatively larger ascomata (500–600 *vs*. 300–400 µm) and the sheathed ascospore of *Ophiosphaerella sasicola* make it readily distinguishable from *O. herpotricha*, and the latter is morphologically similar to the generic type of *Ophiosphaerella* (*O. graminicola*). The identification of the strain of *Wettsteinina lacustris* (CBS 618.86) used here could not be verified. According to Shoemaker & Babcock (1989, p. 1596) however, the collections studied by Leuchtmann (collector of CBS 618.86) under this name, represent "a good *Massarina*", which is "not conspecific with *Wettsteinina*". Thus the strain of CBS 618.86 most likely is of *Massarina s. l.*, which is closely related to *Lentithecium*. Both *Ophiosphaerella sasicola* and *Katumotoa bambusicola* are bambusicolous, and they have lenticular ascomata with a simple peridium structure, as well as numerous persistent pseudoparaphyses. All of these characters fit in the traditional concept of *Lentithecium*. However, their ascospores are asymmetrical (*K. bambusicola*) or filiform (*Ophiosphaerella sasicola*), which differs from the symmetrical and cylindrical to fusiform ascospores possessed by other species of *Lentithecium* (Nagasawa & Otani 1997, Tanaka & Harada 2005a).

***Lentitheciaceae*** Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, **fam. nov.** MycoBank MB515470.

Aquaticus vel terrestris. Saprophyticus. Ascomata immersa, lenticulare, solitaria vel disseminata, nigra. Asci bitunicati, fissitunicati, clavati vel oblongati- cylindrici, pedicellati. Ascosporae cylindrica vel fusiforme vel filiforme, uniseptatae vel aliquando 3-septatae cum supra-maturae, parce multiseptatum, hyalinae vel fulvum.

Freshwater or terrestrial habitat. Saprobic. *Ascomata* immersed, lenticular, solitary or scattered. *Peridium* comprising a few layers of thin-walled cells. *Asci* bitunicate, fissitunicate, cylindro-clavate to cylindro-oblong, short pedicellate. *Ascospores* fusiform or filiform, hyaline to pale yellow, 1-septate, constricted at the septum, sometimes becoming 3-septate when mature, rarely multiseptate.

*Type genus*: *Lentithecium* K.D. Hyde, J. Fourn. & Yin. Zhang.

*Notes*: *Lentithecium* was introduced to accommodate some freshwater taxa with lenticular ascomata and hyaline, 1-septate ascospores (Zhang *et al*. 2009b). *Wettsteinina lacustris*, *Ophiosphaerella sasicola*, and the anamorphic *Stagonospora macropycnidia*, as well as *Keissleriella cladophila* and *Katumotoa bambusicola* are also included in this clade. The strain of *Wettsteinina lacustris* used here may be misidentified (see comments above). However, they all have immersed and lenticular ascomata, with thin peridium usually almost equal in thickness, short pedicellate asci and fusiform or filiform, hyaline or rarely lightly pigmented, 1- to multi-septate ascospores. Phylogenetically, they form a robust clade separating them from all other pleosporalean families. Thus a new family, *Lentitheciaceae,* is introduced to accommodate these species of *Massarina s. l.*, a "genus" which should contain species from numerous genera.

*Currently accepted genera*: *Lentithecium*, *Katumotoa* and ? *Keissleriella*.

*Anamorph*: ? *Stagonospora macropycnidia*.

The genus *Stagonospora* is polyphyletic and considered as the anamorph of *Phaeosphaeria* (Leuchtmann 1984), while a strain of *Stagonospora macropycnidia* used here clusters in *Lentitheciaceae* in this study.

### Clade VI *Massarinaceae*

The *Massarinaceae* clade comprises the generic types of *Massarina* (*M. eburnea*) and *Byssothecium* (*B. circinans*), as well as *M. cisti* and *M. igniaria*, and receives high bootstrap support (MLB = 100 %, JK = 97 %). *Massarinaceae* was introduced to accommodate species having immersed, flattened or sphaerical ascomata with or without clypeus, trabeculate or cellular pseudoparaphyses, clavate to cylindro-clavate asci, hyaline, fusiform to narrowly fusiform, 1- to 3-septate ascospores with or without sheath. Five genera were accepted, *i.e. Keissleriella*, *Massarina, Metasphaeria, Pseudotrichia* and *Trichometasphaeria* (Munk 1956). This family name has not been commonly used and the familial type — *Massarina* has usually been placed under the *Lophiostomataceae* (Bose 1961, Eriksson & Yue 1986, Barr 1987b, 1990). The polyphyletic nature of *Massarina* has been noted (Liew *et al*. 2002, Zhang *et al*. 2009b), and a narrow concept of *Massarina* was accepted, which comprises the generic type (*M. eburnea*) and morphologically similar species (*e.g. M. cisti*) (Zhang *et al*. 2009b). The strain of *Byssothecium circinans* (CBS 675.92) in this clade is unverified, thus its status remains unresolved (see comments by Zhang *et al*. 2009b). *Massarina s. str.* comprising *M. cisti*, *M. eburnea* and *M. igniaria* is confirmed based on these five nuclear loci, which represents a separate branch in *Pleosporales*.

***Massarinaceae*** Munk, Friesia 5: 305. 1956. **emend.**

Terrestrial habitat. Saprobic. *Ascomata* immersed, erumpent to superficial with small to wide papilla, solitary or scattered. *Pseudoparaphyses* cellular to narrowly cellular. *Asci* clavate to cylindrical, with short pedicels. *Ascospores* fusiform to broadly fusiform, hyaline or brown, 1- to 3-septate, with or without sheaths.

*Currently accepted genera*: ? *Byssothecium* and *Massarina*.

*Anamorph*: *Periconia*.

The hyphomycete genus *Periconia* is polyphyletic, and in *the Massarinaceae* associated with *Didymosphaeria* (Booth 1968). The coelomycete genus *Neottiosporina* has not been associated with a teleomorph. In this study however, a strain of *N. paspali* grouped in the *Massarinaceae*.

### Clade VII *Montagnulaceae*

The well-supported clade of *Montagnulaceae* (MLB = 100 %, JK = 100 %) comprises the generic types of *Bimuria* (*B. novae-zelandiae*), *Didymocrea* (*D. sadasivanii*), *Karstenula* (*K. rhodostoma*) and *Paraphaeosphaeria* (*P. michotii*), as well as some species of *Kalmusia*, *Paraconiothyrium*, *Letendraea* and *Montagnula*. Based on the morphological and ecological similarities, *Phaeosphaeria brevispora* was assigned to *Kalmusia* (see comments below). Species in this clade can be saprobic (*Kalmusia scabrispora*, *Phaeosphaeria brevispora* and *Bimuria novae-zelandiae*), plant pathogenic (*Paraphaeosphaeria michotii*) or mycoparasitic (*Paraconiothyrium minitans*) (Fukuhara 2002, Verkley *et al*. 2004). *Montagnulaceae* was introduced by promoting the heterogeneric *Montagnula* to familial level, which contains species with three types of ascospores, *i.e.* muriform (*Montagnula*), phragmosporous (*Kalmusia*) and didymosporous (*Didymosphaerella*) (Barr 2001).

*Paraphaeosphaeria* has been treated as a segregate of *Leptosphaeria* based on its swollen cell above the A1 septum and a longer more highly septate upper part and *Coniothyrium s. l.* anamorphs (Eriksson 1967). By analysing the ITS and 18S rDNA sequences, *Paraphaeosphaeria* was shown to be polyphyletic, and a narrow generic concept accepted (Câmara *et al*. 2001). The familial placement of *Paraphaeosphaeria* under *Montagnulaceae* is verified in this study.

Remarkably, our phylogenetic results indicated that the generic type of *Bimuria*, *B. novae-zelandiae* is included in this group. *Bimuria novae-zelandiae* was initially isolated from soil in a barley field in New Zealand, and is characterised by a very thin peridium, mostly 2-spored, fissitunicate asci and muriform, dark brown, verrucose ascospores, which is considered somewhat comparable with *Montagnula* (Hawksworth *et al*. 1979). The thick carbonaceous peridium, however, distinguishes *Montagnula* from *Bimuria*. In addition, the ascospores of *Montagnula* are discharged forcibly through the ostiole instead of simply deliquescing and gathering at the apex of the ascomata as happens in *Bimuria* (Hawksworth *et al*. 1979). Because of its unique morphological characters, the familial placement of this genus has been debatable and it has been placed in *Pleosporaceae* by Hawksworth *et al*. (1979), in *Phaeosphaeriaceae* by Barr (1987b) and in *Melanommataceae* by Lumbsch & Huhndorf (2007). In agreement with previous phylogenetic studies (Schoch *et al*. 2006), its affinity to other members of *Montagnulaceae* is noted here.

The generic type of *Karstenula* (*K. rhodostoma*) clusters in this group, which is characterised by immersed ascomata, usually with a wide ostiolar opening, narrowly cellular pseudoparaphyses, cylindrical asci with short pedicels, and reddish-brown, muriform ascospores (information obtained from type material). Traditionally, *Karstenula* has been assigned to *Melanommataceae*, but the immersed ascomata, narrowly cellular pseudoparaphyses and reddish-brown, muriform ascospores fit the definition of *Montagnulaceae* (Barr 2001), and this placement is confirmed by the present phylogenetic data (Fig. 1). The clade also contains sequences of *Didymocrea sadasivanii* (*Zopfiaceae*) obtained from GenBank, confirming the polyphyly of *Zopfiaceae*, and its placement in relation to *Bimuria*, as noted before (Kruys *et al*. 2006). The fact that this species produces ostensibly unitunicate asci within ascostromatic ascomata makes it especially interesting (Rogerson 1970, Parguey-Leduc & Janex-Favre 1981).

*Notes*: The 2- or 3-spored asci possessed by *Bimuria novae-zelandiae* is another unique character in *Montagnulaceae*.

*Currently accepted genera*: *Bimuria*, *Didymocrea*, ? *Kalmusia*, *Karstenula*, ? *Letendraea*, ? *Montagnula* and *Paraphaeosphaeria*.

*Anamorph*: *Paraconiothyrium* (Verkley *et al.* 2004).

***Kalmusia brevispora*** (Nagas. & Y. Otani) Yin. Zhang, Kaz. Tanaka, C.L. Schoch, **comb. nov.** MycoBank MB515474.

*Basionym*: *Phaeosphaeria arundinacea* var. *brevispora* Nagas. & Y. Otani, Rep. Tottori Mycol. Inst. 15: 38. 1977.

≡ *Phaeosphaeria brevispora* (Nagas. & Y. Otani) Shoemaker & C.E. Babc., Canad. J. Bot. 67: 1523. 1989.

*Notes*: Morphological characters of *Phaeosphaeria brevispora*, such as the immersed ascomata with clypei, thin peridium, clavate asci with relatively long pedicels, and the reddish-brown, verrucose ascospores constricted at the primary septum, fit *Kalmusia* well. Phylogenetically, *P. brevispora* and *K. scabrispora* form a robust clade. In particular, both of these two species occur on *Sasa* sp. (Tanaka & Harada 2004, Tanaka *et al*. 2005b).

### Clade VIII *Trematosphaeriaceae*

The generic type of *Trematosphaeria* (*T. pertusa*) and the marine fungus, *Halomassarina thalassiae*, form a well supported clade (MLB = 100 %, JK = 100 %), and represent a pleosporalean family, *Trematosphaeriaceae*. Details of this family are addressed by Suetrong *et al*. 2009; this volume).

### Clade IX *Melanommataceae* (syn. *Pleomassariaceae*)

The generic types of *Melanomma* (*M. pulvis-pyrius*) and *Pleomassaria* (*P. siparia*), and some other species, *e.g. Monotosporella tuberculata*, *Herpotrichia diffusa* and *H. juniperi*, representing *Melanommataceae*, form a well-supported clade (MLB = 100 %, JK = 100 %). The *Melanommataceae* is one of the largest families in *Pleosporales*, which comprises 21 genera and 265 species (Kirk *et al*. 2008). Traditionally, *Melanommataceae* comprises immersed, erumpent to superficial, gregarious and black, mostly thick-walled ascomata, trabeculate pseudoparaphyses, and cylindrical asci, brown, septate or muriform ascospores. Presence of trabeculate pseudoparaphyses have been emphasised in *Melanommataceae* and several related families, but this proposal was not supported by molecular phylogenetic results (Barr 1990, Liew *et al*. 2000). The strains of *M. pulvis-pyrius* and *P. siparia* were verified by checking the voucher specimens connected to these cultures (Zhang *et al*. 2008a). As the familial type, *Pleomassaria* is characterised by its cellular pseudoparaphyses (Sivanesan 1984). This study further indicates that morphology of pseudoparaphyses has little significance at familial level classification (Liew *et al*. 2000). Herein *Pleomassariaceae* is treated as a synonym of *Melanommataceae*.

Differing from other terrestrial members of this clade, both *Beverwykella pulmonaria* and *Monotosporella tuberculata* are from freshwater. A *Phoma*-like anamorph (*Aposphaeria* ?) has been reported for *Melanomma pulvis-pyrius* (Chesters 1938, Sivanesan 1984). Both *Beverwykella pulmonaria* and *Monotosporella tuberculata* are aquatic hyphomycetous fungi isolated from Europe (Netherlands and U.K., respectively), which indicates that the anamorphs of *Melanommataceae* should include hyphomycetes as well.

*Genera currently accepted*: ? *Herpotrichia*, *Melanomma* and *Pleomassaria*.

*Anamorphs*: *Aposphaeria* (or *Phoma*-like according to Chesters 1938), *Beverwykella pulmonaria*, *Monotosporella tuberculata*, *Prosthemium* and ? *Pyrenochaeta* (Sivanesan 1984, Paavolainen *et al.* 2000).

The genus *Pyrenochaeta* is polyphyletic (de Gruyter *et al.* 2009), and the generic type species *P. nobilis* grouped in the *Leptosphaeriaceae* in this study.

### Clade X *Sporormiaceae*

The *Sporormiaceae* including the generic types of *Preussia* (*P. funiculata*) and *Westerdykella* (*W. ornata*), and some other species such as *Sporormiella minima*, *Preussia lignicola*, *P. terricola* and *Westerdykella cylindrica* form a well-supported clade (MLB = 98 %, JK = 82 %). The *Sporormiaceae* is the largest coprophilous family of *Pleosporales*, which contains 10 genera and 143 species (Kirk *et al*. 2008). The absence of periphyses and well-developed apical rings together with ascomata with or without ostioles, ascospores with or without germ slits have been used to distinguish the *Sporormiaceae* from other coprophilous families, such as the *Delitschiaceae* and the *Phaeotrichaceae* (Barr 2000, Kruys *et al*. 2006). Phylogenetic analysis based on ITS-nLSU rDNA, mtSSU rDNA and ß-tubulin sequences indicated that compared to the shape of the asci or ascospores, the substrate choice, presence or absence of ostiole, and presence or absence of germ slits have less phylogenetic significance within *Sporormiaceae* (Kruys & Wedin 2009). In particular, the presence of periphyses was verified in the generic type of *Sporormiella* (*S. nigropurpurea*, type, NY), which belongs in *Sporormiaceae* (as *Preussia*) (Kruys & Wedin 2009). Currently, after modifying their concept, three genera, *i.e. Sporormia*, *Preussia* and *Westerdykella* are accepted under *Sporormiaceae* (Kruys & Wedin 2009).

***Sporormiaceae*** Munk, Dansk Bot. Ark. 17: 450. 1957.

*Note*: Although strains of *Eremodothis* and *Pycnidiophora* are not included in current analysis, their familial status in *Sporormiaceae* has been well demonstrated (Kruys & Wedin 2009), and the cleistothecioid ascomata of *Eremodothis* and *Pycnidiophora* is another striking character of this family.

*Currently accepted genera*: ? *Sporormia* (including *Sporormiopsis*), *Preussia* (including *Sporormiella* and *Spororminula*) and *Westerdykella* (including *Eremodothis* and *Pycnidiophora*) (Kruys & Wedin 2009).

*Anamorphs*: *Phoma*-like (von Arx 1974).

### Clade XI *Amniculicolaceae*

*Amniculicolaceae* (clade XI) comprises all three species of *Amniculicola* together with *Murispora rubicunda*, *Neomassariosphaeria grandispora* and *N. typhicola*, and receives high bootstrap support (MLB = 100 %, JK = 100 %). This clade is closely related to *Anguillospora longissima*, *Spirosphaera cupreorufescens* and *Repetophragma ontariense* (Zhang *et al*. 2009a). Compared with *Massariosphaeria grandispora* (as *N. grandispora*) and *M. typhicola* (as *N. typhicola)*, the generic type of *Massariosphaeria* (*M. phaeospora*) cluster with *Thyridaria rubronotata*, and its familial status is undetermined (Fig. 1). *Amniculicola* was first introduced to accommodate the freshwater fungus *A. lignicola* isolated from France, which is characterised by its ascomata with slit-like ostioles, thin, branching and anastomosing hamathecium, cylindrical asci, and hyaline, 1–3-septate ascospores (Zhang *et al*. 2008b). Subsequently, two additional new species of *Amniculicola*, *i.e. A. immersa* and *A. parva* were recovered from Denmark and France, respectively (Zhang *et al*. 2009a). In particular, the paraphyletic nature of *Amniculicola* was revealed in Fig. 1, which indicated that more genes or phylogenetic analyses are needed to separate those genera. All three species were collected in Europe, and stain the woody substrate purple, which could be indicative of metabolite activity (Zhang *et al*. 2009a). Metabolites have rarely been used in the phylogeny and taxonomy of *Pleosporales*, but it is widely used in the taxonomy of xylariaceous taxa (Stadler *et al*. 2004, Bitzer *et al*. 2008). In addition, all species in this clade are from freshwater environments, which may indicate this as a unique ecological habit for the *Amniculicolaceae*.

***Amniculicolaceae*** Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, **fam. nov.** MycoBank MB515469.

Aquaticus. Saprobicus. Ascomata globosa vel subglobosa vel lenticular, nigra, solitaria, immersa vel partim immersa vel superficialia. Apex productum. Peridium exilis. Trabeculae, hyalinae, gelatina circumdatae. Asci, 8-spori, cylindrico vel clavati, fissitunicati, breve pedicellati. Ascosporae, fusiforme vel peranguste fusiforme, uniseptatae vel multiseptatae vel muriforme, hyalinae vel pallide brunneus vel rufobrunneus, tunica gelatinosa praeditae. Substratum malvaceo purpureus.

Freshwater habitat. Saprobic. *Ascomata* solitary, scattered, or in small groups, immersed, erumpent, or nearly superficial, globose, subglobose to lenticular; surface black, roughened; apex elongated. *Peridium* thin. *Pseudoparaphyses* trabeculate, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate, with an ocular chamber. *Ascospores* fusiform or narrowly fusiform, hyaline, pale or reddish-brown, one to multi-septate or muriform, constricted at the median septum, usually surrounded by an irregular, hyaline gelatinous sheath. Ascomata usually stain the woody substrate in shades of purple.

*Type genus*: *Amniculicola* Yin. Zhang & K.D. Hyde.

*Currently accepted genera*: *Amniculicola*, *Murispora* and *Neomassariosphaeria*.

*Anamorphs*: ? *Anguillospora longissima*, *Spirosphaera cupreorufescens* and *Repetophragma ontariense* (Zhang *et al*. 2009a).

***Murispora*** Yin. Zhang, J. Fourn. & K.D. Hyde, **gen. nov.** MycoBank MB515472.

*Etymology*: Named after its muriform ascospores.

Aquaticus. Saprobicus. Ascomata immersa vel partim immersa vel superficialia. Peridium exilis. Trabeculae, hyalinae, gelatina circumdatae. Asci, 8-spori, clavati vel late clavati, fissitunicati, breve pedicellati. Ascosporae, fusiforme, muriforme, brunneus, tunica gelatinosa praeditae. Substratum malvaceo purpureus.

Freshwater habitat. Saprobic. *Ascomata* scattered, or in small groups, immersed, erumpent, or nearly superficial, globose to subglobose, wall black, roughened; apex weakly papillate, conical to laterally flattened. *Peridium* thin. *Pseudoparaphyses* trabeculate, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, oblong to clavate, short pedicellate, with an ocular chamber. *Ascospores* fusiform, pale or reddish brown, muriform, constricted at the median septum, usually surrounded by an irregular, hyaline, gelatinous sheath. Ascomata stain the woody substrate purple.

*Type species*: *Murispora rubicunda* (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde.

*Note*: The studied specimens from which the cultures were obtained are identified in the sense used by Webster (1957), who studied the type specimens, while they might be referred to *Pleospora rubelloides sensu* Crivelli (1983).

***Murispora rubicunda*** (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde, **comb. nov.** MycoBank MB515477.
*Basionym*: *Pleospora rubicunda* Niessl, Notiz. Pyr.: 31. 1876.
≡ *Massariosphaeria rubicunda* (Niessl) Crivelli, Über die Heterogene Ascomycetengattung *Pleospora* Rabh.: 144. 1983.
≡ *Karstenula rubicunda* (Niessl) M.E. Barr, N. Amer. Fl., Ser. 2 (New York): 52. 1990.

***Neomassariosphaeria*** Yin. Zhang, J. Fourn. & K.D. Hyde, **gen. nov.** MycoBank MB515473.
*Etymology*: "*Neo*-" meaning "new", named after its similarity with *Massariosphaeria*.

Aquaticus. Saprophyticus. Ascomata dispergere vel gregariculus, immersa vel partim immersa. Apex productum. Peridium exilis. Trabeculae, hyalinae, gelatina circumdatae. Asci, 8-spori, clavati vel late clavati, fissitunicati, breve pedicellati. Ascosporae, peranguste fusiforme, multiseptatae, hyalinae vel rufobrunneus, tunica gelatinosa praeditae. Substratum plerumque purpureus.

Aquatic. Saprobic. *Ascomata* scattered or in small groups, immersed to erumpent, subglobose to lenticular; wall black, apex elongated. *Peridium* thin. *Pseudoparaphyses* trabeculate, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, clavate to broadly clavate, short pedicellate. *Ascospores* narrowly fusiform, hyaline to reddish brown, multi-septate, constricted at the median septum, usually surrounded by an irregular, hyaline, gelatinous sheath. Ascomata or hyphae usually stain the woody substrate or cultural medium purple.

*Type species*: *Neomassariosphaeria typhicola* (P. Karst.) Yin. Zhang, J. Fourn. & K.D. Hyde.

***Neomassariosphaeria typhicola*** (P. Karst.) Yin. Zhang, J. Fourn. & K.D. Hyde, **comb. nov.** MycoBank MB515479.
*Basionym*: *Leptosphaeria typhicola* P. Karst., Bidrag Kännedom Finlands Natur Folk. 23: 100. 1873.
≡ *Phaeosphaeria typhicola* (P. Karst.) Hedjar., Sydowia 22: 86. 1969.
≡ *Massariosphaeria typhicola* (P. Karst.) Leuchtm., Sydowia 37: 168. 1984.
≡ *Chaetomastia typhicola* (P. Karst.) M.E. Barr, Mycotaxon 34: 514. 1989.

***Neomassariosphaeria grandispora*** (Sacc.) Yin. Zhang, J. Fourn. & K.D. Hyde, **comb. nov.** MycoBank MB515478.
*Basionym*: *Leptosphaeria grandispora* Sacc., Michelia 1: 341. 1878.
≡ *Metasphaeria grandispora* (Sacc.) Sacc., Syll. Fung. 2: 181. 1883.
≡ *Massariosphaeria grandispora* (Sacc.) Leuchtmann, Sydowia 37: 172. 1984.
≡ *Lophiotrema grandispora* (Sacc.) Shoemaker & C.E. Babc., Sydowia 37: 172. 1989.

*Notes*: Although the living habit of *Neomassariosphaeria grandispora* (CBS 613.86) can not be clarified, the freshwater habit of species under this clade seems characteristic (see comments by Zhang *et al*. 2009a). In addition, the ascomata of telemorphs usually stain the woody substrate purple. Their morphological characters, however, vary greatly. For instance, *Amniculicola* species have cylindrical asci, while *N. grandispora*, *N. typhicola* and *Murispora rubicunda* have clavate asci. *Amniculicola* species have hyaline, fusiform 1- or rarely 3-septate ascospores, while the ascospores of *N. typhicola* and *N. grandispora* are narrowly fusiform and multiseptate, but ascospores of *N. typhicola* are brown and *N. grandispora* are hyaline. The ascospores of *M. rubicunda* are brown and muriform. Based on their phylogenetic affinity and morphological distinctions, two new genera, *i.e. Murispora* (based on *Pleospora rubicunda*) and *Neomassariosphaeria* (based on *Massariosphaeria typhicola*) and a new family, *Amniculicolaceae,* are introduced.

### Clade XII *Lophiostomataceae* (uncertain)

The *Lophiostomataceae* comprises some *Lophiostoma* species, such as *L. caulium*, *L. semiliberum*, *L. arundinis*, *L. crenatum*, *L. compressum*, *L. viridarium* and *L. macrostomoides* (MLB = 100 %, JK = 89 %) while *L. fuckelii* is basal (MLB = 94 %, JK = 77 %), as previously reported (Tanaka & Hosoya 2008, Zhang *et al*. 2009b). Traditionally, *Lophiostomataceae* comprised some other genera with various morphological characters, such as *Entodesmium* and *Lophionema* with filiform ascospores, and *Herpotrichia* and *Lophiotrema* with fusiform, brown or hyaline, 1-septate ascospores are usually multiseptate when senescent (Sivanesan 1984, Holm & Holm 1988). The present phylogeny does not support their placement in *Lophiostomataceae*. The paraphyletic nature of *Lophiostomataceae* has been previously noted (Schoch *et al*. 2006), and Clade XII is likely to represent the narrow concept of *Lophiostomataceae*, although it is still too early to draw this conclusion until verified sequences of the generic type of *Lophiostoma* (*L. macrostomum*) are obtained (see comments by Zhang *et al*. 2009b).

Geographically, most species used in this study are from European locations such as Switzerland (*Lophiostoma caulium*, *L. arundinis* and *L. crenatum*), Sweden (*L. semiliberum*) and France (*L. viridarium*, *L. compressum* and *L. macrostomoides*). *Lophiostoma fuckelii*, the only strain from South Africa, diverged earlier than all other members (Fig. 1).

***Lophiostomataceae*** *s. str.* Sacc., Syll. Fung. 2: 672. 1883. **emend.**

Terrestrial or aquatic habitat. Saprobic. *Ascomata* perithecioid, medium to large-sized, solitary or scattered, immersed to erumpent or rarely superficial with protruding, compressed papilla and slite-like ostioles. *Pseudoparaphyses* numerous, narrowly cellular. *Asci* cylindrical to cylindro-clavate, with short pedicels. *Ascospores* fusiform to narrowly fusiform, and mostly multiseptate and heavily pigmented, sometimes with longitudinal septa in one or two cells, rarely 1-septate and hyaline, with or without sheath.

*Currently accepted genus*: *Lophiostoma s. str.*

*Anamorphs*: Reported as *Pleurophomopsis*-like (Leuchtmann 1985).

### Clade XIII *Massariaceae*

The well-supported clade of the *Massariaceae* comprises the generic type of *Massaria* (*M. inquinans*) as well as species of *Roussoella* and *Arthopyrenia* that form a robust clade. The phylogeny in Fig. 1 includes the generic type of *Massaria — M. inquinans*. Morphologically, all of them have immersed ascomata,

pseudoparaphyses from abundant to rare, asci from cylindrical to clavate, ascospores from hyaline to reddish-brown, 1- or 3- septate.

Traditionally, *Massariaceae* (*Melanommatales*) is defined as having large ascomata, a peridium comprising compact, small cells, trabeculate pseudoparaphyses, large, and symmetric distoseptate ascospores usually surrounded with a sheath (Barr 1979). Based on these characters, six genera were included, *i.e. Aglaospora*, *Caryospora*, *Dothivalsaria*, *Massaria*, *Titanella* and *Zopfia* (Barr 1979). *Massaria inquinans* and *Aglaospora profusa* are the generic types of *Massaria* and *Aglaospora* respectively, and they share numerous morphological characters, such as the large, immersed ascomata, trabeculate pseudoparaphyses, cylindrical asci with large and conspicuous apical rings and large, reddish-brown, 3-distoseptate ascospores (Shoemaker & Leclair 1975). The phylogenies here exclude the placement of *Aglaospora* under *Massariaceae*, and the placement of other four traditional genera under *Massariaceae*, *i.e. Caryospora*, *Dothivalsaria*, *Titanella* and *Zopfia* can not be verified here either.

***Massariaceae*** Nitschke, Verh. Naturhist. Vereines Preuss. Rheinl. 26: 73. 1869.

*Note*: Members of this clade are mostly saprobic.

*Currently accepted genera*: ? *Arthopyrenia*, *Massaria*, ? *Roussoella*.

*Anamorph*: ? *Torula herbarum*.

### Clade XIV

The current phylogenetic data show that *Lophiotrema* as well as the generic types of *Lophiotrema* (*L. nucula*), *Verruculina* (*V. enalia*), *Ulospora* (*U. bilgramii*), *Lepidosphaeria* (*L. nicotiae*) *and Xenolophium* (*X. applanatum*) cluster apart from the clade of *Lophiostomataceae s. str*. Members of this clade are all saprobes, but have diverse morphological characters. *Lophiotrema* was introduced as a genus closely related to *Lophiostoma*, but having hyaline ascospores, and was assigned to *Lophiostomataceae* (Saccardo 1878, Holm & Holm 1988). The relatively smaller ascomata, peridium of almost equal in thickness, and the hyaline, 1-septate ascospores have been used to distinguish *Lophiotrema* from *Lophiostoma* (Holm & Holm 1988, Yuan & Zhao 1994, Kirk *et al*. 2001). The peridium concept, however, is not supported by the lectotype specimen, which has a flattened, thin-walled base (Zhang *et al*. 2009b). Species with brown ascospores are found in *Lophiotrema* based on molecular phylogenetic results (Zhang *et al*. 2009b).

*Lepidosphaeria*, *Ulospora* and *Verruculina* are all genera of the *Testudinaceae*, which is characterised by the cleistothecioid ascomata, 1-septate, brown, glabrous or ornamented ascospores (von Arx 1971). The size, shape and ornamentation of the ascospores serve as the distinguishing character between different genera (von Arx 1971, von Arx & Müller 1975, Hawksworth 1979). Based on the present phylogenetic result, these three genera of *Testudinaceae* are closely related. In addition, the non-ostiolate ascomata of the *Testudinaceae* provides evidence that taxa with cleistothecioid fruiting bodies have evolved from taxa with perithecioid ones in the *Pleosporales*.

The diverse morphological characters possessed by members of clade XIV might indicate that they are from more than one family. A more firmly stated hypothesis can only be obtained by further phylogenetic study which should include more genera and related species.

***Lophiotrema*** Sacc., Michelia 1: 338, 1878. **emend.**

Saprobic. *Ascomata* perithecioid, mostly immersed, rarely erumpent; globose, subglobose or ovoid. *Hamathecium* of broadly to narrowly trabeculate or cellular pseudoparaphyses, persistent. *Asci* bitunicate, fissitunicate, cylindrical to clavate. *Ascospores* mostly hyaline, rarely brown, 1-septate, smooth.

*Anamorphs*: unknown.

***Lophiotrema neoarundinaria*** (Ellis & Everh.) Yin. Zhang, Kaz. Tanaka & K.D. Hyde, **comb. nov.** MycoBank MB515475.
*Basionym*: *Didymosphaeria arundinariae* Ellis & Everh., N. Amer. Pyren. (Newfield): 732. 1892.

≡ *Microthelia arundinariae* (Ellis & Everh.) Kuntze, Revis. gen. pl. (Leipzig) 3: 498. 1898.
≡ *Massarina arundinariae* (Ellis & Everh.) M.E. Barr, Mycotaxon 45: 211. 1992.
≡ *Lophiostoma arundinariae* (Ellis & Everh.) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Diversity Res. Ser. 7: 107. 2002.

*Note*: To avoid the duplication with *Lophiotrema arundinariae* Rehm, a new name – *Lophiotrema neoarundinaria* is proposed here.

***Lophiotrema rubi*** (Fuckel) Yin. Zhang, C.L. Schoch & K.D. Hyde, **comb. nov.** MycoBank MB515476.
*Basionym*: *Massaria rubi* Fuckel, Jahrb. Nassauischen Vereins Naturk. 25–26: 303. 1871.

≡ *Massarina rubi* (Fuckel) Sacc., Syll. Fung. (Abellini) 2: 155. 1883.
= *Didymellina rhaphithamni* Keissl., Nat. Hist. Juan. Fernandez Easter Lsl. 2: 480. 1927.
= *Mycosphaerella rhaphithamni* (Keissl.) Petr., Ann. Mycol. 38: 221. 1940.
= *Massarina emergens* (P. Karst.) L. Holm, *Les Pleosporaceae*: 149. 1957.
≡ *Lophiostoma rubi* (Fuckel) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94: 812. 2002.

### Clade XV *Aigialaceae*

The generic type of *Aigialus* (*A. grandis*) and *Lophiostoma mangrovei* form a well-supported cluster, which represents a marine pleosporalean family, *Aigialaceae*. This new family is addressed by Suetrong *et al*. (2009; this volume).

### Clade XVI *Delitschiaceae*

The generic type of *Delitschia* (*D. didyma*) and *D. winteri*, represent *Delitschiaceae* and form a robust clade that diverges before all other members of *Pleosporales*. The *Delitschiaceae* is a small group of coprophilous fungi, which comprises three genera (*i.e. Delitschia*, *Ohleriella* and *Semidelitschia*) and 54 species (Barr 2000, Kirk *et al*. 2008). This family was introduced to accommodate coprophilous pleosporalean species with periphysate ostiole, wide ascus endotunica, conspicious apical ring and heavily pigmented 1- to multiseptate ascospore with germ slits in each cell (Barr 2000).

The presence of a large ocular chamber with an apical ring in the ascus is the most striking character of most members of *Delitschiaceae* as well as species in clade XVII, *Aglaospora profusa*. These two clades are consistently the earliest diverging lineage in *Pleosporales* as in several other phylogenies (Kruys *et al*. 2006, Schoch *et al*. 2006).

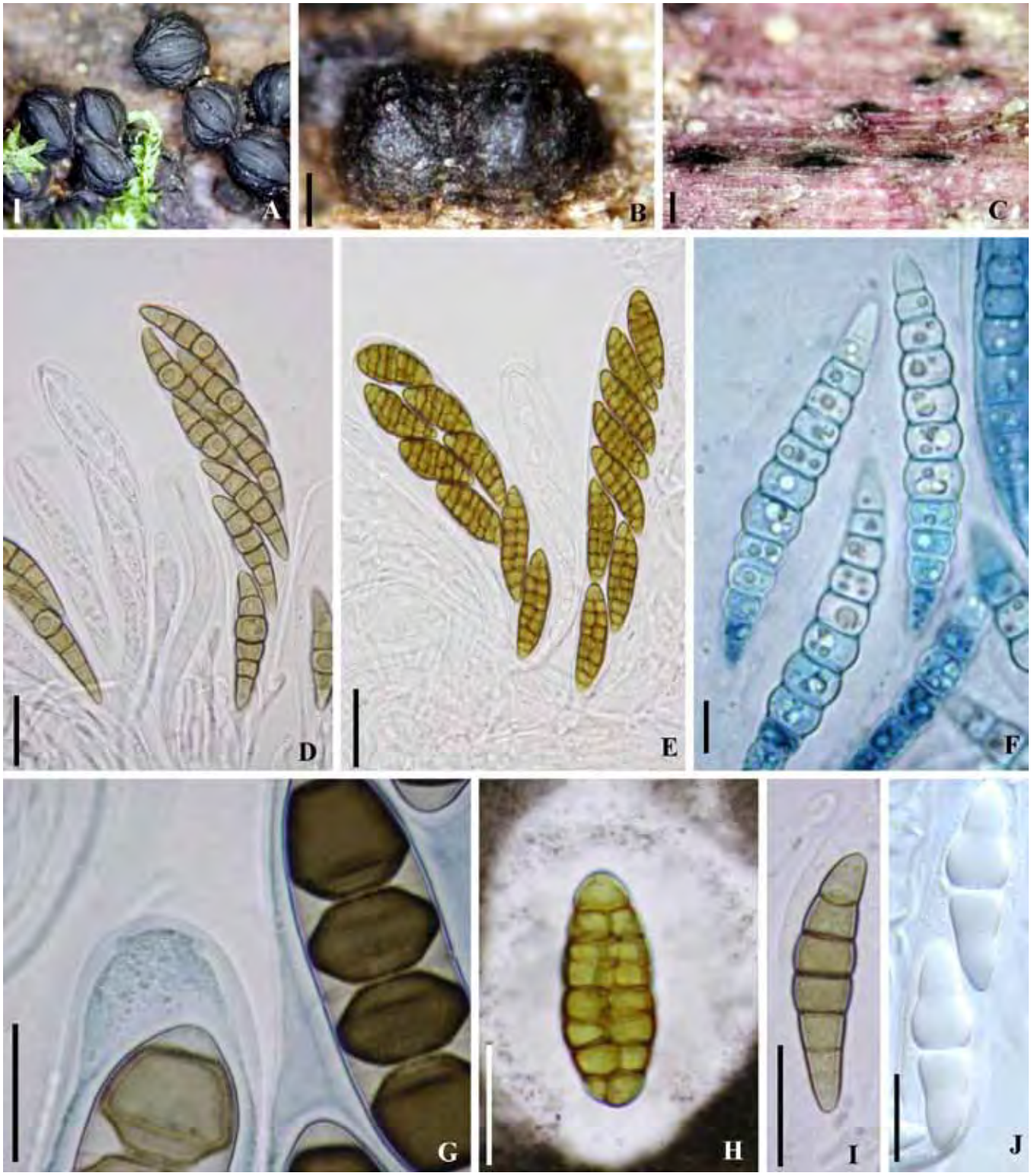


**Fig. 2.** A. *Xenolophium applanatum*. Ascomata on the host surface. Note the slit-like ostiole. B. *Trematosphaeria pertusa*. Ascomata on the host surface. Note the pore-like ostiole. C, E, H. *Murispora rubicunda*. C. Ascomata on the host surface. Note the purple woody substrate. E. Clavate 8-spored asci with short pedicels in pseudoparaphyses. H. Muriform ascospore with wide mucilaginous sheath. D. I. *Trematosphaeria* sp. D. Fusiform mature or immature 8-spored asci with pseudoparaphyses. I. multisepate dark brown ascospore. F. *Neomassariosphaeria grandispora*. Ascospores with sheath. G. *Aglospora profusa*. Apical aparatus. Note the conspicuous apical ring. J. *Amniculicola immersa*. Hyaline fusiform ascospores in ascus. Scale bars; A–C = 100 µm, D–J = 20 µm.

## SUMMARY

### Phylogeny

The results presented here indicate that nutritional modes and environmental habits may have phylogenetic significance in *Pleosporales*, although more extensive statistical analyses remain to be done. Host spectrum (monocotyledon/dicotyledon) appears closely related to the phylogeny of plant associated fungi or plant pathogens (*e.g.* in *Pleosporineae*). Of the morphological characters, the size, shape and immersion degree of ascomata, ostiole characters and ascal shape can be of phylogenetic significance to varying degrees. The purple staining nature of the substrate found in some *Amniculicolaceae* might indicate that secondary metabolites have phylogenetic significance for this group.

However, even closely related species can exhibit diverse morphologies. Ascospores can vary from 1- to multiseptate to even muriform, hyaline to pigmented in many families, such as *Amniculicolaceae* (given as an example in Fig. 2), *Lophiostomaceae s. str.*, *Melanommataceae* and *Didymellaceae*. From an evolutionary perspective, the "bipolar symmetrical ascospore tends to be correlated to passive dispersal", and "the colour, size, shape and texture of spores should be viewed as probable functional adaptations modified in evolution by requirements of liberation, of flotation in fluids, and ultimately of deposition and survival" (Ingold 1971, Gregory 1973, Hawksworth 1987). Thus ascospore shape should be viewed as a highly adaptive character that can obscure underlying relationships.

### Evolutionary trends

Most plant pathogens in *Pleosporales* belong to *Pleosporineae*, which tends to occupy the terminal branches on the *Pleosporales* tree (Fig. 1). On the other hand, a clade of coprophilous fungi — *Delitschiaceae* — consistently occurs as an early-diverged lineage compared to all other pleosporalean members, with numerous other saprotrophic members interspersed. Parasitic fungi are usually considered as "highly specialised", and may require nutritional shifts from several other modes (Cain 1972, Heath 1987, Berbee 2001, Sung *et al*. 2008). This may indicate that *Pleosporales* originated from saprotrophic fungi, and that the transition from saprotrophic to necrotrophic and hemibiotrophic (or biotrophic) is likely, in agreement with earlier ideas (Lewis 1974, Cooke 1977, Cooke & Whipps 1986), also mirroring what is seen in the *Capnodiales* phylogeny (Crous *et al*. 2009a, Schoch *et al.* 2009a; this volume).

It is remarkable that as with the *Delitschiaceae*, *Aglaospora profusa* is also an early diverging lineage. Members of both *Delitschiaceae* and *Aglaospora* have a striking morphological character in having a large apical apparatus, which is rare in *Pleosporales*. According to the hypothesis of Hawksworth (1987), "……foremost of these trends is the loss of apical apparatus associated with a change from active to passive discharge of the ascospores……". Thus this striking apical apparatus might further indicate the plesiomorphic status of both *Delitschiaceae* and *Aglaospora*, supporting the premise that the ancestor of *Pleosporales* was saprobic with a well-developed apical ring.

### Shortcomings and further work

Attempts to write a familial dichotomous key based on the present phylogenetic data has proven to be unsuccesful. The traditional keys rely on single morpho-characters, which are polyphyletic. Thus it appears to be impossible to find any single criterion which can be used to key out a family in such a way as to include all genera or species belonging to it, without incorporating the genus or species in several places in the key, as have been mentioned by Cain (1972).

Compared with the *ca*. 3 000 reported species in *Pleosporales*, the 130 species (< 5 %) used in present investigation are far from sufficient to obtain a comprehensive phylogenetic survey for the genetic diversity in the order, but will hopefully provide a framework for directing further work. Members of some families, such as *Cucurbitariaceae* and *Diademaceae*, are absent from our analysis, thus their status remains unresolved. In particular, erroneous strains or names in databases and culture collections necessitate verification, and circumscriptions of families within the clades currently remain preliminary. Importantly, this data set is geographically biased as most strains originated from temperate areas in the Northern Hemisphere, mainly Europe. Obtaining correctly identified fungal strains from various locations is crucial for further molecular phylogenetic investigations, necessitating the consistent analysis and interperpretation of large taxon datasets. It seems clear that most morphological criteria used by traditional taxonomy for *Pleosporales* at various taxonomic levels (such as genus or family) do not strictly correlate with distinct evolutionary groups. We will therefore have to rely on expanding our base of knowledge in ecology, biochemistry and other biological fields, to supplement the genetic information. The expected expansion in pleosporalean genome sequences makes this especially important.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Isolates used in this study and their GenBank accession numbers. Name changes from their originals are indicated in brackets and newly generated sequences are indicated in bold.

| Classification | Species name | Culture/voucher[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|
| *Aigialaceae* | *Aigialus grandis* | JK 5244A | **GU296131** | **GU301793** | | **GU371762** | |
| | *Astrosphaeriella aggregata* | MAFF 239486 | AB524450 | AB524591 | AF242264 | AB539092 | AB539105 |
| | *Rimora mangrovei* (as *Lophiostoma mangrovei*) | JK 5246A | **GU296193** | **GU301868** | | **GU371759** | |
| *Amniculicolaceae* | *Amniculicola immersa* | CBS 123083 | **GU456295** | FJ795498 | | **GU456358** | **GU456273** |
| | *Amniculicola lignicola* | CBS 123094 | EF493861 | EF493863 | | EF493862 | **GU456278** |
| | *Amniculicola parva* | CBS 123092 | **GU296134** | FJ795497 | | | **GU349065** |
| | *Neomassariosphaeria grandispora* | CBS 613.86 | **GU296172** | **GU301842** | **GU357747** | **GU371725** | **GU349036** |
| | *Neomassariosphaeria typhicola* | CBS 123126 | **GU296174** | FJ795504 | | GU371795 | |
| | *Murispora rubicunda* | IFRD 2017 | **GU456308** | FJ795507 | | | **GU456289** |
| *Delitschiaceae* | *Delitschia didyma* 1 | UME 31411 | | DQ384090 | | | |
| | *Delitschia didyma* 2 (duplicate) | UME 31411 | AF242264 | DQ384090 | | DQ677975 | DQ677922 |
| | *Delitschia winteri* | CBS 225.62 | DQ678026 | DQ678077 | | DQ677975 | DQ677922 |
| *Didymellaceae* | *Ascochyta pisi* | CBS 126.54 | DQ678018 | DQ678070 | | DQ677967 | DQ677913 |
| | *Didymella exigua* | CBS 183.55 | **GU296147** | | **GU357800** | **GU371764** | |
| | *Didymella bryoniae* | CBS 133.96 | | **GU456335** | | GU371767 | |
| | *Leptosphaerulina argentinensis* | CBS 569.94 | | AY849947 | **GU357759** | | **GU349008** |
| | *Leptosphaerulina australis* 1 | CBS 311.51-T | | FJ795500 | | **GU456357** | **GU456272** |
| | *Leptosphaerulina australis* 2 | CBS 317.83 | **GU296160** | **GU301830** | | **GU371790** | **GU349070** |
| | *Macroventuria anomochaeta* | CBS 525.71 | AY787936 | **GU456315** | | **GU456346** | **GU456262** |
| | *Monascostroma innumerosum* | CBS 345.50 | **GU296179** | **GU301850** | | | **GU349033** |
| | *Phoma complanata* | CBS 268.92 | EU754081 | EU754180 | | **GU371778** | **GU349078** |
| | *Phoma exigua* | CBS 431.74 | EU754084 | EU754183 | | **GU371780** | **GU349080** |
| | *Phoma glomerata* | CBS 528.66 | EU754085 | EU754184 | | **GU371781** | **GU349081** |
| | *Phoma herbarum* | CBS 276.37 | DQ678014 | DQ678066 | **GU357792** | DQ677962 | DQ677909 |
| | *Phoma zeae-maydis* | CBS 588.69 | EU754093 | EU754192 | | **GU371782** | **GU349082** |
| | *Platychora ulmi* | CBS 361.52 | EF114726 | EF114702 | | | |
| *Lentitheciaceae* | *Katumotoa bambusicola* | JCM 13131, MAFF 239641 | AB524454 | AB524595 | | **AB539095** | **AB539108** |
| | *Keissleriella cladophila* | CBS 104.55 | **GU296155** | **GU301822** | | **GU371735** | **GU349043** |
| | *Lentithecium aquaticum* | CBS 123099 | **FJ795477** | FJ795434 | | FJ795455 | **GU349068** |
| | *Lentithecium arundinaceum* 1 | CBS 123131 | **GU456298** | **GU456320** | | | **GU456281** |
| | *Lentithecium arundinaceum* 2 | CBS 619.86 | DQ813513 | DQ813509 | | FJ795473 | |
| | *Lentithecium fluviatile*(as *Massarina fluviatile*) | CBS 122367 | FJ795493 | FJ795451 | | | **GU456290** |
| | *Ophiosphaerella sasicola* | JCM 13134, MAFF 239644 | AB524458 | AB524599 | | **AB539098** | **AB539111** |
| | *Stagonospora macropycnidia* | OSC 100965 | **GU296198** | **GU301873** | | | **GU349026** |
| | *Wettsteinina lacustris* | CBS 618.86 | DQ678023 | | | DQ677972 | DQ677919 |
| *Leptosphaeriaceae* | *Coniothyrium palmarum* | CBS 400.71 | DQ678008 | DQ767653 | | DQ677956 | DQ677903 |
| | *Leptosphaeria biglobosa* | CBS 303.51 | | **GU301826** | | | **GU349010** |
| | *Leptosphaeria doliolum* | CBS 505.75 | **GU296159** | FJ795499 | | | **GU349069** |
| | *Leptosphaeria dryadis* | CBS 643.86 | | **GU301828** | | **GU371733** | **GU349009** |
| | *Leptosphaeria maculans* | DAOM 229267 | DQ470993 | DQ470946 | DQ471136 | DQ470894 | DQ471062 |
| | *Neophaeosphaeria filamentosa* | CBS 102202 | GQ387516 | GQ387577 | | **GU371773** | **GU349084** |

**Table 1.** (Continued).

| Classification | Species name | Culture/voucher[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|
| | *Phoma heteromorphospora* | CBS 115.96 | EU754089 | EU754188 | | **GU371775** | **GU349077** |
| | *Pyrenochaeta nobilis* | CBS 407.76 | | DQ678096 | | DQ677991 | DQ677936 |
| *Lophiostomataceae s. str.* | *Lophiostoma arundinis* | CBS 621.86 | DQ782383 | DQ782384 | | DQ782386 | DQ782387 |
| | *Lophiostoma caulium* 1 | CBS 623.86 | FJ795479 | FJ795436 | | FJ795456 | |
| | *Lophiostoma caulium* 2 | CBS 624.86 | | **GU301832** | | | **GU349007** |
| | *Lophiostoma compressum* 1 | IFRD 2014 | FJ795480 | FJ795437 | | FJ795457 | |
| | *Lophiostoma compressum* 2 | IFRDCC2081 | FJ795486 | **GU456321** | | **GU456349** | **GU456264** |
| | *Lophiostoma crenatum* | CBS 629.86 | DQ678017 | DQ678069 | | DQ677965 | DQ677912 |
| | *Lophiostoma fuckelii* | CBS 101952 | FJ795496 | DQ399531 | | FJ795472 | |
| | *Lophiostoma macrostomoides* | CBS 123097 | FJ795482 | FJ795439 | | FJ795458 | **GU456277** |
| | *Lophiostoma semiliberum* | CBS 626.86 | FJ795484 | FJ795441 | | FJ795460 | |
| | *Lophiostoma viridarium* | IFRDCC2090 | FJ795486 | FJ795443 | | FJ795468 | |
| *Massariaceae* | *Arthopyrenia salicis* 1 | CBS 368.94 | AY538333 | AY538339 | **GU371814** | | |
| | *Arthopyrenia salicis* 2 | 1994Coppins | | AY607730 | AY607742 | | |
| | *Massaria inquinans* | CBS 122369 | **GU456300** | **GU456322** | | | **GU456282** |
| | *Pleosporales* sp. 1 (as *Thelenella luridella*) | CBS 101277 | **GU456309** | | | **GU456361** | |
| | *Roussoella hysterioides* 1 | JCM 13126, MAFF 239636 | AB524480 | AB524621 | | **AB539101** | **AB539114** |
| | *Roussoella hysterioides* 2 | CBS 125434 | AB524481 | AB524622 | | **AB539102** | **AB539115** |
| | *Roussoella pustulans* | JCM 13127, MAFF 239637 | AB524482 | AB524623 | | **AB539103** | **AB539116** |
| | *Roussoellopsis tosaensis* | NBRC 106245 | | AB524625 | | **AB539104** | **AB539117** |
| | *Torula herbarum* | CBS 379 58 | | | | **GU456362** | |
| *Massarinaceae* | *Byssothecium circinans* | CBS 675.92 | AY016339 | AY016357 | | DQ767646 | |
| | *Massarina cisti* | CBS 266.62 | FJ795490 | FJ795447 | | FJ795464 | |
| | *Massarina eburnea* | CBS 473.64 | AF164367 | FJ795449 | **GU357755** | FJ795466 | **GU349040** |
| | *Massarina igniaria* | CBS 845.96 | FJ795494 | FJ795452 | | FJ795469 | |
| | *Neottiosporina paspali* | CBS 331.37 | EU754073 | EU754172 | | **GU371779** | **GU349079** |
| *Melanommataceae* | *Beverwykella pulmonaria* | CBS 283.53 | | **GU301804** | | **GU371768** | |
| | *Herpotrichia diffusa* | CBS 250.62 | DQ678019 | DQ678071 | | DQ677968 | DQ677915 |
| | *Herpotrichia juniperi* | CBS 200.31 | DQ678029 | DQ678080 | | DQ677978 | DQ677925 |
| | *Melanomma pulvis-pyrius* 1 | CBS 109.77 | FJ201987 | FJ201986 | | **GU456359** | **GU456274** |
| | *Melanomma pulvis-pyrius* 2 | CBS 124080 | **GU456302** | **GU456323** | | **GU456350** | **GU456265** |
| | *Monotosporella tuberculata* | CBS 256.84 | | **GU301851** | | | **GU349006** |
| | *Pleomassaria siparia* | CBS 279.74 | DQ678027 | DQ678078 | | DQ677976 | AY544726 |
| *Sporormiaceae* | *Preussia funiculata* | CBS 659.74 | **GU296187** | **GU301864** | | **GU371799** | **GU349032** |
| | *Preussia lignicola* (as *Sporormia lignicola*) | CBS 264.69 | **GU296197** | **GU301872** | | **GU371765** | **GU349027** |
| | *Preussia terricola* | DAOM 230091 | AY544726 | AY544686 | DQ471137 | DQ470895 | DQ471063 |
| | *Sporormiella minima* | CBS 524.50 | DQ678003 | DQ678056 | | DQ677950 | DQ677897 |
| | *Westerdykella cylindrica* | CBS 454.72 | AY016355 | AY004343 | DQ471168 | DQ470925 | DQ497610 |
| | *Westerdykella ornata* | CBS 379.55 | **GU296208** | **GU301880** | | **GU371803** | **GU349021** |
| *Montagnulaceae* | *Bimuria novae-zelandiae* | CBS 107.79 | AY016338 | AY016356 | DQ471159 | DQ470917 | DQ471087 |
| | *Didymocrea sadasivanii* | CBS 438.65 | DQ384066 | DQ384103 | | | |
| | *Kalmusia brevispora* 1 | NBRC 106240 | AB524459 | AB524600 | | **AB539100** | **AB539113** |
| | *Kalmusia brevispora* 2 | MAFF 239276 | AB524460 | AB524601 | | **AB539099** | **AB539112** |
| | *Kalmusia scabrispora* 1 | NBRC 106237 | AB524453 | AB524594 | | **AB539094** | **AB539107** |

**Table 1.** (Continued).

| Classification | Species name | Culture/voucher[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|
| | *Kalmusia scabrispora* 2 | JCM 12851, MAFF 239517 | AB524452 | AB524593 | | **AB539093** | **AB539106** |
| | *Karstenula rhodostoma* | CBS 690.94 | **GU296154** | **GU301821** | | **GU371788** | **GU349067** |
| | *Letendraea helminthicola* | CBS 884.85 | AY016345 | AY016362 | | | |
| | *Letendraea padouk* | CBS 485.70 | **GU296162** | AY849951 | | | |
| | *Montagnula opulenta* | CBS 168.34 | AF164370 | DQ678086 | | DQ677984 | |
| | *Paraconiothyrium minitans* | CBS 122788 | EU754074 | EU754173 | | **GU371776** | **GU349083** |
| | *Paraphaeosphaeria michotii* 1 | CBS 652.86 | **GU456304** | **GU456325** | | **GU456351** | **GU456266** |
| | *Paraphaeosphaeria michotii* 2 | CBS 591.73 | **GU456305** | **GU456326** | | **GU456352** | **GU456267** |
| *Phaeosphaeriaceae* | *Chaetosphaeronema hispidulum* | CBS 216.75 | EU754045 | EU754144 | | **GU371777** | |
| | *Entodesmium rude* | CBS 650.86 | | **GU301812** | | | **GU349012** |
| | *Leptosphaeria derasa* | CBS 184.57 | **GU456299** | | | **GU456360** | **GU456275** |
| | *Ophiosphaerella herpotricha* 1 | CBS 620.86 | DQ678010 | DQ678062 | | DQ677958 | DQ677905 |
| | *Ophiosphaerella herpotricha* 2 | CBS 240.31 | DQ767650 | DQ767656 | | DQ767645 | DQ767639 |
| | *Phaeosphaeria ammophilae* | CBS 114595 | **GU296185** | **GU301859** | **GU357746** | **GU371724** | **GU349035** |
| | *Phaeosphaeria avenaria* | CBS 602.86 | AY544725 | AY544684 | | DQ677941 | DQ677885 |
| | *Phaeosphaeria caricis* | CBS 120249 | | **GU301860** | | | **GU349005** |
| | *Phaeosphaeria elongata* | CBS 120250 | **GU456306** | **GU456327** | **GU456340** | **GU456345** | **GU456261** |
| | *Phaeosphaeria eustoma* | CBS 573.86 | DQ678011 | DQ678063 | | DQ677959 | DQ677906 |
| | *Phaeosphaeria juncicola* | CBS 595.86 | | | | | **GU456291** |
| | *Phaeosphaeria juncophila* | CBS 575.86 | **GU456307** | **GU456328** | | | **GU456283** |
| | *Phaeosphaeria luctuosa* | CBS 308.79 | | **GU301861** | | | **GU349004** |
| | *Phaeosphaeria nigrans* | CBS 576.86 | | **GU456331** | | **GU456356** | **GU456271** |
| | *Phaeosphaeria nodorum* 1 | CBS 259.49 | | **GU456332** | | | **GU456285** |
| | *Phaeosphaeria nodorum* 2 | Genome (Broad) | Genome | Genome | Genome | Genome | Genome |
| | *Phaeosphaeria spartinae (as Leptosphaeria albopunctata)* | CBS 254.64 | AF439506 | **GU456314** | **GU456337** | | **GU456279** |
| | *Phaeosphaeria spartinicola* | CBS 176.91 | | **GU456333** | | | **GU456286** |
| | *Phaeosphaeria typharum* | CBS 296.54 | | **GU456334** | | | **GU456287** |
| | *Phoma radicina* | CBS 111.79 | EU754092 | EU754191 | | | **GU349076** |
| | *Setomelanomma holmii* | CBS 110217 | **GU296196** | **GU301871** | | **GU371800** | **GU349028** |
| *Pleosporaceae* | *Allewia eureka* | DAOM 195275 | DQ677994 | DQ678044 | | DQ677938 | DQ677883 |
| | *Alternaria alternata* | CBS 916.96 | DQ678031 | DQ678082 | | DQ677980 | DQ677927 |
| | *Alternaria maritima* | CBS 126.60 | **GU456294** | **GU456317** | | **GU456347** | |
| | *Cochliobolus heterostrophus* | CBS 134.39 | AY544727 | AY544645 | | DQ247790 | DQ497603 |
| | *Cochliobolus sativus* | DAOM 226212 | DQ677995 | DQ678045 | | DQ677939 | |
| | *Phoma betae* | CBS 109410 | EU754079 | EU754178 | | **GU371774** | **GU349075** |
| | *Pleospora herbarum* | CBS 714.68 | DQ767648 | DQ678049 | DQ471163 | DQ677943 | DQ677888 |
| | *Pyrenophora phaeocomes* | DAOM 222769 | DQ499595 | DQ499596 | | DQ497614 | DQ497607 |
| | *Pyrenophora tritici-repentis* 1 (as *Pyrenophora trichostoma*) | OSC 100066 | | AY544672 | | | DQ677882 |
| | *Pyrenophora tritici-repentis* 2 (as *Pyrenophora trichostoma*) | CBS 392.54 | | | | | **GU349017** |
| | *Pyrenophora tritici-repentis* 3 | CBS 328.53 | | | | | **GU456292** |
| | *Scolecobasidium arenarium* (as *Dendryphiella arenaria*) | CBS 181.58 | DQ471022 | DQ470971 | GU349071 | DQ470924 | DQ677890 |
| | *Setosphaeria monoceras* | CBS 154.26 | AY016352 | AY016368 | | | |

**Table 1.** (Continued).

| Classification | Species name | Culture/voucher[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|
| *Trematosphaeriaceae* | *Asteromassaria pulchra* | CBS 124082 | **GU296137** | **GU301800** | | **GU371772** | **GU349066** |
| | *Halomassarina thalassiae* (as *Massarina thalassiae*) | JK 5262D | | **GU301816** | | | **GU349011** |
| | *Trematosphaeria pertusa* 1 | CBS 122368 | FJ201991 | FJ201990 | | FJ795476 | **GU456276** |
| | *Trematosphaeria pertusa* 2 | CBS 122371 | FJ201992 | FJ201993 | | **GU371801** | **GU349085** |
| *Pleosporales Incertae sedis* | *Aglaospora profusa* 1 | CBS 123109 | **GU296130** | **GU301792** | | | **GU349062** |
| | *Aglaospora profusa* 2 | CBS 123129 | **GU456293** | **GU456316** | | | **GU456280** |
| | *Byssolophis sphaerioides* | IFRDCC2053 | **GU296140** | **GU301805** | | **GU456348** | **GU456263** |
| | *Lepidosphaeria nicotiae* | CBS 101341 | | DQ678067 | | DQ677963 | DQ677910 |
| | *Lophiotrema brunneosporum* | CBS 123095 | FJ795487 | FJ795444 | | | GU349071 |
| | *Lophiotrema lignicola* | CBS 123094 | FJ795488 | FJ795445 | | FJ795462 | **GU349072** |
| | *Lophiotrema neoarundinaria* 1 | NBRC 106238 | AB524455 | AB524596 | | **AB539097** | **AB539110** |
| | *Lophiotrema neoarundinaria* 2 | MAFF 239461 | AB524456 | AB524597 | | **AB539096** | **AB539109** |
| | *Lophiotrema nucula* | CBS 627.86 | FJ795489 | FJ795446 | | FJ795463 | **GU349073** |
| | *Massaria anomia* | CBS 591.78 | **GU296169** | **GU301839** | | **GU371769** | |
| | *Massarina rubi* | CBS 691.95 | **GU456301** | FJ795453 | | FJ795470 | |
| | *Massariosphaeria phaeospora* | CBS 611.86 | **GU296173** | **GU301843** | | **GU371794** | |
| | *Munkovalsaria rubra* | CBS 109505 | **GU456303** | **GU456324** | **GU456339** | **GU456344** | **GU456260** |
| | *Thyridaria rubronotata* | CBS 419.85 | | **GU301875** | | **GU371728** | **GU349002** |
| | *Ulospora bilgramii* | CBS 110021 | DQ678025 | DQ678076 | | DQ677974 | DQ677921 |
| | *Valsaria insitiva* | CBS 123098 | **GU456310** | **GU460204** | | | **GU456284** |
| | *Valsaria insitiva* | CBS 123125 | **GU456311** | **GU460205** | | **GU456353** | **GU456268** |
| | *Verruculina enalia* | JK 5235A | DQ678028 | DQ678079 | | DQ677977 | DQ677924 |
| | *Xenolophium applanatum* | CBS 123123 | **GU456312** | **GU456329** | | **GU456354** | **GU456269** |
| | *Xenolophium applanatum* | CBS 123127 | **GU456313** | **GU456330** | | **GU456355** | **GU456270** |
| *Botryosphaeriales* (outgroup) | *Botryosphaeria dothidea* | CBS 115476 | DQ677998 | DQ678051 | **GU357802** | DQ677944 | DQ767637 |
| | *Botryosphaeria tsugae* | CBS 171.55 | DQ678009 | DQ678061 | | DQ677957 | DQ677904 |
| | *Guignardia gaultheriae* | CBS 447.70 | | DQ678089 | **GU357796** | DQ677987 | |
| | *Guignardia bidwellii* | CBS 237.48 | DQ678034 | DQ678085 | **GU357794** | DQ677983 | |
| Dothideales (outgroup) | *Dothidea hippophaës* | CBS 188.58 | U42475 | DQ678048 | **GU357801** | DQ677942 | DQ677887 |
| | *Phaeosclera dematioides* | CBS 157.81 | **GU296184** | **GU301858** | **GU357764** | | **GU349047** |
| | *Dothidea sambuci* | DAOM 231303 | AY544722 | AY544681 | | DQ522854 | DQ497606 |
| *Hysteriales* (outgroup) | *Psiloglonium clavisporum* | CBS 123339 | FJ161157 | FJ167526 | | FJ161124 | FJ161105 |
| | *Hysteriales* sp. 1 | CBS 243.34 | **GU456297** | **GU456319** | **GU456338** | **GU456343** | **GU456259** |
| | *Hysterium angustatum* | CBS 236.34 | **GU397359** | FJ161180 | **GU456341** | FJ161117 | FJ161096 |
| *Jahnulales* (outgroup) | *Jahnula seychellensis* | SS2113.1 | EF175644 | EF175665 | | | |
| | *Jahnula aquatica* | R68-1 | EF175633 | EF175655 | | | |
| | *Aliquandostipite khaoyaiensis* | CBS 118232 | AF201453 | **GU301796** | | FJ238360 | **GU349048** |
| *Mytilinidiales* (outgroup) | *Mytilinidion andinense* | CBS 123562 | FJ161159 | FJ161199 | | FJ161125 | FJ161107 |
| | *Lophium mytilinum* | CBS 269.34 | DQ678030 | DQ678081 | **GU456342** | DQ677979 | DQ677926 |

**Table 1.** (Continued).

| Classification | Species name | Culture/voucher[1] | SSU | LSU | *RPB1* | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|
| *Venturiaceae* (outgroup) | *Venturia pyrina* | ATCC 38995 | | EF114714 | | | |
| | *Venturia inaequalis* | CBS 476.61 | | **GU456336** | | | **GU456288** |
| | *Metacoleroa dickei* | medipc | | EF114695 | | | |
| Arthoniomycetes (outgroup) | *Opegrapha dolomitica* | DUKE 0047528 | DQ883706 | | DQ883717 | DQ883714 | DQ883732 |
| | *Opegrapha varia* | DUKE 0047526 | | | FJ772242 | FJ772243 | FJ772244 |

[1]**Public culture collections and herbaria** ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; DUKE: Duke University Herbarium Durham, North Carolina, U.S.A.; IFRD: International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunmin, People's Republic of China; JCM: Japan Collection of Microorganism, RIKEN BioResource Center, Japan; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; OSC: Oregon State University Herbarium, Corvallis, Oregon, U.S.A.; NBRC: National Institute of Technology and Evaluation, Chiba, Japan; UME: Umeå University Herbarium, Umeå, Sweden.

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# Molecular phylogenetics of *Pleosporales*: *Melanommataceae* and *Lophiostomataceae* re-circumscribed (*Pleosporomycetidae, Dothideomycetes,* Ascomycota)

G.K. Mugambi[1–3] and S.M. Huhndorf[1]

[1]*Botany Department, Field Museum, 1400 S. Lake Shore Dr, Chicago, IL 60605, U.S.A.;* [2]*Department of Biological Sciences, University of Illinois at Chicago, 845 W. Taylor St (MC 066), Chicago, IL 60607, U.S.A.;* [3]*National Museums of Kenya, Botany Department, P.O. Box 45166, 00100, Nairobi, Kenya*

**Correspondence*: G.K. Mugambi, gkmugambi@gmail.com

**Abstract:** The classification of *Pleosporales* has posed major challenges due to the lack of clear understanding of the importance of the morphological characters used to distinguish between different groups in the order. This has resulted in varied taxonomic treatments of many families in the group including *Melanommataceae* and *Lophiostomataceae*. In this study we employ two nuclear DNA gene markers, nuclear ribosomal large subunit DNA and translation elongation factor 1-alpha in order to examine the molecular phylogenetics of *Pleosporales* with strong emphasis on the families *Melanommataceae* and *Lophiostomataceae*. Phylogenetic analyses recovered *Melanommataceae*, *Lophiostomataceae*, *Hypsostromataceae*, and a few others as strongly supported clades within the *Pleosporales*. *Melanommataceae* as currently circumscribed was found to be polyphyletic. The genera *Byssosphaeria, Melanomma,* and *Pseudotrichia* were recovered within the family, while others such as *Ostropella* and *Xenolophium* nested outside in a weakly supported group along with *Platystomum compressum* and *Pseudotrichia guatopoensis* that may correspond to the family *Platystomaceae*. The genus *Byssosphaeria* was recovered as a strongly supported group within the *Melanommataceae* while *Melanomma* was weakly supported with unclear relationships among the species. The genera *Herpotrichia* and *Bertiella* were also found to belong in the *Melanommataceae*. *Lophiostomataceae* occurs as a strongly supported group but its concept is here expanded to include a new genus *Misturatosphaeria* that bears morphology traditionally not known to occur in the family. The strongly supported clade of *Misturatosphaeria* contains nine species that have gregarious, papillate ascomata with lighter coloured apices and plugged ostioles and that vary in ascospore morphology from 1- to 3-septate to muriform. Along with a strongly supported *Lophiostoma* clade, also within the family are *Thyridaria macrostomoides* based on new sequences from Kenyan collections and *Massariosphaeria triseptata, M. grandispora, Westerdykella cylindrica* and *Preussia terricola* based on GenBank sequences. The family *Hypsostromataceae* was recovered as a strongly supported monophyletic group nested within the *Pleosporales*.

**Key words:** *Eumycota*, evolution, fungi, *Hypsostromataceae*, phylogeny, taxonomy.
**Taxonomic novelties:** *Misturatosphaeria* Mugambi & Huhndorf, gen. nov., *M. aurantonotata* Mugambi & Huhndorf, sp. nov., *M. claviformis* Mugambi & Huhndorf, sp. nov., *M. cruciformis* Mugambi & Huhndorf, sp. nov., *M. kenyensis* Mugambi & Huhndorf, sp. nov., *M. minima* Mugambi, A.N. Mill. & Huhndorf, sp. nov., *M. tennesseensis* Mugambi, A.N. Mill. & Huhndorf, sp. nov., *M. uniseptata* Mugambi, A.N. Mill. & Huhndorf, sp. nov., *M. uniseriata* Mugambi, A.N. Mill. & Huhndorf, sp. nov.

## INTRODUCTION

*Pleosporales* is one of the largest orders of loculoascomycetous fungi and includes a complex array of organisms (Schoch *et al*. 2009, Zhang *et al*. 2009). Consequently, Barr (1987) considered arrangement of the genera and families to be far from satisfactory and work continues to this day to try to clarify the relationships. Luttrell (1955) included seven families and Barr (1987) recognised 18 families in her revised concept of the group. Presently it contains 20 families encompassing roughly 167 genera (Lumbsch & Huhndorf 2007). In synonymy with *Pleosporales* is the order *Melanommatales*, created by Barr (1983) for taxa that had a combination of centrum (peripherally occurring asci) and hamathecium (trabeculate pseudoparaphyses) characters she believed were important at the ordinal level. Recent molecular phylogenetic studies (*e.g.*, Berbee 1996, Liew *et al.* 2000, Winka 2000, Lumbsch & Lindemuth 2001, del Prado *et al.* 2005, Schoch *et al.* 2006, Kruys *et al.* 2006, Wang *et al.* 2007) have not supported the separation of *Melanommatales* from *Pleosporales*.

Although the concept of *Pleosporales* has recently attained some consensus (*e.g.* Winka 2000, Lumbsch & Lindemuth 2001, Kruys *et al.* 2006, Schoch *et al.* 2006, Lumbsch & Huhndorf 2007, Kirk *et al.* 2008, Zhang *et al.* 2008), many authors have differed on the circumscription of the families therein (*e.g.* Chesters & Bell 1970, Holm & Holm 1988, Barr 1984, 1987, 1990a, b, Lumbsch & Huhndorf 2007, Kirk *et al.* 2008). In *Melanommataceae*, Barr (1990a) accepted five genera, Kirk *et al.* (2008) accepted 21 genera, while Lumbsch & Huhndorf (2007) accept 18 genera with six of questionable placement. The taxonomy of *Lophiostomataceae*, another family in *Pleosporales*, has followed a similar path with Barr (1987) recognising six genera, Holm & Holm (1988) five genera, while Kirk *et al*., (2008) treated 15 genera and Lumbsch & Huhndorf (2007) 12 genera in the family.

Barr's (1990a) treatment of *Melanommataceae* included the following genera: *Ostropella*, *Keissleriella*, *Strickeria*, *Byssosphaeria* and *Melanomma* united on the basis of similar erumpent to superficial ascomata with walls composed of small, thick-walled cells. *Byssosphaeria* was re-instated by Barr (1984) for species that are separable from *Herpotrichia*, where it had been in synonymy for many years. The classification of *Byssosphaeria*, *Herpotrichia* and *Pseudotrichia* has posed major challenges to many authors because the morphological characters used to distinguish between the genera are not necessarily obvious (Samuels & Müller 1978). This has resulted in varied taxonomic treatments of the groups (*e.g.* Bose 1961, Samuels 1973, Samuels

& Müller 1978, Barr 1984). Detailed taxonomic revision on the genera is offered by Bose (1961) and Barr (1984). The two studies provide detailed morphological characters distinguishing the genera. *Pseudotrichia* differs from *Herpotrichia* in its rather large ascomata, often with compressed apices, while in *Herpotrichia* smaller ascomata are often covered with long flexuous hyphae and with subiculum that sometimes overgrows the fruiting bodies (Bose 1961, Barr 1984). *Byssosphaeria* on the other hand possesses superficial ascomata that are turbinate with a rounded pore and apical area that is usually light coloured (Barr, 1984). Barr (1984) initially segregated *Herpotrichia* into *Massarinaceae* but later transferred it to *Lophiostomataceae* (Barr 1987). Bose's (1961) study on *Massarina* and related genera concluded that *Massarina*, *Herpotrichia* and *Keissleriella* are distinct but closely related, and he placed them in the *Pleosporaceae* citing lack of striking characters to justify the creation of a new family. *Pseudotrichia* was described for ascomycete fungi with immersed-erumpent to superficial ascomata with rounded or laterally compressed apex. On the basis of the shape of the apex, Petrak (1940) placed it in *Lophiostomataceae*. However, Barr (1990a) accepted the genus in *Platystomaceae* and noted substantial variability in ascomatal apical morphology. She observed that even in a single collection it could be rounded or compressed to somewhat triangular.

The genus *Ostropella* was originally named as a subgenus of *Ostropa* for *O. albocincta* and was raised to generic status and redescribed by von Höhnel (1918). Müller & von Arx (1962) reduced *Schizostoma*, *Xenolophium* and *Ostreionella* all to synonymy under *Ostropella*. Barr (1990a) provided the history of *Ostropella* and its relationship to these other taxa. Holm & Yue (1988) presented work on *Schizostoma* in which they tried to clarify some misconceptions that have surrounded the genus since its establishment and to resolve the placement of some species. Chesters & Bell (1970) reduced *Xenolophium* into synonymy under *Lophiostoma* and treated it in their "*L. pachythele* group". Barr (1990a) pointed to the close relationship of *Xenolophium* to *Ostropella* and proposed it be accommodated under *Ostropella*. She also noted the differences between the low apical crest observed in *Ostropella* (that appears almost ornamental) from the compressed papilla in *Lophiostoma* and thus placed the genus in *Melanommataceae*. *Xenolophium* was established for two species of fungi from Hawaii by Sydow (in Stevens 1925). However, the genus was in synonymy for a long time until resurrected by Huhndorf (1993) who also added two new species to the group. She treated *Ostropella* and *Xenolophium* in *Melanommataceae* and distinguished the two genera based on their ascomatal surface structure, morphology of the ascomatal wall in longitudinal section and ascospore morphology.

*Lophiostomataceae* was first erected by Nitschke (1869) and in its long existence the group has greatly increased in size. The overall character that has historically been used to distinguish the family is the slit-like ostiolar opening on a laterally compressed papilla (Chesters & Bell 1970, Holm & Holm 1988). Chesters & Bell (1970) considered the family to comprise taxa with laterally compressed apices, hence they included *Lophiostoma*, and *Platystomum* in the group. However, the variability in the papillate form in the lophiostomataceous fungi had been noted for quite some time with certain species exhibiting mixed morphologies even within a single collection (Holm 1957, Eriksson 1981, Holm & Holm 1988, Barr 1990a). Holm & Holm (1988) took a broad concept of the family including taxa with laterally compressed and rounded apices in the group. They accepted five genera in the family, *Lophiostoma*, *Lophiotrema*, *Massariosphaeria*, *Navicella* and *Trematosphaeria*, while Barr (1987) accepted *Dangeardiella*, *Herpotrichia*, *Massarina*, *Lophiostoma*, *Lophidiopsis*, *Trichometasphaeria*, and *Cilioplea*. Recent molecular studies seem to support the view that the family is not exclusively composed of taxa with compressed papillae and that some taxa traditionally placed in this group belong elsewhere (*e.g.* Wang *et al.* 2007, Zhang *et al.* 2008).

*Lophiostoma* was circumscribed by Holm & Holm (1988) to include taxa that have immersed-erumpent ascomata with a distinctly flattened neck and opening by a slit-like ostiole. Asci are mostly clavate and ascospores are 1-septate, multiseptate or muriform, hyaline to dark brown. Recent phylogenetic work carried out on *Lophiostoma* species bearing these typical characters including the type species, *L. macrostomum*, indicated that the genus formed a monophyletic group (Tanaka & Hosoya 2008). *Lophiotrema* on the other hand was erected by Saccardo for "Hyalophragmiae" and has been used in this sense for a long time. Its circumscription is thus highly heterogeneous (Holm & Holm 1988). Chester & Bell (1970) did not recognise *Lophiotrema* but Holm & Holm (1988) accepted it in the strict sense for the group comprising the type species. *Massariosphaeria* was revised by Crivelli (1983) and Leuchtmann (1984) and its principal characteristics are the gelatinous ascospore sheath and tendency towards formation of red pigment, especially in mycelial cultures (Holm & Holm 1988). Leuchtmann (1984) transferred *Lophiotrema microthecum* to the group as *M. grandispora*. Recent molecular study of *Massariosphaeria* by Wang *et al.* (2007) indicated that the genus is highly polyphyletic with only *M. grandispora* among the species included in the analyses grouping in *Lophiostomataceae*.

*Hypsostromataceae* (Huhndorf 1994) was described for two tropical genera, *Hypsostroma* and *Manglicola*. In setting up the family Huhndorf (1994) noted its affinities to taxa in the *Melanommatales* (= *Pleosporales*) where she suggested it belonged but appeared unrelated to any known families. Characters that united the two genera in the family included superficial, large, elongate ascomata, soft-textured pseudoparenchymatous wall, trabeculate pseudoparaphyses, asci with an apical chamber and fluorescing ring and stipitate, basally arranged and fusiform, septate ascospores (Huhndorf 1994). *Hypsostroma* was erected by Huhndorf (1992) for two tropical wood-inhabiting species *H. saxicola* and *H. caimitalensis*. The two species bear close morphological resemblance, only slightly differing in their ascomatal and ascospore characters, with *H. caimitalensis* bearing long papillate ascomata and ascospores constricted at septum. Currently the family resides in the *Dothideomycetes*, family *incertae sedis* (Lumbsch & Huhndorf 2007).

Many of the recent molecular phylogenetic studies involving the *Pleosporales* seem to reject the monophyly of the families in the order (*e.g.* Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Kruys *et al.* 2006, Schoch *et al.* 2006, Wang *et al.* 2007, Zhang *et al.* 2008). This polyphyly witnessed in major lineages within *Pleosporales* indicates that the order is in urgent need for revision. This study contributes to this endeavor and it employs two nuclear gene markers, nuclear ribosomal large subunit DNA (LSU) and translation elongation factor 1-alpha (TEF) in order to: 1) assess the generic constitution and relationships within *Melanommataceae* and *Lophiostomataceae*, 2) verify the phylogenetic placement of the genus *Hypsostroma*, 3) discuss phylogenetic findings with respect to morphological-based classification schemes.

## MATERIALS AND METHODS

### Taxon sampling and morphological analyses

The taxa used in this study are listed in Table 1 - see online Supplementary Information. Those newly sequenced together with their collection information are indicated in bold while the others were obtained from GenBank. Representative species covering 10 families in the *Dothideomycetes* were targeted for analyses. A total of 149 taxa were included in the analyses with 75 taxa newly sequenced during this study (Table 1). The microscopy and image capture follow methods outlined in Huhndorf & Fernández (1998). The ascomata were squash-mounted in water and images of anatomical structures captured with a Dage DC-330 video system (Dage-MTI®, U.S.A.) mounted on a Zeiss Axioskop microscope (Carl Zeiss®, U.S.A.). Format of the individual figures for most of the species follow those produced for the pyrenomycete website (Pyrenomycetes of the World: www-s.life.illinois.edu/pyrenos/).

### DNA extraction, PCR amplification, sequencing and sequence alignment

Total fungal DNA was extracted from whole fruiting bodies using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Phylogenetic analyses were conducted using partial sequences of two genes, the Translation Elongation Factor 1-Alpha (TEF) and nuclear ribosomal large subunit (LSU) DNA. Nuclear LSU was amplified using the primers LR0R, LR6 and LR3 (Vilgalys & Hester 1990) and TEF was amplified using primers EF1-526F, EF1-983F, EF1-1567R, Ef-df and EF-gr obtained from D. Hibbett's website (www.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.htm).

Polymerase chain reaction (PCR) was carried out using the following protocol: The final volume of the PCR reaction was 25 µL and contained 2.5 µL buffer, 2.5 µL dNTP mix, 1 µL of each primer (10 µM), 5 µL of Bovine Serum Albumin (BSA), 1.5 µL *Taq* polymerase (Roche®, U.S.A.), 2 µL genomic DNA extract and 9.5 µL deionised water. The reaction was then allowed to run for 34 cycles. The annealing temperature was 50 ˚C for LSU, and initial 58 ˚C for TEF and then reduced by 1 ˚C during each of the first eight cycles and maintained at 50 ˚C for the remaining cycles. The fragments were sequenced using the Big Dye® Terminator Cycle Sequencing kit v. 3.1 (ABI PRISM, Applied Biosystems, Forster City, U.S.A.). Sequencing was performed using the same set of primers as the PCR. The other sequences used in the analyses were obtained from GenBank. Sequences were aligned using multiple sequence program Muscle® v. 3.6 (Edger 2004). The alignments were further manipulated manually and those regions, which could not be aligned with confidence were excluded from analyses. The final data matrices comprised of LSU data set with 140 taxa with 1179 unambiguously aligned characters, and a TEF data set with 57 taxa and 750 unambiguously aligned characters while the combined data set comprised of 49 taxa for which both genes were available with 1929 unambiguously aligned characters. Voucher specimens are deposited in the Field Museum Herbarium (F), while Kenyan specimens are deposited at the East Africa Herbarium (EA).

### Phylogenetic analyses

MODELTEST v. 3.7 (Posada & Crandall, 1998) following Akaike Information Criterion was used to determine the best-fit model of evolution for each data set for Bayesian and Maximum Likelihood analyses. Bayesian analyses employing Markov Chain Monte Carlo (MCMC) were carried out using MrBayes v. 3.1 (Huelsenbeck & Ronquist, 2001). Four MCMC chains were ran simultaneously for 5–7 million generations for single-gene and combined gene analyses, the temperature of the heated chains was set at 0.05 for LSU and at 0.2 for TEF and combined gene analyses. Trees were sampled every 100$^{th}$ generation. The TEF gene matrix was partitioned into three parts to take into account the codon positions, while combined gene matrix had four partitions. Independent models of evolution were applied on the partitions for Bayesian analyses. AWTY was used to check the stationarity of the Bayesian tree sampling procedure (Nylander *et al.* 2007). All the trees obtained before the MCMC chains attained stationarity in each analysis were discarded and posterior clade probabilities were determined from the consensus tree generated from the rest. The majority rule consensus tree was obtained by executing the MrBayes sumt command. Maximum likelihood (ML) analyses were carried out for each of the three data sets using RAxML (Stamatakis *et al*. 2008) employing mixed models of evolution settings of the program and Bootstrap support obtained by running 1000 pseudo replicates. Five independent ML tree searches were done in RAxML (Stamatakis *et al*. 2008) each one starting from randomised tree.

Test of conflict was based on single gene analyses and doing comparison based on Bootstrap and Bayesian posterior probabilities support. Clades with greater than or equal to 70 % bootstrap support (BS) and 95 % posterior probabilities (PP) were considered strongly supported. There were no major conflicts in the phylogenies obtained from single-gene analyses. The differences observed were mainly the family relationships in the Pleosporales, which nonetheless received low support. As a result the data sets were combined for Maximum Likelihood (ML) and Bayesian analyses.

## RESULTS

The best-fit model of evolution for LSU, TEF and combined gene data sets was GTR+I+G (Rodriguez *et al.* 1990) following Akaike Information Criterion implemented by ModelTest v. 3.7 (Posada & Crandall, 1998). Analyses using Maximum Likelihood and Bayesian methods resulted in phylogenies with similar topologies. Consequently only results of ML phylograms of single-gene analyses (Figs 1–2) and combined gene matrix (Fig. 3) are presented. The gene genealogies recovered a strongly supported *Pleosporales* that is composed of strongly supported clades for *Melanommataceae*, *Pleosporaceae*, *Lophiostomataceae*, *Delitschiaceae*, *Arthopyreniaceae* and *Hypsostromataceae* in at least one of the trees (Figs 1–3). The LSU tree showed clades with stronger BS support than the TEF tree. *Melanommataceae* includes *Melanomma*, *Byssosphaeria*, *Herpotrichia*, and *Pseudotrichia.* Nested in the family are collections representing *Bertiella macrospora* and *Pleomassaria siparia*. Taxa in *Ostropella* and *Xenolophium* that were previously placed in *Melanommataceae*, group in an unsupported clade distant from the family (Figs 1–3). The genus *Herpotrichia* was recovered as polyphyletic using LSU, with *H. juniperi* grouping separate from a well-supported clade that includes *H. macrotricha* and *H. herpotrichoides* (Fig. 1), but the genus resolves as monophyletic in the TEF and combined gene trees (Figs 2–3). A well-supported clade for *Byssosphaeria* was recovered in all three trees. Only LSU sequences were obtained for taxa in *Melanomma* and they did not resolve in a monophyletic

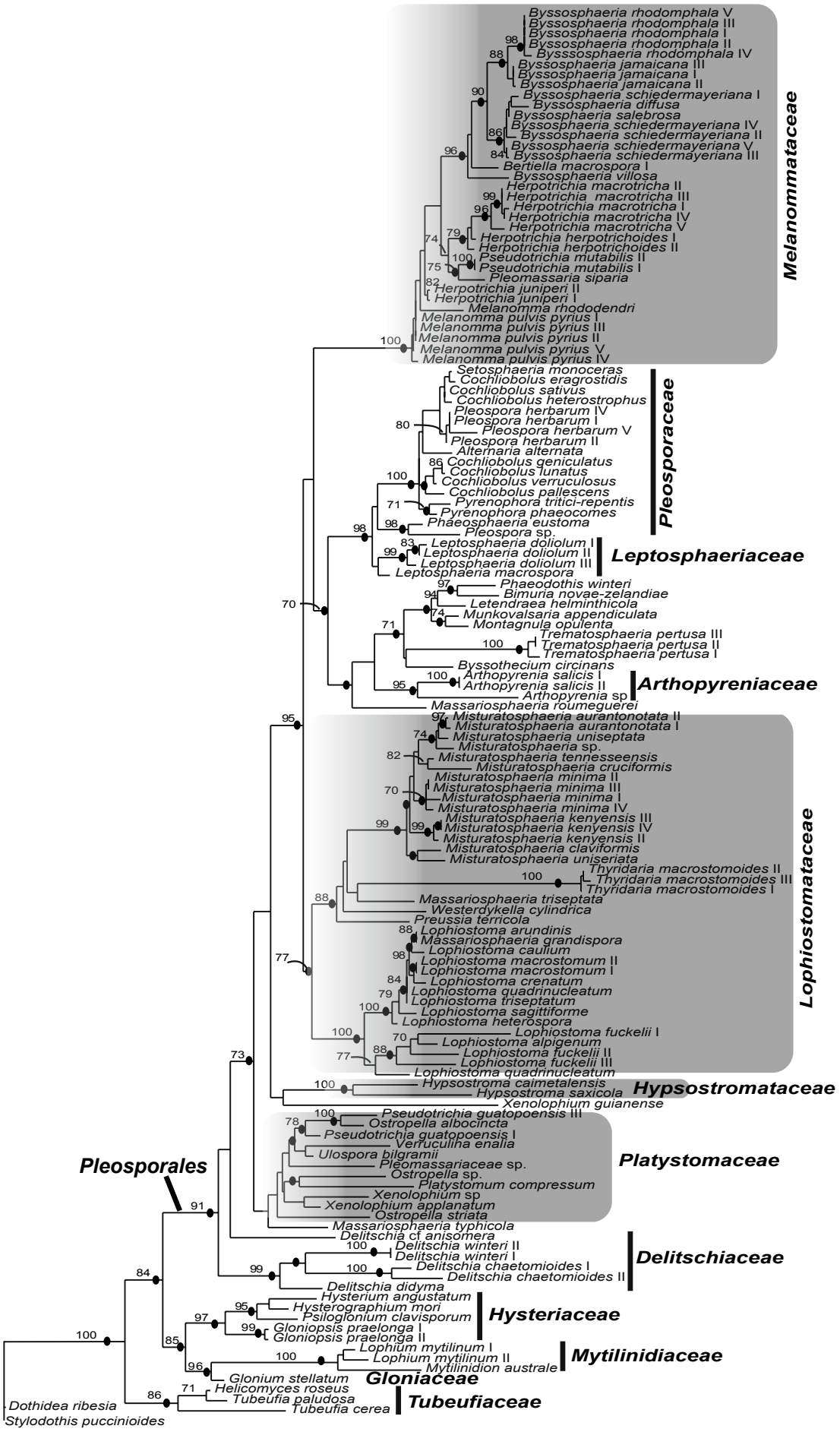



**Fig. 1.** Phylogram of the maximum likelihood analyses generated from LSU sequences. Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).

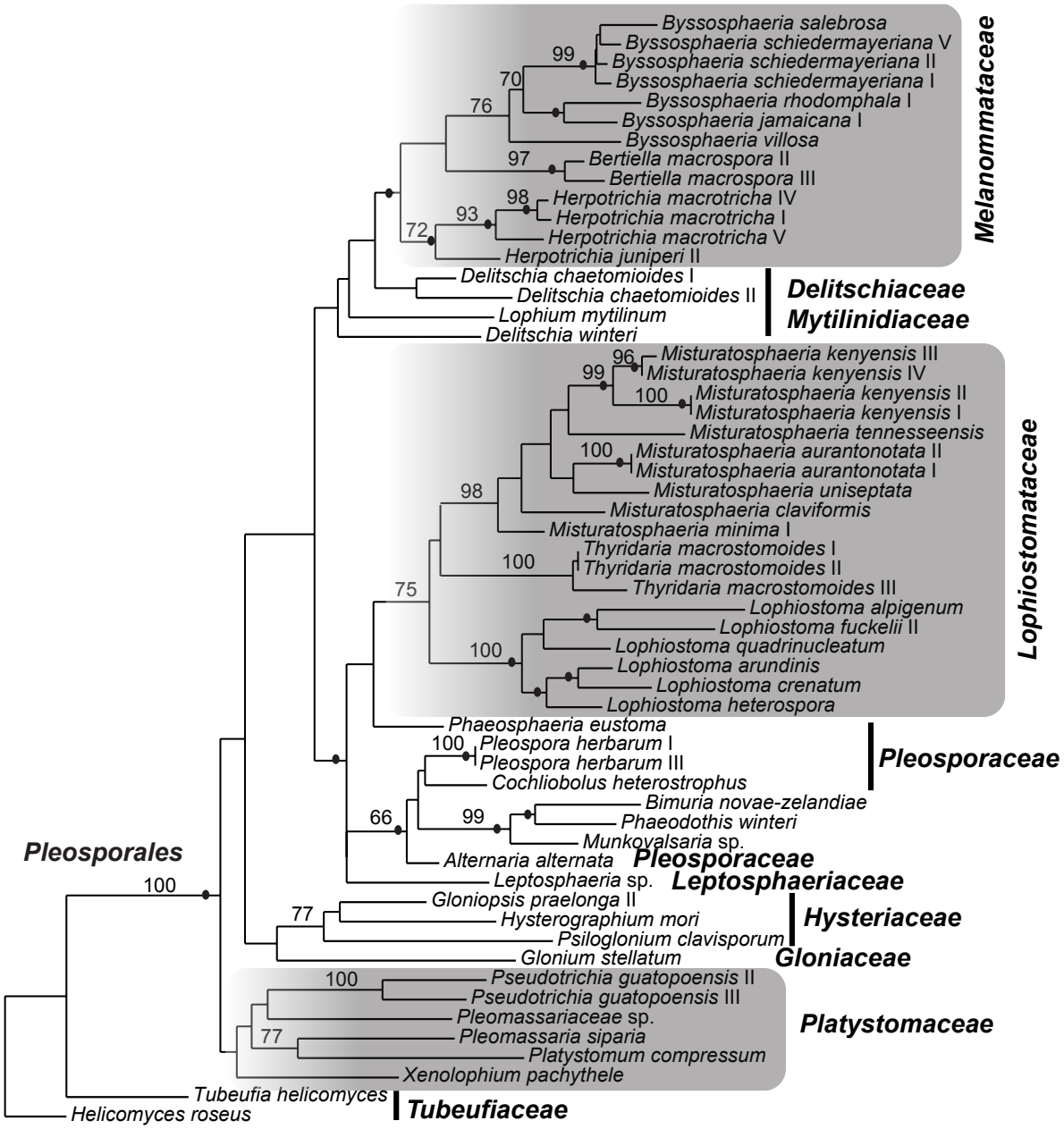



**Fig. 2.** Phylogram of the maximum likelihood analyses generated from TEF sequences. Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).

clade (Fig. 1). *Pseudotrichia* is polyphyletic with the type species, *P. mutabilis* occurring in the *Melanommataceae* and *P. guatopoensis* grouping with *Ostropella* spp. (Figs 1–2). A strong BS and PP supported clade composed of a new genus with nine species was recovered nested within *Lophiostomataceae* (Figs 1, 3) and the taxa are described below. The clade was obtained in TEF analyses but was not strongly supported (Fig. 2). Sister to the new genus are three collections of *Thyridaria macrostomoides* (Figs 1–3). A strongly supported clade for *Lophiostoma* was recovered (Figs 1–3) including two collections of *Lophiostoma macrostomum*. These grouped together in a strongly supported clade that included nine other species in the genus (Fig. 1). *Massariosphaeria grandispora,* a sequence obtained from GenBank, was found nested within *Lophiostoma* (Fig. 1). Single collections of *Preussia terricola* and *Westerdykella cylindrica* whose sequences were obtained from GenBank were found nested within *Lophiostomataceae* (Fig. 1).

*Hypsostromataceae* was recovered as a well-supported clade within *Pleosporales* comprising two species accepted in the family (Fig. 1). Species of *Ostropella*, *Xenolophium* (except *Xenolophium pachythele* that groups separate in TEF tree) and other taxa assemble in a mostly unsupported clade together with a collection of *Platystomum compressum*, possibly representing the *Platystomaceae*. The taxa group together in all three trees but only obtain PP support in the combined gene tree (Fig. 3). A monophyletic clade for *Delitschiaceae* was recovered within *Pleosporales*; *Hysteriaceae*, *Mytilinidiaceae*, *Gloniaceae* and *Tubeufiaceae* were recovered as strongly supported monophyletic groups outside the *Pleosporales* (Figs 1–3).

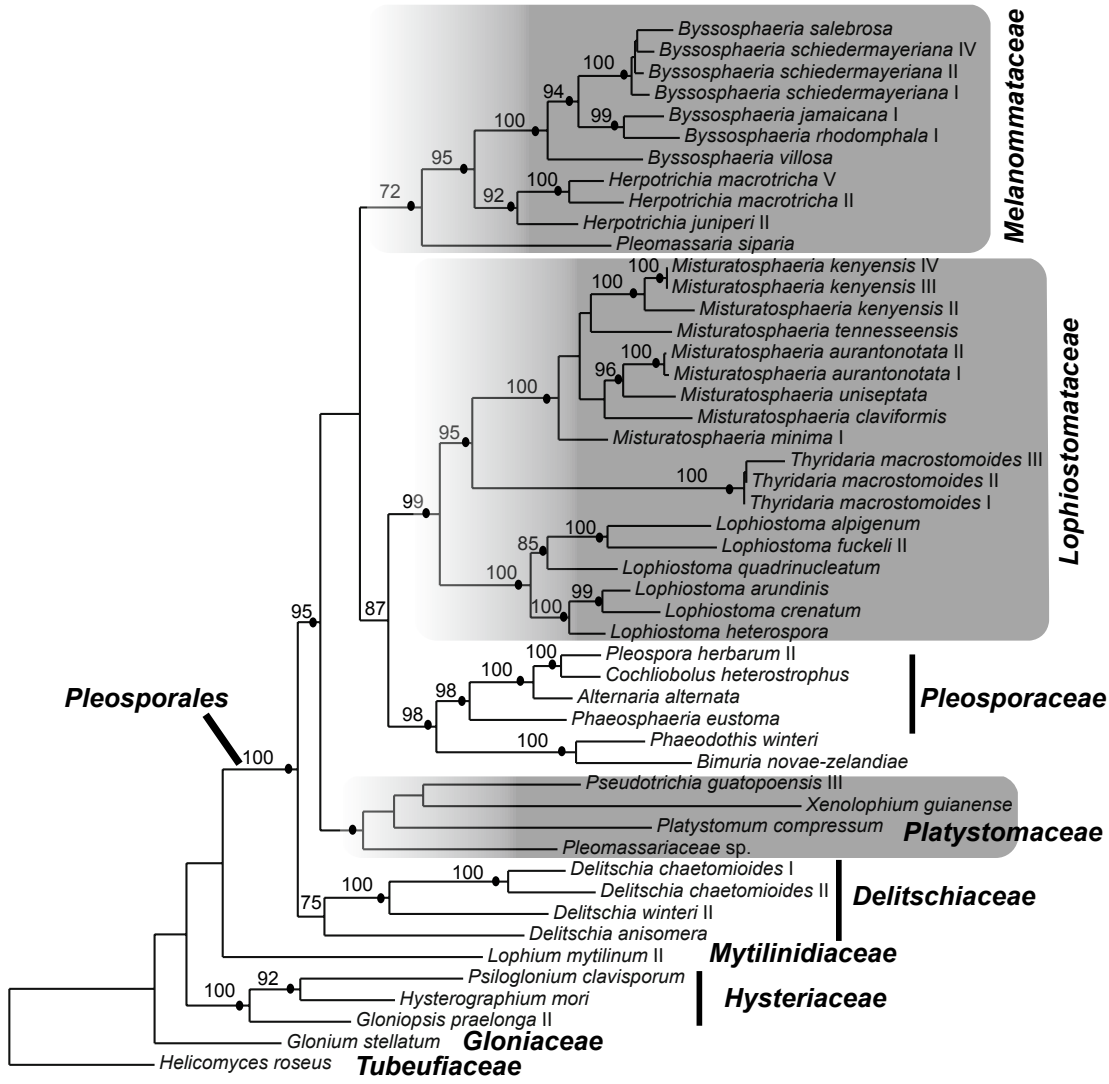



**Fig. 3.** Phylogram of the maximum likelihood analyses generated from the combined genes (LSU and TEF). Bootstrap support values ≥ 70 % are shown above or below the branches. Black circles indicate branches with Bayesian posterior probabilities ≥ 95 %. The families treated in this study are indicated (shaded).

## TAXONOMY

Images of sequenced taxa are included and grouped together to facilitate comparison of morphological characteristics. The species of *Byssosphaeria* and *Bertiella* are arranged together in the first plate: *Byssosphaeria jamaicana* (Figs 4–5), *B. rhodomphala* (Figs 6, 8), *Bertiella macrospora* (Fig. 7), and *Byssosphaeria villosa* (Fig. 9). The second plate contains species of *Byssosphaeria*, *Melanomma* and *Pseudotrichia*: *Byssosphaeria schiedermayeriana* (Figs 10–13, 15), *B. salebrosa* (Fig. 14), *Melanomma pulvis-pyrius* (Fig. 16), *M. rhododendri* (Fig. 17), and *Pseudotrichia mutabilis* (Fig. 18). The third plate contains *Herpotrichia macrotricha* (Figs 19–22) and *H.* cf. *herpotrichoides* (Figs 23–24).

***Misturatosphaeria*** Mugambi & Huhndorf, **gen. nov.** MycoBank MB515583.

*Etymology*: *Misturatus* (*L.*) = mixed, refers to the mixed ascospore morphology in the group.

Ascomata erumpentia ad superficialia, solitaria vel aggregata, cum subiculum vel sine subicolo, apicibus rotundatis pallide coloratis vel incoloratis. Asci claviti vel cylindrici, breve stipitati, octospori, pseudoparaphysibus numerosis, hyalinis et septatis, in matrice mucosa. Ascosporae hyalinae vel brunneae, septatae, cum vagina mucosa vel sine vagina.

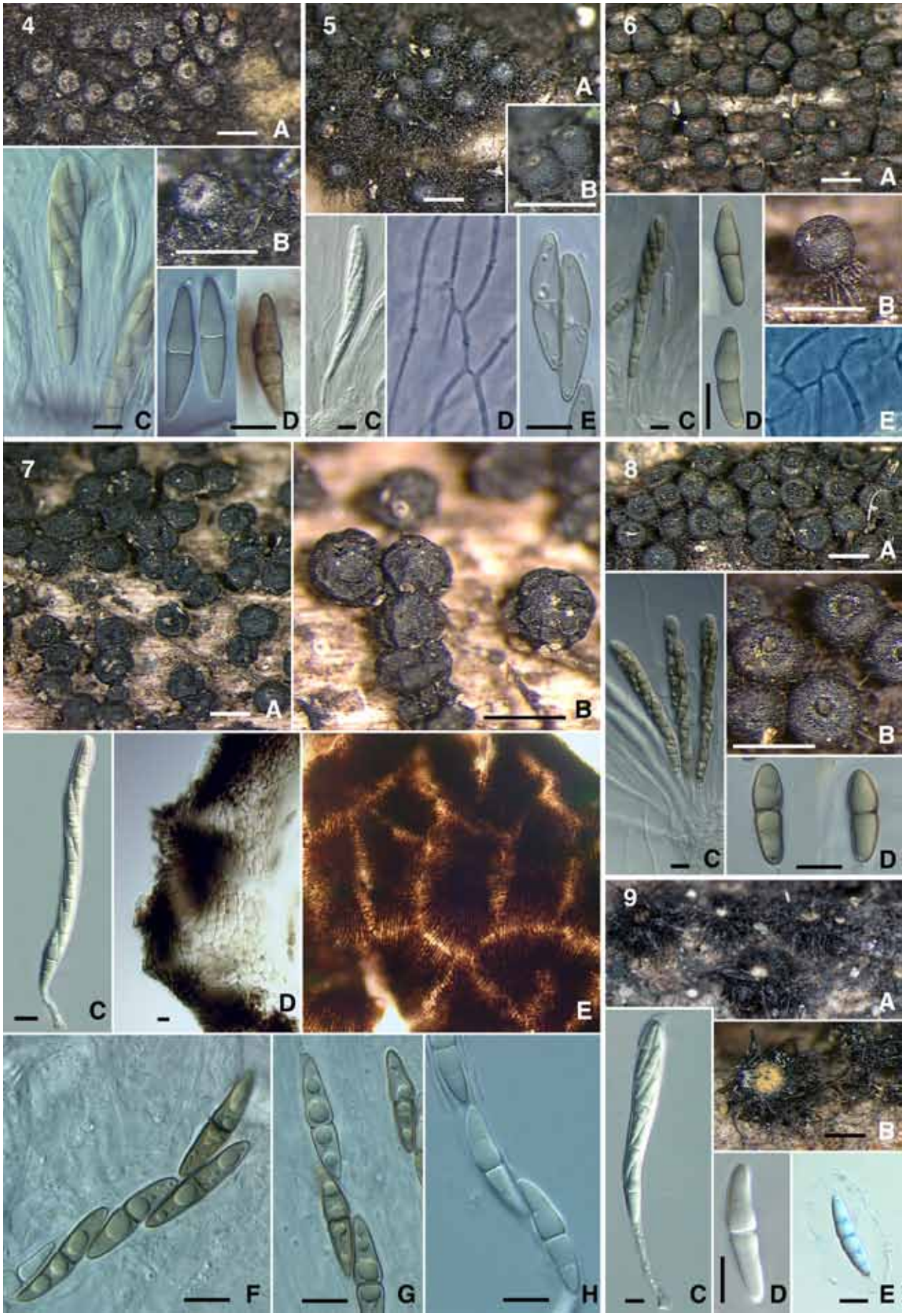
*Typus*: *Misturatosphaeria aurantonotata* Mugambi & Huhndorf, sp. nov.

*Ascomata* erumpent to superficial, occurring singly or aggregated in clusters, subiculum present or lacking, apex rounded, with raised papilla or not, ostiole area light coloured or not and ostiole opening appearing plugged by gelatinous tissue. *Asci* cylindrical or clavate, short stipitate, 8-spored, held in gelatinous matrix, pseudoparaphyses numerous, septate, branching and anastomosing between and above the asci. *Ascospores* brown or hyaline, phragmosporous or dictyosporous, external walls roughened or smooth, with or without a gelatinous sheath covering.

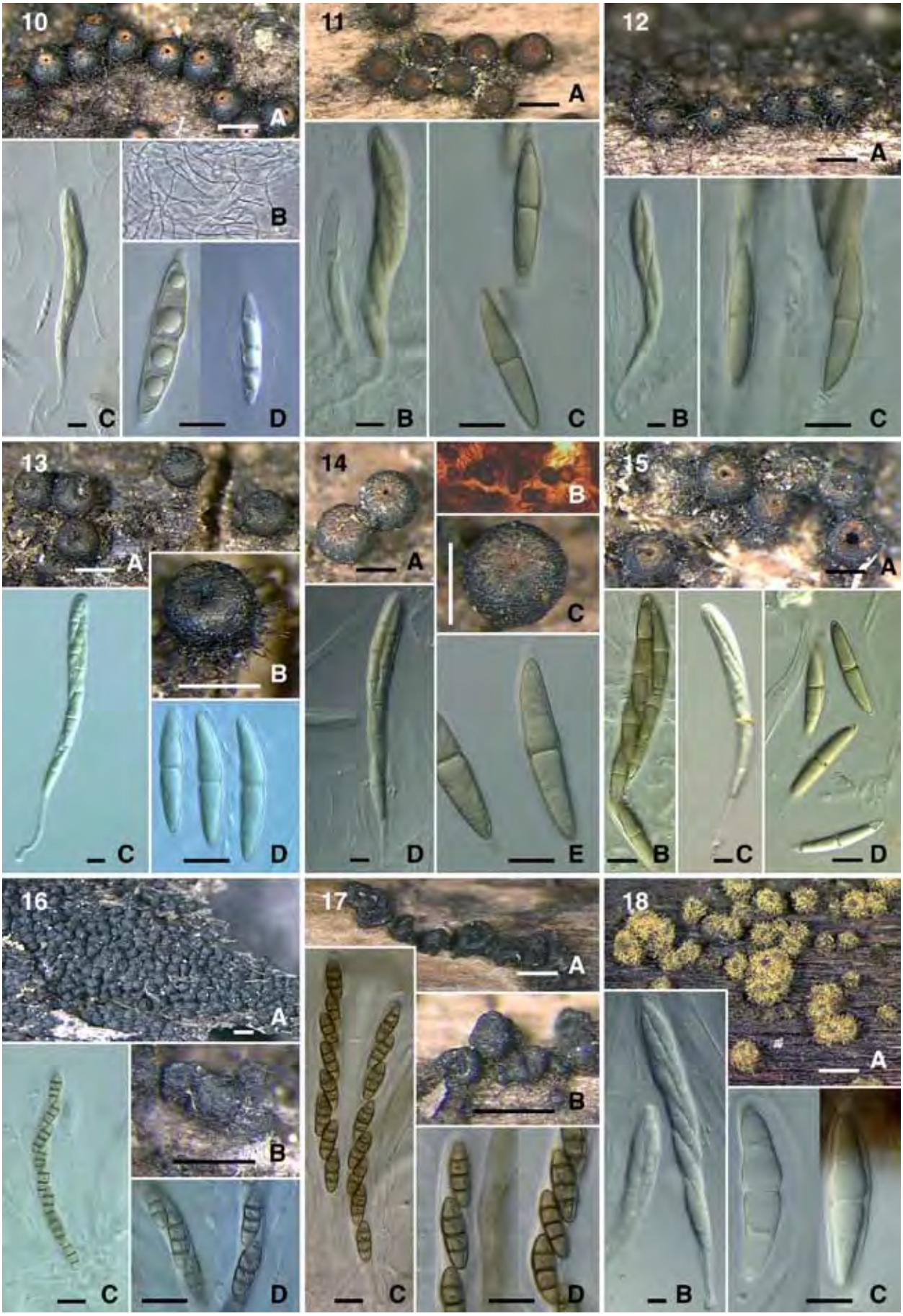
***Misturatosphaeria aurantonotata*** Mugambi & Huhndorf, **sp. nov.** MycoBank MB516007. Fig. 25.

*Etymology*: *Aurantiacum* (*L.*) = orange, *notatus* (*L.*) = marked, refers to the orange colour markings at the ascomata apices.

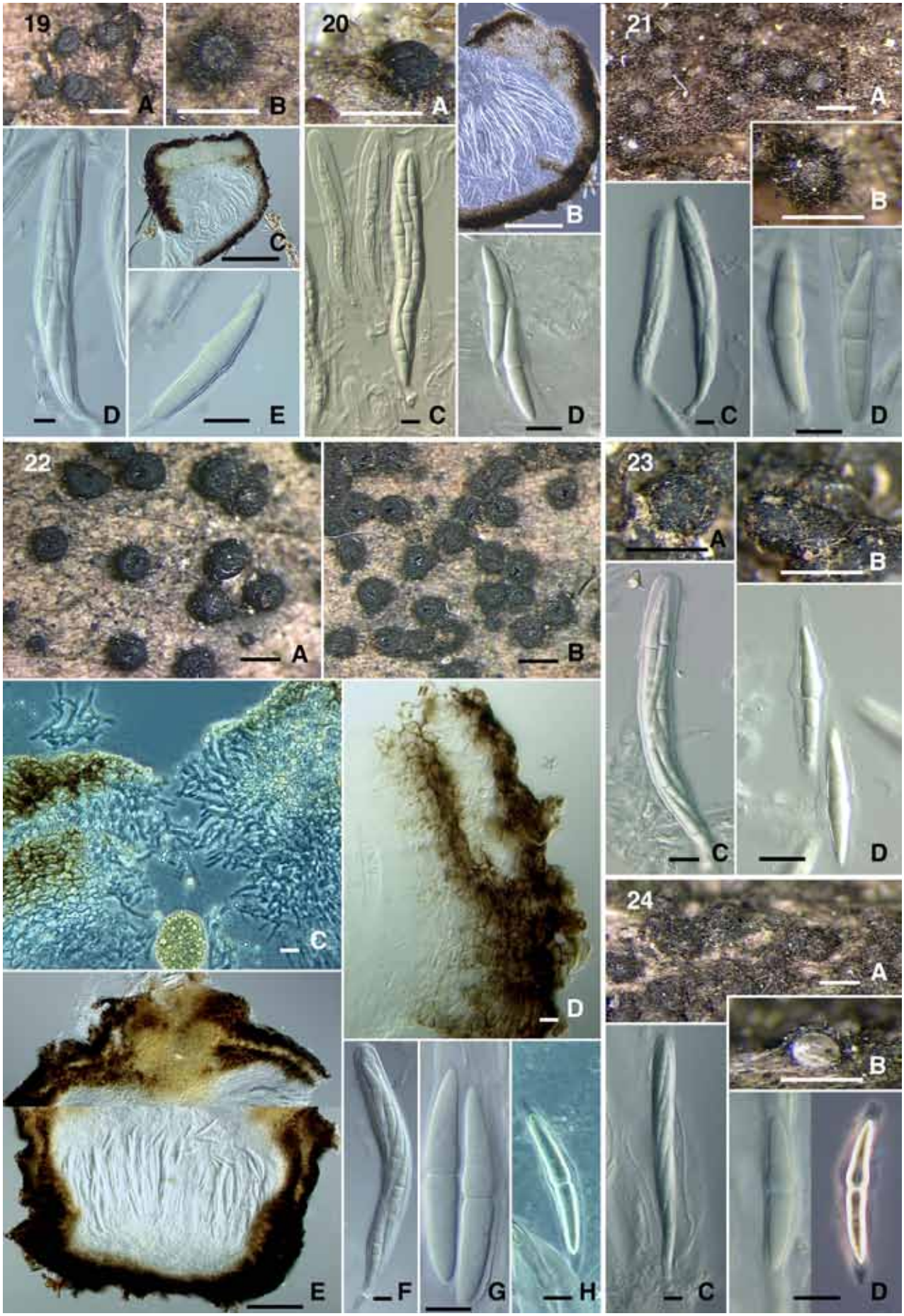
Ascomata superficialia, atrobrunnea, solitaria vel dense aggregata, in subiculo sparso ex hyphis brunneis, pyriformia, 441–710 µm alta, 461–573 µm diam, apicibus rotundatis, saepe aurantiacis. Asci cylindrici-clavati, breve stipitati, octospori, 103–122 x 8–12 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm. Ascosporae fusoides, primo hyalinae, deinde atrobrunnae, 3-septatae, cum vagina mucosa, 17–22 x 5–6 µm.



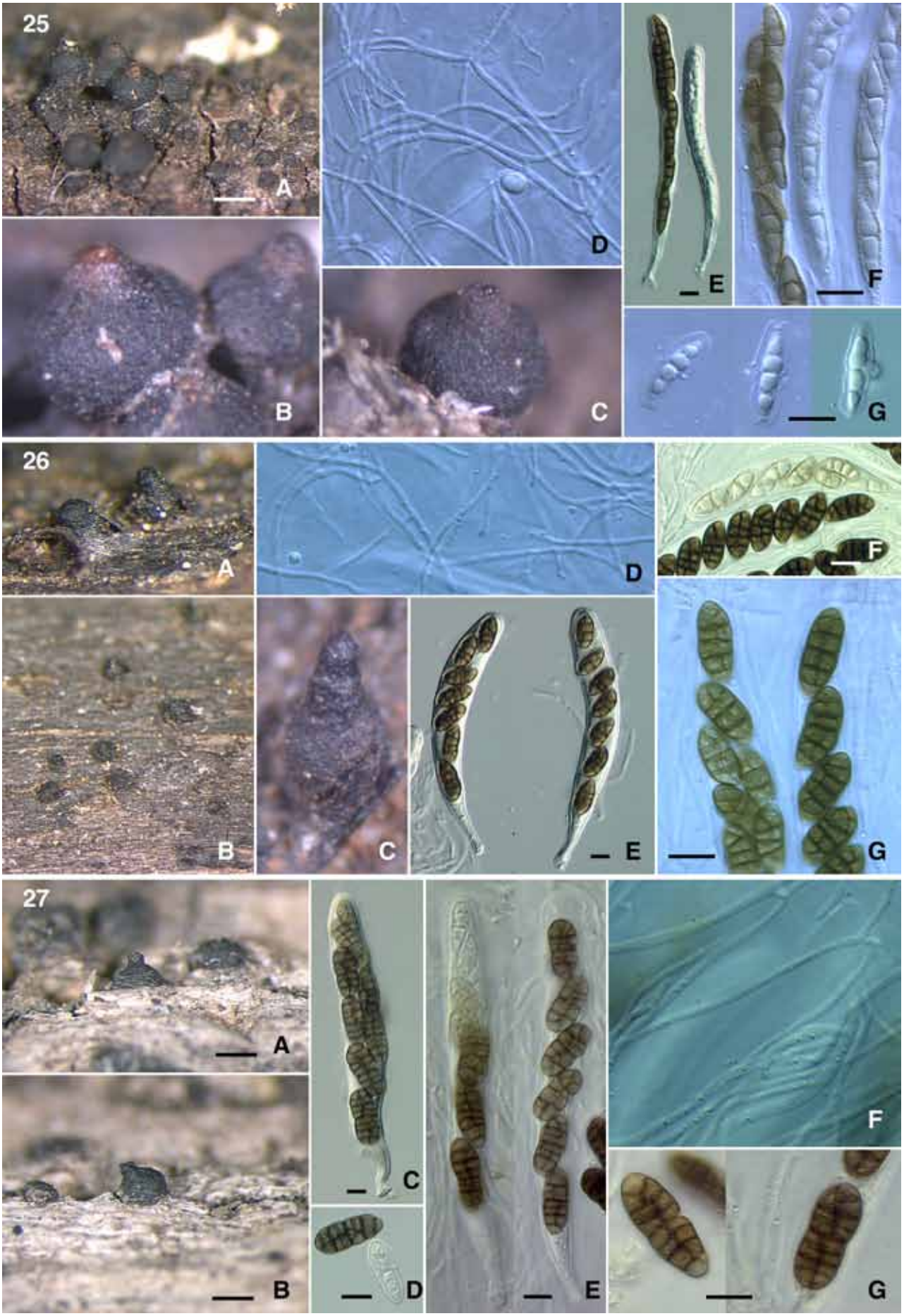

**Figs 4–9. 4.** *Byssosphaeria jamaicana* (SMH1403) A–B. Ascomata. C. Ascus. D. Ascospores. **5.** *B. jamaicana* (SMH3464/3085) A–B. Ascomata. C. Ascus. D. Pseudoparaphyses. E. Ascospores. **6.** *B. rhodomphala* (ANM942) A, B. Ascomata. C. Ascus. D. Ascospores. E. Pseudoparaphyses. **7.** *Bertiella macrospora* (IL5005) A–B. Ascomata. C. Ascus. D. Ascomatal wall section. E. Ascomatal wall surface. F–H. Ascospores. **8.** *B. rhodomphala* (SMH3402) A. Ascomata. B. Asci. C. Ascospores. **9.** *B. villosa* (GKM204N) A–B. Ascomata. C. Ascus. D–E. Ascospores. Scale bars: Ascomata = 500 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.




**Figs 10–18. 10.** *Byssosphaeria schiedermayeriana* (SMH1296) A. Ascomata. B. Pseudoparaphyses. C. Ascus. D. Ascospores. **11.** *B. schiedermayeriana* (SMH3157) A. Ascomata. B. Ascus. C. Ascospores. **12.** *B. schiedermayeriana* (SMH1816) A. Ascomata. B. Ascus. C. Ascospores. **13.** *B. schiedermayeriana* (GKM1197) A–B. Ascomata. C. Ascus. D, E. Ascospores. **14.** *B. salebrosa* (SMH2387) A, C. Ascomata. B. Ascomatal wall surface. D. Ascus. E. Ascospore. **15.** *B. schiedermayeriana* (GKM152N) A. Ascomata. B–C. Asci. D. Ascospores. **16.** *Melanomma pulvis-pyrius* (SMH3291) A–B. Ascomata. C. Ascus. D. Ascospores. **17.** *M. rhododendri* (ANM73) A–B. Ascomata. C. Asci. D. Ascospores. **18.** *Pseudotrichia mutabilis* (SMH5288) A. Ascomata. B. Ascus. C. Ascospores. Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.




**Figs 19–24. 19.** *Herpotrichia macrotricha* (SMH269) A–B. Ascomata. C. Ascomatal section. D. Ascus. E. Ascospore. **20.** *H. macrotricha* (GKM196N) A. Ascoma. B. Ascomatal section. C. Ascus. D. Ascospores. **21.** *H. macrotricha* (GKM1128) A–B. Ascomata. C. Ascus. D. Ascospores. **22.** *H. macrotricha* (SMH4913) A–B. Ascomata. C. Ascomatal neck section. D. Ascomatal wall section. E. Ascomatal section. F. Ascus. G, H. Ascospores. **23.** *H.* cf. *herpotrichoides* (GKM212N) A–B. Ascomata. C. Ascus. D. Ascospores. **24.** *H.* cf. *herpotrichoides* (SMH5167) A–B. Ascomata. C. Ascus. D. Ascospores. Scale bars: Ascomata = 500 µm. Section = 100 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.




**Figs 25–27. 25.** *Misturatosphaeria aurantonotata* (GKM1238) A–C. Ascomata. D. pseudoparaphyses. E. Asci. F–G. Ascospores. **26.** *Misturatosphaeria claviformis* (GKM1210) A–C. Ascomata. D. Pseudoparaphyses. E. Asci. F–G. Ascospores. **27.** *Misturatosphaeria cruciformis* (SMH5151) A–B. Ascomata. C, E. Asci. D, G. Ascospores. F. Pseudoparaphyses. Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.

*Ascomata* superficial, occurring singly or aggregated into large clusters, occasionally even growing on old ascomatal tissue, often sited on sparse brown subiculum, pyriform in shape, dark brown, ascomatal wall smooth, 441–710 µm high, 461–573 µm wide. Apices rounded, usually with raised papillae, ostiole area orange in colour or the colouring lacking all together. *Asci* are cylindrical–clavate with short stipes, 8-spored partially biseriate in arrangement, 103–122 x 8–12 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform, often slightly curved, first hyaline later becoming brown to dark brown, the outer walls are thick and roughened, 3-septate at maturity, one of the middle cells is often larger than the rest, only slightly constricted at the middle septum. Mucilaginous sheath present when the spores are young and falls off upon attaining maturity, 17–22 x 5–6 µm.

*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Presently only known from two tropical forests in the central highlands of Kenya.

*Specimens examined*: **Kenya**, Rift Valley Province, Kajiando District, Ngong hills forest res., 1˚23'934''S, 36˚38'287''E, 12 July 2006, on woody branch, G.K. Mugambi, GKM1238, **holotype** EA, **isotype** F; Koibatek District, Lembus forest along Eldoret-Eldama Ravine Rd, 0˚04'N, 35˚35'E, 19 Jan. 2007, on branch on the ground, G.K. Mugambi, GKM1280, EA, F.

### ***Misturatosphaeria claviformis*** Mugambi & Huhndorf, **sp. nov.** MycoBank MB516008. Fig. 26.

*Etymology*: *Clavatus* (*L.*) = club, refers to the club-shaped ascomata usually witnessed in the species.

Ascomata erumpentia ad superficialia, atrobrunnea, solitaria vel sparse aggregata, sine subiculo, pyriformia ad obclavata, 235–520 µm alta, 195–322 µm diam, apicibus rotundatis, ostiolata. Asci cylindrici-clavati, breve stipitati, octospori, 85–134 x 12–18 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporae ellipticae, brunneae ad atrobrunnae, muriformes, since vagina mucosa, 12–20 x 7–9 µm.

*Ascomata* erumpent to superficial, occurring singly or aggregated into small groups, subiculum absent, pyriform to obclavate in shape, dark brown, ascomatal wall smooth, 235–520 µm high and 195–322 µm wide. Apices rounded, usually possess raised, broad rounded papillae also with rounded openings. *Asci* cylindrical-clavate, with short stipes, 8-spored partially biseriate or uniseriate, oblique or sometimes irregularly arranged in an oblique fashion or irregular, 85–134 x 12–18 µm. *Pseudoparaphyses* numerous, septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* elliptical, straight or inequilateral, brown to dark brown in colour, outer wall smooth, dictyosporous, with no mucilaginous sheath, 12–20 x 7–9 µm.

*Substratum*: Found on decorticated woody branches on the ground in forested areas.

*Anamorph*: Unknown.

*Distribution*: Currently known only from a tropical highland forest in central Kenya.

*Specimen examined*: **Kenya**, Central Province, Nyeri District, Mt Kenya forest, behind Bantu lodge, 0˚6'907''S, 37˚2'699''E, 30 Nov. 2006, on woody branch, G.K. Mugambi, GKM1210, **holotype** EA, **isotype** F.

### ***Misturatosphaeria cruciformis*** Mugambi & Huhndorf, **sp. nov.** MycoBank MB516009. Fig. 27.

*Etymology*: *Cruciatus* (*L.*) = cross-wise, refers to the ascospore septation, transverse and longitudinal septa.

Ascomata erumpentia, atrobrunnea, solitaria vel sparse aggregata, pyriformia, globosa, 500–535 µm alta, 545–649 µm diam, apicibus rotundatis, ostiolata, since subiculo. Asci cylindrici-clavati, breve stipitati, octospori, 127–154 x 14–17 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporae oblongae ad ellipticae, brunneae ad atrobrunnae, muriformes, saepe constrictae ad septum medium, sine vagina mucosa, 19–26 x 8–13 µm.

*Ascomata* erumpent, usually occurring singly rarely clustered into small groups, subiculum lacking, pyriform to globose in shape, dark brown, ascomatal wall smooth, 500–535 µm high and 545–649 µm wide. Apices are rounded with papillae that are usually raised, ostiole opening rounded. *Asci* cylindrical-clavate in shape and bearing short stipes, 8-spored partially biseriate to sometimes overlapping uniseriate, 127–154 x 14–17 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in a gelatinous matrix, 1–2 µm diam. *Ascospores* oblong to elliptical, brown becoming dark brown with age, outer wall smooth, dictyosporous, usually constricted at the middle transverse septum, possess no mucilaginous sheath, 19–26 x 8–13 µm.

*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Currently only known from a forest reserve in Illinois, U.S.A.

*Specimen examined*: **U.S.A.**, Illinois, Cook Co., Swallow Cliff Woods Forest Preserve, 21 May 2004, on woody branch, G.K. Mugambi, SMH5151, **holotype**, F.

### ***Misturatosphaeria kenyensis*** Mugambi & Huhndorf, **sp. nov.** MycoBank MB516010. Figs 28–29.

*Etymology*: Refers to the country the species was collected, Kenya.

Ascomata erumpentia ad superficialia, solitaria vel dense aggregata, atrobrunnea, pyriformia ad globosa, 185–305 µm alta, 245–334 µm diam, apicibus rotundatis pallide coloratis, ostiolo rotundato. Asci cylindrici-clavati, breve stipitati, octospori, 71–79 x 8–9 µm. Pseudoparaphyses, numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporae fusoides, hyalinae, 1–3-septatae vulgo 1–septatae, cum vagina mucosa parva, 15–24 x 4–6 µm.

*Ascomata* erumpent to superficial, occurring singly or aggregated into large clusters, without subiculum, pyriform to globose in shape, dark brown, ascomatal wall smooth, 185–305 µm high and 245–334 µm wide. Apices rounded, usually with raised papillae, opening rounded and the ostiole area often of lighter colour. *Asci* cylindrical-clavate in shape, short stipitate, 8-spored, partially biseriate in arrangement, 71–79 x 8–9 µm. *Pseudoparaphyses* numerous and septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform in shape, hyaline, outer wall smooth, usually 1–3-septate but commonly 1-septate, occasionally one of the middle cells broader than others, possess small mucilaginous sheaths that extends at the tip of spores, 15–24 x 4–6 µm.

*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Presently only known from a tropical cloud forest in Kenya.

*Specimen examined*: **Kenya**, Coast Province, Taita Taveta District, Taita hills, Ngangao forest res., 3˚22'30''S, 38˚20'45''E, 30 Oct. 2006, on woody branch, G.K. Mugambi, GKM1195, **holotype** EA, **isotype** F.

***Misturatosphaeria minima*** Mugambi, A.N. Mill. & Huhndorf, **sp. nov.** MycoBank MB516011. Figs 30–32.

*Etymology*: *Minus* (*L.*) = less, refers to the relatively smaller size ascomata observed in this species.

Ascomata erumpentia ad superficialia, solitaria vel sparse aggregata, atrobrunnea, pyriformia ad globosa, 194–389 µm alta, 244–355 µm diam, since subiculo, apicibus rotundatis saepe aurantiacis, ostiolo rotundato. Asci cylindrici-clavati, breve stipitati, octospori, 72–112 x 8–11 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosae, 1–2 µm diam. Ascosporae fusoides, hyalinae, 1–3-septatae, constrictae ad septum medium, cum vagina mucosa parva, 18–22 x 3–4 µm.

*Ascomata* erumpent through the bark or sometimes appearing superficial after the breakdown of the surrounding plant tissue, occurring singly or aggregated into small clusters usually less than five individuals, possesses no subiculum, pyriform to subglobose in shape, dark brown, ascomatal wall smooth, 194–389 µm high and 244–355 µm wide. Apices rounded, raised with rounded openings, occassionally the pore area appear orange in colour or the colouring is lacking. *Asci* cylindrical-clavate in shape bearing short stipes, 8-spored partially biseriate in arrangement, 72–112 x 8–11 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in a gelatinous matrix, 1–2 µm diam. *Ascospores* are fusiform, hyaline, the outer wall smooth, 1–3-septate and constricted at the middle septum, one of the middle cells is broader than the rest, possesses a small mucilaginous sheath that extends at the tips of the spore, 18–22 x 3–4 µm.

*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Currently known from forested areas in Kenya, Costa Rica and U.S.A.

*Specimens examined*: **Kenya**, Coast Province, Taita Taveta District, Taita hills, Ngangao forest res., 3˚ 22' 30'' S, 38˚ 20' 45'' E, 1800 m elev., 13 Apr. 2005, on woody branch, G.K. Mugambi, GKM169N, **holotype** EA, **isotype** F. **Costa Rica**, San Jose, Albergue de Montagne, Savegre, Sendero la Cataracta, 9˚ 32' 38'' N, 83˚ 48' 51'' W, 13 May 1996, on woody branch on the ground, S.M. Huhndorf, F. Fernández, SMH2448, F. **U.S.A.**, North Carolina, Smoky Mountains National Park, Cataloochee, Rough Fork Trail, 35˚ 61' N, 83˚ 12' W, 868 m elev., 23 May 2006, 1 cm diam woody branch on ground, A.N. Miller, A.Y. Rossman, M. Sogonov, L. Vasilyeva, G.K. Mugambi, ANM933, ILLS; Tennessee: Smoky Mountains National Park, vic. of Gatlinburg, Grotto Falls Trailhead, 35˚ 68' N, 83˚ 46' W, 944 m elev., 12 July 2004, 1 cm diam woody branch on ground, A.N. Miller, S.M. Huhndorf, G.K. Mugambi, L. Ruiz-Sanchez, ANM60, ILLS.

***Misturatosphaeria tennesseensis*** Mugambi, A.N. Mill. & Huhndorf, **sp. nov.** MycoBank MB516012. Fig. 33.

*Etymology*: Refers to the locality where the species was collected.

Ascomata erumpentia, solitaria vel dense aggregata, atrobrunnea, pyriformia, 265–398 µm alta, 201–401 µm diam, apicibus rotundatis. Asci cylindrici-clavati, breve stipitati, octospori, 84–118 x 10–11 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporae fusoides, brunneae, 3-septatae, constrictae ad septum medium, sine vagina mucosae vagina, 14–19 x 5–6 µm.

*Ascomata* erumpent through the bark, usually occurring singly or aggregated into small to large clusters, subiculum lacking, pyriform in shape, dark brown, ascomatal wall smooth, 265–398 µm high and 201–401 µm wide. Apices rounded, papillae are usually raised and often slightly sulcate with rounded opening. *Asci* cylindrical-clavate in shape, bearing short stipes, 8-spored partially biseriate, 84–118 x 10–11 µm. *Pseudoparaphyses* are numerous, septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform, sometimes slightly curved, pale brown in colour, outer wall smooth, 3-septate with the septal area often of darker colour than rest of the spore, slightly constricted at the middle septum, one half of the spore composed cells that are slightly broader, possess no mucilaginous sheath, 14–19 x 5–6 µm.

*Substratum*: On decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Presently only known from a forested area in Tennessee, U.S.A.

*Specimen examined*: **U.S.A.**, Tennessee, Cocke Co., Great Smoky Mountains National Park, Lower Mount Cammerer Trail, 35˚ 45' 256'' N, 83˚12' 329'' W, 686 m elev., 19 May 2006, on woody branch, A.N. Miller, G.K. Mugambi, ANM911, **holotype** F, **isotype** ILLS.

***Misturatosphaeria uniseptata*** Mugambi, A.N. Mill. & Huhndorf, **sp. nov.** MycoBank MB516013. Fig. 34.

*Etymology*: *Unicus* (*L.*) = one, refering to one septate nature of the ascospores in the species.

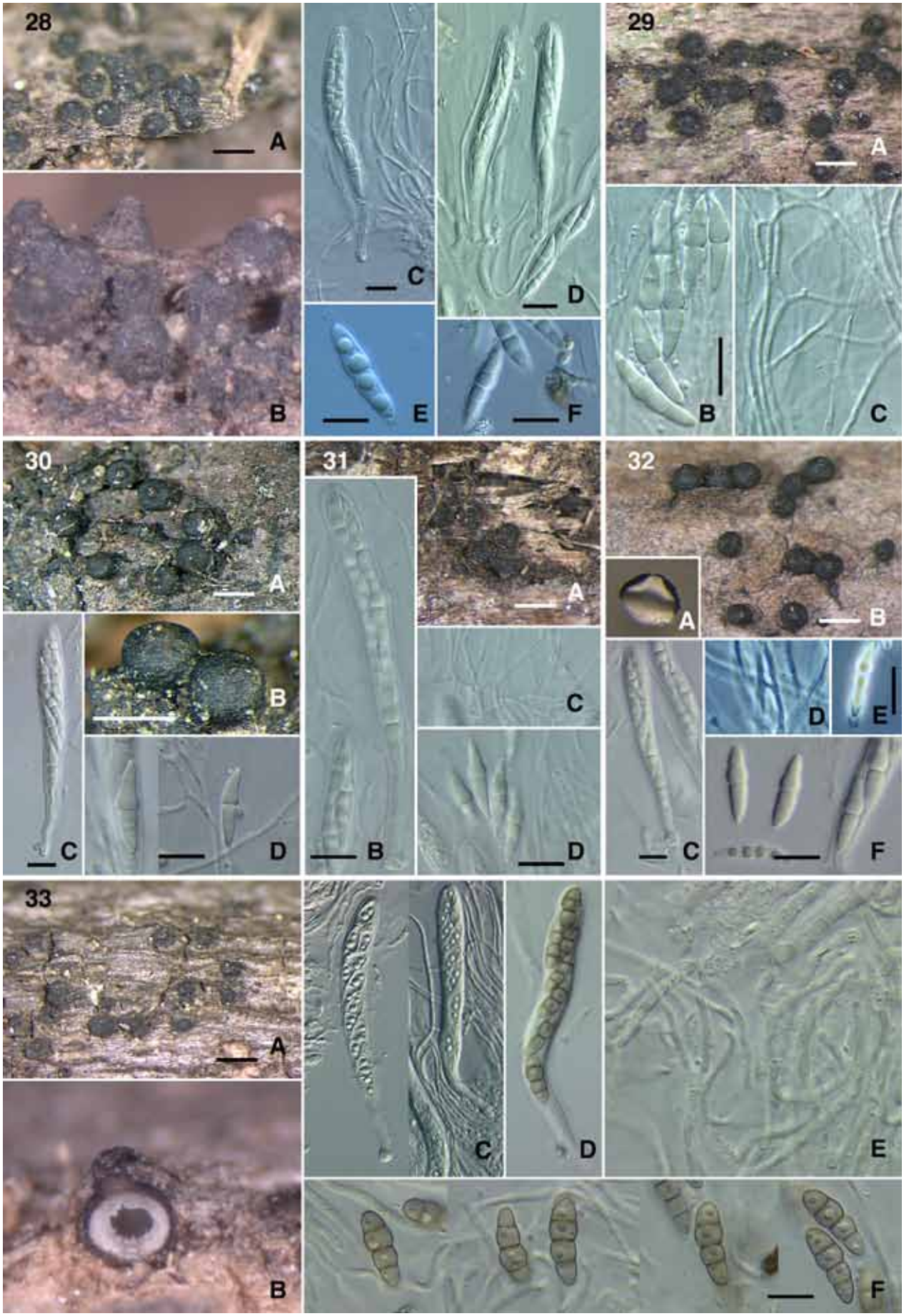
Ascomata superficialia, solitaria vel sparse ad dense aggregata, atrobrunnea, pyriformia ad globosa, 230–293 µm alta, 318–385 µm diam, sine subiculo, apicibus rotundatis pallide coloratis. Asci cylindrici-clavati, breve stipitati, octospori, 70–82 x 5–6 µm. Pseudoparaphyses numerosae, septatae, in matrice mucosa, 1–2 µm diam. Ascosporae fusoides, brunneae, 1-septatae, constrictae ad septum medium, since vagina mucosa, 12–14 x 3–4 µm.

*Ascomata* superficial, occurring singly or aggregated into small to large clusters, subiculum absent, pyriform to globose in shape, dark brown, ascomatal wall smooth, 230–293 µm high and 318–385 µm wide. Apices rounded, often with slightly raised papillae, ostiole area often of lighter colour, ostiole openings often quite prominent but appear plugged by centrum tissue. *Asci* cylindrical-clavate in shape and bearing short stipes, 8-spored partially biseriate or sometimes overlapping uniseriate in arrangement, 70–82 x 5–6 µm. *Pseudoparaphyses* numerous, septate, branching and anastomosing between and above asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform, sometimes slightly curved or straight, brown, outer wall smooth, 1-septate with the septal area of darker colour, upper cell usually shorter and broader than the basal cell, slightly constricted at septum, possess no mucilaginous sheath, 12–14 x 3–4 µm.

*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Currently only known from a tropical forest in Ecuador.




**Figs 28–33. 28.** *Misturatosphaeria kenyensis* (GKM1195) A–B. Ascomata. C–D. Asci. E, F. Ascospores. **29.** *M. kenyensis* (GKM L100Na) A. Ascomata. B. Ascospores. C. Pseudoparaphyses. **30.** *Misturatosphaeria minima* (SMH2448) A, B. Ascomata. C. Ascus. D. Ascospores. **31.** *M. minima* (GKM169N) A. Ascomata. B. Ascus. C. Pseudoparaphyses. D. Ascospores. **32.** *M. minima* (ANM60) A, B. Ascomata. C. Ascus. D. Pseudoparaphyses. E–F. Ascospores. **33.** *Misturatosphaeria tennesseensis* (ANM911) A–B. Ascomata. C–D. Asci. E. Pseudoparaphyses. F. Ascospores. Scale bars: Ascomata = 500 µm. Ascus = 10 µm. Ascospore = 10 µm.

*Specimen examined*: **Ecuador**, Orellana Province, Yasuni National Park, Botanico trail, 5 Mar. 2001, on woody branch, F.A. Fernández, A.N. Miller, R. Briones, SMH4330, **holotype** F.

***Misturatosphaeria uniseriata*** Mugambi, A.N. Mill. & Huhndorf, **sp. nov.** MycoBank MB516014. Fig. 35.

*Etymology*: *Unicus* (*L.*) = one, *Serialis* (*L.*) = row, refers to single-row arrangement of the ascospores in the asci.

Ascomata erumpentia, solitaria vel dense aggregata, atrobrunnea, pyriformia ad globosa, 332–343 µm alta, 309–379 µm diam, sine subiculo, apicibus rotundatis, pallide coloratis. Asci cylindrici ad cylindrici-clavati, breve stipitati, octospori, 100–130 x 8–10 µm. Pseudoparaphyses numerosae, septatae in matrice mucosa, 1–2 µm diam. Ascosporae fusoides ad ellipticae, brunneae ad atrobrunnae, 1–3-septatae, vulgo 3-septatae, constrictae ad septa omnia, since vagina mucosa, 14–19 x 4–7 µm.

*Ascomata* erumpent, occurring aggregated into large clusters, subiculum lacking, pyriform to subglobose in shape, dark brown, ascomatal wall smooth, 332–343 µm high and 309–379 µm wide. Apices rounded, may possess slightly raised papillae, the ostiole area usually of lighter colour, possess prominent pore opening that appears plugged by centrum tissue. *Asci* cylindrical to cylindrical-clavate, bearing short stipes, 8-spored, overlapping uniseriate in arrangement, 100–130 x 8–10 µm. *Pseudoparaphyses* are numerous and septate, branching and anastomosing between and above the asci, held in gelatinous matrix, 1–2 µm diam. *Ascospores* fusiform to ellptical, brown to dark brown in colour, outer wall smooth, 1–3-septate but commonly 3-septate, occasionally constricted at all three septa, with no mucilaginous sheath, 14–19 x 4–7 µm.
*Substratum*: Found on decorticated woody branches on the ground.

*Anamorph*: Unknown.

*Distribution*: Currently only known from forested area in the Great Smoky Mountains National Park in Tennessee, U.S.A.

*Specimen examined*: **U.S.A.**, Tennessee, Cocke Co., Great Smoky Mountains National Park, Lower Mount Cammerer Trail, 35˚ 45' 256'' N, 83˚ 12' 329'' W, 686 m elev., 19 May 2006, on woody branch, A.N. Miller, G.K. Mugambi, ANM909, **holotype** F.

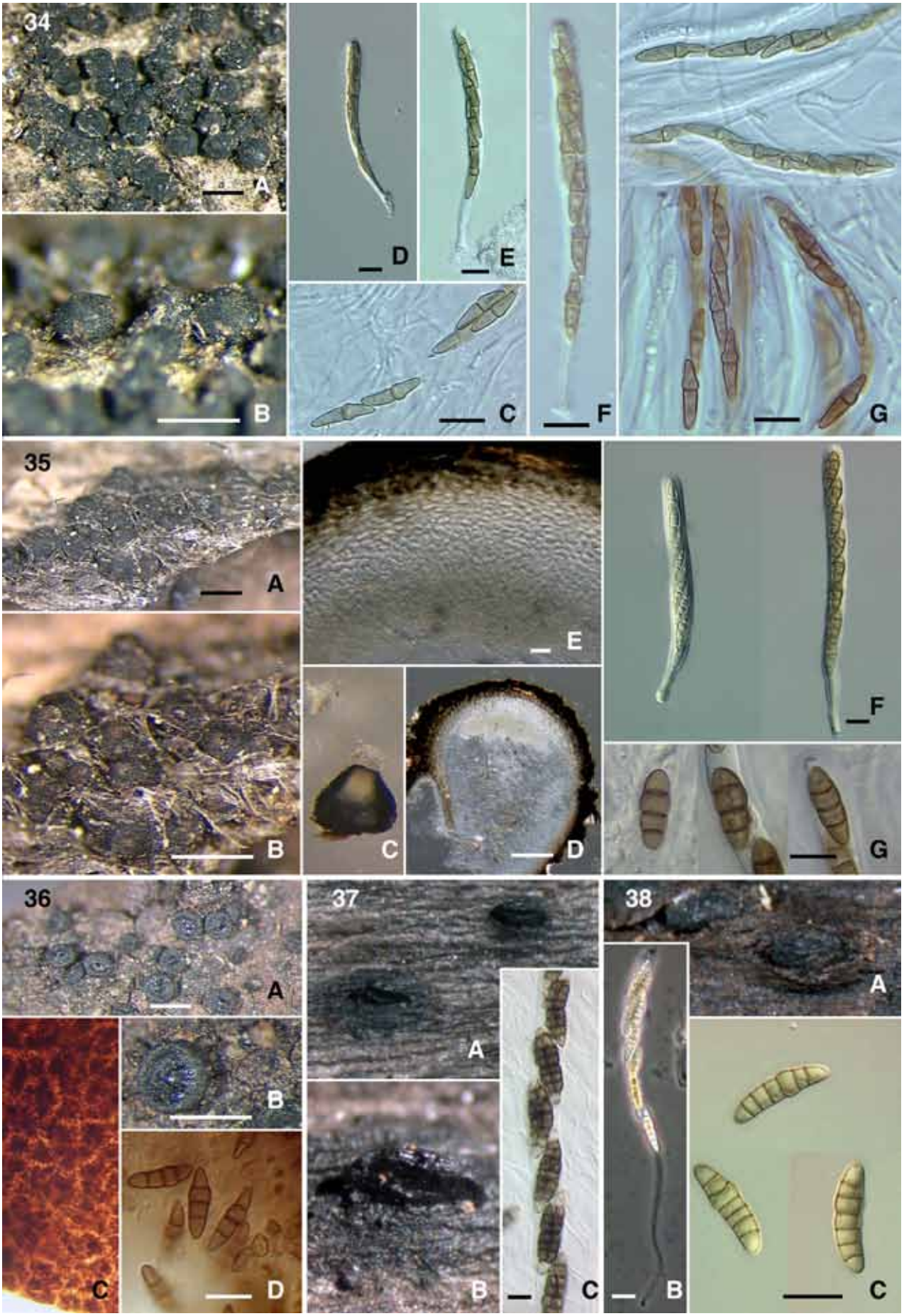
*Notes*: The nine species newly described in *Misturatosphaeria* (Figs 25–35) were collected from wide geographic localities from Africa, North, Central and South America. An additional collection was included in the analyses (*Misturatosphaeria* sp., Fig. 1) that probably represents another species but asci were not seen so the specimen is not described at this time (Fig. 36). *Misturatosphaeria aurantonotata* bears some similarity with *M. tennesseensis* and *M. uniseriata* but differs from both in its much larger superficial ascomata and quite verruculose ascospores. It also differs in its phylogenetic placement (Figs 1–3). *Misturatosphaeria tennesseensis* differs from *M. uniseriata* in the shape and loose aggregation of the ascomata, asci that are predominantly partially biseriate in arrangement and ascospores that are paler brown and strongly constricted at the mid-septum. In contrast *M. uniseriata* ascomata tend to occur in large clusters, asci have predominantly overlapping uniseriate ascospore arrangement, ascospores are dark brown at maturity and are only rarely slightly constricted. Molecular data also support the separation of these two species (Figs 1–3). *Misturatosphaeria uniseptata* differs from all the other species in the group in its pale brown, 1-septate ascospores. *Misturatosphaeria minima* and *M. kenyensis* are quite similar in their morphologies but the former differs in its ascomata that are solitary or in small groups usually less than 5 individuals. *Misturatosphaeria kenyensis* ascomata are aggregated in larger clusters and ascospores are slightly broader. *Misturatosphaeria cruciformis* shares ascospore morphology with *M. claviformis*, but the former differs by having much larger ascomata that are predominantly pyriform and ascospores that are often constricted at the middle septum. *Misturatosphaeria claviformis* has smaller pyriform to obclavate ascomata, and ascospores that are very rarely slightly constricted. Molecular data also indicate the two species are distantly related (Figs 1–3). In addition to species of *Misturatosphaeria*, the sixth plate of figures contains two taxa illustrated for morphological comparison: *Platystomum compressum* (Fig. 37), and *Thyridaria macrostomoides* (Fig. 38). Both species share features with members of the Lophiostomataceae but only *T. macrostomoides* finds placement in the family based on molecular data (Figs 1–3).

## DISCUSSION

### *Melanommataceae*

Phylogenetic analyses recovered *Melanommataceae*, *Lophiostomataceae*, *Hypsostromataceae*, and a few others as strongly supported clades within the *Pleosporales* (Figs 1–3). Although some genera currently accepted in the *Melanommataceae*, such as *Melanomma, Pseudotrichia* and *Byssosphaeria*, were recovered within the family, others such as *Ostropella* and *Xenolophium* are nested outside the family (Figs 1–3). *Herpotrichia* and *Bertiella* currently reside in other families (Kirk *et al*. 2008) but they, along with a single representative of *Pleomassaria siparia*, find their placement within the *Melanommataceae* based on our data. *Byssosphaeria*, *Herpotrichia*, and *Pseudotrichia* are taxa that have been united in the past, mostly under *Herpotrichia* and all share the distinctive characteristic that at least some species bear subiculate ascomata (Bose 1961, Sivanesan 1971, Barr 1984). Previous analyses of the *Pleosporales* have included the GenBank sequences *Lophiostoma macrostomum* (DQ384094) and / or *Trematosphaeria pertusa* (DQ678020) that nested within *Melanommataceae*. Although voucher specimens were not obtained to verify their identity, we strongly believe that these collections were misidentified. *Lophiostoma macrostomum*, the type species of the *Lophiostoma*, has already been confirmed to reside outside *Melanommataceae* (Tanaka & Hosoya 2008), which was corroborated by the results of this study (Figs 1–3). Recently, Zhang *et al.* (2008) using the epitype strain of *Trematosphaeria pertusa* have also demonstrated that this species is not closely related to *Melanomma* and belongs outside *Melanommataceae s. str*. This is corroborated here by our own collection of *T. pertusa* (Fig. 1).

The genus *Melanomma* formed the weakest structure in the clade. While the family was strongly supported, the genus did not unite in a strong clade. All five collections of *Melanomma pulvis-pyrius* clustered at the base of the family clade in the LSU tree, separated by very short branches (two collections appeared to be identical). The other represented species, *M. rhododendri* was on a long branch near *M. pulvis-pyrius*. Morphologically our collections fit within the genus, both species having the clustered, superficial ascomata and small 3-septate brown ascospores in a uniseriate arrangement in the asci (Figs 16–17). It appears that additional collections and other genes might be necessary to understand how the specimens and species relate to each other.




**Figs 34–38. 34.** *Misturatosphaeria uniseptata* (SMH4330) A–B. Ascomata. C, G. Ascospores. D–F Asci. **35.** *Misturatosphaeria uniseriata* (ANM909) A–C. Ascomata. D. Ascomatal section. E. Ascomatal wall section. F. Asci. G. Ascospores. **36.** *Misturatosphaeria sp.* (SMH3747) A–B. Ascomata. C. Ascomatal wall surface. D. Ascospores. **37.** *Platystomum compressum* (GKM1048) A–B. Ascomata. C. Ascospores. **38.** *Thyridaria macrostomoides* (GKM1033) A. Ascomata. B. Ascus. C. Ascospores. Scale bars: Ascomata = 500 µm. Section = 100 µm. Wall = 10 µm. Ascus = 10 µm. Ascospore = 10 µm.

In contrast, a strongly supported, monophyletic *Byssosphaeria* was recovered, which in this study was represented by six species, three of them with multiple collections (Figs 1-3). *Byssosphaeria* was described for taxa bearing superficial ascomata, separate or usually gregarious, turbinate, globose to ovoid and with rounded or minute papilla and opening by rounded pore. The pore and surrounding cells are pallid, whitish, or grey, or bright yellow, orange or red pigmented and the pore region appearing sulcate or plicate at times. Ascomatal wall surfaces are often irregular or slightly roughened, with protruding cells and often bear hyphal hairs (described as appendages in Barr 1984). Asci are clavate to cylindric, peripherally arranged and 8-spored. Ascospores are at first hyaline becoming light reddish brown or clear brown, ellipsoid or fusoid (Barr, 1990a). *Byssosphaeria schiedermayeriana*, the type species of the genus, is represented by five collections that form a strongly supported clade. The collections are neither morphologically nor molecularly identical. Morphologically the main differences seen are in the ascomatal structure. The collections differ in their amount of subiculum or hyphal hairs, amount of vertical collapsing (tendency toward becoming collabent, see Fig. 11A) and in the amount of colouration around the pore area (Figs 10A, 11A, 12A, 13A, 14A, 15A). The ascospores of these collections have measurements within the size range for the species given by Barr (1984), 25–42 x 5–9 µm and a sheath is seen in some of the collections (Figs 10D, 13D). Nested within the clade is the sequence of *B. diffusa* from GenBank (as *Herpotrichia* in GenBank) and one collection representing *B. salebrosa*. *Byssosphaeria diffusa* is reported to differ from *B. schiedermayeriana* in having a pallid or whitish pore area and smaller ascospores (Sivanesan 1971, Barr 1984). *Byssosphaeria salebrosa* is distinguished by non-subiculate ascomata with a surface that is roughened by projecting masses of cells, a feature present in our collection (Fig. 14B, C). Additional collections with the characteristics of these two species should be added to see if these morphological characters are phylogenetically informative.

*Byssosphaeria rhodomphala* and *B. jamaicana*, each represented by several collections, occur in a strongly supported sister relationship (Fig. 1). The collections of *Byssosphaeria jamaicana* fit well within the decription of the species (Figs 4–5). The subiculate, clustered ascomata, varying in the amount of hyphal hairs, have a pale-coloured pore area, the ascospores appear to lack appendages and in at least one of our collections, the ascospores turn somewhat darker brown and become 3-septate (Fig. 4D) (as was illustrated for the species by Sivanesan 1971). Five collections represent *B. rhodomphala* and morphologically and molecularly they are remarkably consistent given that they are geographically distant (Figs 1, 6, 8). Among the collections, the pore region of the ascomata can show variation from red to orange to yellowish granular deposits and can macroscopically appear quite dark. The most distinct characteristic of this species and the most useful aid in identification is the oblong-ellipsoid ascospore with obtuse ends. *Byssosphaeria rhodomphala* differs from *B. jamaicana* by the colouration of the ascomatal pore region. Hyphal appendages are lacking in *B. rhodomphala* and the ascospores are smaller than in *B. jamaicana* (Barr 1984).

On a basal branch in the *Byssosphaeria* clade is a single collection of *B. villosa*. The species is distinguished by ascomata bearing a dense covering of outwardly-projecting, long villose hairs and a pale coloured apex (Samuels & Müller 1978). Its general appearance was described as being "similar to *Lasiosphaeria phyllophila*" (= *Iodosphaeria*) (Samuels & Müller 1978), an observation that aided us in correctly identifying our own collection. Our collection differs from the description by having a distinctly yellow pore area (Fig. 9). However, Samuels & Müller's (1978) description of a bright yellow colour in the colonies they obtained in pure culture allowed us to conclude that the colour of the pore area might vary among collections of the species.

*Bertiella* also found its placement in the *Melanommataceae* in contrast to the *Teichosporaceae* where it was previously placed (Lumbsch & Huhndorf 2007). *Bertiella macrospora* is represented by three collections (Costa Rica, Kenya, U.S.A.) and slightly different placements in the LSU and TEF trees. It was unfortunate that we were not able to sequence one of the same collections for both genes. The collections show the distinctive arrangement of thick-walled and highly melanised ascomatal wall cells bearing bands of less pigmented, thin-walled cells (Fig. 7) that was described by Eriksson & Yue (1986) as "resembling cephalothecioid". As reported for the type specimen (Eriksson & Yue 1986), all three recent collections have ascomata that are superficial, gregarious and non-papillate (Fig. 7) with a roughened surface and lacking appendages or subiculum. The main difference occurs in the size of the ascospores. Whereas Eriksson & Yue (1986) describe the type specimen as having ascospores in the size range of 37–43 x 8–9 µm, our collections have smaller ascospores, ranging 22–30 x 5–7 µm in size. Based on the description given by Barr (1984), another taxon that may be similar to these collections is *Byssosphaeria semen*. This species differs from other *Byssosphaerias* in having a roughened ascomatal surface that lacks hairs or subiculum and has ascospores in the same range as our collections. The main difference is that no cephalothecioid cell arrangement is reported for this species and this is quite a prominent feature of our collections.

Barr (1984) revised the genus *Herpotrichia* and in the process re-established several genera, including *Byssosphaeria* and *Pseudotrichia* that had been in synonymy. She suggested that *Herpotrichia* belonged in *Massarinaceae*, a family later synonymised with Lophiostomataceae (Barr 1987). *Herpotrichia* is characterised by immersed, erumpent or superficial ascomata, with tomentum, rounded apex opening by broad pore. The asci are clavate or cylindrical, basal and pseudoparaphyses are narrowly cellular. The ascospores are fusoid, ellipsoid or oblong, usually surrounded by a mucilaginous sheath, and are hyaline becoming light yellow to reddish brown, mostly 1-septate but developing more septa with age. *Herpotrichia* is represented in our analyses by *H. herpotrichoides*, *H. juniperi* and *H. macrotricha,* and these taxa collectively do not form a monophyletic group in the LSU analyses. Subsets of these taxa group together in the TEF and combined gene trees. Two collections are tentatively identified as *H. herpotrichoides*, the type species of the genus. Morphologically the collections match this species and they do not differ significantly from each other (Figs 23–24); however in the LSU tree they occur in the same clade but separate from each other. The collections are geographically distant (one from the U.S.A. and one from Kenya) so a sequenced European collection would be useful to aid in the placement of the species. *Herpotrichia juniperi,* represented by two strains obtained from GenBank grouped consistently separate from the other two species. They also did not appear to closely align with the other taxa in the clade but occupied a position near *Melanomma* (Fig. 1). Assuming these collections were correctly identified, the results presented suggested that *H. juniperi* may belong outside the genus and thus may bear affinities to *Melanomma*. However, this will only become clear with broader taxon sampling in the groups. The described morphologies of *Melanomma* and *Herpotrichia* overlap in many ways, with both sharing immersed, erumpent to superficial ascomata that are tomentose and usually

sit on an ample subiculum. Asci and ascospore morphologies are also similar, although in *Herpotrichia* ascospores tend to initially start hyaline and 1-septate later become brown and more septate. Since the *Melanomma* clade did not receive strong support the true placement of *H. juniperi* or its relationships to the taxa in the genus remain unclear. *Herpotrichia macrotricha* is reported to have a wide distribution (Barr 1984) and the five collections of *H. macrotricha* (representatives from Costa Rica, Kenya and Puerto Rico) form a strongly supported clade (Fig. 1). The species is easily recognised by its distinctive broad cap-like ascomatal apex with a thick inner layer of hyaline pseudoparenchymatous cells (Figs 19–22). One collection stands out from the rest both in sequence data and morphology. The Costa Rican collection (SMH4913, V on Fig. 1) differs from the others in having an upper, ascomal wall with a central hyaline layer that gives the ascomata a wrinkled, collapsed appearance in the dried collections (Fig. 22). It was thought perhaps to be a separate species but other known species should be checked before making that decision.

*Pseudotrichia* was considered to be in the family *Platystomaceae* by Barr (1990b), but is correctly placed in the *Melanommataceae* as in the most recent outline of the *Ascomycota* in Myconet (Lumbsch & Huhndorf 2007). The type species, *P. mutabilis*, is represented by two U.S.A. collections and in our LSU tree occurs as a sister taxon to the single collection of *Pleomassaria*. *Pseudotrichia mutabilis* is distinctive and easily identified with its gregarious, yellow-green tomentose ascomata and its hyaline, fusiform, septate ascospores (Fig. 18). It is a common entity on decaying wood in temperate forests. As currently circumscribed however, the genus is not monophyletic as our other included species, *P. guatopoensis*, finds a placement among the taxa in the unsupported *Platystomaceae* clade.

*Pleomassaria siparia* (lectotype species of the genus) is represented by two LSU sequences in GenBank (one used here), both coming from the same CBS 279.74 culture. In our analyses the species is nested within the *Melanommataceae* in the clade with *Pseudotrichia mutabilis*. According to Barr (1982: 370) *Pleomassaria siparia* is characterised by immersed, depressed globose ascomata containing oblong asci with simple muriform ascospores. The ascospores are dark brown with a verruculose surface, with 5–7 transverse septa and one longitudinal septum in several cells. The characteristics this species shares with others in the clade are not obvious. Our other collection identified as belonging in the family (*Pleomassariaceae*; SMH5232) does not cluster with *P. siparia* but rather finds a placement among the taxa in the unsupported *Platystomaceae* clade. Additionally, our other unpublished phylogenetic analyses using sequences from putative *Asteromassaria* and *Splanchnonema* species (other taxa that are arranged in the morphologically defined *Pleomassariaceae*) found that these species also do not cluster with *P. siparia*. It will be necessary to have additional sequences from other collections of the species to confirm its placement.

In the LSU tree, *Ostropella* and *Xenolophium* occur distantly related to *Melanommataceae* in a clade lacking significant support that also includes *Pseudotrichia guatopoensis*, *Platystomum compressum* (Fig. 37) and an unnamed *Pleomassariaceae* (Fig. 1). The clade, however, received significant PP support in the combined-gene analysis (Fig. 3). *Ostropella* and *Xenolophium* share a combination of morphological characters that appears distinct from that observed in *Melanommataceae s. str*. They possess relatively large ascomata, bearing raised apices with slit-like ostiolar openings. The asci in these two genera are clavate with quite long stipes, which is unlike taxa that are here treated in *Melanommataceae*. The mostly tropical collections of *Ostropella*, *Xenolophium*, and *P. guatopoensis* all share the distinctive, extensive network of trabeculate pseudoparaphyses that Barr (1983, 1990a) emphasised as an important diagnostic character that separates *Melanommatales* from *Pleosporales*. Subsequent studies using DNA sequence data have established that pseudoparaphysis type is not a phylogenetically informative character at the ordinal level and hence separation of *Melanommatales* from *Pleosporales* based on this character is not supported (Liew *et al.* 2000, Lumbsch & Lindemuth 2001, Kruys *et al.* 2006, Schoch *et al.* 2006, Wang *et al.* 2007). The results of the phylogenetic analyses presented in this study corroborate these findings. Additional genera in this clade are *Ulospora bilgramii* and *Verruculina enalia* whose sequences were obtained from GenBank. Since the clade for the most part did not receive significant support, further studies involving more collections and more markers are needed to confirm the observed relationships. Furthermore, the occurrence of *P. guatopoensis* and *P. compressum* in this clade, which were previously not thought to be related to *Ostropella* and *Xenolophium*, underscores the need for more work to establish with confidence the nature of the relationships.

## *Lophiostomataceae*

Our analyses recovered a strongly supported *Lophiostomataceae* comprised of *Lophiostoma* and some species currently placed in *Thyridaria*. Sister to the *Lophiostoma* clade is the strongly supported clade of the genus *Misturatosphaeria* comprising nine new species (Figs 1–3). The genus *Lophiostoma* was established by Fries (1849) to accommodate taxa that possess mostly erumpent ascomata, bearing apices that are raised and laterally compressed with phragmosporous hyaline or brown ascospores. Since its inception, many more species have been added to the genus but the taxonomy of the group remains uncertain, requiring urgent revision. Chesters & Bell (1970) and Holm & Holm (1988) provide comprehensive information on the taxonomic history and morphology of the genus. Barr (1990a) transferred several species of *Lophiostoma* into *Thyridaria*, which she placed in the *Platystomaceae* that included *T. macrostomoides*. We had among our specimens, collections that bear the morphology of the species and in analyses *T. macrostomoides* groups separate from taxa in *Lophiostoma* (Figs 1–3). *Thyridaria macrostomoides* (Fig. 38) collections occur in an unsupported sister relationship with *Misturatosphaeria* in single-gene trees but receives significant PP in the combined-gene tree (Figs 1–3). Barr (1990a) described *Thyridaria* to include taxa with the following morphology: ascomata that are immersed or erumpent, in valsoid groups or separate or gregarious; ascomata globose with a well developed papilla or short beak that is rounded or compressed; ostioles that are rounded or slit-like and periphysate or filled with pallid or brightly pigmented hyphal ends. Asci were described as clavate or cylindrical with trabeculate pseudoparaphyses held in gelatinous matrix. Ascospores are brown, symmetric or asymmetric, phragmosporous, three or more septate, smooth, verruculose or striate. The results presented here demonstrate that at least one of the species of *Thyridaria* resides in *Lophiostomataceae*. However, since we did not include the type species of *Thyridaria* in our analyses the generic placement remains unclear.

*Misturatosphaeria* differs from other genera in *Lophiostomataceae* by possessing ascomata that are erumpent to superficial, with rounded apices that are often raised. Ostiolar openings are rounded and plugged by gelatinous tissue and

occasionally lighter coloured. Asci are cylindrical to clavate with phragmosporous or dictyosporous ascospores (Figs 25–36). Despite morphological differences of *Misturatosphaeria* from other lophiostomataceous fungi, we feel justified in placing it in *Lophiostomataceae* at this point due to the strong support the clade received in our analyses (Figs 1–3). The only other genus with dictyospores accepted by Barr (1990b) in this family is *Cilioplea*. This genus was not included in our analyses but it differs markedly from the dictyosporous members of *Misturatosphaeria* and others in the group in general. It possesses ascomata with thick walled brown or dark brown setae around the apex and a narrow peridium of few internal rows of compressed pallid cells, surrounded by brown hyphae into the substrate. It also differs in the ascospore shape and septation, with the ascospore longitudinal septum in this genus primarily limited to the central cells (Barr 1990b).

### *Hypsostromataceae*

Huhndorf (1992) described the genus *Hypsostroma* for two tropical species, *H. saxicola* and *H. caimitalensis*. She did not prefer familial placement at the time but suggested they bear affinities in the *Melanommatales* (= *Pleosporales*). Later, Huhndorf (1994) erected family *Hypsostromataceae* for the genera *Hypsostroma* and *Manglicola*. The two species of *Hypsostroma* were included in our analyses and they grouped together in a strongly supported clade within *Pleosporales* distinct from other families in the order (Fig 1). This study is only the second time the two specimens used for sequencing have been collected and this expands their range outside of the Caribbean and South America. *Hypsostroma* is distinctive in having asci with extremely long stipes, similar to those found in *Ostropella* and *Xenolophium* but much longer (Huhndorf 1992, 1994). Although the clade shows no supported relationship with the *Platystomaceae* taxa, *X. guianense* tentatively joins the clade in the LSU tree and the morphological similarities of these long-stipitate taxa suggests that they may in some way be related.

## CONCLUSION

The *Melanommataceae* is found to contain taxa that have gregarious, superficial ascomata and the ascomata may be smooth (*Byssosphaeria p.p.*), roughened (*Melanomma*, *Bertiella macrospora*), or clothed in hyphal hairs (*Herpotrichia*, *Byssosphaeria p.p.*) or coloured tomentum (*P. mutabilis*). Subicular hyphae may be present in some taxa in the family (*Herpotrichia*, *Byssosphaeria*) or absent (*Melanomma*, *Bertiella*). Cephalothecioid-like ascomatal wall structure (*Bertiella macrospora*), versicolourous-layered walls (*H. macrotricha p.p.*) or uniformly brown-pigmented walls (*Byssosphaeria*, *Melanomma*) occur in the family. The ascospores that occur in the group are hyaline or brown, mostly fusiform in shape and 1–3-septate. Anomalous in the family is the presence of *Pleomassaria siparia*, differing from the other sequenced taxa by having immersed ascomata and muriform ascospores. Former family members, *Ostropella* and *Xenolophium* are found to occur outside the *Melanommataceae* in a weakly supported group along with *Platystomum compressum* and *Pseudotrichia guatopoensis*, that may correspond to the family *Platystomaceae*.

*Lophiostomataceae* occurs as a strongly supported monophyletic group but its concept is here expanded to include a new genus *Misturatosphaeria* that bears morphology traditionally not known to occur in the family. The gregarious ascomata of *Misturatosphaeria*, especially *M. minima*, suggests a resemblance and potential relationship to *Byssosphaeria* species or other taxa in the *Melanommataceae*. The molecular data shows this not to be the case. The ascomata differ with papillate apices present in *Misturatosphaeria* versus plane to collapsed apices in *Byssosphaeria*, along with the lack of hyphal hairs or subiculum in the former. Distinctive characteristics of *Misturatosphaeria* are the tendencies towards lighter coloured apices (*M. aurantonotata*) and plugged ostioles (*M. minima*, *M. uniseriata*). Additionally the ascomata in the group are actually mostly erumpent from the substrate and not superficial as in *Byssosphaeria*. The mixture of didymosporous, phragmosporous to dictyosporous ascospore morphologies that gives the genus its name was problematic and for awhile we thought that more than one generic clade would resolve but the molecular data did not provide that satisfaction. Some of the phragmosporous species have ascospores that resemble those in *Lophiostoma s. l.*

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## SUPPLEMENTARY INFORMATION

**Table 1.** Taxa used in the study with those newly sequenced for LSU and TEF genes shown in bold, the rest obtained from GenBank.

| Taxon | Collection locality | Collector and accession number | LSU | TEF |
|---|---|---|---|---|
| *Alternaria alternata* | | | DQ678082 | DQ677927 |
| *Arthopyrenia salicis* I | | | AY538339 | |
| *Arthopyrenia salicis* II | | | AY607730 | |
| ***Arthopyrenia* sp.** | **Costa Rica, Puntarenas, Monteverde** | **S.M. Huhndorf, SMH4900** | **GU385149** | |
| ***Bertiella macrospora* I** | **Costa Rica, Puntarenas, Monteverde** | **I. Lopez, IL5005** | **GU385150** | |
| ***Bertiella macrospora* II** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM L122N** | | **GU327743** |
| ***Bertiella macrospora* III** | **U.S.A., Michigan, Huron Mt. Club** | **S.M. Huhndorf, SMH3953** | | **GU327744** |
| *Bimuria novae-zelandiae* | | | AY016356 | DQ471087 |
| ***Byssosphaeria jamaicana* I** | **U.S.A., Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH1403** | **GU385152** | **GU327746** |
| ***Byssosphaeria jamaicana* II** | **U.S.A., Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH3085** | **GU385154** | |
| ***Byssosphaeria jamaicana* III** | **Panama, Barro Colorado Island** | **S.M. Huhndorf, SMH3464** | **GU385153** | |
| ***Byssosphaeria rhodomphala* I** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM L153N** | **GU385157** | **GU327747** |
| ***Byssosphaeria rhodomphala* II** | **U.S.A., North Carolina, Smoky Mts.** | **A.N. Miller, ANM942** | **GU385160** | |
| ***Byssosphaeria rhodomphala* III** | **U.S.A., Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH3086** | **GU385155** | |
| ***Byssosphaeria rhodomphala* IV** | **Panama, Barro Colorado Island** | **S.M. Huhndorf, SMH3402** | **GU385170** | |
| ***Byssosphaeria rhodomphala* V** | **Ecuador, Yasuni** | **F.A. Fernández, A.N. Miller, SMH4363** | **GU385156** | |
| ***Byssosphaeria salebrosa*** | **Costa Rica, San Jose, San Gerardo de Dota** | **S.M. Huhndorf SMH2387** | **GU385162** | **GU327748** |
| ***Byssosphaeria schiedermayeriana* I** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM152N** | **GU385168** | **GU327749** |
| ***Byssosphaeria schiedermayeriana* II** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM1197** | **GU385161** | **GU327750** |
| ***Byssosphaeria schiedermayeriana* III** | **U.S.A. Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH1296** | **GU385158** | |
| ***Byssosphaeria schiedermayeriana* IV** | **U.S.A. Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH1816** | **GU385159** | |
| ***Byssosphaeria schiedermayeriana* V** | **U.S.A. Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf, SMH3157** | **GU385163** | **GU327745** |
| ***Byssosphaeria villosa*** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.M. Mugambi, GKM204N** | **GU385151** | **GU327751** |
| *Byssothecium circinans* | | | AY016357 | |
| *Cochliobolus eragrostidis* | | | AB288215 | |
| *Cochliobolus geniculatus* | | | AB444670 | |
| *Cochliobolus heterostrophus* | | | AY544645 | DQ497603 |
| *Cochliobolus lunatus* | | | AB444681 | |
| *Cochliobolus pallescens* | | | AB288225 | |
| *Cochliobolus sativus* | | | DQ678045 | |
| *Cochliobolus verruculosus* | | | AB444680 | |
| ***Delitschia* cf. *anisomera*** | **Kenya, Mt. Kenya Forest , along the track past Sirimon entrance** | **G.K. Mugambi, GKM1205** | **GU385171** | |
| ***Delitschia chaetomioides* I** | **Kenya, Rift Valley Province, Lembus forest along Eldoret – Elderma Ravine road** | **G.K. Mugambi, GKM1283** | **GU385172** | **GU327752** |
| ***Delitschia chaetomioides* II** | **Costa Rica, Guanacaste, Bosque Encantado** | **S.M. Huhndorf, SMH3253.2** | **GU390656** | **GU327753** |
| *Delitschia didyma* | | | DQ384090 | |
| *Delitschia winteri* I | | | DQ384091 | |

**Table 1.** (Continued).

| Taxon | Collection locality | Collector and accession number | LSU | TEF |
|---|---|---|---|---|
| *Delitschia winteri* II | | | DQ678077 | DQ677922 |
| *Dothidea ribesia* | | | AY016360 | |
| *Gloniopsis praelonga* I | | | FJ161193 | |
| *Gloniopsis praelonga* II | | | FJ161195 | FJ161103 |
| *Glonium stellatum* | | | FJ161179 | FJ161095 |
| *Helicomyces roseus* | | | DQ678083 | DQ677928 |
| ***Herpotrichia* cf. *herpotrichoides* I** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM212N** | **GU385169** | |
| ***Herpotrichia* cf. *herpotrichoides* II** | **U.S.A., Wisconsin, Upham Woods** | **S.M. Huhndorf, SMH5167** | **GU385175** | |
| *Herpotrichia diffusa (= Byssosphaeria)* | | | DQ678071 | |
| *Herpotrichia juniperi* I | | | DQ384093 | |
| *Herpotrichia juniperi* II | | | DQ678080 | DQ677925 |
| ***Herpotrichia macrotricha* I** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM196N** | **GU385176** | **GU327755** |
| ***Herpotrichia macrotricha* II** | **Kenya, Rift Valley Province, Kajiando District, Ngong hills forest** | **G.K. Mugambi, GKM1128** | **GU385178** | |
| ***Herpotrichia macrotricha* III** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM1193** | **GU385179** | |
| ***Herpotrichia macrotricha* IV** | **U.S.A., Puerto Rico, Toro Negro Forest** | **S.M. Huhndorf, SMH269** | **GU385177** | **GU327756** |
| ***Herpotrichia macrotricha* V** | **Costa Rica, Puntarenas, Monteverde** | **S.M. Huhndorf, SMH4913** | **GU385164** | **GU327754** |
| ***Hypsostroma caimetalensis*** | **Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park** | **G.K. Mugambi, GKM1165** | **GU385180** | |
| ***Hypsostroma saxicola*** | **Costa Rica, San Jose, INBio Parque** | **S.M. Huhndorf, SMH5005** | **GU385181** | |
| *Hysterium angustatum* | | | FJ161194 | |
| *Hysterographium mori* | | | FJ161196 | FJ161104 |
| *Leptosphaeria doliolum* I | | | U43473 | |
| *Leptosphaeria doliolum* II | | | U43474 | |
| *Leptosphaeria doliolum* III | | | U43475 | |
| *Leptosphaeria macrospora* | | | DQ384092 | |
| ***Leptosphaeria* sp.** | **Kenya, Rift Valley Province, Kajiando District, Ngong hills forest** | **G.K. Mugambi, GKM1090** | | **GU327757** |
| *Letendraea helminthicola* | | | AY016362 | |
| ***Lophiostoma alpigenum*** | **Kenya, Rift Valley Province, Kajiando District, Ololua forest** | **G.K. Mugambi, GKM1091b** | **GU385193** | **GU327758** |
| *Lophiostoma arundinis* | | | DQ782384 | DQ782387 |
| *Lophiostoma caulium* | | | DQ528763 | |
| *Lophiostoma crenatum* | | | DQ678069 | DQ677912 |
| ***Lophiostoma fuckelii* I** | **U.S.A., Puerto Rico, Luquillo Mts.** | **S.M. Huhndorf SMH1371** | **GU385186** | |
| ***Lophiostoma fuckelii* II** | **Kenya, Nairobi Province, Nairobi Museum Botanic Garden grounds** | **G.K. Mugambi, GKM1063** | **GU385192** | **GU327759** |
| *Lophiostoma fuckelii* III | | | DQ399531 | |
| *Lophiostoma heterospora* | | | AY016369 | DQ497609 |
| *Lophiostoma macrostomum* I | | | AB433273 | |
| *Lophiostoma macrostomum* II | | | AB433274 | |
| ***Lophiostoma quadrinucleatum*** | **Kenya, Central Province, Nyeri district, Mt. Kenya forest, behind Bantu lodge** | **G.K. Mugambi, GKM1233** | **GU385184** | **GU327760** |
| *Lophiostoma sagittiforme* | | | AB369267 | |
| ***Lophiostoma triseptatum* I** | **U.S.A., Michigan, Huron Mt. Club** | **S.M. Huhndorf, SMH2591** | **GU385183** | |
| ***Lophiostoma triseptatum* II** | **U.S.A., Michigan, Headland Park** | **S.M. Huhndorf, SMH5287** | **GU385187** | |

**Table 1.** (Continued).

| Taxon | Collection locality | Collector and accession number | LSU | TEF |
|---|---|---|---|---|
| *Lophium mytilinum* I | | | EF596819 | |
| *Lophium mytilinum* II | | | DQ678081 | DQ677926 |
| *Massariosphaeria grandispora* | | | EF165034 | |
| *Massariosphaeria roumeguerei* | | | EF165032 | |
| *Massariosphaeria triseptata* | | | EF165031 | |
| *Massariosphaeria typhicola* | | | EF165033 | |
| *Melanomma pulvis pyrius* I | | | DQ384095 | |
| *Melanomma pulvis pyrius* II | | | FJ201984 | |
| *Melanomma pulvis pyrius* III | | | FJ201986 | |
| *Melanomma pulvis pyrius* IV | | | FJ201988 | |
| ***Melanomma pulvis-pyrius* V** | **U.S.A., North Carolina, Highlands Biological Station** | **S.M. Huhndorf, SMH3291** | **GU385197** | |
| ***Melanomma rhododendri*** | **U.S.A., Tennessee, Smoky Mts** | **A.N. Miller, ANM73** | **GU385198** | |
| ***Misturatosphaeria aurantonotata* I** | **Kenya, Rift Valley Province, Kajiando District, Ngong hills forest** | **G.K. Mugambi, GKM1238** | **GU385173** | **GU327761** |
| ***Misturatosphaeria aurantonotata* II** | **Kenya, Rift Valley Province, Lembus forest, along Eldoret – Elderma Ravine road** | **G.K. Mugambi, GKM1280** | **GU385174** | **GU327762** |
| ***Misturatosphaeria claviformis*** | **Kenya, Central Province, Nyeri District, Mt. Kenya forest, behind Bantu lodge** | **G.K. Mugambi, GKM1210** | **GU385212** | **GU327763** |
| ***Misturatosphaeria cruciformis*** | **U.S.A., Illinois, Swallow Cliff Woods** | **S.M. Huhndorf, SMH5151** | **GU385211** | |
| ***Misturatosphaeria kenyensis* I** | **Kenya, Coast Province, Taita District, Taita Hills, Ngango forest** | **G.K. Mugambi, GKM194N** | | **GU327764** |
| ***Misturatosphaeria kenyensis* II** | **Kenya, Coast Province, Taita District, Taita hills, Ngangao forest** | **G.K. Mugambi, GKM234N** | **GU385188** | **GU327765** |
| ***Misturatosphaeria kenyensis* III** | **Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park** | **G.K. Mugambi, GKM L100Na** | **GU385189** | **GU327766** |
| ***Misturatosphaeria kenyensis* IV** | **Kenya, Coast Province, Taita District, Taita hills, Ngangao forest** | **G.K. Mugambi, GKM1195** | **GU385194** | **GU327767** |
| ***Misturatosphaeria minima* I** | **Kenya, Coast Province, Taita District, Taita hills, Ngangao forest** | **G.K. Mugambi, GKM169N** | **GU385165** | **GU327768** |
| ***Misturatosphaeria minima* II** | **U.S.A., North Carolina, Smoky Mts** | **A.N. Miller, ANM60** | **GU385182** | |
| ***Misturatosphaeria minima* III** | **U.S.A., North Carolina, Smoky Mts** | **A.N. Miller, ANM933** | **GU385195** | |
| ***Misturatosphaeria minima* IV** | **Costa Rica, San Jose, San Gerardo de Dota** | **S.M. Huhndorf, SMH2448** | **GU385166** | |
| ***Misturatosphaeria* sp.** | **French Guiana, Saül** | **S.M. Huhndorf, SMH3747** | **GU385196** | |
| ***Misturatosphaeria tennesseensis*** | **U.S.A., Tennessee, Smoky Mts** | **A.N. Miller, ANM911** | **GU385207** | **GU327769** |
| ***Misturatosphaeria uniseptata*** | **Ecuador, Yasuni** | **F.A. Fernández, A.N. Miller, SMH4330** | **GU385167** | **GU327770** |
| ***Misturatosphaeria uniseriata*** | **U.S.A., Tennessee, Smoky Mts** | **A.N. Miller, ANM909** | **GU385206** | |
| *Montagnula opulenta* | | | DQ678086 | |
| *Munkovalsaria appendiculata* | | | AY772016 | |
| ***Munkovalsaria* sp. I** | **Kenya, Nairobi Province, Nairobi Museum Botanic Garden grounds** | **G.K. Mugambi, GKM1286** | | **GU327771** |
| *Mytilinidion australe* | | | FJ161183 | |
| ***Ostropella albocincta* I** | **Panama, Barro Colorado Island** | **S.M. Huhndorf, SMH3536** | **GU385200** | |
| ***Ostropella striata*** | **Costa Rica, Arenal** | **SMH1854** | **GU385203** | |
| *Phaeodothis winteri* | | | DQ678073 | DQ677917 |
| *Phaeosphaeria eustoma* | | | DQ678063 | DQ677906 |
| ***Platystomum* sp.** | **U.S.A., Wisconsin, Upham Woods** | **S.M. Huhndorf, SMH5174** | **GU385199** | |

**Table 1.** (Continued).

| Taxon | Collection locality | Collector and accession number | LSU | TEF |
|---|---|---|---|---|
| ***Platystomum compressum*** | **Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park** | **G.K. Mugambi, GKM1048** | **GU385204** | **GU327772** |
| *Pleomassaria siparia* | | | DQ678078 | DQ677923 |
| ***Pleomassariaceae*** | **New Zealand, Auckland, Wenderholm Regional Park** | **S.M. Huhndorf, SMH5232** | **GU385205** | **GU327773** |
| *Pleospora herbarum* I | | | DQ247804 | DQ471090 |
| *Pleospora herbarum* II | | | DQ678049 | |
| *Pleospora herbarum* III | | | | DQ677888 |
| *Pleospora herbarum* IV | | | AF382386 | |
| *Pleospora herbarum* V | | | U43476 | |
| *Pleospora* sp. | | | EF177848 | |
| *Preussia terricola* | | | DQ471137 | |
| ***Pseudotrichia guatopoensis* I** | **U.S.A., Puerto Rico, Luquillo Mts** | **S.M. Huhndorf, SMH1288** | **GU385208** | |
| ***Pseudotrichia guatopoensis* II** | **Costa Rica, San Jose, San Gerardo de Dota** | **S.M. Huhndorf, SMH2383** | | **GU327775** |
| ***Pseudotrichia guatopoensis* III** | **Costa Rica, Alajuela, Volcan Arenal** | **S.M. Huhndorf, SMH4535** | **GU385202** | **GU327774** |
| ***Pseudotrichia mutabilis* I** | **U.S.A., Wisconsin, New Glarus State Park** | **S.M. Huhndorf, SMH1541** | **GU385209** | |
| ***Pseudotrichia mutabilis* II** | **U.S.A., Michigan, Headlands Park** | **S.M. Huhndorf, SMH5288** | **GU385210** | |
| *Psiloglonium clavisporum* II | | | FJ167526 | FJ161105 |
| *Pyrenophora phaeocomes* | | | DQ499596 | |
| *Pyrenophora tritici repentis* | | | AY544672 | |
| *Setosphaeria monoceras* | | | AY016368 | |
| *Stylodothis puccinioides* | | | AY004342 | |
| ***Thyridaria macrostomoides* I** | **Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park** | **G.K. Mugambi, GKM1033** | **GU385190** | **GU327776** |
| ***Thyridaria macrostomoides* II** | **Kenya, Coast Province, Taita District, Taita Hills, Ngangao forest** | **G.K. Mugambi, GKM224N** | **GU385191** | **GU327777** |
| ***Thyridaria macrostomoides* III** | **Kenya, Coast Province, Malindi District, Arabuko-Sokoke National Park** | **G.K. Mugambi, GKM1159** | **GU385185** | **GU327778** |
| ***Trematosphaeria pertusa* I** | **U.S.A., Wisconsin, Madison, Picnic Point Park** | **S.M. Huhndorf, SMH1448** | **GU385213** | |
| *Trematosphaeria pertusa* II | | | FJ201990 | |
| *Trematosphaeria pertusa* III | | | FJ201992 | |
| *Tubeufia cerea* | | | DQ470982 | |
| *Tubeufia helicomyces* | | | | DQ767638 |
| *Tubeufia paludosa* | | | AY849966 | |
| *Ulospora bilgramii* | | | DQ678076 | |
| *Verruculina enalia* | | | DQ678079 | |
| *Westerdykella cylindrica* | | | AY004343 | |
| ***Xenolophium* sp.** | **Panama, Barro Colorado Island** | **S.M. Huhndorf, SMH3537** | **GU385201** | |
| ***Xenolophium applanatum*** | **U.S.A., Puerto Rico, Luquillo Mts** | **S.M. Huhndorf, SMH2055** | **GU385214** | |
| ***Xenolophium guianense*** | **Ecuador, Yasuni** | **F.A. Fernández, A.N. Miller, SMH4711** | **GU385215** | |
| ***Xenolophium pachythele*** | **French Guiana, Saül** | **S.M. Huhndorf, SMH996** | | **GU327779** |

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# Phylogeny of rock-inhabiting fungi related to *Dothideomycetes*

C. Ruibal[1*], C. Gueidan[2], L. Selbmann[3], A.A. Gorbushina[4], P.W. Crous[2], J.Z. Groenewald[2], L. Muggia[5], M. Grube[5], D. Isola[3], C.L. Schoch[6], J.T. Staley[7], F. Lutzoni[8], G.S. de Hoog[2]

[1]*Departamento de Ingeniería y Ciencia de los Materiales, Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (UPM), José Gutiérrez Abascal 2, 28006 Madrid, Spain;* [2]*CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, Netherlands;* [3]*DECOS, Università degli Studi della Tuscia, Largo dell'Università, Viterbo, Italy;* [4]*Free University of Berlin and Federal Institute for Materials Research and Testing (BAM), Department IV "Materials and Environment", Unter den Eichen 87, 12205 Berlin, Germany;* [5]*Institute für Pflanzenwissenschaften, Karl-Franzens-Universität Graz, Holteigasse 6, A-8010 Graz, Austria;* [6]*NCBI/NLM/NIH, 45 Center Drive, Bethesda MD 20892, U.S.A.;* [7]*Department of Microbiology, University of Washington, Box 357242, Seattle WA 98195, U.S.A.;* [8]*Department of Biology, Duke University, Box 90338, Durham NC 27708, U.S.A.*

**Correspondence*: Constantino Ruibal, tinoruibal@yahoo.com

**Abstract:** The class *Dothideomycetes* (along with *Eurotiomycetes*) includes numerous rock-inhabiting fungi (RIF), a group of ascomycetes that tolerates surprisingly well harsh conditions prevailing on rock surfaces. Despite their convergent morphology and physiology, RIF are phylogenetically highly diverse in *Dothideomycetes*. However, the positions of main groups of RIF in this class remain unclear due to the lack of a strong phylogenetic framework. Moreover, connections between rock-dwelling habit and other lifestyles found in *Dothideomycetes* such as plant pathogens, saprobes and lichen-forming fungi are still unexplored. Based on multigene phylogenetic analyses, we report that RIF belong to *Capnodiales* (particularly to the family *Teratosphaeriaceae s.l.*), *Dothideales*, *Pleosporales*, and *Myriangiales*, as well as some uncharacterised groups with affinities to *Dothideomycetes*. Moreover, one lineage consisting exclusively of RIF proved to be closely related to *Arthoniomycetes*, the sister class of *Dothideomycetes*. The broad phylogenetic amplitude of RIF in *Dothideomycetes* suggests that total species richness in this class remains underestimated. Composition of some RIF-rich lineages suggests that rock surfaces are reservoirs for plant-associated fungi or saprobes, although other data also agree with rocks as a primary substrate for ancient fungal lineages. According to the current sampling, long distance dispersal seems to be common for RIF. *Dothideomycetes* lineages comprising lichens also include RIF, suggesting a possible link between rock-dwelling habit and lichenisation.

**Key words:** *Arthoniomycetes*, *Capnodiales*, *Dothideomycetes*, evolution, extremotolerance, multigene phylogeny, rock-inhabiting fungi.

## INTRODUCTION

The *Dothideomycetes* constitute the largest class of ascomycetes with approximately 19 000 species, which are currently classified in 11 orders and 90 families (Kirk *et al.* 2008). This class is ecologically diverse, with many pathogens or saprobes on plants, some coprophilous species, and a few lichen-forming fungi (Schoch *et al.* 2009b; this volume). Early studies have shown that a large part of the non-lichenised, slow-growing melanised fungi isolated from rock surfaces (here referred to as rock-inhabiting fungi) also belong to this class (Sterflinger *et al.* 1997, 1999). Subsequent sampling efforts revealed a higher diversity of species than expected for these rock-inhabiting fungi (Ruibal 2004, Ruibal *et al.* 2005, 2008, Selbmann *et al.* 2005, 2008).

Rock-inhabiting fungi (RIF) are peculiar organisms that apparently lack sexual reproductive structures and form compact, melanised colonies on bare rock surfaces (Fig. 1). Although very common, RIF have often been overlooked due to their small size, their slow growth and the lack of diagnostic features. First discovered in hot and cold deserts (Krumbein & Jens 1981, Friedmann 1982, Staley *et al.* 1982), RIF are now known to be ubiquitous on hard surfaces, in extreme as well as in temperate climates (Urzì *et al.* 1995, Sterflinger & Prillinger 2001, Gorbushina 2007, Gorbushina & Broughton 2009). RIF are well adapted to nutrient-poor and dry habitats where they are particularly successful colonisers due to restricted competition with other microbes (Gorbushina 2007) and their extremotolerance.

Extremotolerance comprises some specific universally present adaptations that enable these fungi to tolerate surprisingly wide ranges of temperatures, irradiation and osmotic stresses (Palmer *et al.* 1990, Sterflinger 1998, Gorbushina *et al.* 2003, Ruibal 2004, Onofri *et al.* 2007, Gorbushina *et al.* 2008). Melanisation protects cells against UV radiations (Dadachova & Casadevall 2008), whereas the typical isodiametrical (meristematic) growth form ensures an optimal volume : surface ratio and, therefore, allows them to survive extreme temperatures and desiccation (Wollenzien *et al.* 1995). These oligotrophic organisms are able to rely only on sparse, airborne nutrients available on rock surfaces. Their growth on these substrates is limited, and, for some of them, the production of internal asexual spores further allows to save energy. All adaptations contribute to the amazing survival capabilities of RIF in hostile habitats. The environmental tolerance of these fungi, and, in some cases, their capacity to penetrate minerals, make them an attractive subject for studies in microbial ecophysiology and applied research, such as biodeterioration of monuments and exobiology (Gorbushina *et al.* 1993, Diakumaku *et al.* 1995, Wollenzien *et al.* 1997, Gorbushina *et al.* 2002, Gorbushina 2003, Onofri *et al.* 2008).

Sterflinger *et al.* (1997) provided the first molecular evidence of RIF phylogenetic affiliations, and they are known to belong to two groups of ascomycetes, namely *Dothideomycetes* and *Eurotiomycetes* (de Hoog *et al.* 1999, Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008, Sert *et al.* 2007a). In *Eurotiomycetes*, multigene phylogenetic analyses have shown that


**Fig 1.** Rock-inhabiting fungi related to *Dothideomycetes*. A–C: sampling localities (photos C. Ruibal and L. Selbmann). A. Metamorphic black slate from Atazar, Central Mountain System, Spain. B. Limestone from Cala Sant Vicenç, Serra de Tramuntana, Mallorca, Spain. C. Sandstone from Alatna Valley, McMurdo Dry Valleys, Antarctica. D–G: *Coniosporium apollinis*, a rock-inhabiting species from the Mediterranean region (CBS 100213, photos C. Gueidan). D. Colony on MEA. E. Melanised torulose hyphae. F. Hypha disarticulating into bi- to multi-cellular clumps; G. Meristematic growth. H–J: Antarctic rock-inhabiting fungi (photos L. Selbmann). H. RIF growing on a crystal of sandstone. I. Melanised hypha of *Friedmanniomyces endolithicus*. J. Meristematic growth of *Cryomyces antarcticus*. K–L: *Cystocoleus ebeneus*, a lichenised species assigned to *Capnodiales* (photos L. Muggia). K. Microfilamentous thallus. L. Melanised hyphae of the mycobiont forming a furrow around the filamentous algae. Scale bars: D = 2 mm, E–G and I–J = 10 µm, H = 0.5 mm, K–L = 20 µm.

RIF cluster in early diverging lineages of *Chaetothyriales*, whereas two species seem to be more closely related to the lichenised order *Verrucariales*, the sister group of *Chaetothyriales* (Gueidan *et al.* 2008). Gueidan *et al.* (2008) also demonstrated that the most recent common ancestor of both lichenised *Verrucariales* and pathogen-rich *Chaetothyriales* was probably a rock-inhabiting fungus. It was hypothesised that adaptations to life in extreme conditions might have been a prerequisite for the evolution of human pathogenicity (de Hoog 1993, Haase *et al.* 1999, Gueidan *et al.* 2008) and lichenisation in this class (Gueidan *et al.* 2008). In contrast, despite the high diversity of RIF within *Dothideomycetes*, only very few human pathogens are known from this class of *Ascomycota* (de Hoog *et al.* 2000). Alternatively, associations with plants and in particular plant pathogenicity are very common (Schoch *et al.* 2006, Arzanlou *et al.* 2007, Crous *et al.* 2007a–c, 2009; this volume). Additionally, lichenised species also appeared to be nested within *Dothideomycetes* (Lutzoni *et al.* 2004, James *et al.* 2006, Del Prado *et al.* 2006, Muggia *et al.* 2008, Nelsen *et al.* 2009). Presently no strong phylogenetic hypothesis is available to assess the placement of RIF within *Dothideomycetes*. Moreover, no studies have investigated phylogenetic relationships among RIF, lichen-forming fungi and plant-associated fungi within *Dothideomycetes*. Our main goal was to infer phylogenetic relationships of RIF within Dothideomyceta, a lineage including *Dothideomycetes* and *Arthoniomycetes*, to explore more specifically their diversity, origins and evolution.

## MATERIAL AND METHODS

### Taxon and gene sampling

Representative taxa of most of the main orders and families of Dothideomyceta (*Dothideomycetes* and *Arthoniomycetes*) were sampled. Two separate sets of data matrices were assembled. The first set (three-gene analysis; Table 1 - see online Supplementary Information) is composed of 182 taxa (including 102 rock-inhabiting strains) for which DNA sequences of three ribosomal genes have been obtained: the large and small subunits of the nuclear ribosomal RNA gene (nucLSU and nucSSU, respectively) and the small subunit of the mitochondrial ribosomal RNA gene (mtSSU). Because this first set of data matrices included only ribosomal genes, low phylogenetic confidence was expected for deep relationships within Dothideomyceta. To overcome this problem, a second set of data matrices was assembled (five-gene analysis; Table 1 in Supplementary Information) consisting of DNA sequences of five loci from 113 taxa (including 40 rock-inhabiting strains): the largest and second largest subunits of the RNA polymerase II (*RPB1* and *RPB2*, respectively), nucLSU, nucSSU and mtSSU. The outgroup for the three-gene analysis included *Hyphozyma lignicola, Symbiotaphrina buchneri* and *S. kochii*, whereas only the latter two species were selected as outgroup for the five-gene analysis. These species were chosen because they constituted a sister group to Dothideomyceta in a previous study (Schoch *et al.* 2009a).

### DNA isolation and sequencing

Different laboratories contributed data using various protocols, but most DNA sequence information was produced as follows: genomic DNA was isolated from cultures grown on MEA. Fungal biomass was transferred to a tube with 500 µL of TES buffer and ground with a micro-pestle for 1–2 min, with or without silica-mix (2/3 silica-gel, 1/3 Celite® 545). A volume of 140 µL of 5 M NaCl was then added, followed by 65 µL of 10 % (w/v) CTAB (cetyltrimethylammoniumbromid). After an incubation of 30 min at 65 °C, 700 µL of (24:1) chloroform/isoamylalcohol was added, the tubes were mixed carefully by hand, stored on icy water for 30 min, and centrifuged for 10 min at 4 °C (10 000 x g). The supernatant was recovered and the genomic DNA precipitated using isopropanol. After washing the pellets with 70 % ethanol, they were dried in a vacuum centrifuge and re-suspended in 60 µL of TE buffer (protocol modified from Möller *et al.* 1992).

Six regions covering five genes were amplified: nucLSU, nucSSU, mtSSU, *RPB1* region A–D, *RPB2* region 5–7, and *RPB2* region 7–11 (see Table 2 for primers used). Genomic DNA (1 µL of a 1/10 or 1/100 dilution) was added to a PCR mix comprising 2.5 µL of PCR buffer (buffer IV with 15 mM $MgCl_2$, Abgene, Epsom, U.K.), 2.5 µL of dNTPs (2 mM), 2.5 µL of BSA (10 mg/mL), 2.0 µL of primers (10 µM), 0.15 µL *Taq* polymerase (5 U/µL, Denville, Metuchen NJ, U.S.A.), and water for a total volume of 25 µL. Amplification cycles for nucLSU, nucSSU and *RPB1* (same conditions applied for *RPB2*) are described in Gueidan *et al.* (2007), and in Zoller *et al.* (1999) for mtSSU. The PCR products were purified using Microcon PCR cleaning kits (Millipore, Billerica MA, U.S.A.). Sequencing was carried out using Big Dye Terminator Cycle sequencing Kits (ABI PRISM version 3.1, Perkin-Elmer, Applied Biosystems) on ABI 3730xl DNA Analyzers (Applied Biosystems, Foster City CA, U.S.A.) from the Duke Center for Evolutionary Genomics (Durham NC, U.S.A.) and the Hubrecht Institute (Utrecht, Netherlands).

**Table 2.** List of primers for the five genes used in this study (*RPB2* was amplified in two regions).

| Gene regions | PCR primers | Additional primers used for sequencing |
|---|---|---|
| nucLSU | LR0R[a], LR7[b] | LR3, LR3R, LR5, LR5R, LR6, LR6R[b] |
| nucSSU | nssu131[c], NS24[d] | nssu1088, nssu1088R, nssu897R, nssu634[c], SR11R[e], NS23, NS22[d], SR7R, SR7, SR10R[f] |
| mtSSU | mtSSU1, mtSSU3R[g] | mtSSU2, mtSSU2R[g] |
| *RPB1* region A–D | *RPB1*-AF[h], *RPB1*-6R1asc[i] | – |
| *RPB2* region 5–7 | *RPB2*-5F, *RPB2*-7cR[i] | – |
| *RPB2* region 7–11 | *RPB2*-7cF, *RPB2*-11aR[i] | – |

[a]Rehner & Samuels (1994), [b]Vilgalys & Hester (1990), [c]Kauff & Lutzoni (2002), [d]Gargas & Taylor (1992), [e]Spatafora *et al.* (1995), [f]Vilgalys (unpubl.; www.biology.duke.edu/fungi/mycolab/primers.htm), [g]Zoller *et al.* (1999), [h]Hall (unpubl.; http://faculty.washington.edu/benhall/), [i]Hofstetter *et al.* (2007), [j]Liu *et al.* (1999).

## Alignments and phylogenetic analyses

Sequences were assembled and edited using Sequencher (Gene Codes Corporation, Ann Arbor MI, U.S.A.). Manual alignments were performed using MacClade v. 4.08 (Maddison & Maddison 2003). Ambiguous regions (*sensu* Lutzoni *et al.* 2000) and introns were delimited manually and excluded from the alignments. Congruence was tested using a 70 % reciprocal bootstrap criterion (Mason-Gamer & Kellogg 1996, Reeb *et al.* 2004). For the three-gene dataset, the test was performed using Compat (Kauff & Lutzoni 2002) on all possible gene pairs (mtSSU vs. nucSSU, mtSSU vs. nucLSU, and nucLSU vs. nucSSU) and based on bootstrap consensus trees. Bootstrap trees were obtained using Neighbor-Joining bootstrap analyses with Maximum Likelihood distances in PAUP v. 4.0b10 (Swofford 2003). Models of molecular evolution were estimated using the Akaike Information Criterion implemented in Modeltest v. 3.7 (Posada & Crandall 1998). For the five-gene dataset, congruence was also tested using a 70 % reciprocal bootstrap criterion, but the comparison was done manually based on trees obtained with 500 bootstrap replicates using RAxML VI-HPC (Stamatakis *et al.* 2005, 2008) on the Cipres Web Portal (www.phylo.org/sub_sections/portal/). Taxa or sequences responsible for incongruence were removed from the dataset, and the markers were combined. Final phylogenetic analyses of the three-gene and five-gene datasets were performed using RAxML on the Cipres Web Portal. The ML search followed a GTRMIX model of molecular evolution applied to the following nine partitions: *RPB1* first, second and third codon positions, *RPB2* first, second and third codon positions, nucLSU, nucSSU and mtSSU. Support values were obtained with bootstrap analyses of 1 000 pseudoreplicates using RAxML.

# RESULTS

## DNA sequence alignments

Not all markers were recovered or available for all taxa. For the three-gene dataset, 20 nucLSU, 11 nucSSU and 54 mtSSU sequences were missing. Among the 182 taxa, 119 had sequences for three genes, 61 for two genes, and 12 for one gene (Table 1 in Supplementary Information). After exclusion of ambiguous regions and introns, the combined dataset included 3 274 characters (1 106 for nucLSU, 1 616 for nucSSU and 552 for mtSSU). Among these, 2 063 were constant while 931 were parsimony-informative. For the five-gene dataset, missing data comprised 5 nucLSU, 8 nucSSU, 30 mtSSU, 48 *RPB1* and 30 *RPB2* sequences. Among the 113 taxa, 32 had sequences for five genes, 46 for four genes, 30 for 3 genes, and 5 for 2 genes (Table 1 in Supplementary Information). After exclusion of ambiguous regions and introns, the combined dataset included 6 045 characters (1 133 for nucLSU, 1 607 for nucSSU, 593 for mtSSU, 1 011 for *RPB1* and 1 701 for *RPB2*). Among these, 2 912 were constant while 2 693 were parsimony-informative.

## Phylogenetic inference

For the three-gene analysis (Figs 2–3), results show that, within the two classes *Dothideomycetes* and *Arthoniomycetes*, rock-inhabiting fungi belong to 13 groups, either well-known orders or families, or lineages that have not previously been characterised. Among the rock-inhabiting fungi clustering with well-known groups of *Dothideomycetes*, two strains are found in the order *Dothideales*, four in the order *Pleosporales*, one in *Myriangiales*, 12 forming a monophyletic group sister to the remaining members of *Davidiellaceae*, and one in the family *Capnodiaceae*. The family *Teratosphaeriaceae* is not monophyletic in this analysis (also see Crous *et al.* 2009; this volume). In a first group including the generic type *Teratosphaeria fibrillosa* (*Teratosphaeriaceae* 1, Fig. 3), many rock-inhabiting strains are present, including taxa from the three genera *Friedmanniomyces*, *Elasticomyces* and *Recurvomyces*. The second group (*Teratosphaeriaceae* 2, Fig. 3), including the three leaf-colonising species *Devriesia strelitziae, Mycosphaerella eurypotami* and *Tripospermum myrti*, an unknown species of *Capnodiales*, the lichen species *Cystocoleus ebeneus* as well as 20 undescribed rock inhabiting strains, is supported as sister to the family *Mycosphaerellaceae* (91 % bootstrap). The two rock-inhabiting species *Coniosporium uncinatum* and *C. apollinis* are well supported (100 % bootstrap), but their sister relationship is not. Neither these two species of *Coniosporium* nor the Antarctic genus *Cryomyces* can be assigned to any known family or order sampled here. Amongst the unknown lineages, one does not seem to be part of *Dothideomycetes* (lineage 1, Fig. 2), and appears as sister to *Arthoniomycetes* (98 % bootstrap). Due to the lack of support for many deep internodes, it is not possible to determine if lineages 2 and 3 can be accommodated by the expansion of known groups of *Dothideomycetes*, or if the recognition of new taxonomical entities are needed. Finally, the rock isolates A6, AN13, TRN 437 and CCFEE 5413 do not significantly cluster with any other taxa.

With the five-gene analysis (Fig. 4), the inferred deep branching pattern within Dothideomyceta is still poorly supported, but additional well-supported nodes are recovered (*e.g.*, *Capnodiaceae* as sister to the lineage including *Mycosphaerellaceae* and *Teratosphaeriaceae*, and the monophyly of *Teratosphaeriaceae* 1). As in the three-gene analysis, the sister relationship between lineage 1 and *Arthoniomycetes* obtains high support (100 % bootstrap), even though the two rock-inhabiting strains included do not seem to form a monophyletic group. The placement of the lichen family *Trypetheliaceae* as sister to *Arthoniomycetes* (70 % bootstrap) might be an artifact, as this relationship was not recovered in any other studies (Del Prado *et al.* 2006, Spatafora *et al.* 2006, Nelsen *et al.* 2009). Within *Dothideomycetes*, the orders *Dothideales* and *Myriangiales* form a sister group (100 % bootstrap), and are sister to the well-supported *Capnodiales* (100 % bootstrap), which includes most of the rock-inhabiting strains. Within *Capnodiales*, the second group of *Teratosphaeriaceae* (*Teratosphaeriaceae* 2; Fig. 4) is still supported as sister to *Mycosphaerellaceae* (89 % bootstrap). Other lineages comprising exclusively RIF (*Cryomyces*, *Coniosporium uncinatum*, and *C. apollinis*) do not significantly cluster with any known group of *Dothideomycetes*.

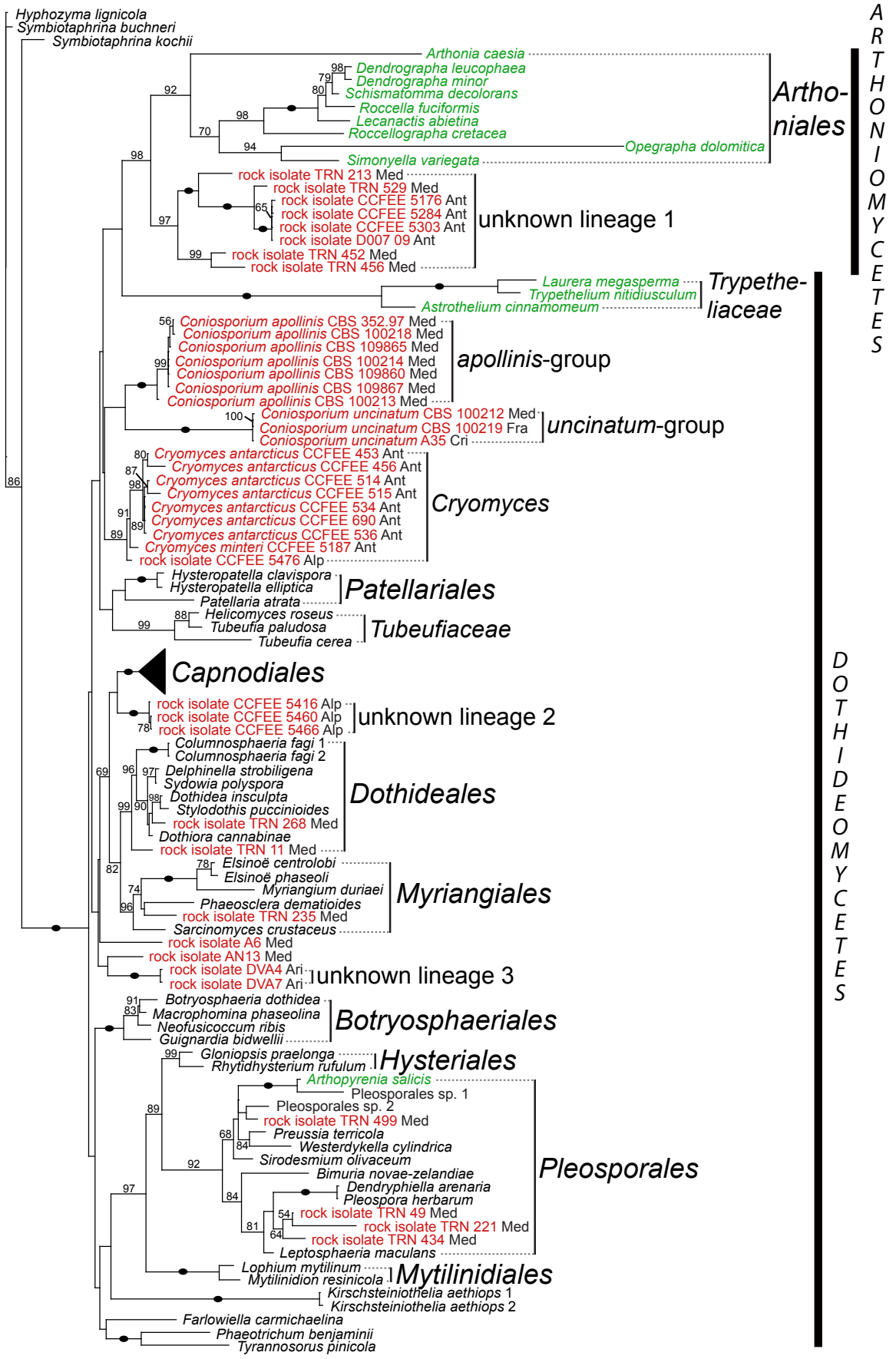


**Fig. 2.** Phylogenetic placement of 102 rock-inhabiting strains within *Dothideomyceta* (*Dothideomycetes* and *Arthoniomycetes*). The tree is based on a Maximum Likelihood analysis of the combined nucLSU, nucSSU and mtSSU (three-gene analysis). A black oval on a branch indicates a bootstrap support value of 100 %. Other bootstrap values ≥ 50 % are shown below or above branches. RIF are highlighted in red and lichens in green. Geographical origins are also labeled for RIF (Alp = Alps, And = Andes, Ant = Antarctica, Ari = Arizona desert, Cri = Crimea, Fra = France, Med = Mediterranean region, including Greece, Israel, Italy, Slovenia, Spain and Turkey). Phylogenetic relationships within *Capnodiales* are detailed in Fig. 3.


**Fig. 3.** Phylogenetic placement of RIF within the order *Capnodiales*. The tree is based on a Maximum Likelihood analysis of the combined nucLSU, nucSSU and mtSSU (three-gene analysis). A black oval on a branch indicates a bootstrap support value of 100 %. Other bootstrap values ≥ 50 % are shown below or above branches. RIF are highlighted in red and lichens in green. Geographical origins are also labeled for RIF (Alp = Alps, And = Andes, Ant = Antarctica, Ari = Arizona desert, Cri = Crimea, Fra = France, Med = Mediterranean region, including Greece, Israel, Italy, Slovenia, Spain and Turkey).

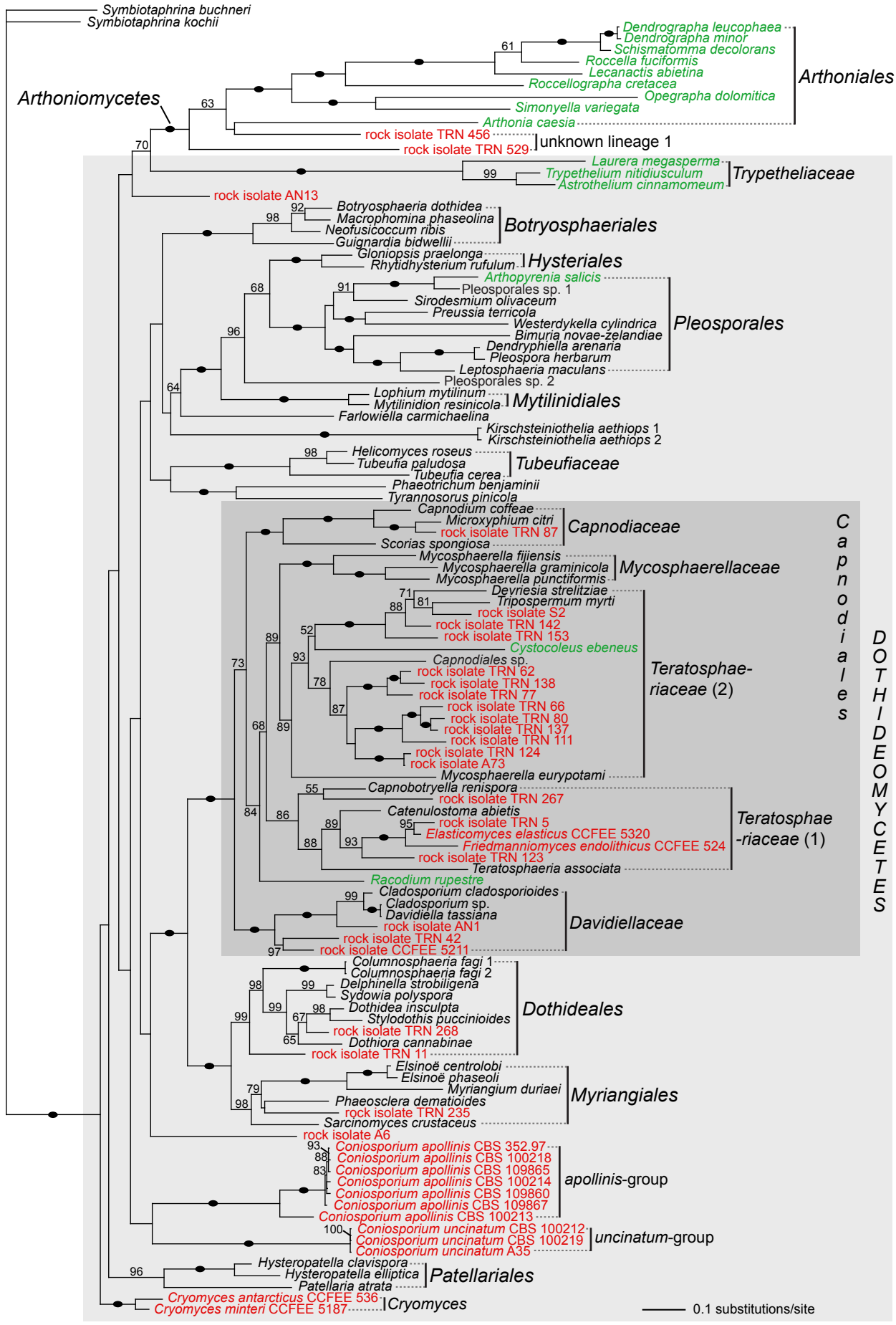


**Fig. 4.** Phylogenetic relationships of rock-inhabiting lineages with known groups of Dothideomyceta based on a Maximum Likelihood analysis of the combined nucLSU, nucSSU, mtSSU, *RPB1* and *RPB2* (five-gene analysis). A black dot on a branch indicates a bootstrap support value of 100 %. Other bootstrap values ≥ 50 % are shown below or above the branches. RIF are highlighted in red and lichens in green.

## DISCUSSION

### Species diversity in *Dothideomycetes*

The *Dothideomycetes* are very diverse in term of species, some of which are well known for their pathogenicity on crops (*e.g.*, *Mycosphaerella fijiensis*, the agent of the leaf spot disease of banana, or *Leptosphaeria maculans*, the agent of the blackleg disease of cabbage). Whilst many species are associated with plants (either as pathogens or as epiphytes), saprobic, coprophilous, lichen-forming and rock-inhabiting fungi are also present in this class. The importance of RIF in term of species richness is still under-investigated. A thorough sampling of dothideomycetous RIF from few localities in Mallorca and Central Spain formed the basis of the analyses described here (Ruibal 2004, Ruibal *et al.* 2005, 2008). RIF from Antarctica, the Alps and the Andes (Selbmann *et al.* 2005, 2008), as well as the Arizona and Negev deserts (Staley *et al.* 1982, A.A. Gorbushina, unpubl. data) extended the geographical range of the sampled taxa. Finally, isolates from monuments in the Mediterranean area supplemented the sampling (Gorbushina *et al.* 1996, Sterflinger *et al.* 1997, Volkmann & Gorbushina 2006). In comparison to known RIF habitats (Gorbushina 2007), our sampling was very restricted and does not permit a realistic overview of fungal diversity on rock surfaces. Nevertheless, an impressive number of rock-inhabiting species is already evident. Our data show that rock-inhabiting fungi are not only present in well-known orders, such as *Capnodiales* or *Pleosporales*, but also in novel lineages (*e.g.*, lineage 1, Fig. 2). Moreover, very few species with overlapping distribution were recovered from neighbouring geographical localities in Mallorca and Central Spain (Ruibal *et al.* 2005, 2008). Therefore, we can hypothesise that species richness within *Dothideomycetes* remains woefully underestimated, and that many more species will need to be described within this class in the future, especially for fungi colonising rocky substrates.

### Classification of rock fungi related to *Dothideomycetes*

Although very diverse within *Dothideomycetes*, RIF have not been included in recent phylogenetic studies of this class (Lumbsch *et al.* 2001, Schoch *et al.* 2006). Only very few of these rock-inhabiting species have been taxonomically described (Sterflinger *et al.* 1997, Bills *et al.* 2005, Sert *et al.* 2007b), and the molecular marker available for most of these species (ITS) does not allow their inclusion in large-scale phylogenetic analyses. The few attempts to produce phylogenies involving RIF have shown that they belong to two diverse classes of *Ascomycota*, namely *Eurotiomycetes* (particularly the order *Chaetothyriales*) and *Dothideomycetes* (preponderantly the orders *Capnodiales*, *Dothideales* and *Pleosporales*) (Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008).

Our results confirm the placement of RIF in the same orders of *Dothideomycetes*, although some lineages are shown to belong to additional groups. Based on our results, many RIF should be classified within *Dothideales*, *Pleosporales* and *Capnodiales*, the latter order holding the largest number in rock-colonising species. The genera *Elasticomyces* and *Recurvomyces,* as well as the Antarctic genus *Friedmanniomyces,* were previously attributed to *Capnodiales* based on nucSSU data (Selbmann *et al.* 2008). Our multigene analyses confirm this placement, and show that these three genera belong to *Teratosphaeriaceae s. str.*, the family currently showing the highest diversity in RIF (Fig. 3). We also showed that one RIF (TRN 235) previously thought to be related to the *Dothideales* (Ruibal *et al.* 2008) actually belongs to *Myriangiales*, along with *Sarcinomyces crustaceus*, a species similarly melanised and meristematic, but isolated from plant material (Sigler *et al.* 1981).

Several well-supported groups of RIF could not be attributed to any known families and orders according to our data. As a consequence, *Cryomyces* should still be considered as *Dothideomycetes incertae sedis*, as no close relationship was recovered for this enigmatic Antarctic genus (Selbmann *et al.* 2005). The positions of RIF-rich genera *Coniosporium* and *Sarcinomyces* are also problematic. Previous studies placed them either in *Dothideales* or *Chaetothyriales* based on ITS or nucSSU data (de Leo *et al.* 1999, Sterflinger *et al.* 1999, Sert *et al.* 2007a). Yet, the limited taxon and gene sampling on which these analyses were based was probably insufficient to demonstrate clear phylogenetic relationships. Our results show that *Coniosporium apollinis* (including the type strain CBS 352.97), *C. uncinatum* (including the type strain CBS 100219) and *Sarcinomyces crustaceus* belong to Dothideomyceta (Fig. 4). However, a previous multigene analysis showed that two other species, *Coniosporium perforans* and *Sarcinomyces petricola*, belong to *Chaetothyriales* (Gueidan *et al.* 2008). These anamorphic genera are therefore not monophyletic, and additional research is required to clarify their status.

Among lineages lacking known reference taxa, two groups seem to belong to *Dothideomycetes* (unknown group 2, a lineage comprising RIF from the Alps, and unknown group 3, a lineage including strains isolated in Arizona; Fig. 2). Another unknown group (lineage 1) clusters outside *Dothideomycetes*, sister to the *Arthoniales* (Figs 2, 4). A previous study had noted the problematic placement of this latter group (Ruibal *et al.* 2008). Many lineages including RIF still need to be named. In the past, several melanised meristematic species and genera have been described such as *Lichenothelia* (Hawksworth 1981; see also Henssen 1987), which could potentially correspond to some of these RIF lineages. However, little is known about these formerly named taxa, and no molecular data or cultures are available for many of them. Naming RIF will therefore require an extensive study of both rock-inhabiting species and formerly described melanised meristematic species, whether they grow on rock or not.

### Rock surfaces: "terroirs" for ancient lineages or reservoirs for plant-associated fungi?

Despite the prevailing extreme conditions, rock surfaces host a large variety of specialised fungi. Fungal colonisation of subaerial rocks can be explained by two non-exclusive hypotheses. Firstly, atmosphere-exposed rock substrates could constitute "terroirs" for ancient fungal lineages. Rock surfaces were among the first terrestrial substrates available for living organisms on earth (Gorbushina & Broughton 2009). It is therefore likely that, early on, some species became adapted to colonise rock surfaces. RIF are persistent to different types of physical stress, but are poor competitors and surrender to more combative organisms (Gorbushina *et al.* 2008). Increasing competition with other rock-inhabiting organisms living under more permissive conditions may have restricted some of these ancient, morphologically reduced, slow-growing, fungal relicts to extreme habitats. The presence of lineages comprising exclusively RIF that diverged early in the evolution of Dothideomyceta (*e.g.*, *Cryomyces* and lineage 1, Fig. 2) supports this hypothesis of rock surfaces as substrates for ancient fungal lineages.

Secondly, rock surfaces could form reservoirs for plant-associated or saprobic fungi. Through spore or propagule dispersal, some species of various unrelated groups of plant pathogens, epiphytes or saprobes can reach rock substrates. Their ability to survive in these environments will depend on some key features, namely oligotrophy, melanisation and pleiomorphism (or diversity of growth forms, amongst which meristematic growth). Under extreme conditions prevailing on rock surfaces, fungi possessing these key features can survive due to their slow, meristematic, clumpy growth and thick-walled, heavily melanised cells. These key features seem to have evolved several times in *Dothideomycetes*, allowing different lineages to colonise rock substrates. In *Dothideales*, phyllosphere fungi such as *Aureobasidium pullulans* and relatives, which have a filamentous or yeast-like growth under moist conditions, but convert to a meristematic form when colonising inert substrates, have also been isolated from rock surfaces (Ruibal *et al.* 2008). The family *Teratosphaeriaceae s. l.* is another example of a group in which some leaf-colonising species can also grow meristematically and form dark, thick-walled cells. According to our results, this family (as traditionally delimited; *i.e.*, including *Teratosphaeriaceae* 1 and 2) is also extremely diverse in RIF (Fig. 3). Rocks supporting growth of subaerial biofilms (Gorbushina & Broughton, 2009) may be viewed as a reservoir for all types of melanised meristematic fungi, from where other habitats can be re-colonised. Survival of new comers is probably additionally facilitated by the existing microbial community on rocks (Gorbushina & Broughton 2009) in a fashion known for immigrant bacteria on leaf surfaces (Monier & Lindow 2005).

Alternatively, rock-colonising lichens may supply buffered environments and refugia for RIF or organisms otherwise occupying other niches (Selbmann *et al*. 2010). Recent studies have shown that lichens harbour an amazing diversity of ascomycetous endophyte-like (endolichenic) fungi (Arnold *et al.* 2009), and phylogenetic relatedness was found between some endolichenic fungi isolated from saxicolous lichens and RIF (Harutyunyan *et al.* 2008). If in most cases, species from rock surfaces can still go back to their primary habitats, in some cases, these fungi keep specialising and get trapped in these extreme habitats. This may be the case for groups with no close relationships with plant-associated fungi, such as the genus *Friedmanniomyces* (Fig. 3).

## Geographical distribution of rock-inhabiting fungi

The large majority of rock-inhabiting strains isolated thus far originated from rocks in the Mediterranean region or Antarctica (Sterflinger *et al.* 1999, Ruibal 2004, Ruibal *et al.* 2005, 2008 Selbmann *et al.* 2005, 2008). In Antarctica, RIF tend to grow within rocks, together with the cryptoendolithic lichen communities, finding shelter from extreme conditions prevailing on rock surfaces. In the Mediterranean area, RIF tend to grow on the rock surface or in cracks, causing damages to the substrate (*e.g.*, biopitting of marble). Despite differences in temperature, they share similar morphological and physiological adaptations, such as melanisation, meristematic growth and oligotrophism.

Similarly to previous studies (Selbmann *et al.* 2005, Ruibal *et al.* 2008), our results show that Antarctic RIF often share an evolutionary history with RIF from semi-arid areas. In our study, RIF sampled in geographically disjoint localities (Antarctica versus Mediterranean region) cluster together in *Davidiellaceae*, the two groups of *Teratosphaeriaceae*, and unknown lineage 1 (Figs 2–3). In some cases, Antarctic and Mediterranean strains are even phylogenetically very closely related, showing a recent common evolutionary history (*e.g.*, in *Teratosphaeriaceae* 2, the Mediterranean rock isolates TRN 124 and A73 with the Antarctic strain CCFEE 5489). Likewise, some strains of *Recurvomyces mirabilis* and *Elasticomyces elasticus* have been recorded in the Antarctic as well as in high peaks of the Alps and Andes (Selbmann *et al.* 2008). Therefore, it seems that an efficient mechanism of dispersal, most probably wind-mediated (Gorbushina *et al.* 2007, Gorbushina & Broughton 2009), have led to a colonisation spanning different continents.

## Rock-dwelling habit and evolution of lichenisation

Most of the diversity in lichen-forming fungi is found in *Lecanoromycetes*, a large and diverse class of ascomycetes including approximately 14 000 species (Miadlikowska *et al.* 2006, Kirk *et al.* 2008). Yet, the classes *Lichinomycetes* (with the single order *Lichinales*), *Eurotiomycetes* (with the orders *Pyrenulales* and *Verrucariales*), *Arthoniomycetes* (with the single order *Arthoniales*), and *Dothideomycetes* also include lichens. Although *Lichinales*, *Pyrenulales*, *Verrucariales* and *Arthoniales* are monophyletic lineages containing mostly lichenised species, lichens in *Dothideomycetes* seem to encompass a broader phylogenetic spectrum: the *Trypetheliaceae*, a family of mostly tropical bark-colonising lichens, forms a monophyletic group within *Dothideomycetes* (Del Prado *et al.* 2006, Nelsen *et al.* 2009, Schoch *et al.* 2009a). *Arthopyrenia salicis*, a corticolous, temperate lichen species nests within the order *Pleosporales* (Del Prado *et al.* 2006, Nelsen *et al.* 2009). Two melanised micro-filamentous lichens, *Cystocoleus ebeneus* and *Racodium rupestre*, were assigned to the order *Capnodiales* (Muggia *et al.* 2008, Nelsen *et al.* 2009). Finally, the two lichen families *Strigulaceae* (mostly leaf-colonising tropical species) and *Monoblastiaceae* (temperate and tropical species) are now shown to belong to *Dothideomycetes* (Nelsen *et al.* 2009; this volume).

Whether these lichen lineages, that are unrelated to *Lecanoromycetes*, originated from independent gains of lichenisation is not clear (Lutzoni *et al.* 2001, James *et al.* 2006, Gueidan *et al.* 2008, Arnold *et al.* 2009, Schoch *et al.* 2009a, b). Within *Eurotiomycetes*, phylogenetic data suggest that the lineage including *Pyrenulales* and *Verrucariales* possibly results from an independent gain of lichenisation (Gueidan *et al.* 2008, Schoch *et al.* 2009a). Phylogenetic data suggest that lichens in *Verrucariales* may have evolved from rock-inhabiting fungi (Gueidan *et al.* 2008), a result in agreement with experimental data demonstrating that some RIF and one melanised lichen-colonising fungus could form associations with lichen-associated algae (Gorbushina *et al.* 2005, Brunauer *et al.* 2007). This rock-inhabiting ancestor may have evolved associations with epilithic microalgae in order to get a more constant supply in nutrients. If the evolution of fungal-algal associations occurred in *Eurotiomycetes*, it most likely also occurred in different fungal groups. It is therefore interesting to see if in *Dothideomycetes*, where rock fungi are so diverse, similar transitions in lifestyles can be suggested.

Although many lichenised species in *Dothideomycetes* are either corticolous or only secondarily or occasionally saxicolous, *Cystocoleus ebeneus* and *Racodium rupestre* are true rock inhabitants. Amongst lichens in *Dothideomycetes*, these two species are the most likely to have evolved from a rock-inhabiting ancestor. They share substrate preference and some morphological features, such as their melanised hyphae, with RIF. Strikingly, in our result, *Cystocoleus ebeneus* is nested within a lineage comprising almost exclusively RIF (*Teratosphaeriaceae* 2, Fig. 3).

*Racodium rupestre* is also related to a RIF, but this relationship is not supported (Fig. 3). This result agrees with a rock-inhabiting ancestor for these two lichenised species, but further data will however be necessary to test this hypothesis. Also of interest is the close phylogenetic relationship between the lichen order *Arthoniales* and the lineage 1 of RIF (Figs 2, 4). Although mostly corticolous or foliicolous, *Arthoniales* also comprises saxicolous species (Ertz *et al.* 2009). Further data is needed to explore the relationships between saxicolous species of *Arthoniales* and RIF. In conclusion, these preliminary results suggest that there might be a link between rock-dwelling habit and lichenisation. However, additional taxon and gene sampling are needed to confirm the phylogenetic placements of some of the lichenised taxa and to clarify their relationships to RIF. Only then the hypothesis of RIF as ancestors of lichenised lineages can be adequately tested.

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**Table 1.** Taxon and gene sampling for the three- and five- gene analyses. Geographical origins are also mentioned for RIF. A dash indicates missing sequences. Newly produced sequences are shown in bold. A column also indicates if taxa were included in the three-gene (3) or in both three- and five-gene analyses (3 & 5).

| Taxon | Collection # | Additional information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis |
|---|---|---|---|---|---|---|---|---|---|
| *Hyphozyma lignicola* | CBS 325.93 | Outgroup | | AF353595 | AJ496239 | – | | | 3 |
| *Symbiotaphrina buchneri* | CBS 6902 | Outgroup, AFTOL 1836 | | FJ176887 | FJ176831 | – | FJ238370 | FJ238442 | 3 & 5 |
| *Symbiotaphrina kochii* | CBS 250.77 | Outgroup, AFTOL 1902 | | AY227719 | FJ176833 | – | GU397369 | FJ238443 | 3 & 5 |
| ***Arthoniomycetes*** | | | | **LSU** | **SSU** | **mtSSU** | **RPB2** | **RPB1** | **Analysis** |
| *Arthonia caesia* | – | AFTOL 775 | *Arthoniales* | FJ469668 | – | FJ469671 | FJ469670 | FJ772241 | 3 & 5 |
| *Dendrographa leucophaea* | – | AFTOL 308 | *Arthoniales* | AY548810 | AY548803 | AY548811 | EU704017 | – | 3 & 5 |
| *Dendrographa minor* | – | AFTOL 355 | *Arthoniales* | AF279382 | AF279381 | **GU561843** | AY641034 | **GU561849** | 3 & 5 |
| *Lecanactis abietina* | – | AFTOL 305 | *Arthoniales* | AY548812 | AY548805 | AY548813 | AH013900 | **GU561850** | 3 & 5 |
| *Opegrapha dolomitica* | – | AFTOL 993 | *Arthoniales* | – | DQ883706 | – | DQ883714 | DQ883717 | 3 & 5 |
| *Roccella fuciformis* | – | AFTOL 126 | *Arthoniales* | AY584654 | AY584678 | EU704082 | DQ782866 | – | 3 & 5 |
| *Roccellographa cretacea* | – | AFTOL 93 | *Arthoniales* | DQ883696 | DQ883705 | FJ772240 | DQ883713 | DQ883716 | 3 & 5 |
| *Schismatomma decolorans* | – | AFTOL 307 | *Arthoniales* | AY548815 | AY548809 | AY548816 | DQ883715 | – | 3 & 5 |
| *Simonyella variegata* | – | AFTOL 80 | *Arthoniales* | – | AY584669 | AY584631 | DQ782861 | DQ782819 | 3 & 5 |
| ***Dothideomycetes*** | | | | **LSU** | **SSU** | **mtSSU** | **RPB2** | **RPB1** | **Analysis** |
| *Botryosphaeria dothidea* | CBS 115476 | AFTOL 946 | *Botryosphaeriales* | DQ678051 | DQ677998 | FJ190612 | DQ677944 | EU186063 | 3 & 5 |
| *Guignardia bidwellii* | CBS 237.48 | AFTOL 1618 | *Botryosphaeriales* | DQ678085 | DQ678034 | – | DQ677983 | – | 3 & 5 |
| *Macrophomina phaseolina* | CBS 227.33 | AFTOL 1783 | *Botryosphaeriales* | DQ678088 | DQ678037 | FJ190645 | DQ677986 | – | 3 & 5 |
| *Neofusicoccum ribis* | CBS 115475 | AFTOL 1232 | *Botryosphaeriales* | DQ678053 | DQ678000 | – | DQ677947 | – | 3 & 5 |
| *Capnodium coffeae* | CBS 147.52 | AFTOL 939 | *Capnodiales*, *Capnodiaceae* | DQ247800 | DQ247808 | FJ190609 | DQ247788 | DQ471162 | 3 & 5 |
| *Capnodium salicinum* | CBS 131.34 | AFTOL 937 | *Capnodiales*, *Capnodiaceae* | DQ678050 | DQ677997 | – | | | 3 |
| *Microxyphium citri* | CBS 451.66 | | *Capnodiales*, *Capnodiaceae* | **GU301848** | **GU296177** | – | **GU371727** | **GU357750** | 3 & 5 |
| *Scorias spongiosa* | CBS 325.33 | AFTOL 1594 | *Capnodiales*, *Capnodiaceae* | DQ678075 | DQ678024 | FJ190643 | DQ677973 | – | 3 & 5 |
| *Cladosporium cladosporioides* | CBS 170.54 | AFTOL 1289 | *Capnodiales*, *Davidiellaceae* | DQ678057 | DQ678004 | FJ190628 | DQ677952 | EU186064 | 3 & 5 |
| *Cladosporium* sp. | CBS 180.53 | AFTOL 1035 | *Capnodiales*, *Davidiellaceae* | AY016367 | AY016351 | AY350576 | DQ677945 | – | 3 & 5 |
| *Davidiella tassiana* | CBS 399.80 | AFTOL 1591 | *Capnodiales*, *Davidiellaceae* | DQ678074 | DQ678022 | – | DQ677971 | – | 3 & 5 |
| *Cercospora beticola* | CBS 116456 | AFTOL 1788 | *Capnodiales*, *Mycosphaerellaceae* | DQ678091 | DQ678039 | FJ190647 | | | 3 |
| *Mycosphaerella fijiensis* | OSC 100622 | AFTOL 2021 | *Capnodiales*, *Mycosphaerellaceae* | DQ678098 | DQ767652 | FJ190656 | DQ677993 | – | 3 & 5 |
| *Mycosphaerella graminicola* | CBS 292.38 | AFTOL 1615 | *Capnodiales*, *Mycosphaerellaceae* | DQ678084 | DQ678033 | DQ677982 | DQ677982 | – | 3 & 5 |
| *Mycosphaerella punctiformis* | CBS 113265 | AFTOL 942 | *Capnodiales*, *Mycosphaerellaceae* | DQ470968 | DQ471017 | FJ190611 | DQ470920 | DQ471165 | 3 & 5 |

**Table 1.** (Continued).

| *Dothideomycetes* | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis |
|---|---|---|---|---|---|---|---|---|---|
| *Capnobotryella renispora* | CBS 214.90 | | *Capnodiales*, *Teratosphaeriaceae* | EU019248 | Y18698 | – | – | – | 3 & 5 |
| *Catenulostroma abietis* | CBS 459.93 | AFTOL 2210 | *Capnodiales*, *Teratosphaeriaceae* | DQ678092 | DQ678040 | FJ190648 | – | GU357797 | 3 & 5 |
| *Catenulostroma microsporum* | CBS 110890; CPC 1832 | | *Capnodiales*, *Teratosphaeriaceae* | EU019255 | **GU214520** | – | | | 3 |
| *Hortaea werneckii* | CBS 107.67 | mtSSU from CBS 708.76 | *Capnodiales*, *Teratosphaeriaceae* | EU019270 | Y18693 | **GU561844** | | | 3 |
| *Teratosphaeria associata* | CBS 112224 | ex *Teratosphaeria fibrillosa* | *Capnodiales*, *Teratosphaeriaceae* | **GU301874** | **GU296200** | – | – | **GU357744** | 3 & 5 |
| *Teratosphaeria destructans* | CBS 111370 | | *Capnodiales*, *Teratosphaeriaceae* | **GU214702** | **GU214702** | – | | | 3 |
| *Teratosphaeria juvenalis* | CBS 110906 | | *Capnodiales*, *Teratosphaeriaceae* | AY720715 | FJ493217 | – | | | 3 |
| *Capnodiales* sp. 1 | CBS 101364 | ex *Anisomeridium consobrinum* | *Capnodiales*, *incertae sedis* | **GU323215** | **GU561840** | – | **GU561853** | – | 3 & 5 |
| *Devriesia strelitziae* | CBS 122379 | | *Capnodiales*, *incertae sedis* | **GU296146** | **GU301810** | **GU561845** | **GU371738** | – | 3 & 5 |
| *Mycosphaerella eurypotami* | JK 5586J | | *Capnodiales*, *incertae sedis* | **GU301852** | **GU479761** | – | **GU371722** | **GU561851** | 3 & 5 |
| *Tripospermum myrti* | CBS 437.68 | | *Capnodiales*, *incertae sedis* | **GU323216** | – | **GU561846** | **GU561854** | **GU561852** | 3 & 5 |
| *Columnosphaeria fagi* 1 | CBS 171.93 | AFTOL 1582 | *Dothideales* | AY016359 | AY016342 | – | DQ677966 | – | 3 & 5 |
| *Columnosphaeria fagi* 2 | CBS 584.75 | AFTOL 912 | *Dothideales* | DQ470956 | DQ471004 | FJ713608 | DQ470906 | DQ471148 | 3 & 5 |
| *Delphinella strobiligena* | CBS 735.71 | AFTOL 1257 | *Dothideales* | DQ470977 | DQ471029 | – | DQ677951 | DQ471175 | 3 & 5 |
| *Dothidea insculpta* | CBS 189.58 | AFTOL 921 | *Dothideales* | DQ247802 | DQ247810 | FJ190602 | AF107800 | DQ471154 | 3 & 5 |
| *Dothiora cannabinae* | CBS 737.71 | AFTOL 1359 | *Dothideales* | DQ470984 | DQ479933 | FJ190636 | DQ470936 | DQ471182 | 3 & 5 |
| *Stylodothis puccinioides* | CBS 193.58 | AFTOL 902 | *Dothideales* | AY004342 | AY016353 | – | – | FJ238427 | 3 & 5 |
| *Sydowia polyspora* | CBS 116.29 | AFTOL 1300 | *Dothideales* | DQ678058 | DQ678005 | FJ190631 | DQ677953 | – | 3 & 5 |
| *Gloniopsis praelonga* | CBS 112415 | | *Hysteriales* | FJ161173 | FJ161134 | – | – | – | 3 & 5 |
| *Rhytidhysterium rufulum* | CBS 306.38 | | *Hysteriales* | FJ469672 | AF164375 | – | – | FJ238444 | 3 & 5 |
| *Elsinoë centrolobi* | CBS 222.50 | AFTOL 1854 | *Myriangiales* | DQ678094 | DQ678041 | FJ190651 | – | – | 3 & 5 |
| *Elsinoë phaseoli* | CBS 165.31 | AFTOL 1855 | *Myriangiales* | DQ678095 | DQ678042 | FJ190652 | – | – | 3 & 5 |
| *Myriangium duriaei* | CBS 260.36 | AFTOL 1304 | *Myriangiales* | DQ678059 | AY016347 | AY571389 | DQ677954 | – | 3 & 5 |
| *Phaeosclera dematoides* | CBS 157.81 | | *Myriangiales* | **GU301858** | **GU296184** | – | – | **GU357764** | 3 & 5 |
| *Lophium mytilinum* | CBS 269.34 | AFTOL 1609 | *Mytilinidiales* | DQ678081 | DQ678030 | **GU456342** | DQ677979 | – | 3 & 5 |
| *Mytilinidion resinicola* | CBS 304.34 | | *Mytilinidiales* | FJ161185 | FJ161145 | – | FJ161101 | – | 3 & 5 |
| *Hysteropatella clavispora* | CBS 247.34 | AFTOL1305 | *Patellariales* | AY541493 | DQ678006 | AY571388 | DQ677955 | – | 3 & 5 |
| *Hysteropatella elliptica* | CBS 935.97 | AFTOL 1790 | *Patellariales* | DQ767657 | EF495114 | FJ190649 | DQ767647 | – | 3 & 5 |
| *Patellaria atrata* | CBS 958.97 | | *Patellariales* | **GU301855** | **GU296181** | – | DQ767647 | **GU357749** | 3 & 5 |
| *Arthopyrenia salicis* | CBS 368.94 | mtSSU from GenBank | *Pleosporales* | AY538339 | AY538333 | AY538345 | – | FJ941893 | 3 & 5 |

**Table 1.** (Continued).

| *Dothideomycetes* | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis |
|---|---|---|---|---|---|---|---|---|---|
| *Bimuria novae–zelandiae* | CBS 107.79 | AFTOL 931 | *Pleosporales* | AY016356 | AY016338 | FJ190605 | DQ470917 | DQ471159 | 3 & 5 |
| *Dendryphiella arenaria* | CBS 181.58 | AFTOL 995 | *Pleosporales* | DQ470971 | DQ471022 | FJ190617 | DQ470924 | DQ842036 | 3 & 5 |
| *Leptosphaeria maculans* | DAOM 229267 | AFTOL 277 | *Pleosporales* | DQ470946 | DQ470993 | – | DQ470894 | DQ471136 | 3 & 5 |
| *Pleospora herbarum* | CBS 541.72 | AFTOL 940 | *Pleosporales* | DQ247804 | DQ247812 | FJ190610 | DQ247794 | DQ471163 | 3 & 5 |
| *Preussia terricola* | DAOM 230091 | AFTOL 282 | *Pleosporales* | AY544686 | AY544726 | AY544754 | DQ470895 | DQ471137 | 3 & 5 |
| *Sirodesmium olivaceum* | CBS 395.59 | | *Pleosporales* | **GU250894** | **GU250915** | **GU250904** | **GU250947** | **GU250958** | 3 & 5 |
| *Westerdykella cylindrica* | CBS 454.72 | AFTOL 1037 | *Pleosporales* | AY004343 | AY016355 | AF346430 | DQ470925 | DQ471168 | 3 & 5 |
| *Pleosporales* sp. 1 | CBS 101277 | ex *Thelenella luridella* | *Pleosporales* | – | **GU456309** | – | **GU456361** | – | 3 & 5 |
| *Pleosporales* sp. 2 | AFTOL 101 | ex *Anisomeridium polypori* | *Pleosporales* | – | DQ782877 | – | DQ782864 | DQ782822 | 3 & 5 |
| *Astrothelium cinnamomeum* | AFTOL 110 | ex *Trypethelium* sp. | *Trypetheliaceae* | AY584652 | AY584676 | AY584632 | AY584690 | DQ782824 | 3 & 5 |
| *Laurera megasperma* | AFTOL 2094 | | *Trypetheliaceae* | FJ267702 | **GU561841** | **GU561847** | **GU561855** | – | 3 & 5 |
| *Trypethelium nitidiusculum* | AFTOL 2099 | | *Trypetheliaceae* | FJ267701 | **GU561842** | **GU561848** | **GU561856** | – | 3 & 5 |
| *Helicomyces roseus* | CBS 283.51 | AFTOL 1613 | *Tubeufiaceae* | DQ678083 | DQ678032 | – | DQ677981 | – | 3 & 5 |
| *Tubeufia cerea* | CBS 254.75 | AFTOL 1316 | *Tubeufiaceae* | DQ470982 | DQ471034 | FJ190634 | DQ470934 | DQ471180 | 3 & 5 |
| *Tubeufia paludosa* | CBS 245.49 | AFTOL 1580 | *Tubeufiaceae* | DQ767654 | DQ767649 | – | DQ767643 | – | 3 & 5 |
| *Cystocoleus ebeneus* | L348 | *RPB*2 from L344; *RPB*1 from L343 | *Dothideomycetes, incertae sedis* | EU048580 | EU048573 | EU048586 | **GU214293** | **GU214204** | 3 & 5 |
| *Farlowiella carmichaelina* | CBS 206.36 | AFTOL 1787 | *Dothideomycetes, incertae sedis* | AY541492 | AY541482 | – | DQ677989 | – | 3 & 5 |
| *Kirschsteiniothelia aethiops* 1 | CBS 109.53 | AFTOL 925 | *Dothideomycetes, incertae sedis* | AY016361 | AY016344 | FJ190604 | DQ470914 | DQ471157 | 3 & 5 |
| *Kirschsteiniothelia aethiops 2* | DAOM 231155 | AFTOL 273 | *Dothideomycetes, incertae sedis* | DQ678046 | DQ677996 | FJ190590 | DQ677940 | – | 3 & 5 |
| *Phaeotrichum benjaminii* | CBS 541.72 | AFTOL 1184 | *Dothideomycetes, incertae sedis* | AY004340 | AY016348 | – | DQ677946 | – | 3 & 5 |
| *Racodium rupestre* | L424 | *RPB*1 from L341 | *Dothideomycetes, incertae sedis* | EU048582 | EU048577 | EU048589 | – | **GU214205** | 3 & 5 |
| *Sarcinomyces crustaceus* | CBS 156.89 | | *Dothideomycetes, incertae sedis* | **GU250893** | – | **GU250905** | **GU250948** | **GU250959** | 3 & 5 |
| *Tyrannosorus pinicola* | CBS 124.88 | AFTOL 1235 | *Dothideomycetes, incertae sedis* | DQ470974 | DQ471025 | FJ190620 | DQ470928 | DQ471171 | 3 & 5 |

| Rock–inhabiting fungi | | | | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis | Locality |
|---|---|---|---|---|---|---|---|---|---|---|
| *Coniosporium apollinis* | CBS 352.97 | ex-type strain | *Dothideomycetes, incertae sedis* | **GU250895** | **GU250916** | **GU250906** | **GU250949** | – | 3 & 5 | Greece |
| *Coniosporium apollinis* | CBS 100213 | | *Dothideomycetes, incertae sedis* | **GU250896** | **GU250917** | **GU250907** | **GU250950** | **GU250960** | 3 & 5 | Greece |
| *Coniosporium apollinis* | CBS 100214 | | *Dothideomycetes, incertae sedis* | **GU250897** | **GU250918** | **GU250908** | **GU250951** | **–** | 3 & 5 | Greece |
| *Coniosporium apollinis* | CBS 100218 | | *Dothideomycetes, incertae sedis* | **GU250898** | **GU250919** | **GU250909** | **GU250952** | **GU250961** | 3 & 5 | Greece |
| *Coniosporium apollinis* | CBS 109860 | | *Dothideomycetes, incertae sedis* | **GU250899** | **GU250920** | **GU250910** | **GU250953** | **GU250962** | 3 & 5 | Spain |

**Table 1.** (Continued).

| Rock–inhabiting fungi | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis | Locality |
|---|---|---|---|---|---|---|---|---|---|---|
| *Coniosporium apollinis* | CBS 109865 | | *Dothideomycetes*, *incertae sedis* | **GU250900** | **GU250921** | **GU250911** | **GU250954** | **GU250963** | 3 & 5 | Greece |
| *Coniosporium apollinis* | CBS 109867 | | *Dothideomycetes*, *incertae sedis* | **GU250901** | – | **GU250912** | **GU250955** | **GU250964** | 3 & 5 | Greece |
| *Coniosporium uncinatum* | CBS 100212 | | *Dothideomycetes*, *incertae sedis* | **GU250902** | **GU250922** | **GU250913** | **GU250956** | – | 3 & 5 | Italy |
| *Coniosporium uncinatum* | CBS 100219 | ex-type strain | *Dothideomycetes*, *incertae sedis* | **GU250903** | **GU250923** | **GU250914** | **GU250957** | **GU250965** | 3 & 5 | France, Paris |
| rock isolate TRN 5 | CBS 118762 | Ruibal *et al.* (2008) | *Capnodiales*, *Teratosphaeriaceae* | **GU323956** | **GU323988** | **GU324017** | – | **GU324051** | 3 & 5 | Central Spain |
| rock isolate TRN 11 | CBS 118281 | Ruibal *et al.* (2008) | *Dothideales* | **GU323957** | – | **GU324018** | – | **GU324052** | 3 & 5 | Central Spain |
| rock isolate TRN 42 | CBS 117958 | Ruibal *et al.* (2008) | *Capnodiales*, *Davidiellaceae* | **GU323958** | – | **GU324019** | – | **GU324053** | 3 & 5 | Central Spain |
| rock isolate TRN 43 | CBS 117950 | Ruibal *et al.* (2008) | *Capnodiales*, *Davidiellaceae* | **GU323959** | **GU323989** | **GU324020** | | | 3 | Central Spain |
| rock isolate TRN 44 | CBS 118324 | Ruibal *et al.* (2008) | *Capnodiales*, *Davidiellaceae* | **GU323960** | **GU323990** | **GU324021** | | | 3 | Central Spain |
| rock isolate TRN 49 | – | Ruibal *et al.* (2008) | *Pleosporales* | – | AY843233 | – | | | 3 | Central Spain |
| rock isolate TRN 62 | CBS 118305 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323961** | **GU323991** | **GU324022** | – | **GU324054** | 3 & 5 | Mallorca |
| rock isolate TRN 66 | CBS 118306 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323962** | **GU323992** | **GU324023** | – | **GU324055** | 3 & 5 | Mallorca |
| rock isolate TRN 77 | CBS 118287 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323963** | **GU323993** | **GU324024** | **GU324066** | **GU324057** | 3 & 5 | Mallorca |
| rock isolate TRN 79 | CBS 117930 | Ruibal *et al.* (2005) | *Capnodiales*, *Teratosphaeriaceae* | **GU323964** | **GU323994** | **GU324025** | | | 3 | Mallorca |
| rock isolate TRN 80 | CBS 118286 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323965** | **GU323995** | **GU324026** | – | **GU324056** | 3 & 5 | Mallorca |
| rock isolate TRN 87 | CBS 118290 | Ruibal *et al.* (2005) | *Capnodiales*, *Capnodiaceae* | **GU323966** | **GU323996** | **GU324027** | – | **GU324058** | 3 & 5 | Mallorca |
| rock isolate TRN 111 | CBS 118294 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323967** | **GU323997** | **GU324028** | – | **GU324059** | 3 & 5 | Mallorca |
| rock isolate TRN 119 | CBS 118250 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323968** | – | **GU324029** | | | 3 | Mallorca |
| rock isolate TRN 122 | CBS 117931 | Ruibal *et al.* (2005) | *Capnodiales*, *Teratosphaeriaceae* | **GU323969** | **GU323998** | **GU324030** | | | 3 | Mallorca |
| rock isolate TRN 123 | CBS 117932 | Ruibal *et al.* (2005) | *Capnodiales*, *Teratosphaeriaceae* | **GU323970** | **GU323999** | **GU324031** | **GU324067** | **GU324060** | 3 & 5 | Mallorca |
| rock isolate TRN 124 | CBS 118283 | Ruibal *et al.* (2005) | *Capnodiales*, *Teratosphaeriaceae* | **GU323971** | **GU324000** | **GU324032** | – | **GU324061** | 3 & 5 | Mallorca |
| rock isolate TRN 129 | CBS 117933 | Ruibal *et al.* (2005) | *Capnodiales*, *Teratosphaeriaceae* | **GU323972** | **GU324001** | **GU324033** | | | 3 | Mallorca |
| rock isolate TRN 137 | CBS 118300 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323973** | **GU324002** | **GU324034** | – | **GU324062** | 3 & 5 | Mallorca |
| rock isolate TRN 138 | CBS 118301 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323974** | **GU324003** | **GU324035** | **GU324068** | **GU324063** | 3 & 5 | Mallorca |
| rock isolate TRN 142 | CBS 118302 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323975** | **GU324004** | **GU324036** | **GU324069** | – | 3 & 5 | Mallorca |
| rock isolate TRN 152 | CBS 118346 | Ruibal *et al.* (2005) | *Capnodiales*, *incertae sedis* | **GU323976** | **GU324005** | **GU324037** | | | 3 | Mallorca |

**Table 1.** (Continued).

| Rock–inhabiting fungi | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis | Locality |
|---|---|---|---|---|---|---|---|---|---|---|
| rock isolate TRN 153 | CBS 118330 | Ruibal *et al.* (2005) | *Capnodiales, incertae sedis* | **GU323977** | **GU324006** | **GU324038** | **GU324070** | – | 3 & 5 | Mallorca |
| rock isolate TRN 211 | CBS 117937 | Ruibal *et al.* (2008) | *Capnodiales, Teratosphaeriaceae* | **GU323978** | **GU324007** | **GU324039** | | | 3 | Central Spain |
| rock isolate TRN 213 | – | Ruibal *et al.* (2008) | related to *Arthoniales* | – | **GU324008** | **GU324040** | | | 3 | Central Spain |
| rock isolate TRN 221 | – | Ruibal *et al.* (2008) | *Pleosporales* | – | AY843241 | – | | | 3 | Central Spain |
| rock isolate TRN 235 | CBS 118605 | Ruibal *et al.* (2008) | *Myriangiales* | **GU323979** | – | **GU324041** | **GU324071** | – | 3 & 5 | Central Spain |
| rock isolate TRN 245 | CBS 117940 | Ruibal *et al.* (2008) | *Capnodiales, Teratosphaeriaceae* | **GU323980** | **GU324009** | **GU324042** | | | 3 | Central Spain |
| rock isolate TRN 267 | CBS 118769 | Ruibal *et al.* (2008) | *Dothideomycetes, incertae sedis* | – | **GU324010** | **GU324043** | **GU324072** | – | 3 & 5 | Central Spain |
| rock isolate TRN 268 | CBS 119305 | Ruibal *et al.* (2008) | *Dothideales* | **GU323981** | – | **GU324044** | – | – | 3 & 5 | Central Spain |
| rock isolate TRN 279 | CBS 117943 | Ruibal *et al.* (2008) | *Capnodiales, Teratosphaeriaceae* | **GU323983** | **GU324012** | **GU324046** | | | 3 | Central Spain |
| rock isolate TRN 434 | – | Ruibal *et al.* (2008) | *Pleosporales* | – | AY843260 | – | | | 3 | Central Spain |
| rock isolate TRN 437 | CBS 118327 | Ruibal *et al.* (2008) | *Dothideomycetes, incertae sedis* | **GU323984** | **GU324013** | **GU324047** | | | 3 | Central Spain |
| rock isolate TRN 452 | – | Ruibal *et al.* (2008) | related to *Arthoniales* | **GU323985** | **GU324014** | **GU324048** | | | 3 | Central Spain |
| rock isolate TRN 456 | – | Ruibal *et al.* (2008) | related to *Arthoniales* | **GU323986** | **GU324015** | **GU324049** | – | **GU324065** | 3 & 5 | Central Spain |
| rock isolate TRN 499 | – | Ruibal *et al.* (2008) | *Pleosporales* | – | AY843278 | – | | | 3 | Central Spain |
| rock isolate TRN 529 | – | Ruibal *et al.* (2008) | related to *Arthoniales* | **GU323987** | **GU324016** | **GU324050** | – | – | 3 & 5 | Central Spain |
| rock isolate A6 | – | Gorbushina (unpublished) | *Dothideomycetes, incertae sedis* | **GU250924** | **GU250932** | – | **GU250939** | – | 3 & 5 | Turkey |
| rock isolate A35 | CBS 123158 | Gorbushina (unpublished) | *Coniosporium uncinatum* | **GU250925** | **GU250933** | – | – | **GU250943** | 3 & 5 | Crimea |
| rock isolate A73 | – | Gorbushina (unpublished) | *Capnodiales, incertae sedis* | **GU250926** | **GU250934** | – | **GU250940** | **GU250944** | 3 & 5 | Greece |
| rock isolate AN1 | – | Gorbushina (unpublished) | *Capnodiales, Davidiellaceae* | **GU250927** | **GU250935** | – | **GU250941** | – | 3 & 5 | Israel, Negev |
| rock isolate AN13 | CBS 125207 | Gorbushina (unpublished) | *Dothideomycetes, incertae sedis* | **GU250928** | **GU250936** | – | **GU250942** | **GU250945** | 3 & 5 | Israel, Negev |
| rock isolate S2 | – | Gorbushina (unpublished) | *Capnodiales, incertae sedis* | **GU250931** | – | – | – | **GU250946** | 3 & 5 | Slovenia |
| rock isolate DVA4 | – | Staley *et al.* (1982) | *Dothideomycetes, incertae sedis* | **GU250929** | **GU250937** | – | | | 3 | U.S.A., Arizona |
| rock isolate DVA7 | – | Staley *et al.* (1982) | *Dothideomycetes, incertae sedis* | **GU250930** | **GU250938** | – | | | 3 | U.S.A., Arizona |
| rock isolate CCFEE 451 | – | Selbmann *et al.* (2005, 2008) | *Capnodiales, incertae sedis* | **GU250360** | **GU250314** | **GU250403** | | | 3 | Antarctica |
| rock isolate CCFEE 453 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | **GU250361** | **GU250315** | **GU250404** | | | 3 | Antarctica |
| rock isolate CCFEE 456 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | – | **GU250316** | **GU250405** | | | 3 | Antarctica |

**Table 1.** (Continued).

| Rock–inhabiting fungi | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis | Locality |
|---|---|---|---|---|---|---|---|---|---|---|
| rock isolate CCFEE 502 | – | Selbmann *et al.* (2005, 2008) | *Capnodiales*, *Teratosphaeriaceae* | **GU250363** | **GU250318** | **GU250406** | | | 3 | Antarctica |
| rock isolate CCFEE 514 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | – | **GU250319** | **GU250407** | | | 3 | Antarctica |
| rock isolate CCFEE 515 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | – | **GU250320** | **GU250408** | | | 3 | Antarctica |
| rock isolate CCFEE 524 | – | Selbmann *et al.* (2005, 2008) | *Friedmanniomyces endolithicus* | **GU250364** | DQ066715 | **GU250409** | – | – | 3 & 5 | Antarctica |
| rock isolate CCFEE 534 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | – | DQ066713 | **GU250410** | | | 3 | Antarctica |
| rock isolate CCFEE 536 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | **GU250365** | **GU250321** | **GU250411** | – | – | 3 & 5 | Antarctica |
| rock isolate CCFEE 670 | – | Selbmann *et al.* (2005, 2008) | *Friedmanniomyces endolithicus* | **GU250366** | **GU250322** | **GU250412** | | | 3 | Antarctica |
| rock isolate CCFEE 690 | – | Selbmann *et al.* (2005, 2008) | *Cryomyces antarcticus* | – | **GU250323** | **GU250413** | | | 3 | Antarctica |
| rock isolate CCFEE 5018 | – | Selbmann *et al.* (2005, 2008) | *Capnodiales*, *Davidiellaceae* | – | **GU250324** | **GU250414** | | | 3 | Antarctica |
| rock isolate CCFEE 5176 | – | Selbmann *et al.* (2005, 2008) | related to *Arthoniales* | – | **GU250325** | – | | | 3 | Antarctica |
| rock isolate CCFEE 5180 | – | Selbmann *et al.* (2005, 2008) | *Friedmanniomyces endolithicus* | **GU250367** | **GU250326** | **GU250415** | | | 3 | Antarctica |
| rock isolate CCFEE 5184 | – | Selbmann *et al.* (2005, 2008) | *Friedmanniomyces simplex* | **GU250368** | DQ066716 | **GU250416** | | | 3 | Antarctica |
| rock isolate CCFEE 5187 | CBS 116302 | Selbmann *et al.* (2005, 2008) | *Cryomyces minteri* | **GU250369** | DQ066714 | **GU250417** | – | – | 3 & 5 | Antarctica |
| rock isolate CCFEE 5205 | – | Selbmann *et al.* (2005, 2008) | *Capnodiales*, *incertae sedis* | **GU250370** | **GU250327** | **GU250418** | | | 3 | Antarctica |
| rock isolate CCFEE 5211 | – | Selbmann *et al.* (2005, 2008) | *Capnodiales*, *Davidiellaceae* | **GU250371** | **GU250328** | **GU250419** | – | – | 3 & 5 | Antarctica |
| rock isolate CCFEE 5264 | – | Selbmann *et al.* (2008) | *Recurvomyces mirabilis* | **GU250372** | **GU250329** | – | | | 3 | Antarctica |
| rock isolate CCFEE 5284 | – | Selbmann (unpublished) | related to Arthoniales | **GU250373** | **GU250330** | – | | | 3 | Antarctica |
| rock isolate CCFEE 5299 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | **GU250374** | – | – | | | 3 | Antarctic Peninsula |
| rock isolate CCFEE 5303 | – | Selbmann (unpublished) | related to *Arthoniales* | – | **GU250331** | – | | | 3 | Antarctica |
| rock isolate CCFEE 5319 | – | Selbmann *et al.* (2008) | *Elasticomyces elasticus* | **GU250375** | **GU250332** | – | | | 3 | Antarctica on lichens |
| rock isolate CCFEE 5320 | CBS 122540 | Selbmann *et al.* (2008) | *Elasticomyces elasticus* | **GU250376** | **GU250333** | **GU250420** | – | – | 3 & 5 | Antarctica on lichens |
| rock isolate CCFEE 5322 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250377** | **GU250334** | – | | | 3 | Antarctica on lichens |
| rock isolate CCFEE 5388 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | **GU250380** | **GU250337** | **GU250422** | | | 3 | Alps |
| rock isolate CCFEE 5389 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250381** | **GU250338** | **GU250423** | | | 3 | Alps |
| rock isolate CCFEE 5398 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | **GU250382** | **GU250339** | – | | | 3 | Alps |
| rock isolate CCFEE 5401 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250383** | **GU250340** | **GU250424** | | | 3 | Alps |

**Table 1.** (Continued).

| Rock–inhabiting fungi | Collection # | Additional Information | Order | LSU | SSU | mtSSU | RPB2 | RPB1 | Analysis | Locality |
|---|---|---|---|---|---|---|---|---|---|---|
| rock isolate CCFEE 5410 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250384** | **GU250341** | **GU250425** | | | 3 | Andes |
| rock isolate CCFEE 5413 | – | Selbmann (unpublished) | *Dothideomycetes*, *incertae sedis* | **GU250385** | **GU250342** | **GU250426** | | | 3 | Alps |
| rock isolate CCFEE 5414 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | **GU250386** | **GU250343** | – | | | 3 | Alps |
| rock isolate CCFEE 5416 | – | Selbmann (unpublished) | *Dothideomycetes*, *incertae sedis* | **GU250387** | **GU250344** | **GU250427** | | | 3 | Alps |
| rock isolate CCFEE 5456 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | **GU250388** | **GU250345** | **GU250428** | | | 3 | Alps |
| rock isolate CCFEE 5457 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250389** | **GU250346** | **GU250429** | | | 3 | Alps |
| rock isolate CCFEE 5458 | – | Selbmann (unpublished) | *Capnodiales*, *Davidiellaceae* | – | **GU250347** | **GU250430** | | | 3 | Alps |
| rock isolate CCFEE 5459 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250390** | **GU250348** | **GU250431** | | | 3 | Alps |
| rock isolate CCFEE 5460 | – | Selbmann (unpublished) | *Dothideomycetes*, *incertae sedis* | **GU250391** | **GU250349** | **GU250432** | | | 3 | Alps |
| rock isolate CCFEE 5466 | – | Selbmann (unpublished) | *Dothideomycetes*, *incertae sedis* | **GU250392** | **GU250350** | **GU250433** | | | 3 | Alps |
| rock isolate CCFEE 5467 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250393** | **GU250351** | – | | | 3 | Alps |
| rock isolate CCFEE 5476 | – | Selbmann (unpublished) | close to *Cryomyces* | **GU250394** | **GU250352** | **GU250434** | | | 3 | Alps |
| rock isolate CCFEE 5489 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250395** | – | **GU250435** | | | 3 | Antarctica |
| rock isolate CCFEE 5490 | – | Selbmann (unpublished) | *Elasticomyces elasticus* | – | **GU250353** | – | | | 3 | Antarctica |
| rock isolate CCFEE 5499 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250398** | **GU250355** | **GU250436** | | | 3 | Alps |
| rock isolate CCFEE 5501 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250399** | **GU250356** | **GU250437** | | | 3 | Aconcagua, Andes |
| rock isolate CCFEE 5502 | – | Selbmann (unpublished) | *Capnodiales*, *incertae sedis* | **GU250400** | **GU250357** | **GU250438** | | | 3 | Aconcagua, Andes |
| rock isolate CCFEE 5508 | – | Selbmann (unpublished) | *Capnodiales*, *Teratosphaeriaceae* | **GU250401** | **GU250358** | – | | | 3 | Aconcagua, Andes |
| rock isolate D007 09 | – | Selbmann (unpublished) | related to *Arthoniales* | **GU250402** | **GU250359** | – | | | 3 | Antarctica |

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# Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta

M.P. Nelsen[1, 2], R. Lücking[2], M. Grube[3], J.S. Mbatchou[2, 4], L. Muggia[3], E. Rivas Plata[2, 5] and H.T. Lumbsch[2]

[1]*Committee on Evolutionary Biology, University of Chicago, 1025 E. 57th Street, Chicago, Illinois 60637, U.S.A.;* [2]*Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.;* [3]*Institute of Botany, Karl-Franzens-University of Graz, A-8010 Graz, Austria;* [4]*Department of Biological Sciences, DePaul University, 1 E. Jackson Street, Chicago, Illinois 60604, U.S.A.;* [5]*Department of Biological Sciences, University of Illinois-Chicago, 845 West Taylor Street (MC 066), Chicago, Illinois 60607, U.S.A.*

**Correspondence*: Matthew P. Nelsen, mpnelsen@gmail.com

**Abstract:** We present a revised phylogeny of lichenised Dothideomyceta (*Arthoniomycetes* and *Dothideomycetes*) based on a combined data set of nuclear large subunit (nuLSU) and mitochondrial small subunit (mtSSU) rDNA data. Dothideomyceta is supported as monophyletic with monophyletic classes *Arthoniomycetes* and *Dothideomycetes*; the latter, however, lacking support in this study. The phylogeny of lichenised *Arthoniomycetes* supports the current division into three families: *Chrysothrichaceae* (*Chrysothrix*), *Arthoniaceae* (*Arthonia s. l.*, *Cryptothecia*, *Herpothallon*), and *Roccellaceae* (*Chiodecton*, *Combea*, *Dendrographa*, *Dichosporidium*, *Enterographa*, *Erythrodecton*, *Lecanactis*, *Opegrapha*, *Roccella*, *Roccellographa*, *Schismatomma*, *Simonyella*). The widespread and common *Arthonia caesia* is strongly supported as a (non-pigmented) member of *Chrysothrix*. *Monoblastiaceae*, *Strigulaceae*, and *Trypetheliaceae* are recovered as unrelated, monophyletic clades within *Dothideomycetes*. Also, the genera *Arthopyrenia* (*Arthopyreniaceae*) and *Cystocoleus* and *Racodium* (*Capnodiales*) are confirmed as *Dothideomycetes* but unrelated to each other. *Mycomicrothelia* is shown to be unrelated to *Arthopyrenia s.str.*, but is supported as a monophyletic clade sister to *Trypetheliaceae*, which is supported by hamathecium characters. The generic concept in several groups is in need of revision, as indicated by non-monophyly of genera, such as *Arthonia, Astrothelium, Cryptothecia, Cryptothelium, Enterographa, Opegrapha,* and *Trypethelium* in our analyses.

**Key words:** *Arthoniomycetes*, Ascolocularous fungi, bitunicate fungi, *Dothideomycetes*, lichens, phylogeny, ribosomal DNA.

## INTRODUCTION

Mutualism is one of the three main modes of nutrition within *Ascomycota*, besides saprotrophism and parasitism. A large number of mutualistic ascomycetes form symbiotic relationships with algae and/or cyanobacteria, so-called lichens. Of the 64 000 species currently accepted in *Ascomycota* (Kirk *et al.* 2008), about almost 30 % (17 600) are lichen-forming fungi (Feuerer & Hawksworth 2007, Kirk *et al.* 2008). Lichenised fungi differ from all other fungi in the formation of complex, persistent vegetative thalli, which makes them a prime subject for evolutionary studies.

It was long believed that lichens evolved several times independently within *Ascomycota* (and *Basidiomycota*), an idea supported by the first molecular study testing this hypothesis (Gargas *et al.* 1995). Lutzoni *et al*. (2001, 2004) were unable to conclusively determine whether there were multiple gains of lichenisation or whether an initial lichenisation event occurred deep within *Ascomycota*, however, Lutzoni *et al*. (2001) found some *Eurotiomycetes* to be secondarily de-lichenised. This is particularly intriguing as *Eurotiomycetes* includes economically important fungi in the genera *Aspergillus* and *Penicillium* that feature a complex secondary chemistry similar to that found in lichens produced by homologous polyketide synthase genes (Grube & Blaha 2003, Kroken *et al.* 2003, Schmitt *et al.* 2005, Schmitt & Lumbsch 2009).

Since then, the phylogeny and classification of *Ascomycota* has further advanced (Lindemuth *et al*. 2001, Lumbsch *et al*. 2001, 2002a, b, 2004, Grube *et al*. 2004, Lücking *et al*. 2004, Lutzoni *et al*. 2004, Persoh *et al*. 2004, Wedin *et al*. 2005, del Prado *et al*. 2006, Miadlikoswka *et al*. 2006, Schmitt *et al*. 2006, Spatafora *et al*. 2006, Hibbett *et al*. 2007, Hofstetter *et al*. 2007, Lumbsch & Huhndorf 2007a, Schoch *et al*. 2006, 2009a–c). Our current understanding suggests that there were several lichenisation events but also some major delichenisation events during the evolution of *Ascomycota* (Gargas *et al*. 1995, Lutzoni *et al*. 2001, Liu & Hall 2004, Gueidan *et al*. 2008, Schoch *et al*. 2009a). The largest clade of lichenised fungi, *Lecanoromycetes*, with 14 000 accepted species, appears to be the result of a single lichenisation event with at least one major delichenisation event in *Ostropales* and several delichenisation events throughout the class (Lumbsch *et al.* 2004, Persoh *et al.* 2004, Wedin *et al.* 2005, Miadlikoswka *et al.* 2006, Hofstetter *et al.* 2007, Schoch *et al.* 2009a, Baloch *et al*. in prep.). A similar pattern is suggested within the second largest lichenised clade, *Arthoniomycetes*, with about 1 500 species (Tehler 1995, Myllys *et al.* 1998, Sundin 2000, Tehler & Irestedt 2007, Ertz *et al*. 2008). This class was recently shown to include the mazaediate genus *Tylophoron* (Lumbsch *et al*. 2009a), previously considered to be related to pyrenocarpous lichens (Aptroot *et al*. 2008). *Arthoniomycetes* is composed primarily of lichenised fungi producing apothecia or apothecioid ascomata with partially ascolocular development and bitunicate asci (Henssen & Jahns 1974, Eriksson & Winka 1997). The base of this clade was reconstructed as lichenised (Schoch *et al*. 2009a) and it is presumed that non-lichenised and lichenicolous species within the class represent reversions to the unlichenised state. One family that has not yet been confirmed within *Arthoniomycetes* using molecular data is *Chrysothrichaceae*, a small family of two genera (*Byssocaulon*, *Chrysothrix*) and little over 20 species (Kirk *et al*. 2008). The third primarily lichenised class is *Lichinomycetes* (350 species).

The remaining lichenised fungi are primarily restricted to *Dothideomycetes* and *Eurotiomycetes* (subclass *Chaetothyriomycetidae*). Gueidan *et al*. (2008) demonstrated that lichenisation may have evolved at least twice within *Eurotiomycetes* (once at base of *Verrucariales* and once at base of *Pyrenulales*), though, this is uncertain as the ancestral state of the common ancestor to *Pyrenulales*, *Verrucariales* and *Chaetothyriales*, is not unambiguously resolved (Gueidan *et al*. 2008, Schoch *et al*. 2009a). Within both *Verrucariales* and *Pyrenulales*, there appears to be at least one loss of lichenisation each. *Dothideomycetes* and *Arthoniomycetes* together form the rankless clade Dothideomyceta, a name introduced by Schoch *et al*. (2009a, b). The ancestral state of Dothideomyceta and *Dothideomycetes* nodes are not resolved with confidence (Gueidan *et al*. 2008, Schoch *et al*. 2009a, b). In this paper we do not aim to resolve this issue but rather attempt to clarify, confirm or reject the placement of lichenised lineages within Dothideomyceta, specifically *Dothideomycetes*.

The following families have been confirmed or are believed to belong in either *Chaeothyriomycetidae* or *Dothideomycetes*: *Verrucariaceae* (930 species), *Pyrenulaceae* (280 species), *Celotheliaceae* (eight species), *Microtheliopsidaceae* (three species), and *Pyrenothrichaceae* (three species) in *Chaetothyriomycetidae* (Herrera-Campos *et al.* 2005, del Prado *et al.* 2006, Lücking 2008), and *Trypetheliaceae* (200 species), *Monoblastiaceae* (130 species), *Strigulaceae* (120 species), and *Arthopyreniaceae* (120 species) in *Dothideomycetes* (Lutzoni *et al.* 2004, del Prado *et al.* 2006, Lumbsch & Huhndorf 2007b). Most of these families have traditionally been placed within *Pyrenulales* (Poelt 1973, Henssen & Jahns 1974, Hafellner 1986, Kirk *et al*. 2001, Eriksson *et al*. 2004, Cannon & Kirk 2007), and much of the confusion regarding previous classifications of these pyrenocarpous lichens stems from the fact that *Pyrenulales* were at some point considered synonymous with the ascolocular *Melanommatales* (currently regarded synonymous with *Pleosporales*; Barr 1980, Harris 1984, 1990, 1991, 1995), whereas other workers considered *Pyrenulales* to be ascohymenial (Henssen & Jahns 1974). The fact that *Trypetheliaceae* have no close relative within *Dothideomycetes* was reflected in the establishment of a separate order, *Trypetheliales* (Aptroot *et al*. 2008).

In addition to the aforementioned families, there are several genera of uncertain position, such as *Cystocoleus* and *Racodium*, both of which belong in *Capnodiales/Dothideomycetes* (Muggia *et al.* 2007), as well as *Julella*, *Mycoporum*, *Collemopsidium* (*Pyrenocollema*), and others, of unconfirmed affinities (Harris 1995). Yet other lineages, such as the recently discovered *Eremithallus* (Lücking *et al.* 2008) or the genera *Thelocarpon* and *Vezdaea* (Reeb *et al.* 2004, Lumbsch *et al.* 2009b) appear to fall outside the currently accepted classes known to contain lichen-forming fungi. The current phylogeny of *Chaetothyriomycetidae* suggests that the two large lichen-forming families in this subclass may have emerged from distinct lichenisation events, however, this could not be resolved with confidence (see node 18 in fig. 1 and table 1 of Gueidan *et al*. 2008, Schoch *et al*. 2009a). It thus appears that *Dothideomycetes*, the largest class of *Ascomycota* with an estimated number of 19 000 species (Kirk *et al.* 2008), a class that has largely been neglected when assessing the phylogeny of lichenised fungi, might be the only class within *Ascomycota* containing several lineages that evolved through independent lichenisation. In addition to *Trypetheliaceae*, at least two other families, which exhibit substantial radiation accompanied with morphological variation at the generic and species level (*Monoblastiaceae* and *Strigulaceae*) have been suggested to belong to *Dothideomycetes*. The only sequenced species of *Strigula* has been suggested to belong to *Eurotiomycetes* (Schmitt *et al*. 2005); however, re-examination of the specimen used in this study showed that it belonged in *Verrucariaceae*. Therefore the phylogenetic position of *Strigulaceae* remains unresolved. In addition, *Anisomeridium polypori* (*Monoblastiaceae*) was suggested to belong to *Dothideomycetes* (James *et al*. 2006).

In this paper, we are using nuclear large subunit (nuLSU) and mitochondrial small subunit (mtSSU) rDNA data, to construct a phylogeny of lichenised fungi with bitunicate asci, focusing on Dothideomyceta. We also present novel data that require adjustments in the systematic classification of taxa within both classes. A further objective was to begin to examine generic concepts within the family *Trypetheliaceae*, which is comprised of 11 genera (Lumbsch & Huhndorf 2007b) and approximately 200 species (Harris 1984, Aptroot 1991b, del Prado *et al*. 2006).

## MATERIAL AND METHODS

### Taxon sampling

Representatives of lichenised Dothideomyceta taxa were obtained through recent field work in the U.S.A., Central and South America, Europe, India, Thailand, and Fiji. Newly generated sequences were supplemented with other lichenised and non-lichenised Dothideomyceta from GenBank plus additional taxa in *Pezizomycetes*, *Leotiomycetes*, *Sordariomycetes, Eurotiomycetes*, and *Lecanoromycetes*, chiefly from a previous alignment published by Schoch *et al*. (2009a). In total, we analysed 162 operational taxonomic units (OTUs) representing 152 species and 111 genera. All OTUs included in the analyses, along with GenBank accession numbers and collection information for newly sequenced samples, are listed in Table 1 - see online Supplementary Information.

### Molecular methods

The Sigma REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) was used to isolate DNA, following the manufacturer's instructions, except only 10 µL of extraction buffer and 10 µL dilution buffer were used, following Avis *et al*. (2003). Dilutions of these extractions (rather than the stock DNA solution) were found to work best for PCR (C. Andrew, pers. comm. 2009), and a 20× DNA dilution was then used in subsequent PCR reactions.

Samples were PCR amplified and/or sequenced using the mrSSU1, mrSSU2, mrSSU2r and mrSSU3r primers (Zoller *et al*. 1999) for the mitochondrial small subunit (mtSSU) and the AL2R (Mangold *et al*. 2008), LR3R, LR3, LR5, LR6, LR7 (Vilgalys & Hester 1990) primers for the nuclear ribosomal large subunit rDNA (nuLSU). The 10 µL PCR reactions consisted of 5 µM of each PCR primer, 3 mM of each dNTP, 2 µL of 10 mg/mL 100x BSA (New England BioLabs, Ipswich, Massachusetts, U.S.A.), 1.5 µL 10× PCR buffer (Roche Applied Science, Indianapolis, Indiana, U.S.A.), 0.5 µL *Taq*, approximately 2 µL diluted DNA, and 2 µL water. The PCR cycling conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, a locus-specific annealing temperature for 1 min, and 72 °C for 1 min, followed by a single 72 °C final extension for 7 min. An annealing temperature of 53 °C was used for mtSSU, while 57 °C was used for nrLSU.

Samples were visualised on a 1 % ethidium bromide-stained agarose gel under UV light and bands were gel extracted, heated at 70 °C for 5 min, cooled to 45 °C for 10 min, treated with 1 µL

GELase (Epicentre Biotechnologies, Madison, WI, U.S.A.) and incubated at 45 °C for at least 24 h. The 10 µL cycle sequencing reactions consisted of 1–1.5 µL of Big Dye v. 3.1 (Perkin-Elmer Applied Biosystems, Foster City, California, U.S.A.), 2.5–3 µL of Big Dye buffer, 6 µM primer, 0.75–2 µL Gelased PCR product and water. The cycle sequencing conditions were as follows: 96 °C for 1 min, followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Samples were precipitated and sequenced in an Applied Biosystems 3730 DNA Analyser (Foster City, California, U.S.A.), and sequences assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

## Phylogenetic analysis

The alignment of Schoch *et al*. (2009a) was used as a starting point, from which a large number of sequences were removed. Newly generated sequences were added and manually aligned (nuLSU), or were separately aligned, added to the Schoch *et al*. (2009a) alignment, and manually adjusted (mtSSU). In addition to a representative set of dothideomycetous fungi, members of several *Ascomycota* classes were retained and *Pezizomycetes* taxa were used as the outgroup. The entire set of sequences generated in the present study plus those from GenBank were aligned in Se-Al v. 2.0a11 (Rambaut 1996) and BioEdit 7.0.9 (Hall 1999). An iterative procedure was used for the nuLSU in which ambiguous regions were aligned with Muscle 3.6 (Edgar 2004) through Mesquite 2.71 (Maddison & Maddison 2009); the alignment was again manually refined and other portions realigned with Muscle. After a final manual refinement, ambiguous regions and introns were removed and the alignment was deposited in TreeBase.

Alignments for each gene were concatenated in Mesquite 2.71 (Maddison & Maddison 2009) and analysed under the maximum likelihood (ML) optimality criterion in RAxML 7.0.4 (Stamatakis 2006). The data set was partitioned by locus and the GTRMIXI model with twenty-five rate parameter categories (default) was used for each partition. In addition, support was estimated by performing 1000 bootstrap replicates, and clades with bootstrap support of 70 % or greater were considered strongly supported. Additionally, the data sets were analyzed in GARLI 0.96 (Zwickl 2006) using the GTR-gamma-invariant model which is similar to the model used in RAxML.

## RESULTS

The final alignment consisted of 1 915 unambiguously aligned characters (1 199: nuLSU; 716: mtSSU). Both ML analyses recovered the major class-level ingroup nodes (Fig. 1) corresponding to other recent studies (*Leotiomycetes, Sordariomycetes, Eurotiomycetes, Lecanoromycetes, Arthoniomycetes, Dothideomycetes*). *Arthoniomycetes* and *Dothideomycetes* form a strongly supported sister-group relationship, corresponding to Dothideomyceta. Individual gene phylogenies suggested some incongruence between loci (unpubl. data), however, the topology in the combined analysis is in agreement with previously reported phylogenies and we did not exclude taxa.

The phylogeny of *Arthoniomycetes* (*Arthoniales*) largely confirmed previous analyses, with *Chrysothrichaceae* forming an additional family within this clade (Fig. 1). *Arthoniaceae s. l*. and *Roccellaceae s. l*. are both monophyletic and well separated. However, several smaller lineages that eventually could be reinstated at the family level show strong support: *Arthoniaceae s. str*., *Cryptothecia*ceae (*Cryptothecia-Herpothallon*), the *Tylophoron* clade, *Roccellaceae s. str*., *Opegraphaceae s. str*., and possibly *Chiodectonaceae* (as *Chiodecton sphaerale* is closely related to *Erythrodecton* and *Dichosporidium* whereas the sequenced *C. natalense* is apparently not a *Chiodecton s. str*.). Surprisingly, *Arthonia caesia* clustered with *Chrysothrichaceae* and not *Arthoniaceae*. *Herpothallon rubrocinctum* is nested within *Cryptothecia s. l*.

Six distinct, lichenised lineages were confirmed as belonging to *Dothideomycetes* (Fig. 1): the order *Trypetheliales*, the families *Arthopyreniaceae, Monoblastiaceae,* and *Strigulaceae*, and the genera *Cystocoleus* and *Racodium*. The latter two (*Cystocoleus and Racodium*) are members of the order *Capnodiales*, whereas *Arthopyreniaceae*, represented by the species *Arthopyrenia salicis*, was confirmed as clustering within *Pleosporales*. However, *Arthopyreniaceae* as currently defined, including the genera *Julella* (not sequenced) and *Mycomicrothelia*, is not monophyletic, as the sequenced species of *Mycomicrothelia* appeared outside *Pleosporales* and form a sister-group to *Trypetheliaceae*.

*Strigulaceae* is represented by five samples of the three genera *Flavobathelium, Phyllobathelium,* and *Strigula,* which formed a supported monophyletic clade sister to *Kirschsteiniothelia aethiops*, but without support. *Monoblastiaceae* was strongly supported and included four genera with one species each in this analysis: *Acrocordia subglobosa*, *Anisomeridium ubianum*, *Megalotremis verrucosa*, and *Trypetheliopsis* (syn. *Musaespora*) *kalbii*. Initially we also included a GenBank sequence of *Anisomeridium polypori* in the data set, but the nuLSU sequence was recovered in *Eurotiomycetes* and the taxon was excluded from the final analysis. It is possible that this sequence is derived from a contaminant or that it was confused with a similar species in an unrelated lineage.

*Trypetheliaceae* was strongly supported as monophyletic, being sister to the genus *Mycomicrothelia*. There was no support for the traditional separation into the perithecial and ascospore core genera *Astrothelium*, *Laurera*, and *Trypethelium*, as species of these genera were found scattered over the *Trypetheliaceae* clade.

## DISCUSSION

This is the first molecular phylogenetic study that includes presumably all major lichenised lineages within Dothideomyceta. This rankless taxon was informally introduced by Schoch *et al*. (2009a, b) for the clade including *Arthoniomycetes* and *Dothideomycetes*. The sister group of Dothideomyceta is not yet resolved but Ruibal *et al*. (2009; this volume) demonstrated an unnamed lineage of melanised rock-inhabiting fungi to be basal to *Arthoniomycetes* (not included in our sampling).

*Arthoniomycetes* is the second largest class of primarily lichenised *Ascomycota* and exhibits considerable morpho-anatomical variation (Fig. 2). The molecular phylogeny presented here confirms the current classification of lichenised *Arthoniomycetes* in three families: *Arthoniaceae, Chrysothrichaceae*, and *Roccellaceae* (Tehler 1995, Grube 1998, Tehler & Irestedt 2007). The morphological concept used to classify the single order included few large genera, with *Arthonia* and *Opegrapha* having the highest number of species (500 and 300, respectively). The infrageneric relationships of these species were repeatedly discussed and there was common agreement that these genera were not monophyletic and include morphologically distinct groups. Similarly the relationships of other genera with fewer species or of monospecific genera in the family *Roccellaceae* was

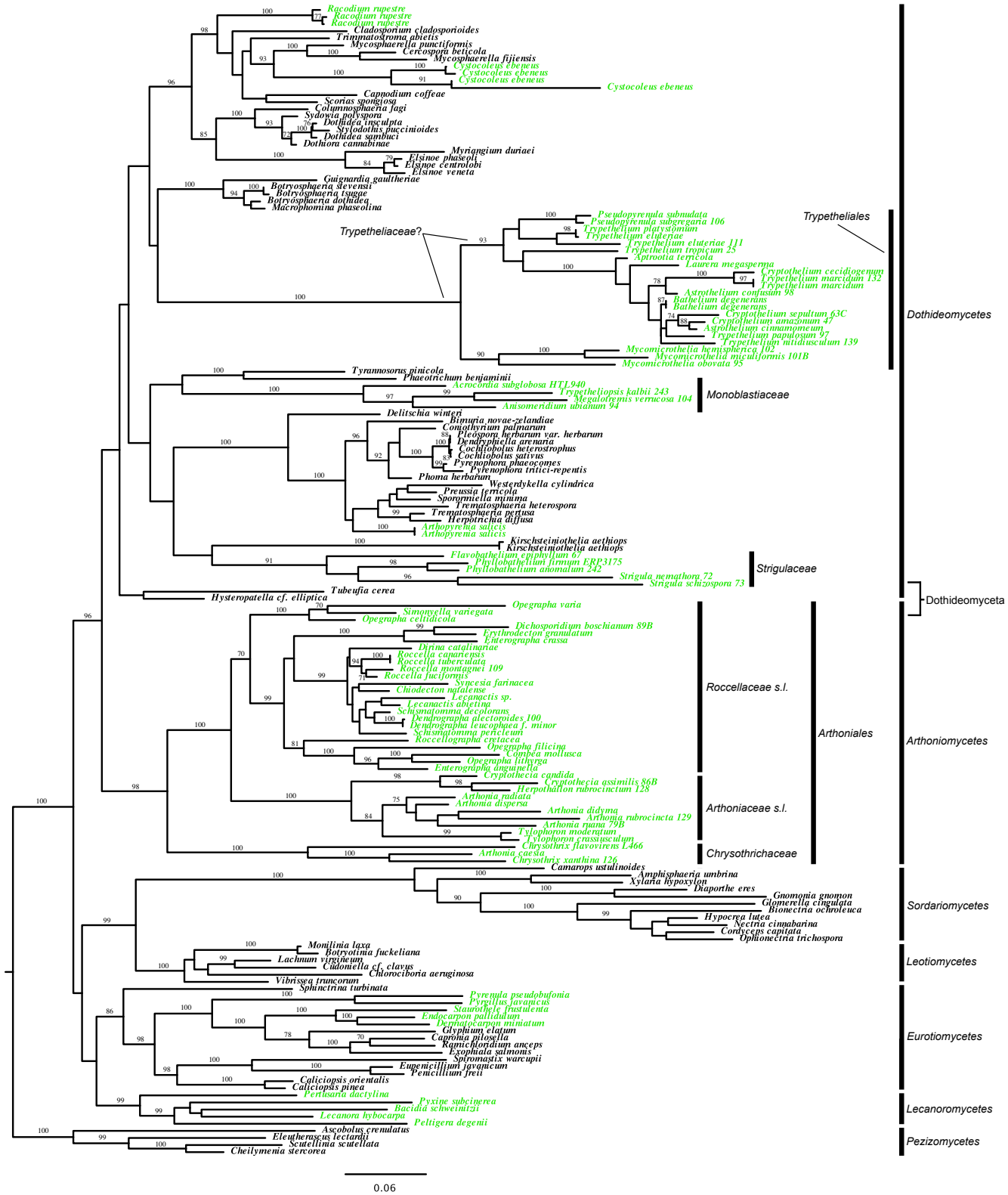


**Fig.1.** The ML tree from RAxML maximum likelihood analysis with bootstrap percentages equal to or greater than 70 are plotted above or below branches. Lichenised taxa are in green, while non-lichenised taxa are in black.

unclear. Along with previous data (Tehler 1995, Myllys *et al*. 1998, Tehler & Irestedt 2007) and recent results by Ertz *et al*. (2009), the present tree is a further step to resolve these questions based on molecular data.

Little can be said regarding generic concepts of most genera, as the taxon sampling is still far too incomplete for this group, but it appears that some of the traditional concepts based on fruit body structure are not supported, which suggests some degree of parallel evolution. An example is the *Chiodecton-Enterographa* complex: while the sequenced *Chiodecton natalense* appears to be unrelated to the morphologically and anatomically similar *Dichosporidium* and *Erythrodecton* (Thor 1990), *Enterographa* and the similar *Schismatomma* (Sparrius 2004) were found in three different clades related to either *Chiodecton natalense* (*Schismatomma*), *Dichosporidium* (*Enterographa crassa*), and *Opegrapha* (*Enterographa anguinella*), respectively. This is in agreement with


**Fig. 2.** Select lichenised *Arthoniomycetes*. A. *Chrysothrix xanthina*; B. *C. septemseptata*; C. *Arthonia caesia*; D. *A. cyanea*; E. *A. pulcherrima*; F. *A. rubrocincta*; G. *Cryptothecia candida*; H. *Herpothallon rubrocinctum*; I. *Tylophoron crassiusculum* (teleomorph); J. *T. crassiusculum* (anamorph); K. *Opegrapha filicina*; L. *O. astraea*; M. *Enterographa anguinella*; N. *Syncesia glyphysoides*; O. *S. byssina*; P. *Lecanactis epileuca*; Q. *Chiodecton sphaerale*; R–S. *Erythrodecton granulatum*; T. *Dichosporidium boschianum*; U. *D. nigrocinctum* (ascomata); V. *D. nigrocinctum* (isidia); W. *Mazosia rotula*; X. *Roccella* spec. Photo credits: R. Lücking.

Ertz *et al*. (2009), who showed that *Enterographa* is not monophyletic and groups either with the core *Opegrapha* clade (here represented by *O. lithyrgica*), or with *Chiodecton*-like species (*Dichosporidium* and *Erythrodecton*). Consequently, Ertz *et al*. (2009) tranferred *Enterographa anguinella* to *Opegrapha*. Not surprisingly, neither *Arthonia* nor *Opegrapha* are monophyletic. Ertz *et al*. (2009) showed convincingly that despite different ascomatal structure, *Opegrapha atra* and *O. calcarea* (with distinct excipulum) are closely related to *Arthonia radiata* (lacking an excipulum), which is confirmed by similarities of ascus structure and pigment type. Subsequently, Ertz *et al*. (2009) suggested these two *Opegrapha* species be recognised as belonging to *Arthonia*. *Opegrapha varia* and *O. celtidicola* form another monophyletic lineage together with *Simonyella variegata*. Most likely this branch also includes other *Opegrapha* species, according to the results of Ertz *et al*. (2009). *Opegrapha s. str*. forms a further lineage including *O. lithyrgica*, which is closely related to the type species *O. vulgata* (Ertz *et al*. 2009), the foliicolous *O. filicina*, as well as *Combea mollusca* and *Roccellographa cretacea*.

*Herpothallon rubrocinctum* is now confirmed as an ascomycete in *Arthoniomycetes*. This seems trivial as the species also morphologically shows clear affinities with *Cryptothecia* (Aptroot *et al*. 2008), but the position of this taxon was questioned long ago and was even considered a basidiomycete (see discussion in Withrow & Ahmadjian 1983, Aptroot *et al*. 2008). Our analysis shows *Herpothallon* nested within *Cryptothecia*, supporting the previous hypothesis that byssoid-isidiate species within this complex are indeed members of *Cryptothecia* rather than forming a separate genus, as proposed by Aptroot *et al*. (2008). However, a larger taxon sampling is needed to resolve the *Cryptothecia-Herpothallon* complex, especially considering that there are other genera such as *Stirtonia* involved and even further new genera have been segregated recently (Aptroot *et al.* 2009, Frisch & Thor 2010). The fruticose *Roccella* species form a clearly monophyletic branch together with several crustose species representing various genera; this assemblage of core *Roccellaceae* has already been recognised previously (Tehler 1995, Myllys *et al*. 1998, Tehler & Irestedt 2007). The placement of *Tylophoron*, a genus that has passive spore dispersal and was previously assigned to *Caliciales*, is here confirmed as a member of *Arthoniaceae s. l*., in agreement with Lumbsch *et al*. (2009a).

The strongly supported placement of *Arthonia caesia* within *Chrysothrix* is unexpected; however, fertile species of *Chrysothrix* are very similar to *Arthonia* in ascoma morphology and anatomy, and particularly *A. caesia* and allies can be easily perceived as non-pigmented species of *Chrysothrix* in apothecial anatomy and morphology and thallus structure (including the chlorococcoid photobiont). Similar *Arthonia* species include *A. cupressina*, which is closely related to *A. caesia*. Further studies are needed to elucidate which additional *Arthonia* taxa need to be placed in *Chrysothrix.* The latter genus was variously placed in its own family *Chrysothrichaceae* mainly due to the presence of pulvinic acids as secondary metabolites but also in *Arthoniaceae* due to similarities in ascus characters (Grube 1998). The present data strongly support *Chrysothrichaceae* as a separate family, especially as it is sister to all remaining *Arthoniales* and not to *Arthoniaceae*. It is therefore necessary to transfer *Arthonia caesia* (which lacks pulvinic acids) and related species to this family. The other *Arthonia* species sampled group form a fairly well supported monophyletic group, which includes a species formerly assigned to *Arthothelium*, *i.e. Arthonia ruana*, because of its muriform ascospores; however, it has been known for some time that most species with muriform ascospores are more closely related to *Arthonia* than to the type of *Arthothelium*, *A. spectabile* (Tehler 1990, Sundin & Tehler 1998, Cáceres 2007, Grube 2007), which has not yet been sequenced. Notably, *Arthonia didyma* and *A. rubrocincta*, two species with reddish pigments, form a weakly supported group. If future efforts confirm this grouping, the name *Coniocarpon* could be used for this clade (Cáceres 2007).

In contrast to *Arthoniomycetes*, the overwhelming majority of *Dothideomycetes* species are non-lichenised. In addition to *Arthopyreniaceae, Trypetheliaceae* and *Cystocoleus* and *Racodium* (Muggia *et al.* 2007), this study confirms the placement of *Monoblastiaceae* and *Strigulaceae* within *Dothideomycetes*. Although our support for the *Dothideomycetes* node is weak, the included non-lichenised taxa are well supported within this class in other studies (Schoch *et al*. 2006, 2009a, b); in addition, placement within Dothideomyceta is strongly supported. Both, *Monoblastiaceae* and *Strigulaceae* are comparatively large with over 100 accepted species each and show substantial morphological and ecological radiation (Fig. 3); both are chiefly tropical. The mostly corticolous *Monoblastiaceae* range from barely lichenised forms with exposed perithecia (many species of *Anisomeridium*) to taxa with well-developed, corticate thalli (*Anisomeridium p.p.*, *Megalotremis*, *Trypetheliopsis*). Ascospores vary from small to large and thick-walled but are always simple or transversely septate only (Harris 1995). Substantial variation is found in the conidiomata, and many species, particularly in the genera *Caprettia*, *Megalotremis*, and *Trypetheliopsis* (= *Musaespora*) have developed unique pycnidia that in part are similar to campylidia or hyphophores found in certain *Lecanoromycetes* (Aptroot & Sipman 1993, Lücking *et al.* 1998, Aptroot *et al.* 2008, Lücking 2008). Secondary substances are few, including lichexanthone and anthraquinones. All species of *Monoblastiaceae* in which conidiomata are known share a particular synapomorphy: the conidia are always embedded in a strongly coherent, gelatinous matrix. Thus, besides the uniform hamathecium and ascus anatomy, there is substantial phenotypic evidence for monophyly of this family, now confirmed by molecular data.

*Strigulaceae* share many characteristics with *Monoblastiaceae*, specifically the ascus type and the mostly 1- or 3-septate ascospores, although some species have muriform ascospores (Harris 1995, Aptroot *et al*. 2008, Lücking 2008). Species in this family are found on a variety of substrata, including rocks, bark, and living leaves. Poorly developed thalli are found in corticolous species with barely lichenised thalli and exposed perithecia (*Strigula p.p.*), whereas the genera *Flavobathelium*, *Phyllobathelium*, and *Phyllocratera* include taxa with well-developed, corticate thalli. Also in this family, the most characteristic synapomorphy are the conidia, which feature terminal gelatinous appendices (Harris 1995, Lücking 2008). Unfortunately, our taxon sampling of this family is poor but sufficient to confirm its monophyly and its placement in *Dothideomycetes*. This is the first molecule-based support for the inclusion of *Phyllobatheliaceae* within *Strigulaceae*, a concept first presented by Harris (1995).

The largest lichenised family within *Dothideomycetes*, *Trypetheliaceae*, contains members that are typically lichen-forming and tropical to subtropical in distribution, with some taxa extending into temperate regions (Aptroot 1991, Harris 1995, Brodo *et al*. 2001, Aptroot *et al*. 2008). The species are almost exclusively corticolous, forming a crustose, endo- or epiperidermal thallus with algae belonging to *Trentepohliaceae*; however, *Anisomeridium* is often found lignicolous and *Aptrootia* grows on bryophytes. Detailed studies in Costa Rica suggest *Trypetheliaceae* to occur primarily on trunks and branches of trees in exposed habitats of lowland to lower montane (200–1000 m) rain and dry forests and savannas with rather distinct dry season (Aptroot *et al*. 2008, Rivas-Plata *et al*. 2008). *Trypetheliaceae* species are quite variable in perithecial morphology (Fig. 3) but have a rather uniform hamathecium composed of thin, anastomosing pseudoparaphyses embedded in a stiff gelatinous matrix. The most characteristic synapomorphy are the usually hyaline ascospores with internal wall thickenings that cause more or less diamond-shaped septa, but these wall thickenings are often reduced or absent in species with multiseptate or muriform ascospores (Harris 1984, 1990, 1995, Aptroot 1991b, Aptroot *et al*. 2008). The secondary chemistry is equally simple, with lichexanthone and pigments as most common substances, *i.e.* polyketide derived aromatic compounds produced through the acetyl-polymalonyl pathway (Elix & Stocker-Wörgötter 2008). However, the number of species with substances present is much higher in *Trypetheliaceae* than any other lineage within *Dothideomycetes*: more than 70 species are known to produce secondary substances in this family. The core genera *Astrothelium*, *Campylothelium*, *Cryptothelium*, *Laurera*, and *Trypethelium*, are


**Fig. 3.** Select lichenised *Dothideomycetes*; A. *Arthopyrenia cinchonae*; B. *Mycomicrothelia modesta*; C. *Anisomeridium subprostans*; D. *Anisomeridium spec.* (pycnidia); E. *A. foliicola* (pycnidia); F. *Caprettia amazonensis* (pycnidia); G. *Megalotremis cauliflora* (pycnidia); H. *Trypetheliopsis* (= *Musaespora*) *coccinea* (campylidia); I. *Strigula viridiseda*; J. *S. laureriformis* (pycnidia); K. *S. smaragdula*; L. *Flavobathelium epiphyllum*; M. *Phyllobathelium firmum*; N. *P. leguminosae* (pycnidia); O. *Pseudopyrenula subnudata*; P. *Trypethelium tropicum*; Q. *T. platystomum*; R. *Bathelium degenerans*; S. *Laurera purpurina*; T. *Astrothelium cinnamomeum*; U. *A. eustomum*; V. *Trypethelium nitidiusculum*; W. *Laurera megasperma*; X. *Campylothelium* spec. Photo credits: R. Lücking.

separated primarily on the basis of perithecial arrangement and ostiolar orientation (solitary *vs*. aggregate, apical *vs*. excentric) and ascospore septation (transverse vs. muriform; Harris 1990, 1995, del Prado *et al*. 2006). Because of the schematic classification, Harris (1995) suggested that these genera may be polyphyletic, and del Prado *et al*. (2006) subsequently illustrated the non-monophyly of *Trypethelium*. Aptroot *et al*. (2008) echoed Harris's (1995) sentiment and stated that generic concepts in *Trypetheliaceae* are in need of revision.

Surprisingly, *Mycomicrothelia* was recovered as sister to *Trypetheliaceae*. *Mycomicrothelia* has traditionally been considered a sister genus to *Arthopyrenia* with brown ascospores (Harris 1995). However, the hamathecium at least of the sequenced species is identical to that found in *Trypetheliaceae*, whereas *Arthopyrenia* has thicker and less branched and anastomosing pseudoparaphyses. Moreover, the ascospores are of a different type, often with internal wall thickenings. It remains to be tested whether *Arthopyrenia* and *Mycomicrothelia* in their current circumscriptions are monophyletic

**Table 2.** Systematic placement of selected pyrenocarpous lichens according to different concepts.

| Genus | Zahlbruckner 1926 | Barr 1987 | Harris 1995 | current |
|---|---|---|---|---|
| ***Celothelium*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Eurotiomycetes* |
| | *(*as *Leptorhaphis)* | *Pleosporales* | *Melanommatales* | *Pyrenulales* |
| | *Pyrenulaceae* | *Pleosporaceae* | *Thelenellaceae* | *Celotheliaceae* |
| ***Lithothelium*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Eurotiomycetes* |
| | *Astrotheliaceae* | *Melanommatales* | *Melanommatales* | *Pyrenulales* |
| ***Pyrenula*** | *Pyrenocarpeae* | *Pyrenulaceae* | *Pyrenulaceae* | *Pyrenulaceae* |
| | *Pyrenulaceae* | | | |
| ***Arthopyrenia*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Dothideomycetes* |
| | *Pyrenulaceae* | *Pleosporales* | *Pleosporales* | *Pleosporales* |
| | | *Arthopyreniaceae* | *Pleosporaceae* | *Arthopyreniaceae* |
| ***Acrocordia*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Dothideomycetes* |
| ***Anisomeridium*** | *(*as *Arthopyrenia)* | *Melanommatales* | *Melanommatales* | *incertae sedis* |
| | *Pyrenulaceae* | *Acrocordiaceae* | *Monoblastiaceae* | *Monoblastiaceae* |
| ***Phyllobathelium*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Dothideomycetes* |
| ***Strigula*** | *Strigulaceae* | *Chaetothyriales* | *Melanommatales* | *incertae sedis* |
| | | *Strigulaceae* | *Strigulaceae* | *Strigulaceae* |
| ***Astrothelium*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Dothideomycetes* |
| | *Astrotheliaceae* | *Melanommatales* | *Melanommatales* | *Trypetheliales* |
| ***Campylothelium*** | *Pyrenocarpeae* | *Trypetheliaceae* | *Trypetheliaceae* | *Trypetheliaceae* |
| | *Paratheliaceae* | | | |
| ***Laurera*** | *Pyrenocarpeae* | | | |
| | *Trypetheliaceae* | | | |
| ***Pseudopyrenula*** | *Pyrenocarpeae* | | | |
| | *Pyrenulaceae* | | | |
| ***Trypethelium*** | *Pyrenocarpeae* | | | |
| | *Trypetheliaceae* | | | |
| ***Mycomicrothelia*** | *Pyrenocarpeae* | *Loculoascomycetes* | *Loculoascomycetes* | *Dothideomycetes* |
| | *(*as *Microthelia)* | *Pleosporales* | *Pleosporales* | *Trypetheliales* |
| | *Strigulaceae* | *Arthopyreniaceae* | *Arthopyreniaceae* | *Trypetheliaceae?* |
| ***Porina*** | *Pyrenocarpeae* | | *Hymenoascomycetes* | *Lecanoromycetes* |
| | *Pyrenulaceae* | | *Trichotheliales* | *Ostropales* |
| ***Trichothelium*** | *Pyrenocarpeae* | | *Trichotheliaceae* | *Porinaceae* |
| | *Strigulaceae* | — | | |

genera or whether at least some species currently assigned to these genera perhaps represent further lichenised lineages within *Dothideomycetes*. Whether *Mycomicrothelia* should be included within *Trypetheliaceae* or receive its own family rank is open to question. *Mycomicrothelia* has primarily thin-walled, dark brown ascospores, whereas in *Trypetheliaceae* they are primarily thick-walled with diamond-shaped lumina and hyaline (brown only in *Aptrootia* and *Architrypethelium*). Understanding the phylogenetic position of *Polymeridium*, which also has thin-walled ascospores, will hopefully help clarify this.

In spite of the many characters in parallel with *Monoblastiaceae* and *Strigulaceae*, also the *Trypetheliaceae* plus *Mycomicrothelia* (*Trypetheliales*) are quite unique genetically and there is no evidence that the three families would be related to each other or with *Arthopyreniaceae*. This supports the notion of several shifts in lichenisation within the *Dothideomycetes* (Aptroot 1991a, 1998). However, the often barely lichenised thalli in certain species of *Anisomeridium*, *Arthopyrenia*, *Julella*, *Mycomicrothelia*, *Mycoporum*, *Pseudopyrenula*, and *Strigula* (Aptroot 1991a, Aptroot 1998, Harris 1995) suggest that these species can possibly switch between being (almost) non-lichenised to distinctly lichenised, a situation also found in the unrelated genus *Stictis* within *Lecanoromycetes* (Wedin *et al.* 2004).

The present study clarifies the systematic position of further pyrenocarpous lichenised lineages within the *Ascomycota* and shows that previous concepts in part diverged widely from our present understanding but also came suprisingly close even without molecular evidence (Table 2). This study emphasises that pyrenocarpous lichens with bitunicate asci are not only not monophyletic, but belong to at least two different classes (*Dothideomycetes* and *Eurotiomycetes*) and several different orders and families; the data at hand also suggest that these

represent several independent lineages of lichenisation. Although we consider this study a contribution to clarify the systematic position of pyrenocarpous lichens and the evolution of lichenisation within *Dothideomycetes*, much remains to be done, considering that at present only a fraction of the presumably 600 species of lichens belonging in this class have been studied using DNA sequences. In particular, clarifying the generic and species concepts within *Monoblastiaceae*, *Strigulaceae*, and *Trypetheliaceae*, speciose families that are important elements of crustose lichen communities especially in the tropics, will be a major challenge in the near future.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Taxa included in this study with GenBank accession numbers and collection information. Numbers following taxon names are DNA identification numbers used in this study.

| Taxon | Collection | *Accession Number* | |
|---|---|---|---|
| | | **nuLSU** | **mtSSU** |
| *Acrocordia subglobosa* (HTL940) | Palice s.n., Poland (F) | | GU327681 |
| *Amphisphaeria umbrina* | | FJ176863 | FJ713609 |
| *Anisomeridium ubianum* (94) | Lumbsch 19845j, Fiji (F) | GU327709 | GU327682 |
| *Aptrootia terricola* | | | DQ328995 |
| *Arthonia caesia* | | FJ469668 | FJ469671 |
| *Arthonia didyma* | | EU704083 | EU704047 |
| *Arthonia dispersa* | | AY571381 | AY571383 |
| *Arthonia radiate* | | | EU704048 |
| *Arthonia ruana* (79B) | Zimmerman 1117, Germany (F) | | GU327683 |
| *Arthonia rubrocincta* (129) | Nelsen 4010, U.S.A. (F) | | GU327684 |
| *Arthopyrenia salicis* | | AY538339 | AY538345 |
| | | AY607730 | AY607742 |
| *Ascobolus crenulatus* | | AY544678 | FJ713607 |
| *Astrothelium cinnamomeum* | | AY584652 | AY584632 |
| *Astrothelium confusum* (98) | Nelsen 4004a, Peru (F) | GU327710 | GU327685 |
| *Bacidia schweinitzii* | | DQ782911 | DQ972998 |
| *Bathelium degenerans* | | | DQ328987 |
| | | | DQ328988 |
| *Bimuria novae-zelandiae* | | AY016356 | FJ190605 |
| *Bionectria ochroleuca* | | AY489716 | FJ713619 |
| *Botryosphaeria dothidea* | | DQ678051 | FJ190612 |
| *Botryosphaeria stevensii* | | DQ678064 | |
| *Botryosphaeria tsugae* | | DQ767655 | |
| *Botryotinia fuckeliana* | | AY544651 | AY544732 |
| *Caliciopsis orientalis* | | DQ470987 | FJ190654 |
| *Caliciopsis pinea* | | DQ678097 | FJ190653 |
| *Camarops ustulinoides* | | DQ470941 | FJ190588 |
| *Capnodium coffeae* | | DQ247800 | FJ190609 |
| *Capronia pilosella* | | DQ823099 | FJ225725 |
| *Cercospora beticola* | | DQ678091 | FJ190647 |
| *Cheilymenia stercorea* | | AY544661 | AY544733 |
| *Chiodecton natalense* | | EU704085 | EU704051 |
| *Chlorociboria aeruginosa* | | AY544669 | AY544734 |
| *Chrysothrix flavovirens* (L466) | Perlmutter 786, U.S.A. (NCU) | GU327711 | GU327686 |
| *Chrysothrix xanthina* (126) | Nelsen 4005, U.S.A. (F) | GU327712 | GU327687 |
| *Cladosporium cladosporioides* | | DQ678057 | FJ190628 |
| *Cochliobolus heterostrophus* | | AY544645 | AY544737 |
| *Cochliobolus sativus* | | DQ678045 | FJ190589 |
| *Columnosphaeria fagi* | | DQ470956 | FJ713608 |
| *Combea mollusca* | | AY571382 | AY571384 |
| *Coniothyrium palmarum* | | DQ767653 | FJ190638 |
| *Cordyceps capitata* | | AY489721 | FJ713628 |
| *Cryptothecia assimilis* (86B) | Lumbsch 19815l, Fiji (F) | | GU327688 |

**Table 1.** (Continued).

| Taxon | Collection | Accession Number | |
|---|---|---|---|
| | | nuLSU | mtSSU |
| *Cryptothecia candida* | | | EU704052 |
| *Cryptothelium amazonum* (47) | Nelsen 4000a, Peru (F) | GU327713 | GU327689 |
| *Cryptothelium cecidiogenum* | | | DQ328991 |
| *Cryptothelium sepultum* (63C) | Nelsen 4001a, Peru (F) | GU327714 | GU327690 |
| *Cudoniella cf. clavus* | | DQ470944 | FJ713604 |
| *Cystocoleus ebeneus* | | EU048578 | EU048584 |
| | | EU048579 | EU048585 |
| | | EU048580 | EU048586 |
| | | | EU048587 |
| *Delitschia winteri* | | DQ678077 | FJ190644 |
| *Dendrographa alectoroides* (100) | Lumbsch 19914g, U.S.A. (F) | GU327715 | GU327691 |
| *Dendrographa leucophaea f. minor* | | AF279382 | AY548811 |
| *Dendryphiella arenaria* | | DQ470971 | FJ190617 |
| *Dermatocarpon miniatum* | | AY584644 | AY584616 |
| *Diaporthe eres* | | AF408350 | FJ190607 |
| *Dichosporidium boschianum* (89B) | Lumbsch 19815a, Fiji (F) | GU327716 | GU327692 |
| *Dirina catalinariae* | | EF081387 | |
| *Dothidea insculpta* | | DQ247802 | FJ190602 |
| *Dothidea sambuci* | | AY544681 | AY544739 |
| *Dothiora cannabinae* | | DQ470984 | FJ190636 |
| *Eleutherascus lectardii* | | DQ470966 | FJ190606 |
| *Elsinoe centrolobi* | | DQ678094 | FJ190651 |
| *Elsinoe phaseoli* | | DQ678095 | FJ190652 |
| *Elsinoe veneta* | | DQ767658 | FJ190650 |
| *Endocarpon pallidulum* | | DQ823097 | FJ225674 |
| *Enterographa anguinella* | | EU704086 | EU704054 |
| *Enterographa crassa* | | EU704088 | EU704056 |
| *Erythrodecton granulatum* | | EU704090 | EU704058 |
| *Eupenicillium javanicum* | | EF413621 | FJ225778 |
| *Exophiala salmonis* | | EF413609 | FJ225745 |
| *Flavobathelium epiphyllum* (67) | Lücking s.n. Panama (F) | GU327717 | |
| *Glomerella cingulata* | | AF543786 | FJ190626 |
| *Glyphium elatum* | | AF346420 | AF346425 |
| *Gnomonia gnomon* | | AF408361 | FJ190615 |
| *Guignardia gaulteriae* | | DQ678089 | FJ190646 |
| *Herpothallon rubrocinctum* (128) | Nelsen 4006, U.S.A. (F) | | GU327693 |
| *Herpotrichia diffusa* | | DQ678071 | DQ384076 |
| *Hypocrea lutea* | | AF543791 | FJ713620 |
| *Hysteropatella cf. elliptica* | | DQ767657 | FJ190649 |
| *Kirschsteiniothelia aethiops* | | AY016361 | FJ190604 |
| | | DQ678046 | FJ190590 |
| *Lachnum virgineum* | | AY544646 | AY544745 |
| *Laurera megasperma* | | FJ267702 | |
| *Lecanactis abietina* | | AY548812 | AY548813 |
| *Lecanactis sp.* | | EU704091 | EU704059 |
| *Lecanora hybocarpa* | | DQ782910 | DQ912273 |
| *Macrophomina phaseolina* | | DQ678088 | FJ190645 |

**Table 1.** (Continued).

| Taxon | Collection | Accession Number | |
|---|---|---|---|
| | | nuLSU | mtSSU |
| *Megalotremis verrucosa* (104) | Lücking 26316, Colombia (F) | GU327718 | GU327694 |
| *Monilinia laxa* | | AY544670 | AY544748 |
| *Mycomicrothelia hemispherica* (102) | Lücking 28641, Nicaragua (F) | GU327719 | GU327695 |
| *Mycomicrothelia miculiformis* (101B) | Lücking 28637, Nicaragua (F) | GU327720 | GU327696 |
| *Mycomicrothelia obovata* (95) | Nelsen 4007a, Peru (F) | GU327721 | GU327697 |
| *Mycosphaerella fijiensis* | | DQ678098 | FJ190656 |
| *Mycosphaerella punctiformis* | | DQ470968 | FJ190611 |
| *Myriangium duriaei* | | DQ678059 | AY571389 |
| *Nectria cinnabarina* | | U00748 | FJ713622 |
| *Opegrapha celtidicola* | | EU704094 | EU704066 |
| *Opegrapha filicina* | | EU704095 | EU704067 |
| *Opegrapha lithyrga* | | EU704096 | EU704068 |
| *Opegrapha varia* | | EU704103 | EU704075 |
| *Ophionectria trichospora* | | AF543790 | FJ713626 |
| *Peltigera degenii* | | AY584657 | AY584628 |
| *Penicillium freii* | | AY640958 | AY584712 |
| *Pertusaria dactylina* | | DQ782907 | DQ972973 |
| *Phaeotrichum benjaminii* | | AY004340 | AY538349 |
| *Phoma herbarum* | | DQ678066 | FJ190640 |
| *Phyllobathelium anomalum* (242) | Lücking s.n., Panama (F) | GU327722 | GU327698 |
| *Phyllobathelium firmum* (HTL3175) | Lücking s.n., Panama (F) | GU327723 | |
| *Pleospora herbarum* var. *herbarum* | | DQ247804 | FJ190610 |
| *Preussia terricola* | | AY544686 | AY544754 |
| *Pseudopyrenula subgregaria* (106) | Lücking 24079, Thailand (F) | GU327724 | GU327699 |
| *Pseudopyrenula subnudata* | | | DQ328997 |
| *Pyrenophora phaeocomes* | | DQ499596 | FJ190591 |
| *Pyrenophora tritici-repentis* | | AY544672 | FJ713605 |
| *Pyrenula pseudobufonia* | | AY640962 | AY584720 |
| *Pyrgillus javanicus* | | DQ823103 | FJ225774 |
| *Pyxine subcinerea* | | DQ883802 | DQ912292 |
| *Racodium rupestre* | | EU048583 | EU048588 |
| | | EU048581 | |
| | | EU048582 | EU048589 |
| *Ramichloridium anceps* | | DQ823102 | FJ225752 |
| *Roccella canariensis* | | AY779328 | |
| *Roccella fuciformis* | | AY584654 | EU704082 |
| *Roccella montagnei* (109) | Lumbsch 19700a, India (F) | GU327725 | GU327700 |
| *Roccella tuberculata* | | AY779328 | |
| *Roccellographa cretacea* | | DQ883696 | FJ772240 |
| *Schismatomma decolorans* | | AY548815 | AY548816 |
| *Schismatomma pericleum* | | AF279408 | AY571390 |
| *Scorias spongiosa* | | DQ678075 | FJ190643 |
| *Scutellinia scutellata* | | DQ247806 | FJ190587 |
| *Simonyella variegate* | | | AY584631 |
| *Sphinctrina turbinate* | | EF413632 | FJ713611 |
| *Spiromastix warcupii* | | DQ782909 | FJ225794 |
| *Sporormiella minima* | | DQ678056 | FJ190624 |

**Table 1.** (Continued).

| Taxon | Collection | *Accession Number* | |
|---|---|---|---|
| | | **nuLSU** | **mtSSU** |
| *Staurothele frustulenta* | | DQ823098 | FJ225702 |
| *Strigula nemathora* (72) | Lücking s.n., Costa Rica (F) | | GU327701 |
| *Strigula schizospora* (73) | Lücking s.n., Costa Rica (F) | | GU327702 |
| *Stylodothis puccinioides* | | AY004342 | AF346428 |
| *Sydowia polyspora* | | DQ678058 | FJ190631 |
| *Syncesia farinacea* | | EF081452 | |
| *Trematosphaeria heterospora* | | AY016369 | AF346429 |
| *Trematosphaeria pertusa* | | DQ678072 | FJ190641 |
| *Trimmatostroma abietis* | | DQ678092 | FJ190648 |
| *Trypetheliopsis kalbii* (243) | Lücking s.n., Panama (F) | | GU327703 |
| *Trypethelium eluteriae* | | | DQ328989 |
| *Trypethelium eluteriae* (111) | Lumbsch 19701a, India (F) | GU327726 | GU327704 |
| *Trypethelium marcidum* | | | DQ329007 |
| *Trypethelium marcidum* (132) | Nelsen 4008, U.S.A. (F) | GU327727 | GU327705 |
| *Trypethelium nitidiusculum* (139) | Nelsen 4002a, U.S.A. (F) | GU327728 | GU327706 |
| *Trypethelium papulosum* (97) | Nelsen 4009a, Peru (F) | GU327729 | GU327707 |
| *Trypethelium platystomum* | | | DQ329009 |
| *Trypethelium tropicum* (25) | Nelsen 4003, Thailand (F) | GU327730 | GU327708 |
| *Tubeufia cerea* | | DQ470982 | FJ190634 |
| *Tylophoron crassiusculum* | | EU670258 | |
| *Tylophoron moderatum* | | EU670256 | |
| *Tyrannosorus pinicola* | | DQ470974 | FJ190620 |
| *Vibrissea truncorum* | | FJ176874 | FJ190635 |
| *Westerdykella cylindrical* | | AY004343 | AF346430 |
| *Xylaria hypoxylon* | | AY544648 | AY544760 |

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# The molecular phylogeny of freshwater *Dothideomycetes*

C.A. Shearer[1*], H.A. Raja[1], A.N. Miller[2], P. Nelson[3], K. Tanaka[4], K. Hirayama[4], L. Marvanová[5], K.D. Hyde[6] and Y. Zhang[7]

[1]*Department of Plant Biology, University of Illinois, 505 S. Goodwin Ave., Urbana, IL 61801, U.S.A.;* [2]*Illinois Natural History Survey, University of Illinois, 1816 South Oak St., Champaign, IL, 61820, U.S.A.;* [3]*University of Minnesota, Ecology, Evolution, and Behavior, 100 Ecology Building, St. Paul, MN 55108, U.S.A.;* [4]*Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan;* [5]*Czech Collection of Mircroorganisms, Institute of Experimental Biology, Faculty of Science, Masaryk University, Tvrdého 14, Brno CZ-602 00, Czech Republic;* [6]*School of Science, Mae Fah Luang University, Tasud, Muang, Chiang Rai 57100, Thailand;* [7]*Division of Microbiology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China*

**Correspondence*: C.A. Shearer, carolshe@uiuc.edu

**Abstract:** The freshwater *Dothideomycetes* species are an ecological rather than taxonomic group and comprise approximately 178 meiosporic and mitosporic species. Due to convergent or parallel morphological adaptations to aquatic habitats, it is difficult to determine phylogenetic relationships among freshwater taxa and among freshwater, marine and terrestrial taxa based solely on morphology. We conducted molecular sequence-based phylogenetic analyses using nuclear ribosomal sequences (SSU and/or LSU) for 84 isolates of described and undescribed freshwater *Dothideomycetes* and 85 additional taxa representative of the major orders and families of *Dothideomycetes*. Results indicated that this ecological group is not monophyletic and all the freshwater taxa, except three aeroaquatic *Tubeufiaceae,* occur in *Pleosporomycetidae* as opposed to *Dothideomycetidae.* Four clades comprised of only freshwater taxa were recovered. The largest of these is the *Jahnulales* clade consisting of 13 species, two of which are the anamorphs *Brachiosphaera tropicalis* and *Xylomyces chlamydosporus.* The second most speciose clade is the *Lindgomycetaceae* clade consisting of nine taxa including the anamorph *Taeniolella typhoides*. The *Lindgomycetaceae* clade consists of taxa formerly described in *Massarina, Lophiostoma, and Massariosphaeria e.g., Massarina ingoldiana*, *Lophiostoma breviappendiculatum*, and *Massariosphaeria typhicola* and several newly described and undescribed taxa. The aquatic family *Amniculicolaceae*, including three species of *Amniculicola*, *Semimassariosphaeria typhicola* and the anamorph, *Anguillospora longissima*, was well supported. A fourth clade of freshwater species consisting of *Tingoldiago graminicola, Lentithecium aquaticum, L. arundinaceum* and undescribed taxon A-369-2b was not well supported with maximum likelihood bootstrap and Bayesian posterior probability. Eight freshwater taxa occurred along with terrestrial species in the *Lophiostoma* clades 1 and 2. Two taxa lacking statistical support for their placement with any taxa included in this study are considered singletons within *Pleosporomycetidae.* These singletons, *Ocala scalariformis,* and *Lepidopterella palustris,* are morphologically distinct from other taxa in *Pleosporomycetidae.* This study suggests that freshwater *Dothideomycetes* are related to terrestrial taxa and have adapted to freshwater habitats numerous times. In some cases (*Jahnulales* and *Lindgomycetaceae*), species radiation appears to have occurred. Additional collections and molecular study are required to further clarify the phylogeny of this interesting ecological group.

**Key words:** Ascomycetes, aquatic, evolution, *Jahnulales, Pleosporales*.

## INTRODUCTION

Freshwater ascomycetes comprise a diverse taxonomic assemblage of about 577 species (Shearer *et al.* 2009). These fungi are mostly saprobic on submerged woody and herbaceous debris and are important in aquatic food webs as decomposers and as a food source to invertebrates (see Gessner *et al.* 2007, Simonis *et al.* 2008). Although in the early ascomycete taxonomic literature some species were reported and/or described from plants in or near aquatic habitats, little was noted about whether the fungi were on aerial or submerged parts of their hosts/substrates. For the purpose of this study, we consider freshwater ascomycetes as only those species that occur on submerged substrates; ascomycetes on aerial parts of aquatic plants are considered terrestrial and not dealt with herein.

Ingold was the first to recognise that a distinctive freshwater ascomycota might exist and published a series of papers about fungi on submerged substrates in the Lake District, England (Ingold 1951, 1954, 1955, Ingold & Chapman 1952). Ingold was collecting from the submerged stems of aquatic macrophytes in the English Lake District when he discovered the magnificent freshwater Dothideomycete, *Macrospora scirpicola* on *Schoenoplectus lacustris*, the lakeshore bulrush (Ingold 1955). This fungus has ascospores equipped with a gelatinous sheath (Fig. 1A) that elongates and becomes sticky after the ascospores are discharged into water (Fig. 1B), a feature thought to improve the probability that ascospores will attach to substrates in moving water (Hyde & Jones 1989, Shearer 1993, Jones 2006). This feature is found in numerous freshwater *Dothideomycetes* (see species monograph, Shearer *et al.* 2009). The ascospores also germinate immediately upon contact with a firm substrate (Fig. 1C), which may help them adhere to substrates in moving water. *Macrospora scirpicola* is one of the earliest known freshwater Dothideomycete species; DeCandolle originally described it in 1832 as *Sphaeria scirpicola*, and Pringsheim first reported it from freshwater in 1858.

The early literature dealing specifically with freshwater ascomycetes, including *Dothideomycetes,* has been reviewed by Dudka (1963, 1985) and Shearer (1993). Since the 1990's, interest in aquatic ascomycetes has grown and the number of species reported and/or described from freshwater habitats has increased by 370 to a total of 577 taxa (Shearer *et al.* 2009). For more recent reviews of the freshwater ascomycetes, see: Goh & Hyde (1996), Wong *et al.* (1998), Shearer (2001), Tsui & Hyde (2003), Shearer *et al.* (2007), and Raja *et al.* (2009b). Approximately 30 % of the 577 freshwater ascomycetes are Dothideomycete species, and based on morphology, belong primarily in *Pleosporales* or secondarily in *Jahnulales.* Exceptions include four species in *Capnodiales (Mycosphaerellaceae)* and four species in *Tubeufiaceae.*

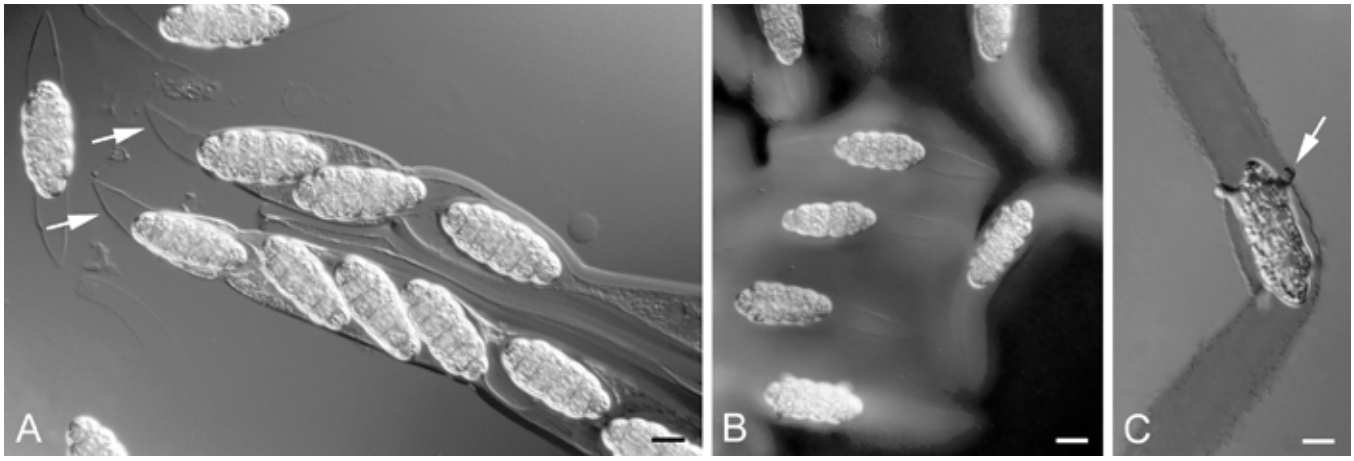


**Fig. 1.** *Macrospora scirpicola* A27-1. A. Ascospores being discharged from bitunicate asci showing bipolar gelatinous appendages. B. Ascospores showing an outer and inner sheath when stained with India ink. C. Ascospore on a glass slide germinating within its gelatinous sheath stained with India ink. Scale Bars: = 20 µm.

Molecular studies of freshwater *Dothideomycetes* have been of four basic types. The first type was to determine the overall taxonomic placement of one or more undescribed taxa (*e.g.,* Inderbitzin *et al.* 2001, Cai & Hyde 2007, Kodsueb *et al.* 2007, Cai *et al.* 2008, Zhang *et al.* 2008a, b, 2009a, c, Raja *et al.* 2010). In these studies one or more nuclear genes were sequenced to place a newly described fungus in an order or family within the *Dothideomycetes* framework. In the second type, the goal was to use single or multi-gene phylogenies to elucidate the evolutionary relationships among a group of closely related taxa, and to evaluate which suite of morphological characters might be informative for predicting evolutionary relationships and which might be misleading or homoplasious (*e.g.,* Liew *et al.* 2002, Pang *et al.* 2002, Campbell *et al.* 2006, 2007, Tsui & Berbee 2006, Zhang *et al.* 2009a, c, Hirayama *et al.* 2010). The third type of molecular study was used to identify relationships between aquatic anamorphic and teleomorphic *Dothideomycetes* (see Baschien 2003, Belliveau & Bärlocher 2005, Baschien *et al.* 2006, Campbell *et al.* 2006, Tsui *et al.* 2006, 2007). Here the goal was to use sequence data to place the aquatic anamorphs within the teleomorph phylogeny to better understand the phylogenetic affinities of freshwater anamorphs. The fourth type addressed the evolution of freshwater ascomycetes (Vijaykrishna *et al.* 2006).

*Dothideomycetes* possess freshwater hyphomycetous anamorphs rather rarely. Approximately only 10 % of 86 aquatic hyphomycete species, which are at least tentatively assigned to an ascomycete family, order or class, have affinity to *Dothideomycetes*. Four of them are connected to known teleomorphs via cultural studies: *Tumularia aquatica* to *Massarina aquatica* (Webster 1965), *Anguillospora longissima* to *Massarina* sp. (Willoughby & Archer 1973), *Clavariopsis aquatica* to *Massarina* sp. (Webster & Descals 1979), and *Aquaphila albicans* to *Tubeufia asiana* (Tsui *et al.* 2007). Four connections are published on the basis of molecular phylogenetic rather than cultural studies, but some of these connections are controversial and require further molecular study using additional genes and/or cultural studies. These connections include: *Anguillospora rubescens* in *Dothideales* (Belliveau & Bärlocher 2005), *Lemonniera pseudofloscula* and *Goniopila monticola* in *Pleosporales* (Campbell *et al.* 2006), and *Mycocentrospora acerina* to *Mycosphaerellaceae* (Stewart *et al.* 1999). (Note: Data on affinity of *Mycocentrospora* is not explicitly given in the text, but is in the GenBank entry AY266155).

Most of the above-mentioned molecular studies have used limited taxon sampling of various orders and families currently in the *Dothideomycetes,* as well as a single gene (either nuc SSU rDNA or nuc LSU rDNA) to understand the phylogenetic affinities of the freshwater taxa. A review of past molecular phylogenetic studies of freshwater *Dothideomycetes* revealed that very few of the approximately 170 freshwater Dothideomycete species have been sequenced. In addition, different genes and different regions of the same genes have been sequenced for different taxa making any comprehensive molecular analysis impossible. Clearly more sequences are needed for taxa already studied and more taxa need to be sequenced if we are to understand the phylogeny of the freshwater *Dothideomycetes.*

The purpose of this study, therefore, was to obtain two gene sequences (nuc SSU rDNA & nuc LSU rDNA) for as many freshwater *Dothideomycetes* (teleomorphs and anamorphs) as possible to conduct molecular sequence analyses to place these taxa within a phylogenetic framework comprised of a broader taxonomic and ecological taxon sampling from major orders and families using the most current classification system proposed for the *Dothideomycetes* (Schoch *et al.* 2006, Hibbett *et al.* 2007).

## MATERIALS AND METHODS

### Taxon sampling

The species used in this study, their isolate numbers, sources and GenBank accession numbers are listed in Table 1 - see online Supplementary Information. The datasets contained 156 taxa for the SSU and 160 taxa for LSU, while the combined dataset consisted of 169 taxa with some missing data. Twenty-two aquatic taxa were newly sequenced for the SSU gene and/or the LSU gene, while sequences of several other aquatic taxa included in the analyses were obtained from very recently published or unpublished phylogenetic studies of freshwater fungi (Zhang *et al.* 2008a, b, 2009a, c, Hirayama *et al.* 2010, Raja *et al.* 2010). Sequences of a wide array of taxa representing various orders and families within the *Dothideomycetes* based on Schoch *et al.* (2006) were included in this study. In addition to taxa from the *Dothideomycetes,* members of *Arthoniomycetes, Lecanoromycetes, Sordariomycetes* and *Leotiomycetes* were also included in the analyses. Members of the *Pezizomycetes* were used as outgroup taxa.

## DNA extraction and PCR amplification

For extraction of genomic DNA, mycelium from axenic cultures was scraped with a sterile scalpel from nutrient agar in plastic Petri dishes and ground to a fine powder in liquid nitrogen using a mortar and pestle. Approximately 400 µL of AP1 buffer from the DNAeasy Plant Mini Kit (QIAGEN Inc., Valencia, California) was added to the mycelial powder and DNA was extracted following the manufacturer's instructions. The DNA was finally eluted in 30 µL distilled water. Fragments of SSU and LSU nrDNA were amplified by PCR using PuReTaq™ Ready-To-Go PCR beads (Amersham Biosciences Corp., Piscataway, New York) according to Promputtha & Miller (2010). Primers NS1 and NS4 for SSU (White *et al.* 1990) and LROR and LR6 for LSU (Vilgalys & Hester 1990, Rehner & Samuels 1995) were used for PCR reactions in addition to 2.5 µL of BSA (bovine serum albumin, New England Biolabs, Ipswich, MA) and/or 2.5 µL of DMSO (dimethyl sulfoxide, Fisher Scientific, Pittsburgh, PA). PCR products were purified to remove excess primers, dNTPs and nonspecific amplification products with the QIAquick PCR Purification Kit (QIAGEN Inc., Valencia, California). Purified PCR products were used in 11 µL sequencing reactions with BigDye Terminators v. 3.1 (Applied Biosystems, Foster City, California) in combination with the following SSU primers: NS1, NS2, NS3, NS4 (White *et al.* 1990), and LSU primers: LROR, LR3, LR3R, LR6 (Vilgalys & Hester 1999, Rehner & Samuels 1995). Sequences were generated on an Applied Biosystems 3730XL high-throughput capillary sequencer at the UIUC Biotech facility. Sequences were also obtained using other methods outlined in Hirayama *et al.* (2010) and Zhang *et al.* (2009c).

## Sequence alignment

Each sequence fragment obtained was subjected to an individual blast search to verify its identity. Individual fragments were edited and contigs were assembled using Sequencher v. 4.9 (Gene Codes Corp., Ann Arbor Michigan). Newly obtained sequences were aligned with sequences from GenBank using the multiple sequence alignment program, MUSCLE® (Edgar 2004) with default parameters in operation. MUSCLE® was implemented using the programs Seaview (Galtier *et al.* 1996) and Geneious Pro v. 4.7.6 (Biomatters) (Drummond *et al.* 2006). Sequences were aligned in MUSCLE using a previous (trusted) alignment made by eye in Sequencher v. 4.9, based on a method called "jump-starting alignment" (Morrisson 2006). The final alignment was again optimised by eye and manually corrected using Se-Al v. 2.0a8 (Rambaut 1996) and McClade v. 4.08 (Maddison & Maddison 2000).

## Phylogenetic analyses

Separate alignments were made for SSU and LSU sequences. The aligned SSU and LSU datasets were first analysed separately and then the individual datasets were concatenated into a combined dataset. Prior to combining the datasets, the possibility of clade conflict was explored. Independent maximum likelihood (ML) analyses were run with a GTR model including invariable sites and discrete gamma shape distribution and 100 bootstrap replicates were performed using the program Seaview (Galtier *et al.* 1996). The individual SSU and LSU phylogenies were then examined for conflict by comparing clades with bootstrap support (Wiens 1998). If clades were < 50 % they were considered weakly supported, whereas 70–100 % indicated a strong support. We combined the datasets since there was no obvious clade conflict for 90 % of the taxa included in our study. Subsequent analyses were then performed on the combined SSU + LSU dataset. In the final combined dataset, 13 ambiguously aligned regions were delimited and excluded from all further analyses.

Modeltest v. 3.7 (Posada & Crandall 1998) was used to determine the best-fit model of evolution for the dataset. ML analyses were performed using RAxML v. 7.0.4 (Stamatakis 2006) with 100 successive searches and the best-fit model, which was the (GTR) model with unequal base frequencies (freqA = 0.2666, freqC = 0.2263, freqG = 0.2664, freqT = 0.2407), a substitution rate matrix (A<–>C = 0.9722, A<–>G = 2.7980, A<–>T = 1.1434, C<–>G = 0.6546, C<–>T = 5.1836, G<–>T = 1.0000), a proportion of invariable sites (– 0.2959) and a gamma distribution shape parameter ( – 0.4649). For the ML analyses constant characters were included and again 13 ambiguously aligned regions were excluded. Each search was performed using a randomised starting tree with a rapid hill climbing option. One thousand fast bootstrap pseudoreplicates (Stamatakis *et al.* 2008) were run under the same conditions.

Bayesian Metropolis Coupled Markov Chain Monte Carlo (B-MCMCMC) analyses were performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) as an additional means of assessing branch support. Constant characters were included. A comparable model to the ML analyses was used to run 10 million generations with trees sampled every 1 000$^{th}$ generation resulting in 10 000 total trees. The first 1 000 trees which extended beyond the burn-in phase in each analysis were discarded and the remaining 9 000 trees were used to calculate posterior probabilities. The consensus of 9 000 trees was viewed in PAUP v. 4.0b10 (Swofford 2002). The analysis was repeated twice each with four Markov Chains for the dataset starting from different random trees.

# RESULTS

## Sequence alignment

The complete dataset (combined SSU and LSU alignment) along with intron regions and ambiguous characters had 169 taxa and 7 264 characters. The dataset consisted of 169 taxa and 3 641 characters after removal of intron regions. We then delimited and removed 548 ambiguous characters from the final alignment along with characters from the 5' and 3' end regions due to missing information in most taxa included in the alignment. The final dataset after removal of all the intron regions and 13 ambiguous regions along with missing data from the 5' and 3' ends consisted of 1816 characters. There were no significant conflicts among the clades in the separate SSU and LSU analyses in either SSU or LSU datasets (data not shown) therefore we used all 169 taxa in the combined SSU and LSU analyses.

## Phylogenetic analyses

The combined matrix analysed in this study produced 852 distinct alignment patterns and the most likely tree (Fig. 2) had a log likelihood of -17187.0385 compared to the average (100 trees) of -17191.7927. Several major clades presented in the multi-gene phylogeny of Schoch *et al.* (2006) were recovered in our combined SSU and LSU phylogeny. *Leotiomycetes* was not monophyletic in our analyses, but this relationship was not supported.

Eighty-four Dothideomycete isolates from freshwater habitats, including meiosporic and mitosporic representatives, were included

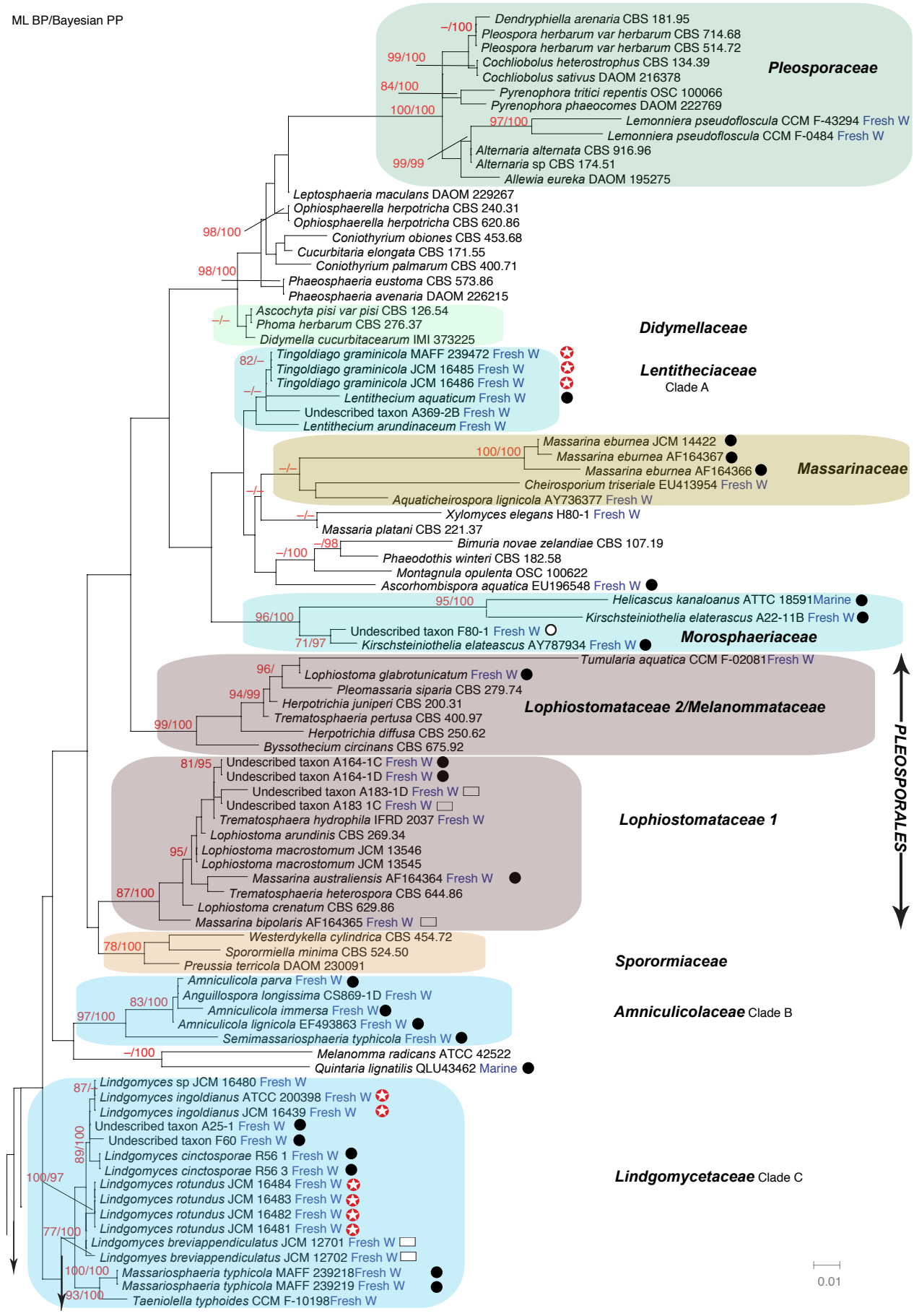


**Fig. 2.** Freshwater *Dothideomycetes* phylogeny. The most likely tree (Ln L = -17187.0385) after 100 replicates of a RAxML analysis of combined SSU and LSU data. Orders, classes, and families are indicated on the tree. ML bootstrap support values greater than 70 % are indicated along with Bayesian posterior probabilities ≥ 95 % for nodes. Members of *Pezizomycetes* are used as outgroup taxa. Freshwater lineages are labeled as Clades A–D and are shaded in blue and taxa isolated and described from freshwater habitats are indicated with Fresh W. Ascospore modifications are indicated by: ✪ = greatly elongating sheath; ● = thin to thick non-elongating sheath; ▭ = apical appendages; ○ = no sheath; ▲ = gelatinous pads. Scale bar indicates nucleotide substitutions per site.

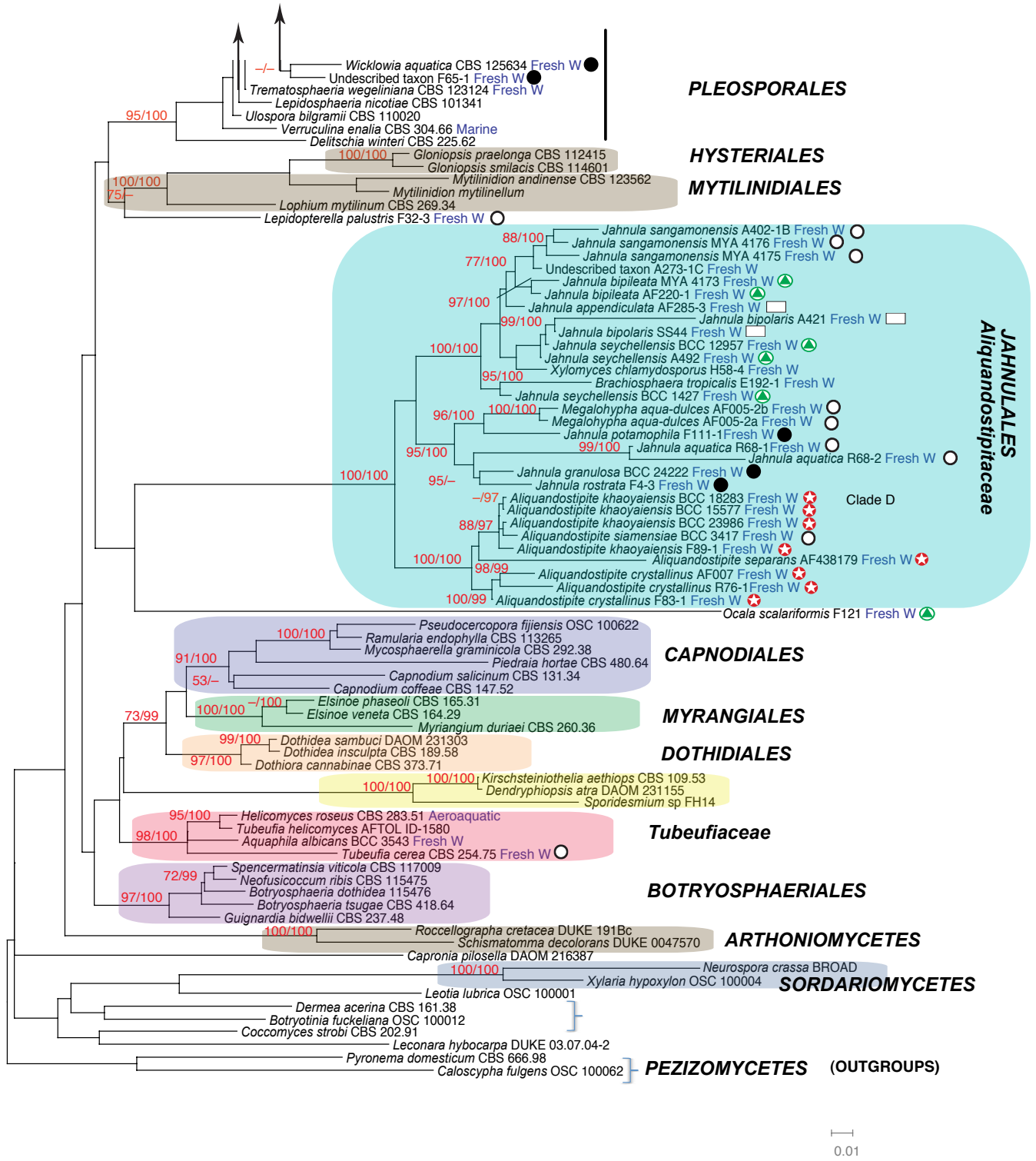


**Fig. 2.** (Continued).

in this study. The majority of freshwater *Dothideomycetes* had phylogenetic affinities to taxa in *Pleosporales* (Fig. 2). Four major clades (A–D) of freshwater fungi were recovered, of which three clades received ≥ 70 % Maximum Likelihood Bootstrap (MLB) support and ≥ 90% Bayesian Posterior Probability (BPP) (Fig. 2). *Lentitheciaceae* (Clade A) included six taxa, together with undescribed taxon A369-2B but was not supported by either MLB or BPP. *Amniculicolaceae* (Clade B) was well supported with 97 % ML bootstrap support and 100 % BPP. *Lindgomycetaceae* (Clade C) was also supported with 77 % MLB and 100 % BPP values. *Jahnulales* (Clade D) received 100 % MLB and 100 % BPP support and formed a strong monophyletic group.

Eight undescribed freshwater *Dothideomycetes* were dispersed throughout the *Pleosporomycetidae* as follows: A369-2B in *Lentitheciaceae;* F80-1 as sister taxon to *K. elaterascus*; A164 and A183 in *Lophiostomataceae* 1; A-25-1, F-60, and F-65 in *Lindgomycetaceae;* and A273-1c in *Jahnulales*. A few singletons such as *Lepidopterella palustris* and *Ocala scalariformis* are on single lineages without any relationships to known groups included in the analyses.

The anamorph genus *Xylomyces* was polyphyletic, with one species, *X. elegans*, placed with *Massarina* species in the *Pleosporales, and the other, X. chlamydosporus*, placed within *Jahnulales* (Fig. 2). The affinity of *Anguillospora longissima* (CS869-1D, Shearer isolate) to *Amniculicola lignicola*, *A. immersa* and *A. parva* (Fig. 2) confirms this relationship reported previously for a different isolate of *A. longissima* (Zhang *et al.* 2009a). *Tumularia aquatica*, originally assigned to *Massarina aquatica* (Webster 1965) was placed with *Lophiostoma glabrotunicatum,* an aquatic fungus collected in mountain streams in France on submerged wood of *Alnus glutinosa, Fagus sylvatica and Salix sp.* (Zhang *et al.* 2009c). *Taeniolella typhoides* occurred in a well-supported group with members of *Lindgomycetaceae* in *Pleosporales. Lemonniera pseudofloscula* isolates occurred among terrestrial taxa as a highly supported sister taxon to a clade of *Alternaria alternata, Alternaria* sp. and *Allewia eureka.* This placement is somewhat controversial and a more detailed study with additional isolates and more gene regions should be carried out.

## DISCUSSION

Within *Dothideomycetes*, the freshwater species occur in *Pleosporomycetidae* but not *Dothideomycetidae*. It is interesting to speculate on possible reasons for this pattern. First, overall there are more taxa in the *Pleosporomycetidae* than *Dothideomycetidae* resulting in a numerical imbalance between subclasses in most ecological and taxonomic groups. Second, many of the orders in *Dothideomycetidae* contain specialised plant pathogens, *e.g., Capnodiales, Myriangiales, and Botryosphaeriales,* many of which grow on leaves. It is possible that such specialised fungi have lost the genetic potential to adapt to a submerged, saprobic lifestyle. Third, the absence of pseudoparaphyses in *Dothideomycetidae* taxa may limit survival in aquatic habitats with fluctuating water levels. Pseudoparaphyses of aquatic species in *Pleosporomycetidae* are often abundant and surrounded by gel, which may protect the asci from desiccation during dry conditions. There is currently no experimental evidence, however, to support this idea.

Freshwater Dothideomycete species are distributed throughout the *Pleosporomycetidae* (Fig. 2). Several clades, however, contain numerous freshwater species and merit discussion. Clade A (*Lentitheciaceae*), which consists entirely of freshwater taxa, is not well supported in this study (Fig. 2). Reasons for this lack of support are not clear at this time. For a discussion of this clade, see Zhang *et al.* (2009b; this volume). The well-supported Clade B (*Amniculicolaceae*) consists of four freshwater teleomorph species and one aquatic hyphomycete anamorph species. This family is established and described in detail by Zhang *et al.* (2009b; this volume).

A third exclusively freshwater lineage is Clade C (*Lindgomycetaceae)* (Fig. 2). This well supported clade was first revealed during a recent molecular sequence-based study of *Massarina ingoldiana* Shearer & Hyde *s. l.* (Hirayama *et al.* 2010). A number of dothideomycetous aquatic species that have 1-septate, hyaline ascospores surrounded by a prominent gelatinous sheath that elongates greatly in water were included in this study. Analyses of a combined dataset of SSU and LSU sequences for a number of aquatic isolates of *M. ingoldiana* and other morphologically similar fungi along with the type specimens of *Massarina* and *Lophiostoma* were conducted. Their results showed that none of the aquatic taxa belonged in *Massarina* or *Lophiostoma* and that convergent evolution in ascospore morphology had occurred, confounding systematic placement based on ascospore morphology. Our results support the study by Hirayama *et al.* (2010) which found that taxa with 1-septate, hyaline ascospores with a large, elongating gelatinous sheath have evolved independently in several lineages within *Dothideomycetes* (*Lentitheciaceae, Lindgomycetaceae*, *and Aliquandostipitaceae*) (Fig. 2). Thus in freshwater *Dothideomycetes*, this form of the gelatinous sheath is not taxonomically informative at the family or genus level.

Clade D (*Jahnulales*) contains the greatest number of freshwater species (Fig. 2). The type species of *Jahnula*, *J. aquatica*, was described as *Amphisphaeria aquatica* by Plöttner and Kirschstein in 1906 from *Salix* wood in a wet ditch in Germany. Kirschstein (1936) subsequently changed the name of this fungus to *Jahnula*. The genus remained monotypic until 1999, when Hyde & Wong (1999) described five new tropical species based on morphological data. Currently, *Jahnula* and *Aliquandostipite*, a genus morphologically similar to *Jahnula* that was established by Inderbitzen *et al.* (2001), represent a well-supported lineage in *Dothideomycetidae* based on molecular and morphological data (Inderbitzen *et al.* 2001, Pang *et al.* 2002, Campbell *et al.* 2007, Suetrong *et al.* 2009, 2010). Pang *et al.* (2002) established a new order, *Jahnulales,* for this group. *Jahnulales* now contains numerous species representing four meiosporic genera and two mitosporic genera from freshwater habitats (Hyde 1992, Hyde & Wong 1999, Pang *et al.* 2002, Pinruan *et al.* 2002, Raja *et al.* 2005, 2008, Ferrer *et al.* 2007, Raja & Shearer 2006, 2007). *Manglicola guatemalensis*, collected from mangroves, was recently confirmed to belong in *Jahnulales* (Suetrong *et al.* 2010). There appear to be four, possibly five, separate lineages within *Jahnulales,* but further molecular work is needed to confirm these lineages. Species in this clade are well adapted for aquatic habitats with large-celled pseudothecia and ascospores filled with lipid guttules and equipped with a variety of gelatinous appendages, pads and sheaths (Fig. 2). Thus far, all members in the order have broad vegetative hyphae (10–40 µm) that attach the fungi to softened, submerged wood.

Clade *Lophiostomataceae* 1 was well supported as a whole in this study and studies by Tanaka & Hosoya (2008) and Zhang *et al.* (2009c), but relationships within this clade were not well resolved. Several taxa within this clade are undescribed and additional morphological and molecular data are needed to further resolve relationships within this group.

Two interesting freshwater taxa in *Dothideomycetidae* included in this study, *Ocala scalariformis* and *Lepidopterella palustris*, did not show strong phylogenetic affinities with any of the major families and orders included in the *Dothideomycetes* (Fig. 2). These so called singletons each has a distinctive combination of morphological characteristics that perhaps make them unique among other *Dothideomycetes* taxa included in the phylogeny. *Ocala scalariformis* possesses morphological characters that include superficial to erumpent, globose to subglobose, hyaline perithecial ascomata with an ostiole; cellular pseudoparaphyses; fissitunicate asci; and hyaline, 1-septate, thick-walled ascospores with appendages (Raja *et al.* 2009a). However, based on the combined SSU and LSU phylogeny, *Ocala scalariformis* is placed as basal to the *Jahnulales,* without any statistical support. *Lepidopterella palustris* has black, cleistothecial ascomata appearing as raised dome-shaped structures on the substrate; hamathecium of hyaline, septate, narrow pseudoparaphyses not embedded in a gel matrix; thick-walled, globose to subglobose, broadly rounded, fissitunicate asci; and brown butterfly shaped ascospores (Shearer & Crane 1980, Raja & Shearer 2008). Based on our phylogeny it forms a single branch by itself, basal to the

*Mytilindiales* with moderate bootstrap support (Fig. 2). It is possible that these singletons represent new lineages currently unknown in the *Dothideomycetes.*

Belliveau & Baerlocher (2005) showed that aquatic hyphomycetes have multiple origins within the ascomycetes. In this study, we included some hyphomycete taxa that had phyologenetic affinities to the *Dothideomycetes* based on previous studies (Belliveau & Bärlocher 2005, Campbell *et al.* 2006, 2007, Zhang et *al.* 2009c). These taxa are: *Anguillospora longissima*, *Lemonniera pseudofloscula, Taeniolella typhoides, Tumularia aquatica, and Brachiosphaera tropicalis.* Previous studies showed that *Anguillospora longissima* had a strong affinity to *Pleosporales* and was a sister species to *Kirschsteiniothelia maritima* (Baschien 2003, Belliveau & Bärlocher 2005). In contrast, Voglmayr (2004) reported a close relationship between an aeroaquatic fungus, *Spirosphaera cupreorufescens*, and *A. longissima*. Baschien *et al.* (2006) confirmed the close relationships of the five isolates of *A. longissima* to *Spirosphaera cupreorufescens*. Zhang *et al.* (2009c) in a maximum parsimony tree generated from partial 28S rDNA gene sequences showed a 91 % bootstrap support for a clade formed by *A. longissima*, *Spirosphaera cupreorufescens, Repetophragma ontariense* and three species of *Amniculicola*. In our analyses, *A. longissima* is placed in the new aquatic family *Amniculicolaceae* (Clade B) Fig. 2 (See Zhang *et al.* 2009b; this volume).

*Taeniolella typhoides* was described without a teleomorph. Here it forms a well-supported sister clade with *Massariosphaeria typhicola.* The epithet of *T. typhoides* may indicate some relationship to *Typha*, but this is a casual coincidence only as "*typhoides*" is for "similar to *Typha*". The teleomorph of *Taeniolella* is *Glyphium, Mytilinidiales* (Kirk *et al.* 2008).

*Tumularia aquatica* is the type species of *Tumularia* and was connected by Webster (1965) to the teleomorph, *Massarina aquatica*. *Massarina aquatica* was later recombined on the basis of morphology in *Lophiostoma* as *L. aquatica* (Hyde *et al.* 2002). In this study, *T. aquatica* is placed with *Lophiostoma glabrotunicatum* in the *Lophiostomataceae 2/Melannomataceae* Clade, but lacks significant bootstrap support (Fig. 2).

*Brachiosphaera tropicalis* has conidia very similar to those of *Actinosporella megalospora* and the two species are sometimes confused with each other. On the basis of pure culture studies Descals *et al.* (1976) pointed out the essentially different conidiogenesis (blastic sympodial in *Brachiosphaera vs.* retrogressive thallic in *Actinosporella*) and also subtle differences in conidial morphology (constricted appendage insertion in *Brachiosphaera vs*. unconstricted in *Actinosporella).* The placement of *Brachiosphaera* within *Jahnulales* (Campbell *et al.* 2007) confirms its unrelatedness to *Actinosporella,* which has been connected to the *Pezizales* by Descals and Webster (1978).

The genus *Lemonniera* is characterised by tetraradiate conidia with long arms, phialidic conidiogenesis, and formation of minute dark sclerotia in culture. Previously, it has been shown to be polyphyletic and different species of *Lemonniera* are placed in two distinct clades, namely the *Leotiomycetes* and the *Dothideomycetes* (Campbell *et al.* 2006). In our study we used two isolates of *L. pseudofloscula* previously sequenced by Campbell *et al.* (2006). These isolates form a strongly supported monophyletic group within the *Pleosporaceae*.

More recently, Prihatini *et al.* (2008) have shown that *Speiropsis pedatospora* (Tubaki 1958) has phylogenetic affinities within the *Jahnulales* based on ITS rDNA data. Also, in another recent study by Jones *et al.* (2009), *Sigmoidea prolifera* and *Pseudosigmoidea cranei*, two aquatic hyphomycetes were shown to have phylogenetic affinities with the *Phaeotrichaceae, Pleosporales* based on SSU data. Sequencing of additional aquatic hyphomycete taxa in the future will continue to shed light on the evolutionary relationships of freshwater aquatic hyphomycetes to different lineages within the *Dothideomycetes.*

## CONCLUSIONS

The freshwater *Dothideomycetes* occur primarily in the *Pleosporomycetidae* as opposed to the *Dothideomycetidae* and appear to have adapted to freshwater habitats numerous times, often through ascospore adaptations, and sometimes, through anamorph conidial adaptations. Ascospores and conidiospores of freshwater fungi are under strong selective pressure to disperse and attach to substrates in freshwater habitats in order for the fungi to complete their life cycles. Thus ascospore features that facilitate dispersal and attachment may not be as reliable as other morphological features such as ascomata and hamathecia in interpreting phylogenetic relationships among freshwater *Dothideomycetes.* This idea is supported by the presence of similar ascospore modifications such as the presence of gelatinous ascospore sheaths in phylogenetically distant taxa. Further support is the presence of tetraradiate conidia present in widely separated clades.

The presence of morphologically unique singletons within the molecular-based phylogenetic tree of *Dothideomycetes* suggests that we need to further sample the freshwater ascomycetes to identify close relatives of these taxa.

We expect that future collections from freshwater habitats will modify the phylogeny presented in this paper by increasing the size and support values of existing clades containing freshwater species and in increasing the number of exclusively freshwater clades.

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## SUPPLEMENTARY INFORMATION

**Table 1.** Species used in this study.

| Species | Isolate number | Source | GenBank No. | |
|---|---|---|---|---|
| | | | SSU | LSU |
| *Aliquandostipite crystallinus** | F83-1 | Raja & Shearer | GU266221 | GU266239 |
| | AF007 | – | EF175631 | EF175652 |
| | R76-1 | – | EF175630 | EF175651 |
| *Aliquandostipite khaoyaiensis* | F89-1 | Raja & Shearer | EF175625 | EF175647 |
| | SS2961 | BCC 15577 | EF175626 | EF175648 |
| | SS3028 | BCC 23986 | EF175627 | EF175649 |
| | SS3321 | BCC 18283 | EF175628 | EF175650 |
| *Aliquandostipite separans* | | – | AF438179 | – |
| *Aliquandostipite siamensiae* | SS81.02 | BCC 3417 | EF175645 | EF175666 |
| *Allewia eureka* | | DAOM 195275 | DQ677994 | DQ678044 |
| *Alternaria alternata* | | CBS 916.96 | DQ678031 | DQ678082 |
| *Alternaria* sp. (as *Clathrospora diplospora*) | | CBS 174.51 | DQ678016 | DQ678068 |
| *Amniculicola immersa* | – | KD Hyde | GU456295 | FJ795498 |
| *Amniculicola lignicola* | – | KD Hyde | EF493863 | EF493861 |
| *Amniculicola parva* | | KD Hyde | GU296134 | FJ795497 |
| *Anguillospora longissima** | CS869-1D | Shearer | GU266222 | GU266240 |
| *Aquaticheirospora lignicola* | | – | AY736377 | AY736378 |
| *Aquaphila albicans* | | BCC 3543 | DQ341093 | DQ341101 |
| *Ascochyta pisi* var. *pisi* | | CBS 126.54 | DQ678018 | DQ678070 |
| *Ascorhombispora aquatica* | | – | – | EU196548 |
| *Bimuria novae-zelandiae* | | CBS 107.19 | AY016338 | AY016356 |
| *Botryosphaeria dothidea* | | CBS 115476 | DQ677998 | DQ678051 |
| *"Botryosphaeria" tsugae* | | CBS 418.64 | AF271127 | DQ767655 |
| *Botryotinia fuckeliana* | | OSC 100012 | AY544695 | AY544651 |
| *Brachiosphaera tropicalis* | E192-1 | Shearer | GU266223 | EF175653 |
| *Byssothecium circinans* | | CBS 675.92 | AY016339 | AY016357 |
| *Caloscypha fulgens* | | OSC 100062 | DQ247807 | DQ247799 |
| *Capnodium coffeae* | | CBS 147.52 | DQ247808 | DQ247800 |
| *Capnodium salicinum* | | CBS 131.34 | DQ6779977 | DQ678050 |
| *Capronia pilosella* | | DAOM 216387 | DQ823106 | DQ823099 |
| *Coccomyces strobi* | | CBS 202.91 | DQ471027 | DQ470975 |
| *Cheirosporium triseriale* | | – | – | EU413954 |
| *Cochliobolus heterostrophus* | | CBS 134.39 | AY544727 | AY544645 |
| *Cochliobolus sativus* | | DAOM 216378 | DQ677995 | DQ678045 |
| *Coniothyrium obiones* | | CBS 453.68 | DQ678001 | DQ678054 |
| *Coniothyrium palmarum* | | CBS 400.71 | DQ678008 | DQ767653 |
| *Cucurbitaria elongata* | | CBS 171.55 | DQ678009 | DQ678061 |
| *Delitschia winteri* | | CBS 225.62 | DQ678026 | DQ678077 |
| *Dendryphiella arenaria* | | CBS 181.85 | DQ471022 | DQ470971 |
| *Dendyphiopsis atra* | | DAOM 231155 | DQ677996 | DQ678046 |
| *Dermea acerina* | | CBS 161.38 | DQ247809 | DQ247801 |
| *Didymella cucurbitacearum* | | IMI 373225 | AY293779 | AY293792 |
| *Dothidea insculpta* | | CBS 189.58 | DQ247810 | DQ247802 |
| *Dothidea sambuci* | | DAOM 231303 | AY544722 | AY544681 |

**Table 1.** (Continued).

| Species | Isolate number | Source | GenBank No. | |
|---|---|---|---|---|
| | | | SSU | LSU |
| *Dothiora cannabinae* | | CBS 373.71 | DQ479933 | DQ470984 |
| *Elsinoë phaseoli* | | CBS 165.31 | DQ678042 | DQ678095 |
| *Elsinoë veneta* | | CBS 164.29 | DQ678007 | DQ678060 |
| *Gloniopsis praelonga* | | CBS 112415 | FJ161134 | FJ161173 |
| *Gloniopsis smilacis* | | CBS 114601 | FJ161135 | FJ161174 |
| *Guignardia bidwelli* | | CBS 237.48 | DQ678034 | DQ678085 |
| *Helicascus kanaloanus* | | ATCC 18591 | AF053729 | – |
| *Helicomyces roseus* | | CBS 283.51 | DQ678032 | DQ678083 |
| *Herpotrichia diffusa* | | CBS 250.62 | DQ678019 | DQ678071 |
| *Herpotrichia juniperi* | | CBS 200.31 | DQ678029 | DQ678080 |
| *Jahnula appendiculata** | AF285-3 | Shearer | GU266224 | GU266241 |
| *Jahnula aquatica* | R68-1 | Raja & Shearer | EF175633 | EF175655 |
| | R68-2 | Raja & Shearer | EF175632 | NA |
| *Jahnula bipileata* | F49-1 | MYA 4173 | EF175635 | EF175657 |
| | AF220-1 | Shearer | EF175634 | EF175656 |
| *Jahnula bipolaris* | SS44 | BCC 3390 | EF175637 | EF175658 |
| | A421 | Shearer | EF175636 | – |
| *Jahnula granulosa* | SS1562 | BCC24222 | EF175638 | EF175659 |
| *Jahnula potamophila** | F111-1 | Raja & Shearer | GU266225 | GU266242 |
| *Jahnula rostrata* | F4-3 | MYA4176 | GU266226 | EF175660 |
| *Jahnula sangamonensis* | A482-1B | MYA 4174 | EF175640 | EF175662 |
| | A402-1B | Shearer | EF175639 | EF175661 |
| | F81 | MYA 4175 | EF175641 | EF175663 |
| *Jahnula seychellensis* | SS2133.1 | BCC 14207 | EF175644 | EF175665 |
| | SS2113.2 | BCC 12957 | EF175643 | EF175664 |
| | A492 | Shearer | EF175642 | GU266243 |
| *Kirschsteiniothelia aethiops* | | CBS 109.53 | AY016344 | AY016361 |
| *Kirschsteiniothelia elaterascus* | A22-11B-/ | – | AF053728 | – |
| | | | – | AY787934 |
| *Lecanora hybocarpa* | | DUKE 03.07.04-2 | DQ782883 | DQ782910 |
| *Lentithecium aquaticum* | | CBS 123099 | FJ795477 | FJ795434 |
| *Lentithecium arundinaceum* | | CBS 619.86 | DQ813513 | DQ813509 |
| *Lemonniera pseudofloscula* | | CCM F-0484 | – | DQ267631 |
| | | CCM F-43294 | – | DQ267632 |
| *Leotia lubrica* | | OSC100001 | AY544687 | AY544644 |
| *Lepidopterella palustris** | F32-3 | Raja & Shearer | GU266227 | GU266244 |
| *Leptosphaeria maculans* | | DAOM 229267 | DQ470993 | DQ470946 |
| *Lepidosphaeria nicotiae* | | CBS 101341 | – | DQ678067 |
| *Lindgomyces cinctosporae* | R56-1 | | AB522430 | AB522431 |
| | R56-3 | Raja & Shearer | GU266238 | GU266245 |
| *Lindgomyces breviappendiculatus* | KT 215 | JCM 12702/MAFF 239291 | AB521733 | AB521748 |
| | KT 1399 | JCM 12701/MAFF 239292 | AB521734 | AB521749 |
| *Lindgomyces ingoldianus* | A39-1 | ATCC200398 | AB521719 | AB521736 |
| | KH 100 | JCM 16479 | AB521720 | AB521737 |
| *Lindgomyces* sp. | KH 241 | JCM16480 | AB521721 | AB521738 |
| *Lindgomyces rotundatus* | KT 966 | JCM 16481/MAFF 239473 | AB521722 | AB521739 |
| | KT 1096 | JCM 16482 | AB521723 | AB521740 |

**Table 1.** (Continued).

| Species | Isolate number | Source | GenBank No. | |
|---|---|---|---|---|
| | | | SSU | LSU |
| | KH 114 | JCM 16484 | AB521725 | AB521742 |
| | KT1107 | JCM 16483 | AB521724 | AB521741 |
| *Lophiostoma arundinis* | | CBS 269.34 | DQ782383 | DQ782384 |
| *Lophiostoma crenatum* | | CBS 629.86 | DQ678017 | DQ678069 |
| *Lophiostoma glabrotunicatum* | | IFRD 2012 | FJ795481 | FJ795438 |
| *Lophiostoma macrostomum* | KT 635 | JCM 13545 | AB521731 | AB433273 |
| | KT 709 | JCM 13546 MAFF 239447 | AB521732 | AB433274 |
| | | | SSU | LSU |
| *Lophium mytilinum* | | CBS 269.34 | DQ678030 | DQ678081 |
| *Massaria platani* | | CBS 221.37 | DQ678013 | DQ678065 |
| *Massarina australiensis* | | – | AF164364 | – |
| *Massarina bipolaris* | | – | AF164365 | – |
| *Massarina eburnea* | H 3953 | JCM 14422 | AB521718 | AB521735 |
| | | – | AF164366 | – |
| | | – | AF164367 | |
| *Massariosphaeria typhicola* | KT 667 | MAFF 239218 | AB521729 | AB521746 |
| | KT 797 | MAFF 239219 | AB521730 | AB521747 |
| *Megalohypha aqua-dulces** | AF005-2a | – | GU266228 | EF175667 |
| | AF005-2b | – | – | EF175668 |
| *Melanomma radicans* | | ATCC 42522 | U43461 | U43479 |
| *Montagnula opulenta* | | CBS 168.34 | AF164370 | DQ678086 |
| *Mycosphaerella graminicola* | | CBS 292.38 | DQ678033 | DQ678084 |
| *Myriangium duriaei* | | CBS 260.36 | AY016347 | DQ678059 |
| *Mytilinidion andinense* | | EB 0330 (CBS 123562 | FJ161159 | FJ161199 |
| *Mytilinidion mytilinellum* | | CBS 303.34 | FJ161144 | FJ161184 |
| *Neofusicoccum ribis* | | CBS 115475 | DQ678000 | DQ678053 |
| *Neurospora crassa* | | BROAD | X04971 | AF286411 |
| *Ocala scalariformis** | F121-1 | Raja & Shearer | GU266229 | – |
| *Ophiosphaerella herpotricha* | | CBS 620.86 | DQ678010 | DQ678062 |
| | | CBS 240.31 | DQ767650 | DQ767656 |
| *Phaeodothis winteri* | | CBS 182.58 | DQ678021 | DQ678073 |
| *Phaeosphaeria avenaria* | | DAOM 226215 | AY544725 | AY544684 |
| *Phaeosphaeria eustoma* | | CBS 573.86 | DQ678011 | DQ678063 |
| *Phoma herbarum* | | CBS 276.37 | DQ678014 | DQ678066 |
| *Piedraia hortae* | | CBS 480.64 | AY016349 | AY016366 |
| *Pleomassaria siparia* | | CBS 279.74 | DQ678027 | DQ678078 |
| *Pleospora herbarum* var. *herbarum* | | CBS 714.68 | DQ767648 | DQ678049 |
| | | CBS 514.72 | DQ247812 | DQ247804 |
| *Preussia terricola* | | DAOM 230091 | AY544726 | AY544686 |
| *Pseudocercospora fijiensis* | | OSC 100622 | DQ767652 | DQ678098 |
| *Pyrenophora phaeocomes* | | DAOM 222769 | DQ499595 | DQ499596 |
| *Pyrenophora tritici-repentis* | | OSC 100066 | AY544716 | AY544672 |
| *Pyronema domesticum* | | CBS 666.98 | DQ247813 | DQ247805 |
| *Quintaria lignatilis* | | – | QLU43462 | – |
| *Ramularia endophylla* | | CBS 113265 | DQ471017 | DQ470920 |
| *Roccellographa cretacea* | | DUKE 191Bc | DQ883705 | DQ883696 |
| *Schismatomma decolorans* | | DUKE 0047570 | AY548809 | AY548815 |

**Table 1.** (Continued).

| Species | Isolate number | Source | GenBank No. | |
|---|---|---|---|---|
| | | | SSU | LSU |
| *Semimassariosphaeria typhicola* ** | | | GU296174 | FJ795504 |
| *Spencermartinsia viticola* | | CBS 117009 | DQ678036 | DQ678087 |
| *Sporormiella minima* | | CBS 524.50 | DQ678003 | DQ678056 |
| *Sporidesmium* sp. | FH14 | – | GU266230 | – |
| *Taeniolella typhoides* | | CCM F-10198/extype | GU266231 | – |
| *Tingoldiago graminicola* | KH 68 | JCM 16485 | AB521726 | AB521743 |
| | KT 891/ | MAFF 239472 | AB521727 | AB521744 |
| | KH 155/ | JCM 16486 | AB521728 | AB521745 |
| *Trematosphaeria hydrophila* | | IFRD 2037 | GU261721 | – |
| *Trematosphaeria heterospora* | | CBS 644.86 | AY016354 | AY016369 |
| *Trematosphaeria pertusa* | | CBS 400.97 | DQ678020 | DQ678072 |
| *Trematosphaeria wegeliniana* | | CBS 123124 | GU261720 | GU261722 |
| | | | SSU | LSU |
| *Tubeufia cerea* | | CBS 254.75 | DQ471034 | DQ470982 |
| *Tubeufia helicomyces* | | – | DQ767649 | DQ767654 |
| *Tumularia aquatica* | | CCM F-02081 | AY357287 | – |
| *Ulospora bilgramii* | | CBS 110020 | DQ678025 | DQ678076 |
| *Verruculina enalia* | | CBS 304.66 | DQ678028 | DQ678079 |
| *Westerdykella cylindrica* | | CBS 454.72 | AY016355 | AY004343 |
| *Wicklowia aquatica** | F76-2 | CBS 125634 | GU266232 | GU045445 |
| *Xylaria hypoxylon* | | OSC 100004 | AY544719 | AY544676 |
| *Xylomyces chlamydosporus** | H58-4 | | GU266233 | EF175669 |
| *Xylomyces elegans** | H80-1 | | GU266234 | – |
| Undescribed taxon A25-1* | | Shearer | – | GU266246 |
| Undescribed taxon R60-1* | | Raja & Shearer | GU266235 | GU266247 |
| Undescribed taxon F65-1 | | Shearer | GU266236 | GU266248 |
| Undescribed taxon A369-1* | | Raja & Shearer | – | GU266249 |
| Undescribed taxon F80-1* | | Shearer | GU266237 | GU266250 |
| Undescribed taxon A164-1C* | | Shearer | – | GU266251 |
| Undescribed taxon A164-1D* | | Shearer | – | GU266252 |
| Undescribed taxon A183-1C* | | Shearer | – | GU266253 |
| Undescribed taxon A183-1D* | | Shearer | – | GU266254 |
| Undescribed taxon A273-1C* | | Shearer | | GU266255 |

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# Molecular systematics of the marine *Dothideomycetes*

S. Suetrong[1, 2], C.L. Schoch[3], J.W. Spatafora[4], J. Kohlmeyer[5], B. Volkmann-Kohlmeyer[5], J. Sakayaroj[2], S. Phongpaichit[1], K. Tanaka[6], K. Hirayama[6] and E.B.G. Jones[2*]

[1]*Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand;* [2]*Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Paholyothin Road, Khlong 1, Khlong Luang, Pathum Thani, 12120, Thailand;* [3]*National Center for Biothechnology Information, National Library of Medicine, National Institutes of Health, 45 Center Drive, MSC 6510, Bethesda, Maryland 20892-6510, U.S.A.;* [4]*Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, 97331, U.S.A.;* [5]*Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557, U.S.A.;* [6]*Faculty of Agriculture & Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan*

**Correspondence*: E.B. Gareth Jones, remispora@gmail.com

**Abstract:** Phylogenetic analyses of four nuclear genes, namely the large and small subunits of the nuclear ribosomal RNA, transcription elongation factor 1-alpha and the second largest RNA polymerase II subunit, established that the ecological group of marine bitunicate ascomycetes has representatives in the orders *Capnodiales, Hysteriales*, *Jahnulales*, *Mytilinidiales*, *Patellariales* and *Pleosporales*. Most of the fungi sequenced were intertidal mangrove taxa and belong to members of 12 families in the *Pleosporales*: *Aigialaceae*, *Didymellaceae, Leptosphaeriaceae*, *Lenthitheciaceae*, *Lophiostomataceae*, *Massarinaceae, Montagnulaceae*, *Morosphaeriaceae*, *Phaeosphaeriaceae*, *Pleosporaceae*, *Testudinaceae* and *Trematosphaeriaceae*. Two new families are described: *Aigialaceae* and *Morosphaeriaceae*, and three new genera proposed: *Halomassarina*, *Morosphaeria* and *Rimora.* Few marine species are reported from the *Dothideomycetidae* (e.g. *Mycosphaerellaceae*, *Capnodiales*), a group poorly studied at the molecular level. New marine lineages include the *Testudinaceae* and *Manglicola guatemalensis* in the *Jahnulales*. Significantly, most marine *Dothideomycetes* are intertidal tropical species with only a few from temperate regions on salt marsh plants (*Spartina* species and *Juncus roemerianus*), and rarely totally submerged (e.g. *Halotthia posidoniae* and *Pontoporeia biturbinata* on the seagrasses *Posidonia oceanica* and *Cymodocea nodosum*). Specific attention is given to the adaptation of the *Dothideomycetes* to the marine milieu, new lineages of marine fungi and their host specificity.

**Key words:** *Dothideomycetes*, ecology, marine fungi, multi-locus, new genera, systematics.
**Taxonomic novelties:** *Aigialaceae* Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & Schoch, fam. nov., *Halomassarina* Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & Schoch, gen. nov., *Halomassarina thalassiae* (Kohlm. & Volkm.-Kohlm.), Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & Schoch, comb. nov., Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm.,comb. nov., Clade V. *Morosphaeriaceae* Suetrong, Sakayaroj, E.B.G. Jones, & Schoch, fam. nov., *Morosphaeria velataspora* (K.D. Hyde & Borse) Suetrong, Sakayaroj, E.B.G. Jones & Schoch, comb. nov., *Morosphaeria ramunculicola* (K.D. Hyde) Suetrong, Sakayaroj, E.B.G. Jones & Schoch, comb. nov., *Rimora* Kohlm., Volkm-Kohlm., Suetrong, Sakayaroj, E.B.G. Jones, gen. nov., *Rimora mangrovei* (Kohlm. & Vittal) Kohlm.,Volkm-Kohlm., Suetrong, Sakayaroj, E.B.G. Jones, comb. nov.

## INTRODUCTION

Most marine *Dothideomycetes* are intertidal, primarily from mangrove habitats and rely on the active discharge of their ascospores. They are frequently found as saprobes of decaying woody materials in the marine environment. The species that occur completely submerged in the sea are mostly parasites or symbionts of seagrasses or marine algae. It is not clear how ascospore discharge occurs in these species as their hosts are often submerged for most of the time. Jones *et al*. (2009) list 64 genera and *ca.* 108 species of marine *Dothideomycetes* that fall into three accepted orders (*Capnodiales*, *Dothideales*, *Pleosporales*), three orders *incertae sedis* (*Hysteriales*, *Patellariales*, *Jahnulales*) and 23 genera not assigned with confidence to any order. Most of these higher order taxa are represented by a single genus or species while most are members of the *Pleosporales* with 25 genera and 61 species (+ 13 genera, 20 species, *incertae sedis*). Taxa that can not be assigned with confidence to either an order or family include *Aigialus*, *Halotthia, Lautospora, Manglicola*, *Mauritiana*, *Passeriniella*, *Pontoporeia*, and *Tirisporella*. A notable feature of the marine *Dothideomycetes* is how few anamorphs are known. Examples include *Amarenographium metableticum*, *Scolecosporiella typhae*, *Stemphylium triglochinicola* and *Phialophora* cf. *olivacea* and molecular data indicates that the teleomorphs of *Amorosia littoralis*, *Dendryphiella salina* and *D. arenaria* may be in the *Pleosporales* (Mantle *et al*. 2006, Jones *et al*. 2008). This paucity of marine anamorphic fungi is in marked contrast to freshwater fungi and terrestrial genera of the class (Cai *et al*. 2006, Shenoy *et al*. 2007, Shearer *et al*. 2009; this volume).

Marine *Dothideomycetes* occur on a wide range of substrata: mangrove wood, twigs and leaves; sea and marsh grasses (especially *Spartina* spp. and *Juncus roemerianus*) (Kohlmeyer *et al*. 1995a–c, 1996, 1997a–b). Culms and leaves of sea and marsh grasses are ideal substrata for saprobic fungi because they may remain standing for several years during and after senescence (Christian *et al*. 1990, Kohlmeyer & Volkmann-Kohlmeyer 2001). Other species are found on brown and red seaweeds, *e.g. Lautitia danica* and *Pleospora gracilariae* (Schatz 1984, Simmons & Schatz 1989), on wood associated with sand *e.g. Caryospora australiensis* and *Decaisnella formosa* (Abdel-Wahab & Jones 2003) or on the brackish water palm *Nypa fruticans*, *e.g. Carinispora nypae*, *Herpotrichia nypicola*, *Tirisporella beccariana* and *Helicascus nypae* (Jones *et al*. 1996, Hyde & Alias 2000). Few marine *Dothideomycetes* produce elaborate appendaged ascospores, and

most possess gelatinous sheaths that swell in water when released from the asci (*Massarina velataspora* and *Tremateia halophila*). Genera with appendaged ascospores, although generally modifications of a gelatinous sheath, include: *Carinispora nypae*, *Decorospora gaudefroyi* and *Falciformispora lignatilis*.

The main objective of this study is to provide information on the taxa that are unique to the marine milieu, *e.g*. *Aigialus* spp., *Manglicola guatemalensis*, *Halotthia posidoniae* and *Pontoporeia biturbinata* and confirm the taxonomic assignment of other marine ascomycetes within the context of a well sampled analysis with other related fungi.

## MATERIAL AND METHODS

### Collection of fungi

Drift and attached wood, culms and leaves of marsh plants, seagrasses and seaweeds were collected from a variety of habitats and geographical locations, placed in clean plastic bags and returned to the laboratory. After washing with freshwater to remove sediments, the samples were examined for fungi. Samples were kept moist by spraying with sterilised distilled water. Sporulating fungi were examined, identified, illustrated and single-spore isolations made. Most of the fungi sequenced in this study were obligate species, but some facultative and halotolerant terrestrial taxa from *Juncus roemerianus* have also been included so as to increase the sampling diversity.

### Fungal isolates and culture characteristics

A selection of specimens were isolated by cutting the top of an ascoma with a sterilised razor blade, removing the contents of the centrum by making a spore suspension and then streaking the spores on antibiotic seawater agar (Kohlmeyer & Kohlmeyer 1979, Schoch *et al*. 2007) and germinating spores picked up. Other single ascospore isolations were made on cornmeal seawater agar (CMA/SW) with added antibiotics (streptomycin sulfate 0.5g/L, penicillin G 0.5 g/L) and allowed to germinate overnight. Germinating spores were transferred to a fresh agar plate and incubated for 2 wk at 25 ºC and deposited in relevant culture collections (Table 1 - see online Supplementary Information).

### DNA extraction, amplification and sequencing

Fungal genomic DNA from a selection of cultures was isolated by filtering mycelia grown in seawater broth at 22 ºC with subsequent lyophilisation (Spatafora *et al*. 1998). DNA was then extracted using the FastDNA kit and cells were ground on the Fast-Prep instrument from MPI Biochemicals (Irvine, CA, U.S.A.) following manufacturer recommendations. Fungal biomass was harvested for a different set of isolates by filtering through cheesecloth, and washed several times with sterile distilled water. The harvested mycelium was stored at -20 °C and ground to a fine powder with a mortar and pestle. Fifty to 100 mg ground fungal mycelium was placed into 400 mL lysis buffer (O'Donnell *et al*. 1997) and DNA extracted as follows: the tube was incubated at 70 °C for 30 min, and an equal volume of phenol-chloroform (PIERCE) added. The upper liquid phase was transferred to a new microtube containing chilled absolute ethanol and 7.5 M ammonium acetate. The mixture was kept at -20 °C for 30 min, or until the DNA had precipitated, and then centrifuged at 14 000 rpm, 4 °C, for 15 min. The DNA pellet was washed twice with chilled 75 % ethanol and air dried. The DNA was resuspended in 50 mL TE buffer and checked for quantity and quality by 1 % agarose gel electrophoresis.

The following four genes were chosen for this study: small (18S) and large subunit (28S) of the nuclear ribosomal DNA (SSU, LSU) plus the gene fragments from the second largest subunit of RNA polymerase (*RPB2*) and the translation elongation factor 1-alpha (*TEF1*) gene. The rDNA was amplified with *Taq* DNA polymerase from FERMENTAS (Cat.No. MBDOEPO402) using PCR Model MJ Research DYAD ALD *ALD* 1244 thermocycler (*MJ Research*, Waltham, MA). Primers used for amplification include the SSU, LSU, *RPB2* and *TEF1* (White *et al*. 1990, Bunyard *et al*. 1994, Liu *et al.* 1999, Rehner 2001, respectively). The PCR products were purified using a NucleoSpin Extraction Kit (Macherey-Nagel, Germany), following the manufacturer's instructions. The characterisation of PCR products was performed via agarose gel electrophoresis on 1 % agarose gel containing ethidium bromide as the staining agent. PCR products were directly sequenced by Macrogen Inc., Korea. The sequencing primers used for as the different regions are SSU: NS1, NS3, NS4, NS6 (White *et al*. 1990); LSU: JS1, JS8, LROR and LR7 (Bunyard *et al.* 1994); *TEF1*: 983F, 2218R, CEFF2 and CEFR2 (Rehner 2001); *RPB2*: 5F1, 5F2, 7cR and 7R (Liu *et al.* 1999). Each sequence was checked for ambiguous bases and assembled using BioEdit v. 6.0.7 (Hall 2004) and SeqMerge, forming part of the GCG v. 10 software suite (Accelrys, San Diego, U.S.A.).

### Sequence alignment and phylogenetic analyses

A total of 51 species (90 new sequences – Table 1) from the *Dothideomycetes*, representing 46 teleomorphic genera and five anamorphic genera were analysed along with reference fungal sequences from fungal families that were downloaded from the GenBank (listed in Table 1).

The consensus sequences for each DNA region were initially aligned with ClustalW v. 1.6 (Thompson *et al*. 1994) and improved in MUSCLE (Edgar 2004) (as part of Geneious Pro v. 4.7.4 (Biomatters, Auckland, N.Z.). When necessary new sequences were added to a core set of seed sequences using MAFFT v. 6.708b (Katoh & Toh 2008) using the e-insi option. Sequence homologies were also analysed using BLAST (Altschul *et al*. 1990) to facilitate the selection of other fungal sequences to be used in the analyses. Alignments were checked and manually optimised along with other sequences obtained from the GenBank nucleotide database. The dataset was refined visually in BioEdit v. 7.0.1 (Hall 2004). Incomplete data at the 5´- and 3´-end of partial sequences were coded as missing. Following Wiens (2006), we included taxa in our multi-locus matrix even if they did not have all genes present. All absent genes were coded as missing data, forming at least 30 % of the total characters. Two members of the *Arthoniomycetes*, namely *Roccella fuciformis* and *Opegrapha dolomitica*, were chosen as outgroup sequences based on their placement as sister to the *Dothideomycetes* (Schoch *et al*. 2009).

Phylogenetic trees based on individual SSU, individual LSU, combined SSU and LSU and combined SSU, LSU and TEF datasets (data not shown) were congruent with the combined SSU, LSU, *RPB2* and *TEF1* data sets. However the position of the taxa *Biatriospora marina* and *Quintaria lignatilis* (in Clades XIV and XVI, respectively) and *Saccardoella rhizophorae* (unresolved taxon) were not constant. The phylogenetic analyses of the combined SSU, LSU, *RPB2* and *TEF1* data were performed using parsimony, Bayesian and maximum likelihood algorithms.

(i) Maximum parsimony (MP) analyses: MP analyses were performed using PAUP v. 4.0b10 (Swofford 2003). Gaps were treated as missing data with 100 replicates of random stepwise addition of sequences and tree-bisection reconnection (TBR) branch-swapping. All characters were given equal weight. The consistency indices (CI; Kluge & Farris 1969), retention indices (RI; Farris 1989) and rescaled consistency indices (RC; Farris 1989) were calculated for each tree generated. Bootstrap support values (Felsenstein 1985) were calculated for all parsimony analyses by 1000 bootstrap replicates (full heuristic searches, 10 replicates of random stepwise addition of sequences). Maximum parsimony bootstrap values (MPBP) equal or greater than 50 % are given above each node (Fig. 1).

(ii) Bayesian analyses (Larget & Simon 1999): The model of substitution used for Bayesian analyses was chosen with MrModeltest v. 2.2 (Nylander 2004). Independent Bayesian phylogenetic analyses were performed in MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) using a uniform [GTR+I+G] model, lset nst = 6 rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The Metropolis-Coupled Markov Chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities (PP). Four Markov chains were run from a random starting tree for 5 000 000 generations and trees sampled every 100 generations. The first 5 000 trees were discarded as burn-in prior to convergence of the four chains. The remaining trees were used to construct a 50 % majority rule consensus tree and to calculate Bayesian Posterior Probabilities (BYPP) with those equal or greater than 0.95 given below each node (Fig. 1).

(iii) Maximum likelihood analyses (ML) were conducted in RAxML v. 7.2.2 (Stamatakis 2006). The dataset was partitioned according to each gene and separate codons (eight partitions) as previously done in Schoch *et al*. (2009). A general time reversible model (GTR) with a discrete gamma distribution and four rate classes was applied to each partition. A tree was obtained by simultaneously running a fast bootstrap search of 1 000 pseudoreplicates followed by a search for the most likely tree under functional setting "a". We also did 100 successive searches in RAxML under the GTR model with gamma rate distribution and starting each search from a randomised tree. Maximum Likelihood bootstrap values (MLBP) equal or greater than 50 % are given above each node (Fig. 1).

Phylogenetic trees were drawn using Treeview v. 1.6.6 (Page 2001) and TreeDyn 198.3 (Chevenet *et al*. 2006). Sequences derived in this study are deposited in GenBank, and the alignments in TreeBASE (www.treebase.org).

## RESULTS

### Molecular phylogenies

The BLAST search based on SSU and LSU sequences revealed the closest matches with taxa in *Dothideomycetes* and SSU, LSU, *TEF1*, and *RPB2* sequences generated as part of this study are listed in Table 1. These sequences were combined with previously published data from various orders of the *Dothideomycetes* (*Botryosphaeriales*, *Capnodiales*, *Dothideales*, *Hysteriales*, *Pleosporales* and *Myriangiales*) obtained from GenBank (Table 1). The data set consisted of 199 taxa, with *Opegrapha dolomitica* and *Roccella fuciformis* included as the outgroup taxa. The maximum parsimony dataset consists of 4 141 total characters, 1 890 (45.6 %) characters are constant, 532 (12.8 %) characters are parsimony informative and 1 791 (41.6 %) characters are parsimony uninformative. The heuristic search resulted in a single most parsimonious tree (MPT) with a length of 18 715 steps (CI = 0.208, RI = 0.623, RC = 0.130; data not shown). One hundred successive searches using a rapid hill-climbing algorithm from distinct randomised starting trees in RAxML yielded a best scoring likely tree (Fig. 1) with a log likelihood –84765.605900. The matrix had 2 985 alignment patterns with 32 % of the characters consisting of gaps or undetermined characters. The alignment patterns were distributed across seven partitions as follows: LSU – 859, SSU – 217, *TEF1* codon1 – 195, *TEF1* codon2 – 309, *TEF1* codon3 – 309, *RPB2* codon1 – 230, *RPB2* codon2 – 203, *RPB2* codon1 – 254.

Phylogenetic trees obtained from maximum likelihood, Bayesian and maximum parsimony analyses yielded trees with similar overall topology at subclass, order and family relationship in agreement with previous work based on maximum likelihood (Schoch *et al*. 2006). However, the internal node relationships of some taxa were resolved differently between the maximum likelihood, Bayesian and maximum parsimony trees. For example: the taxonomic position of *Biatriospora marina* differed between the maximum likelihood, Bayesian and Maximum parsimony trees In the maximum likelihood and Bayesian tree, *B. marina* grouped in a basal part of Clade XIV- Residual paraphyletic assemblage. But in the maximum parsimony tree, *B. marina* grouped in a basal clade to the *Testudinaceae*. This is not unexpected as divergence in evolutionary rates and the presence of missing data affects all these methods differently. Nevertheless, we describe new taxa based on agreement in support for all three computational methods.

### Taxonomy

This study resulted in the sampling of 51 marine dothideomycetous species (Table 1) with most of the marine genera beloning in the *Pleosporomycetidae*, and only two taxa (*Mycosphaerella*, *Scirrhia*) referred to the *Dothideomycetidae*. Only clades with marine taxa (in blue bold in the tree) are discussed in the text.

Marine *Dothideomycetes* show great variation in the morphology of the ascomata, asci and ascospores as illustrated in Figs 2–3. Many genera possess ascospores with a mucilaginous sheath that swells in water, once released from the asci. In others the sheaths are drawn out to form appendages (*e.g. Carinispora nypae*, *Decorospora gaudefroyi*, *Falciformispora lignatilis*).

### *Pleosporomycetidae*

### 1. *Pleosporales*, Fig. 1.

Delineation of families in the *Pleosporales* previously relied extensively on morphological characters which resulted in 17 to 19 families (Kirk *et al.* 2001, Lumbsch & Huhndorf 2007). These were poorly resolved at the molecular level and Schoch *et al*. (2006) could only find reasonable support for seven families in a phylogeny generated from four genes: *Leptosphaeriaceae*, *Lophiostomataceae, Phaeosphaeriaceae*, *Pleosporaceae*, *Sporormiaceae*, *Testudinaceae* and *Trematosphaeriaceae*. A major reassessment of these taxa is needed and attempts are underway to complete this (see Mugambi *et al.* 2009a, and Zhang *et al*. 2009; this volume). As part of this process we attempted to place a diverse selection of marine *Dothideomycetes* using phylogenetic

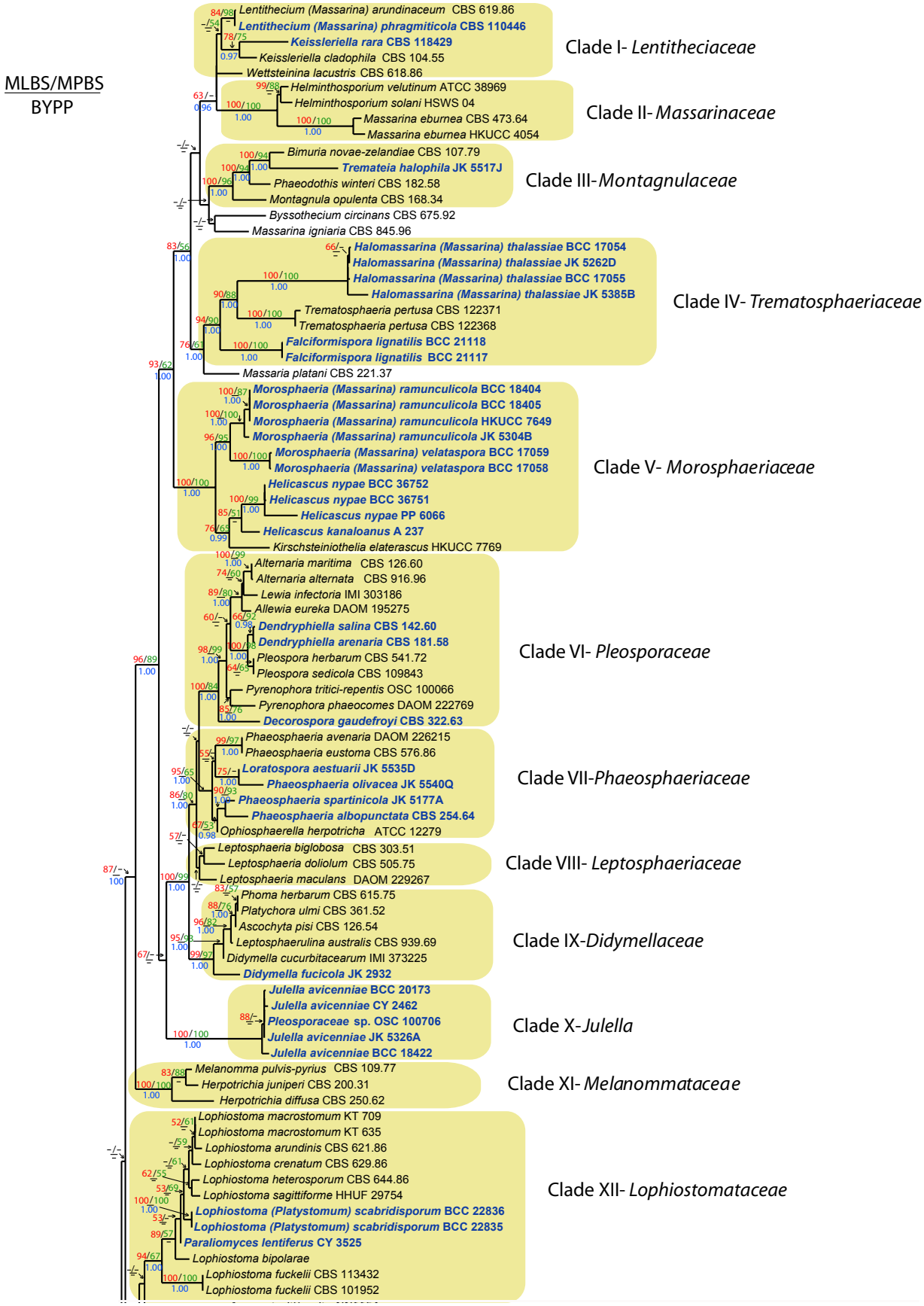


**Fig. 1.** RAxML tree of marine *Dothideomycetes* with bootstrap support values for maximum likelihood and maximum parsimony above the nodes. The values below the nodes are Bayesian posterior probalities. Relevant clades are highlighted in colour.

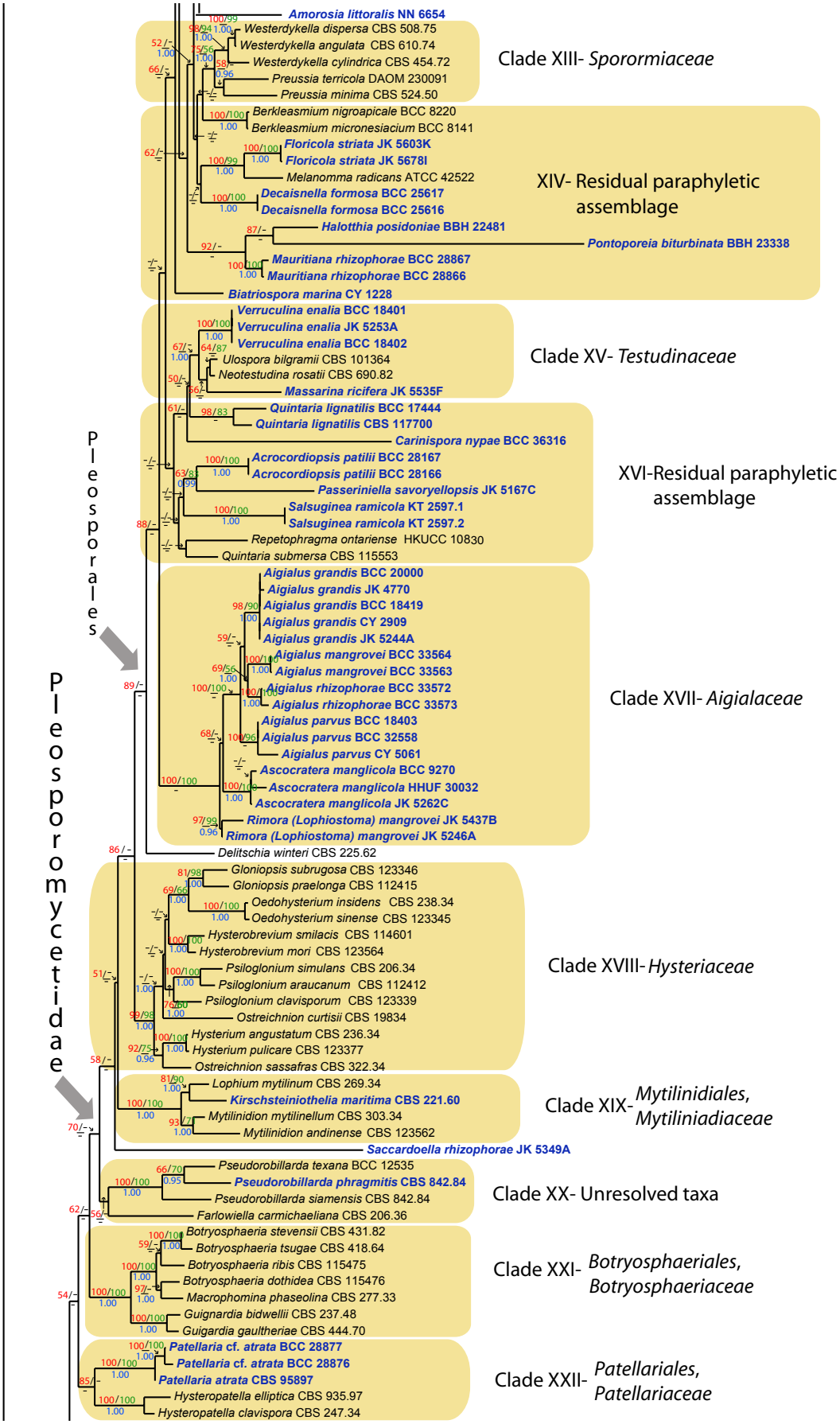


**Fig. 1.** (Continued).

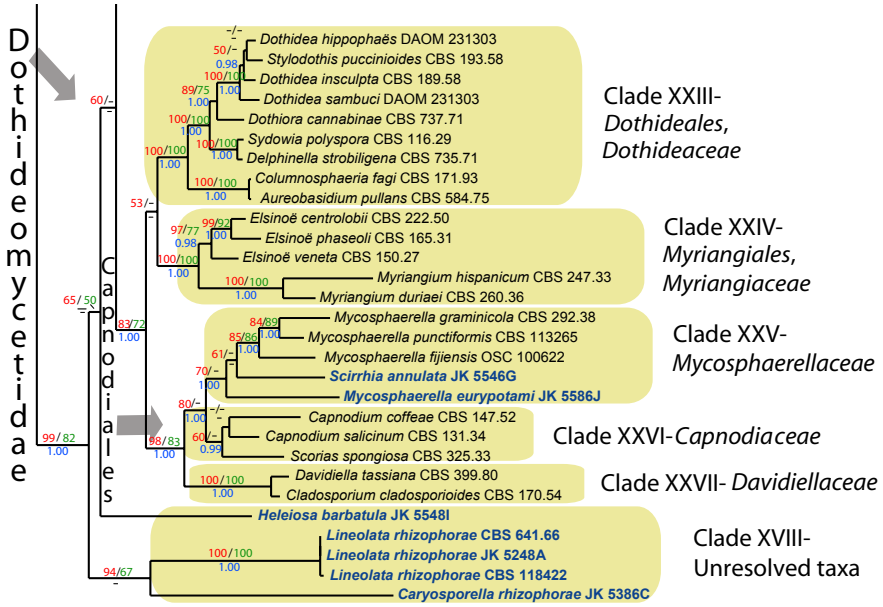


**Fig. 1.** (Continued).

reconstruction. This resulted in 11 supported clades corresponding to families, with marine representatives (Fig. 1) (*Didymellaceae*-Clade IX, *Lentitheciaceae*-Clade I, *Leptosphaeriaceae*-Clade VIII, *Lophiostomataceae*-Clade XII, *Massarinaceae*-Clade II, *Montagnulaceae*-Clade III, *Phaeosphaeriaceae*-Clade VII, *Pleosporaceae*-Clade VI, *Sporormiaceae*-Clade XIII, *Testudinaceae*-Clade XV, *Trematosphaeriaceae*-Clade IV) and two new families: 1) *Aigialaceae* (Clade XVII) for *Aigialus* and related taxa (*Ascocratera manglicola* and *Lophiostoma mangrovei*), and 2) *Morosphaeriaceae* (Clade V) for the species *Morosphaeria* (*Massarina ramunculicola*, *Massarina velataspora*), *Helicascus nypae*, *H. kanaloanus* and *Kirschsteiniothelia elaterascus*. Further clades are also identified, but their position remains unresolved, *e.g.* the familial position of the taxa *Halotthia posidoniae*, *Mauritiana rhizophorae* and *Pontoporeia biturbinata* in clade XIV.

## Clade I. *Lentitheciaceae*

The marine *Massarina* species are not monophyletic which is in agreement with observations on terrestrial and freshwater members of the genus (Zhang *et al.* 2009b). Consequently a number of taxonomic changes are proposed in this chapter. Zhang *et al*. (2009a; this volume) erected the family *Lentitheciaceae*, and the genus *Lentithecium* for *Massarina* that do not group in the *Massarinaceae*. However the monophyly of *Lentithecium* is not supported in the current study. *Massarina phragmiticola* was described from the saltmarsh grass *Phragmites australis* (Poon *et al*. 1998), and groups within this family. It grouped with *M. arundinacea* with 84 % MLBP and 98 % MPBP support (Fig. 1). However Zhang *et al*. (2009a; this volume) refers *M. arundinacea* to the new genus *Lentithecium* and we place *M. phragmiticola* in synonymy with *Lentithecium arundinaceum.*

*Keissleriella* (type species *K. aesculi)* comprises some 25 species (Kirk *et al*. 2008) and two species group with *Lentithecium* in clade I, with high support. *Keissleriella rara* was described from the salt marsh species *Juncus roemerianus*, a rare halotolerant species (Kohlmeyer *et al*. 1995c). Zhang *et al*. (2009a) also included *Keissleriella linearis* in their phylogenetic analysis and transferred it to *Lentithecium.*

## Clade II. *Massarinaceae*

Aptroot (1998) reviewed the genus *Massarina* and reduced the 160 names in the literature to 43 taxa, while others (especially those from aquatic habitats) have been transferred to *Lophiostoma* (Hyde & Aptroot 1998, Hyde *et al.* 2002b, Liew *et al*. 2002). However, subsequent studies indicate that *Massarina* and *Lophiostoma* species are polyphyletic (Zhang *et al*. 2009a; this volume). These genera and the families *Lophiostomataceae* / *Massarinaceae* are difficult to separate and often have overlapping characters (Zhang *et al*. 2009b). In our analysis the type species *Massarina eburnea* forms a well supported clade (Clade II) with two *Helminthosporium* species (*H. velutinum*, *H. solani*) as a sister group.

Jones *et al*. (2009) referred the genus *Massarina* to the *Lophiostomataceae* based on the molecular evaluation of Hyde *et al*. (2002b) and Liew *et al*. (2002). *Lophiostoma* has been reported as a monophyletic genus (Tanaka & Harada 2003, Tanaka & Hosoya 2008) while Zhang *et al.* (2009b) have shown that *Lophiostoma* is phylogenetically divided into two groups: *Lophiostoma* I which includes the type species *L. macrostomum* (voucher Lundqvist 20504), and *Lophiostoma* II which also contains sequences of *L. macrostomum* (voucher HHUF 27293 and HHUF 27290). Zhang *et al*. (2009b) were unable to verify the identity of the different strains of *L. macrostomum* and consequently could not determine the taxonomic position of *Lophiostoma s. str.* The paraphyletic nature of the *Lophiostomataceae* has previously been noted (Schoch *et al.* 2006) and clade XII is likely to represent the narrow concept of the *Lophiostomataceae*, although it is still too early to draw this conclusion until type material of *Lophiostoma* (*L. macrostomum*) is obtained (Zhang *et al.* 2009b). In our analysis we have selected the accession numbers AB433273 and AB433274 from the voucher specimens HHUF 27290 and HHUF 27293, respectively, and regard this clade as representing the family *Lophiostomataceae* (Clade XII).

## Clade III. *Montagnulaceae*

Based on morphological data, Jones *et al.* (2009) referred the genus *Tremateia* to the *Pleosporaceae*, but molecular data places it with high support in the *Montagnulaceae* (100 % MLBP, 94 % MPBP, 1.00 BYPP) with *Bimuria novae-zelandiae* as a sister

taxon. Kohlmeyer *et al.* (1995a) described *Trematei a halophila* from senescent leaves of *Juncus roemerianus* and regarded it as a facultative marine ascomycete. Characteristic features include an apical cap on the ascus, I- ocular chamber, and muriform ascospores with a wide mucilaginous sheath, and a *Phoma*-like anamorph.

### Clade IV. *Trematosphaeriaceae*

This clade comprises four strains of *Massarina thalassiae*, a common species on mangrove wood, from Aldabra, Australia, Belize, Brunei, Florida, Galapagos, India, Malaysia, Mexico, Thailand (Kohlmeyer & Volkmann-Kohlmeyer 1987, Hyde 1992d, 1993, Alias & Jones 2000, Jones *et al*. 2006), with *Trematosphaeria pertusa* as a sister taxon. *Falciformispora lignatilis* (Fig. 2T, W) also groups in this clade with high support (94 % MLBP, 90 % MPBP, 1.00 BYPP); a species found on mangrove wood as well as on the fronds of the terrestrial oil palm (U. Pinruan, pers. comm.). As *Massarina thalassiae* cannot be accommodated in the genus *Massarina* based on molecular evidence, a new genus *Halomassarina,* is described.

***Halomassarina*** Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch, **gen. nov.** MycoBank MB515951. Fig. 2AF.

*Etymology*: From the Greek *hals* = salt, in reference to the marine origin of the fungus.

Ascomata subglobosa ad pyriformia, immersa vel erumpentia, ostiolata, periphysata, papillata vel epapillata, clypeata, coriacea, brunnea, singularia. Peridium cellulis applanatis pachydermisque, texturam angularem formans. Hamathecium pseudoparaphysibus simplicibus, rariter anastomosantibus. Asci octospori, cylindrici ad clavati, pedunculati, pachydermi, fissitunicati, camera oculare, sine apparatu apicali, I non reagentes. Ascosporae distichae, ellipsoideae, triseptatae, hyalinae, tunica gelatinosa tectae.

*Ascomata* subglobose to pyriform, immersed or erumpent, ostiolate, periphysate, papillate or apapillate, clypeate, coriaceous, brown, single. *Peridium* of flattened, thick-walled cells, forming a *textura angularis*. *Hamathecium* of simple, rarely anastomosing pseudoparaphyses. *Asci* 8-spored, cylindrical to clavate, pedunculate, thick-walled, fissitunicate, with ocular chamber but without apical apparatus, I-negative. *Ascospores* distichous, ellipsoidal, 3-septate, hyaline, surrounded by a gelatinous sheath.

*Type species*: *Halomassarina thalassiae* Kohlm. & Volkm.-Kohlm.), Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch.

***Halomassarina thalassiae*** (Kohlm. & Volkm.-Kohlm.) Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch, **comb. nov.** MycoBank MB515952.
*Basionym*: *Massarina thalassiae* Kohlm. & Volkm.-Kohlm. Canad. J. Bot. 65: 575. 1987.

This is a widely collected tropical species from intertidal and subtidal mangrove wood or fishing crafts (Kohlmeyer & Volkmann-Kohlmeyer 1987).

### Clade V. *Morosphaeriaceae*

This clade, comprising four marine species *Massarina ramunculicola*, *M. velataspora*, *Helicascus kanaloanus* and *H. nypae,* is well supported (100 % MLBP, 100 % MPBP, 1.00 BYPP) with the *Massarinaceae*, *Montagnulaceae* and *Trematosphaeriaceae* as sister clades. As *M. ramunculicola* and *M. velataspora* do not group with other *Massarina* species, a new family and genus *Morosphaeria* are proposed.

***Morosphaeriaceae*** Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch, **fam. nov.** MycoBank MB515953.

Familia Pleosporalium, Ascomycetium. Ascomata subglobosa, conica, lenticulara, immersa ad superficialia, ostiolata, papillata, periphysata, brunnea vel nigra, coriacea vel carbonacea, solitaria, vel gregaria, cum 3–4 loculis, ostiolo communi ad centrum. Hamathecium pseudoparaphysibus filamentosis, numerosis, ramosis ad basem, ramosis anastomosantibusque supra ascos. Asci octospori, clavati vel cylindrici pedunculati, pachydermi, fissitunicati, persistentes, camera apicale et disco apicale, IKI non-reagentes. Ascosporae biseriatae, hyalinae ad brunneae, septatae constrictae ad leviter constrictae, tunica vel calyptra gelatinosa tectae, vel sine tunica.

Family in the *Pleosporales*, *Ascomycota*. *Ascomata* subglobose, conical, lenticular, immersed to superficial, ostiolate, papillate, periphysate, brown to black, coriaceaous or carbonaceous, single to gregarious, stromatic with 3–4 loculi with a common central ostiole. *Hamathecium* with filamentous pseudoparaphyses, unbranched to branched at the base, anastomosing above the asci, embedded in a gelatinous matrix. Asci 8-spored, clavate to cylindrical, pedunculate, thick-walled, fissitunicate, with an ocular chamber and apical ring, non-amyloid, persistent. *Ascospores* biseriate, hyaline to brown, septate, with or without a gelatinous sheath or cap.

*Type genus*: *Morosphaeria* Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch.

***Morosphaeria*** Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch, **gen. nov.** MycoBank MB515954.

*Etymology*: Named after *Mor* = sea in Welsh in reference to its marine habitat and *sphaeria* in reference to the perithecial ascomata

Ascomata solitaria vel gregaria, subglobosa vel lenticularia, immersa, erumpentia, ostiolata, papillata, coriacea, brunnea ad nigra, pseudoparaphysibus angusti, hyalinis, simplicibus et numerosis. Asci octospori, clavati vel cylindrici, pedunculati, bitunicati, pachydermi, fissitunicati, cum camera apicale et aparatu apicale, IKI non reagentes. Ascosporae uniseriatae vel biseriatae, fusiformes vel ellipsoidales, 1–3 septatae, constrictae ad septae, cum tunica gelatinosae.

*Ascomata* solitary or gregarious, subglobose to lenticular, immersed becoming superficial, ostiolate, papillate, coriaceous, brown to black, pseudoparaphyses filamenatous, anastomosing, branching, and numerous. *Asci* 8-spored, clavate to cylindrical, short pedunculate, thick-walled, bitunicate, fissitunicate, with an ocular chamber and apical apparatus, persistent. *Ascospores* hyaline, 1–3 septate, constricted at the septa, fusiform to ellipsoidal, surrounded by a mucilaginous sheath.

*Type species*: *Morosphaeria velataspora* (K.D. Hyde & Borse) Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch.

***Morosphaeria velataspora*** (K.D. Hyde & Borse) Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch, **comb. nov.** MycoBank MB515955. Fig. 2 AG.
*Basionym*: *Massarina velataspora* K.D. Hyde & Borse, Mycotaxon 27: 163. 1986.


***Morosphaeria ramunculicola*** (K.D. Hyde) Suetrong, Sakayaroj, E.B.G. Jones & C.L. Schoch, **comb. nov.** MycoBank MB515956. Fig. 3A, H.

*Basionym*: *Massarina ramunculicola* K.D. Hyde, Mycologia 83: 839. 1992.

Both species are common and frequently collected on dead wood of various mangrove trees in tropical and subtropical localities (Hyde & Borse 1986b, Hyde 1992a, Schmit & Shearer 2003, Jones & Abdel-Wahab 2005, Jones *et al*. 2006). Ascospores of both species possess a well-developed sheath (Au *et al*. 2001, Au & Vrijmoed 2002), while in *M. ramunculicola* polar appendages are formed as outgrowth of the fibrillar material within the inner regions of the sheath through polar discontinuities (Read *et al*. 1997a, b).

The taxa *Helicascus kanaloanus* and *H. nypae* form a sister group to *Morosphaeria* species with high bootstrap support. Jones *et al.* (2009) referred this genus to the *Pleosporaceae* as in previous analyses (Tam *et al*. 2003) and grouped it with *Kirschsteiniothelia*

**Fig. 2.** (p. 162) Morphological features of marine *Dothideomycetes*. A. Immersed lenticular ascomata beneath clypeus of *Carinispora nypae*. B. Apothecium of *Patellaria* cf. *atrata* (*Patellariales*). C. Broadly conical ascomata of *Halotthia posidoniae*. D. Immersed ascomata of *Helicascus nypae*. E. Globose ascoma of *Pontoporeia biturbinata*. F. Immersed ascomata of *Quintaria lignatilis*. Released asci (arrow) from ostiole. G. Mature ascomata of *Manglicola guatemalensis* (*Jahnulales*). H. Tangential section of *Helicascus nypae* through stroma with several loculi. I. Longitudinal section (l.s.) of *Manglicola guatemalensis* ascoma with asci and pseudoparaphyses. J. *Pontoporeia biturbinata*, non-ostiolate ascoma, asci originating at the periphery of a hemispherical basal pulvinus. K. Longitudinal section through ascoma of *Verruculina enalia*. Asci. L–U. Ascus tip of *Manglicola guatemalensis*. Ascospores show the apical appendage (arrow) in ascus. M. Ascus tip of *Salsuginea ramicola*, consisting of a large distinctive ocular chamber and prominent ring (arrows). N. Clavate ascus of *Quintaria lignatilis* with apical plate. O. Clavate ascus of *Quintaria lignatilis*, with biseriate ascospores, in Nomarski and Quartz. P. Ovoidal or ellipsoidal ascospores in cylindrical asci of *Acrocordiopsis patilii*. Q. Clavate to long-cylindrical ascus of *Carinispora nypae*. R. Clavate ascus of *Patellaria* cf. *atrata*. S. Subcylindrical asci with pseudoparaphyses of *Helicascus nypae*. T. Clavate asci of *Falciformispora lignatilis* (*Trematosphaeriaceae*). U. Broadly clavate ascus of *Pontoporeia biturbinata*. V–AH. Ascospores of marine *Dothideomycetes*: V. *Carinispora nypae*. Cylindrical and multiseptate ascospore with keel-like mucilaginous sheath (arrows). W. *Falciformispora lignatilis*. Fusiform ascospores surrounded by thin mucilaginous sheath and single scythe-like appendage (arrow) at the base. X. *Salsuginea ramicola*. Ovoid, dark brown ascospore with hyaline apical germ pores. Y. *Manglicola guatemalensis*. Fusiform ascospore with lager, pale brown apical cell and hyaline turbinate basal cell. Z. *Halotthia posidoniae*. Ellipsoidal, dark brown ascospores, darker around septum. AA. *Verruculina enalia*. Ellipsoidal, dark brown ascospore, 1-septate. AB. *Helicascus nypae*. Obovoidal ascospore with persistent mucilaginous sheath. AC. *Mauritiania rhizophorae*. Fusiform ascospore, 9–13-distoseptate. AD. *Patellaria* cf. *atrata*. Clavate ascospore, 5–7-septate. AE. *Julella avicenniae*. Muriform ascospores with dilated sheath (arrows), straining in ink. AF. *Halomassarina (Massarina) thalassiae*. Ellipsoidal ascospores with gelatinous sheath (arrows). AG. *Morosphaeria (Massarina) velataspora*. Fusiform to ellipsoidal ascospores, 3-septate with mucilaginous sheath (arrows). AH. *Morosphaeria (Massarina) ramunculicola*. Fusiform ascospores with fully dilated mucilaginous sheath (arrows). Habitat: A, D, G, H, I, L, Q, S, V, Y, AB. On the surface of *Nypa fruticans*. B, F, K, M–P, R, X, AA, AC–AD, AF–AH. On mangrove wood. C, E, J, U, Z. On rhizomes of *Posidonia oceanica*. T, W. On oil palm (*Elaeis guineensis*). AE. On *Avicennia* spp. Scale bars: A–C, E–H = 500 mm; D = 1000 mm; I = 250 mm; K = 200 mm; J = 150 mm; L–Z, AB, AF–AH = 20 mm; AA, AC–AE = 10 mm.

*elaterascus* (Shearer 1993a). However, *Kirschsteiniothelia* is polyphyletic with the marine species *K. maritima* grouping in our analysis in the *Mytilinidaceae* (Clade XIX, Fig. 1). In addition to this the type species of the genus, *K. aethiops* and its anamorph, *Dendryphiopsis atra,* are placed outside of the *Pleosporales* as currently defined, always in close association with an isolate of *Phaeotrichum benjaminii*, originally isolated from dung (Lumbsch & Lindemuth 2001, Kruys *et al*. 2006, Schoch *et al*. 2009b). This continues to demonstrate the polyphyletic nature of this genus in agreement with clear morphological differences alluded to earlier (Shearer 1993a). There is great morphological variation in the three genera assigned to this family, especially the ascospores, hyaline in *Morosphaeria*, brown to dark-brown in *K. elaterascus* and *Helicascus* species, respectively.

## Clade VI. *Pleosporaceae*

Jones *et al*. (2009) referred five genera with marine representatives in this family: *Decorospora, Helicascus, Falciformispora, Pleospora* and *Trematei*a. The current study confirms the placement of *D. gaudefroyi* in this family (Inderbitzin *et al*. 2002), along with the two anamorphic species, *Dendryphiella arenaria* and *D. salina*, that form a sister group to *Pleospora herbarum* and *Pleospora sedicola* (Jones *et al*. 2008). *Alternaria maritima* groups as a sister taxon with *Alternaria alternata* and *Lewia* species with moderate support (74 % MLBP, 60 % MPBP). The current study refers *Trematei*a to the *Montagnulaceae* (Clade II) and *Helicascus* to the new family *Morosphaeriaceae* (Clade V), respectively, while *Falciformispora* groups in a sister group to *Halomassarina thalassiae* and *Trematosphaeria pertusa* (Clade IV, Fig. 1). (Zhang *et al*. 2009a; this volume). The identity of the *Alternaria maritima* strain is questioned as this taxon was regarded as *nomen dubium* by Kohlmeyer & Kohlmeyer (1979) since there is no type material to verify the original description by Sutherland (1916).

## Clade VII. *Phaeosphaeriaceae*

The families *Leptosphaeriaceae* and *Phaeosphaeriaceae* are closely related as recent sequence data have shown (Khashnobish & Shearer 1996, Cámara *et al*. 2002, Kodsueb *et al*. 2006, Schoch *et al*. 2006). The consensus was that they should both be retained (Câmara *et al*. 2002, Cannon & Kirk 2007).

*Loratospora aestuarii*, *Phaeosphaeria albopunctata, Ph. olivacea,* and *Ph. spartinicola* are the only marine species represented in the *Phaeosphaeriaceae* in this data set. Based on ITS2 and partial 28S nrDNA sequences Khashnobish & Shearer (1996) confirmed the inclusion of *Ph. albopunctata* and *Ph. typharum* in the *Phaeosphaeriaceae*, and suggested that *Leptosphaeria orae-maris* had a closer relationship with *Phaeosphaeria* than *Leptosphaeria*. Jones *et al*. (2009) tentatively referred the genera *Carinispora, Lautitia* and *Phaeosphaeria* to this family, with *Loratospora aestuarii* in the *Planistromellaceae* (*Dothideomycetidae*, family *incertae sedis*), based on morphological observations. Barr (1996) erected the *Planistromellaceae* for six genera in the *Dothideales* based on brown-celled pseudoparenchymatous ascostroma with one or more locules which open schizogeneously and contain asci, which are separated and overtopped by interthecial tissues at maturity. However molecular data suggests that species in some currently accepted genera *sensu* Lumbsch & Huhndorf (2007) *e.g. Comminutispora*, are unrelated (Schoch *et al*. 2009a; this volume).

Zhang *et al*. (2009a; this volume) include the following marine species in the *Phaeosphaeriaceae*: *Leptosphaeria albopunctata, Ph. spartinae*, *Ph. spartinicola, Ph. typharum* as well as *Amarenomyces ammophilae*. Eriksson (1981) established the new genus *Amarenomyces* for *Ph. ammophilae*, but molecular data places it in *Phaeosphaeria* and thus the earlier name as proposed by Kohlmeyer & Kohlmeyer (1965) and Leuchtmann (1984) should be retained. *Phaeosphaeria olivacea* is a facultative marine species collected on *Juncus roemerianus* throughout the year (Kohlmeyer *et al*. 1997a). Of the marine taxa included in this family all occur on salt marsh plants: *L. aestuarii, Ph. olivacea* on *J. roemerianus*, *Ph. spartinae*, and *Ph. spartinicola* on *Spartina* spp., while *Ph. ammophilae* occurs on a range of grasses and sedges, but primarily on *Ammophila arenaria* (Kohlmeyer & Kohlmeyer 1979).

## Clade VIII. *Leptosphaeriaceae*

Currently five *Leptosphaeria* species are referred to this family (Jones *et al*. 2009), but no sequences of marine *Leptosphaeria* are available for any of these, and therefore their taxonomic position cannot be verified.

## Clade IX. *Didymellaceae*

The family *Didymellaceae* was recently described for the teleomorphic genera *Didymella*, *Leptosphaerulina*, including several *Phoma* anamorphs (de Gruyter *et al*. 2009). Four marine *Didymella* species have been described, three from brown or red seaweeds and *D. avicenniae* from wood of *Avicennia* (Patil & Borse 1985, Jones *et al*. 2009). In our analyses it forms a well-supported

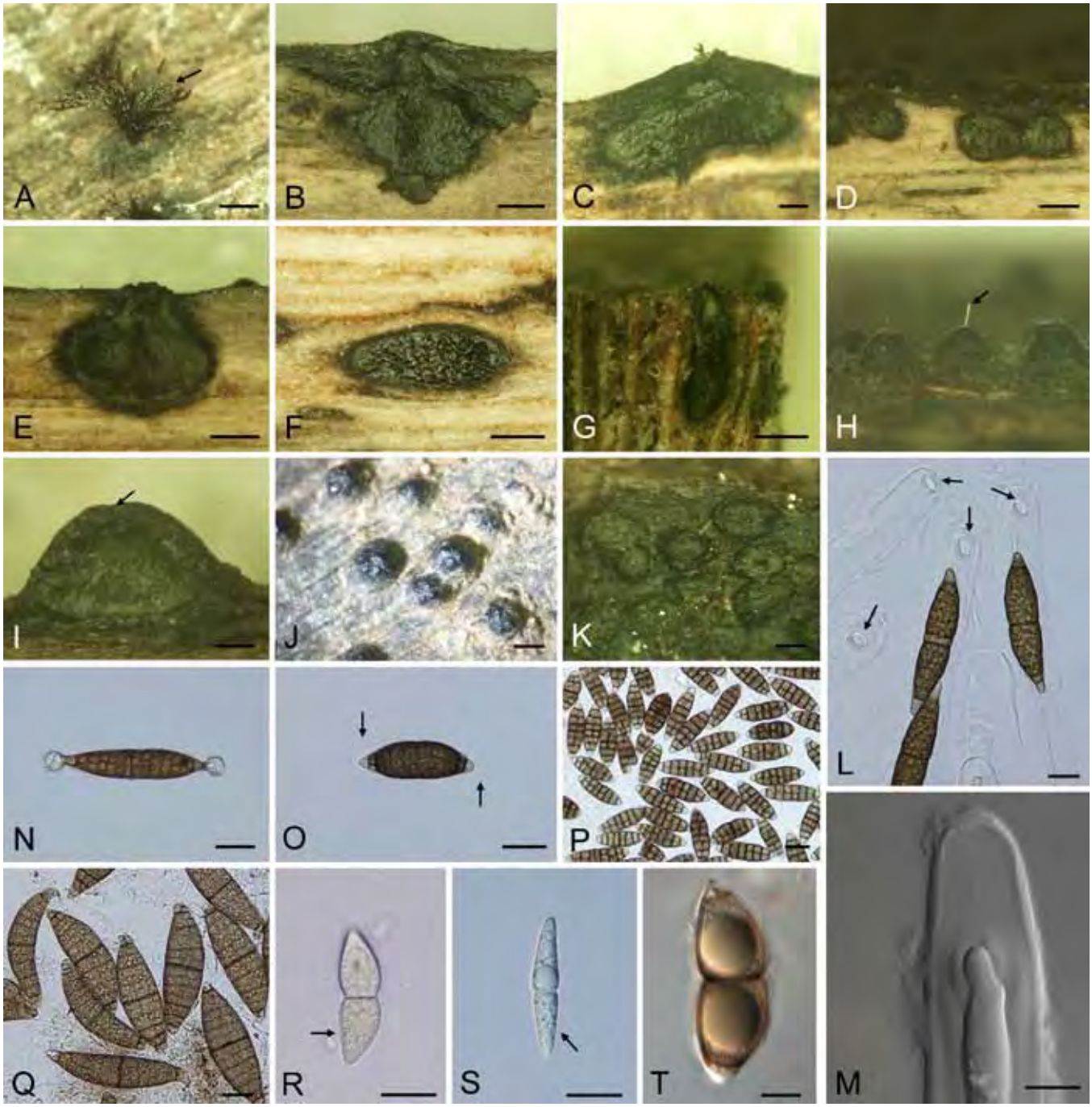


**Fig. 3.** Morphological features of marine *Dothideomycetes* in the *Aigialaceae* and *Coronopapilla mangrovei*. A. *Aigialus grandis*. Immersed ascomata with ascospores (arrow) released from ostiole. B–E. Longitudinal section (l.s.) through ascomata of *Aigialus grandis* (A), *A. parvus* (B), *A. mangrovis* (C) and *A. rhizophorae* (D). F. *A. parvus*. Surface wood showing ascoma with thick peridium. G. *A. parvus.* Sagittal section through ascoma. H. *Ascocratera manglicola*. Crater-like ascomata with released ascus (arrow) from the ostiole. I. *Ascocratera manglicola*. l.s. of ascoma filled with gelatinous matrix. J. *Coronopapilla mangrovei*. Surface view of ascomata. K. *Rimora (Lophiostoma) mangrovei*. Broadly oblong ascomata. L. *Aigialus grandis*. Asci with apical refractive ring (arrows) and ascospores. M. *Coronopapilla mangrovei*. Ascus tip, thick-walled with ocular chamber. N–T. Ascospores of marine *Dothideomycetes* in *Aigialaceae*: N. *Aigialus grandis*. Broadly fusiform (front view), muriform ascospores with drop of mucilage from end cells. O. *Aigialus parvus*. Ellipsoidal to broadly fusiform (front view), muriform ascospores with a gelatinous cap around apical and subapical cells (arrows). P. *Aigialus mangrovis*. Ellipsoidal to fusiform (front view), muriform ascospores. Q. *Aigialus rhizophorae*. Broadly fusiform (front view), muriform ascospores. R. *Ascocratera manglicola.* Ellipsoidal ascospores, initially 1-septate, later becoming 3-septate with gelatinous sheath (arrow). S. *Rimora (Lophiostoma) mangrovei.* Fusiform ascospore with gelatinous sheath (arrow). T. *Coronopapilla mangrovei*. Ellipsoidal ascospore. Habitat A–T. On mangrove wood. Scale bars: A, D–G, J–K = 500 mm; B–C, H = 250 mm; L, N–S = 25 mm; M, T = 10 mm.

basal clade (99 % MLBP, 97 % MPBP, 1.00 BYPP) to the families *Phaeosphaeriaceae*, *Pleosporaceae*, and *Leptosphaeriaceae*. Kohlmeyer & Volkmann-Kohlmeyer (2003) questioned the taxonomic position of *Didymella magnei*, a species found on the red seaweed *Palmaria palmata*, because the ascospores differed morphologically from those of other *Didymella* species.

### Clade X. *Julella clade*

The genus *Julella* was previously assigned to the *Pleosporales incertae sedis* and *Phaeosphaeriaceae*, respectively (Jones *et al*. 2009). *Julella avicenniae* (Fig. 2 AE) was initially described as a *Pleospora* species but because the ascomata develop on woody substrata, immersed beneath a clypeus with narrow pseudoparaphyses, Hyde (1992b) transferred it to *Julella*.

However, ascomata can be superficial on well-decayed mangrove wood. Although regarded as an obligate marine ascomycete (Hyde 1992b), it may be implicated in the dieback of young shoots of *Avicennia marina*, at Morib mangrove, Malaysia, not submerged in seawater (Jones 2007). *Julella avicenniae* strains form a monophyletic clade with an unidentified pleosporaceous sequence (OSC 100706). This forms a moderately supported clade separated from other families in the *Pleosporales* (67 % MLBP).

### Clade XII. *Lophiostomataceae*

In our analyses the families *Lophiostomataceae* and *Massarinaceae* are distinct, and distantly placed within the *Pleosporales*. This is confirmed elsewhere (Zhang *et al*. 2009a; this volume). Jones *et al*. (2009) referred seven genera with marine species to this family (*Decaisnella*-Clade XIV, Unresolved, *Herpotrichia*-Clade XI, *Melanommataceae*, *Lophiostoma*, *Massarina*-Clade II, *Massarinaceae*, *Paraliomyces*, *Platystomum, Quintaria*-Clade XVI Residual assemblage). However, molecular data places some of these in other families, as indicated in the above sentence (Fig. 1). Of these genera, only *Platystomum* and *Paraliomyces* (Tam *et al*. 2003) were included in the present analysis. Currently four marine *Lophiostoma* species are recognised: *L. acrostichi*, *L. armatisporum, L. rhizophorae* and *Platystomum scabridisporum*; however, Suetrong *et al*. (pers. obs.) propose the transfer of the latter species to *Lophiostoma* based on morphological and molecular data. Other *Lophiostoma* species have been transferred to *Astrosphaeriella* (*A. asiana, A. mangrovis*) by Hyde *et al*. (2002b) and Liew *et al*. (2002). In our analysis, based on molecular data, *Lophiostoma mangrovei* is referred to the family *Aigialaceae* (Clade XVII, Fig. 1), while other *Massarina* species are placed in the *Lentitheciaceae* (Clade I) [*Lentithecium* (*Massarina) phragmiticola*], or the new family *Morosphaeriaceae* (clade V) [*Morosphaeria* (*Massarina*) *ramunculicola, M.* (*Massarina*) *velataspora*]. No molecular data is available for the marine species *Herpotrichia nypicola* which occurs on the palm *Nypa fruticosa*, while *Quintaria lignatilis* forms a sister group to the *Testudinaceae* with low support (Schoch *et al*. 2006).

### Clade XIV. *Residual paraphyletic assemblage*

Several unresolved species form part of a poorly resolved group that includes some members of the *Lophiostomataceae* and it is not clear whether missing data influenced this result. One of these is the marine anamorphic species *Amorosia littoralis* (isolated from the littoral zone in the Bahamas) and referred to the *Sporormiacaeae* based on molecular data (Mantle *et al*. 2006). Another anamorphic species, *Floricola striata*, is a facultative marine coelomycete from *Juncus roemerianus,* which grouped with *Melanomma radicans* with high support (100 % MLBP, 99 % MPBP, 1.00 BYPP). The teleomorph genera forming part of this poorly resolved group include: *Decaisnella* (*Lophiostomataceae*), *Halotthia* (Fig. 2C) (*Pleosporales incertae sedis*), *Mauritiana* (*Requienellaceae*) (Fig. 2AC) and *Pontoporeia* (Fig. 2E, J, Z) (*Zopfiaceae*) with weak support and previously assigned to the families listed in brackets (Jones *et al*. 2009). Morphologically they differ radically with perithecioid or cleistothecial ascomata, clavate to cylindrical asci and ascospores that are 3-septate and thick-walled in *Halotthia posidoniae* and *Pontoporeia biturbinata*, muriform in *Decaisnella formosa* and with 9–13 distosepta in *Mauritiana rhizophorae.* They also occur on different substrata: *Decaisnella formosa* on wood associated with sand, *Mauritiana rhizophorae* on mangrove wood, and *Halotthia* and *Pontoporeia* on submerged rhizomes of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa*. The latter are temperate hosts, while *D. formosa* and *M. rhizophorae* are from the tropics.

### Clade XV. *Testudinaceae*

*Verruculina* and *Massarina ricifera* (Fig. 2K, AA) are the only marine genera referred to this family, poorly supported in the current analysis, but confirming the results of a previous study (Schoch *et al*. 2006). In their analysis the family formed the basal node to the *Pleosporales*. Members of the *Testudinaceae* form a monophyletic clade and are characterised by ascospores that are 1-septate, brown without germ slits and with or without ornamentation (Kruys *et al*. 2006). However, *Verruculina enalia* shares few characters with members of the *Testudinaceae*, it differs especially by its marine habitat and persistent asci. *Massarina ricifera* is an obligate marine ascomycete growing on *Juncus roemerianus* and referred by Kohlmeyer *et al*. (1995b) to the *Lophiostomataceae* "with hesitation" as it did not fully agree with the type species *Massarina eburnea*. Molecular data presented here clearly indicates that it does not belong in *Massarina*, but further assignment must await additional collections.

### Clade XVI. *Residual paraphyletic assemblage*

Several unresolved species form part of a poorly resolved group that includes the *Testudinaceae* and it is not clear whether missing data played a role in this. The genera in question include: *Carinispora* (Fig. 2AV), *Massarina ricifera*, *Passeriniella*, *Salsuginea* and *Quintaria* (Fig. 2F). Jones *et al*. (2009) referred *Salsuginea ramicola* (Fig. 2M, X*)* to the *Pleosporales incertae sedis*; a genus with similarities to *Helicascus* (Kohlmeyer 1969, Hyde 1991) while Hyde (1991) suggested the *Dothideales incertae sedis.* Both genera occur on mangrove wood but differ in that *Salsuginea* lacks a stroma, the ascomata form under a clypeus, asci have a distinctive ocular chamber and ascospores with prominent apical pores and lacking a mucilaginous sheath. It is a species collected from various mangrove tree species with ascospore measurements differing, but whether this is in response to the host remains to be evaluated (Hyde 1991).

The genera *Acrocordiopsis* (Fig. 3P) and *Passeriniella* form an unsupported clade with both taxa known from mangrove wood in the tropics (Hyde & Mouzouras 1988, Borse & Hyde 1989, Alias *et al*. 1999) and referred previously to the *Melanommataceae* and *Dothideales incertae sedis*, respectively (Jones *et al*. 2009). Morphologically they would appear to share few common characters. *Acrocordiopsis* species are characterised by large (< 2 mm) ascomata that are conical, superficial on the host and carbonaceous with the asci formed on a thin layer of peridial tissue on the host substratum while the ascospores are hyaline and 1-septate (Alias *et al*. 1999). Currently two *Passeriniella* species are accepted (Jones *et al*. 2009), namely *P. mangrovei* and *P. savoryellopsis*, with coriaceous, globose to subglobose, immersed ascomata, and ascospores that are 3-septate, central cells brown, and hyaline end cells (Hyde & Mouzouras 1988, Maria & Sridhar 2002). The taxonomic characterisation of the genus *Passeriniella* is confusing and has been discussed by Hyde & Mouzouras (1988) and Kohlmeyer & Volkmann-Kohlmeyer (1991).

*Byssothecium (Passeriniella) obiones*, a common species on senescent culms of *Spartina*, has a checkered history, assigned to *Pleospora*, *Leptosphaeria*, *Didymosphaeria*, *Metasphaeria* and *Passeriniella* (Jones *et al*. 2009). Khashnobish & Shearer (1996) showed that based on ITS sequence data, *Byssothecium*

(*Passeriniella*) *obiones* did not belong in either *Leptosphaeria* or *Phaeosphaeria*. Subsequently, Barr (2002) assigned it to *Byssothecium*, based on the vericolourous ascospores in the *Teichosporaceae*. In our original data set, it grouped with *Mycosphaerella* species in the *Capnodiales*. As the origin of this sequence (JK 4748) cannot be verified, and because of the distinctive morphology of *B. obiones* which has little in common with those of *Mycosphaerella* and other members in the *Capnodiales*, we did not present these data here.

Two sequences of *Quintaria lignatilis* form a sister group to the *Testudinaceae* but with moderate support for all analyses. The genus has previously been referred to the *Lophiostomataceae* (Cai *et al*. 2006) and shares features in common with *Trematosphaeria*. *Quintaria* differs from *Trematosphaeria* by having completely immersed ascomata with rounded bases, black incrustations lining the sides of the ostiolar canal, a non-amyloid plate in the ascus and hyaline ascospores (Kohlmeyer & Volkmann-Kohlmeyer 1991).

*Carinispora nypae* is another anomalous taxon whose taxonomic position cannot be resolved at this time. It is placed in the paraphyletic assemblage XVI by maximum likelihood and Bayesian derived phylogenies, but not for those obtained by maximum parsimony. This may be due to artifacts associated with long branch lengths and its placement will require more in depth analysis. *Carinispora nypae* is found growing on the marine palm *Nypa fruticans* and has raised crust-like spots covered in a soft crust-like stroma, with lenticular ascomata under a clypeus, cylindrical and narrow asci, and yellow to pale-brown ascospores with a pronounced sheath drawn out on one side into a spine-like polar appendage (Hyde 1992a). Hyde (1992a) commented that it was close to *Phaeosphaeria,* but our data do not support this view.

**Clade XVII*. Aigialaceae*** Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch, **fam. nov.** MycoBank MB515957.

*Etymology*: Named after the type genus.

Familia Pleosporalium, Ascomycetium. Ascomata subglobosa, conica, immersa ad superficialia, ostiolata, ostiolum rotundum vel fissuriforme, epapillata, periphysata. Hamathecium pseudoparaphysibus trabeculatis, eramosis ad basem, ramosis anastomosantibusque supra ascos. Asci octospori, cylindrici pedunculati, pachydermi, fissitunicati, disco apicale, IKI non-reagentes. Ascosporae biseriatae vel uniseriatae, hyalinae ad atro-brunneae, septatae vel muriformes, constrictae ad leviter constrictae, tunica vel calyptra gelatinosa tectae.

Family in the *Pleosporales*, *Ascomycota*. *Ascomata* subglobose and immersed to superficial or conical, ostiolate, ostiolum round or cleft-like, apapillate, black, carbonaceous to coriaceous, single to gregarious. Periphysate. *Hamathecium* trabeculate, unbranched at the base, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 8-spored, cylindrical, pedunculate, thick-walled, fissitunicate, with a refractive apical ring, non-amyloid. *Ascospores* biseriate or monostichous, hyaline to brown, septate to muriform, with a gelatinous sheath or cap.

*Type genus*: *Aigialus* Kohlm. & Schatz.

***Aigialus*** Kohlm. & S. Schatz, Trans. Brit. Mycol. Soc. 85: 699. 1985.
***A. grandis*** Kohlm. & S. Schatz, Trans. Brit. Mycol. Soc. 85: 699. 1985 (*Type species*). Fig. 3A–B, L, N
***A. mangrovis*** Borse, Trans. Brit. Mycol. Soc. 88: 424. 1987. Fig. 3D, P
***A. parvus*** S. Schatz & Kohlm., Trans. Brit. Mycol. Soc. 85: 704. 1985. Fig. 3C, F–G, O
***A. rhizophorae*** Borse, Trans. Brit. Mycol. Soc. 88 : 424. 1987. Fig. 3E, Q
***A. striatispora*** K.D. Hyde, Mycol. Res. 96: 1044. 1992.

Jones *et al*. (2009) accepted four species in this genus, but rejected *A. rhizophorae* as it shared a number of features with *A. grandis*, but only differed in the vertical septation in the subapical cell. Recent collections made in Thailand have enabled us to sequence this species and it is clearly distinct from *A. grandis*. This is a commonly encountered genus on mangrove wood and widely reported in the literature (Borse 1987, Schmit & Shearer 2003, Abdel-Wahab 2005, Jones *et al*. 2006). *Aigialus striatispora* was described from Ranong mangrove, Thailand, but no further collections have been made (Hyde *et al*. 1990, 1993).

***Ascocratera*** Kohlm., Canad. J. Bot. 64: 3036. 1986.
***A. manglicola*** Kohlm., Canad. J. Bot. 64: 3036. 1986 (*Type species*).

*Ascocratera manglicola* is characterised by carbonaceous, black, gregarious ascomata that are conical, crater-like, superficial on wood, on a black stroma, by trabeculate pseudoparaphyses, by asci with a refractive apical ring, and hyaline ascospores, surrounded by a gelatinous evanescent sheath (Kohlmeyer 1986). It is a common species on mangrove wood in the intertidal zone, and known from various tropical geographic locations (Schmit & Shearer 2003).

***Rimora*** Kohlm., Volkm-Kohlm., Suetrong, Sakayaroj & E.B.G. Jones, **gen. nov.** MycoBank MB515958.

*Etymology*: From the Latin *rima* = cleft, fissure and *os* = mouth, in reference to the cleft-like ostiole, a unique feature among marine ascomycetes.

Ascomata erumpentia, apice plano, elongata, epapillata, ostiolo fissuriforme, periphysata, nigra, gregaria. Peridium cellulis pachydermis, texturam angularem formans. Hamathecium pseudoparaphysibus ramosibus. Asci octospori, cylindrici, pedunculati, pachydermi, fissitunicati, sine apparatu apicali. Ascosporae distichae, fusiformes, triseptatae, hyalinae, tunica gelatinosa tectae.

*Ascomata* erumpent, with flat tops, elongated, apapillate, opening with a periphysate cleft-like ostiole, black, gregarious. *Peridium* of thick-walled cells, forming a *textura angularis. Hamathecium* of branched pseudoparaphyses. *Asci* 8-spored, cylindrical, pedunculate, thick-walled, fissitunicate, without apical apparati. *Ascospores* biseriate, fusiform, 3-septate, hyaline, surrounded by an evanescent sheath.

*Type species*: *Rimora mangrovei* (Kohlm. & Vittal) Kohlm.,Volkm-Kohlm., Suetrong, Sakayaroj, E.B.G. Jones.

***Rimora mangrovei*** (Kohlm. & Vittal) Kohlm.,Volkm-Kohlm., Suetrong, Sakayaroj & E.B.G. Jones, **comb. nov.** MycoBank MB515959. Fig. 3K, S.
*Basionym: Lophiostoma mangrovei* Kohlm. & Vittal, Mycologia 78: 487. 1986.
≡ *Astrosphaeriella mangrovei* (Kohlm. & Vittal) Aptroot & K.D. Hyde, in K.D. Hyde, Fungi in Marine Environments. Fungal Diversity Press 7: 106. 2002.

*Rimora mangrovei* was described from collections of bark and wood of mangrove trees from Belize and India (Kohlmeyer & Vittal 1986) as *Lophiostoma*. It was subsequently transferred to *Astrosphaeriella* (Hyde *et al*. 2002b) based on the trabeculate morphology of

the pseudoparaphyses. However, the aforementioned authors conceded that *A. mangrovis* (and *A. asiana*) differed from other *Astrosphaeriella* species by their round flattened ascomata, slit-like ostioles and non monocotyledonous hosts.

All three genera *Aigialus*, *Ascocratera* and *Rimora* share features such as carbonaceous, apapillate ascomata, trabeculate pseudoparaphyses, cylindrical asci with an apical apparatus and ascospores with a sheath. However, they differ in the morphology of their ascospores: brown and muriform in *Aigialus*, hyaline and 1–3-septate in *Ascocratera* and *Rimora*.

## 2. *Mytilinidiales*, Fig. 1

### Clade XIX. *Mytilinidiaceae*

The common bitunicate ascomycete *Kirschsteiniothelia maritima* groups with *Lophium mytilinum*, with *Mytilinidion mytilinellum* and *Hysterium andinense* as a sister group. The genus *Kirschsteiniothelia* has been referred to the *Pleosporaceae* (Eriksson & Hawksworth 1998, Kirk *et al*. 2001), *Pleomassariaceae* (Barr 1993), and questionably the *Massarinaceae* (Kodsueb *et al*. 2006). The genus appears to be polyphyletic, and Shearer (1993a) and Schoch *et al*. (2006) are of the opinion that *K. aethiops* does not belong in the *Pleosporaceae*. Kodsueb *et al*. (2006) show that *K. elaterascus* (a freshwater species) clusters with *Morosphaeria* (*Massarina) ramunculicola* in a sister clade to the *Melanommataceae* (see also clade XI, Fig. 1). However, *K. elaterascus* differs from *K. maritima,* and other *Kirschsteiniothelia* species in ascus structure, its unusual endoascus with a long, coiled base that uncoils during ascus dehiscence, ascospore measurements, the presence of an ascospore sheath and its freshwater occurrence (Shearer 1993a).

### Clade XX. Unresolved taxa

Included in this clade are three coelomycete species of which *Pseudorobillarda phragmitis* has been reported from pine and yellow poplar test panels from estuarine waters (Salinity 3–16 ppt) (Jones *et al*. 2009). This monophyletic group formed a well-supported clade and a sister group to the *Mytilinidiales*. However in the current study they form a weakly supported clade with *Farlowiella carmichaeliana* and are basal to the *Mytilinidiales* in all analyses.

## 3. *Patellariales*, Fig. 1

### Clade XXII. *Patellariaceae*

*Patellaria cf. atrata* (Fig 2B, R, AD), a species found growing on various mangrove wood species collected in Hong Kong and Thailand, forms a sister group to *Hysteropatella* species, taxa normally assigned to the *Hysteriales*, but recently removed (Boehm *et al.* 2009a, b; this volume). Morphologically, little distinguishes *Gloniella clavatispora* and *Patellaria atrata*; paraphyses in the latter species are distinctly branched and club-shaped (Suetrong & Jones 2006). The paraphyses illustrated by Steinke & Hyde (1997) are simple and not branched (Suetrong & Jones 2006). Boehm *et al*. (2009a; this volume) refer *Gloniella* to the *Hysteriaceae*, and *Patellaria* in the *Patellariaceae*; further collections of the marine taxa are required to resolve their identification.

A number of marine species do not group within existing orders of *Dothideomycetes* and this may indicate new supergeneric taxa not yet circumscribed. The lack of sufficient protein coding gene sequences for these in our analysis and the tendency for these species to be associated with fast evolving branches on our trees further complicates the development of phylogenetic hypotheses for these taxa.

(i) *Biatriospora marina* (Clade XIV), in all analyses, forms a distinct long branch and is a basal taxon to the *Pleosporomycetidae* without any closely related taxa (Fig. 1). It is an unusual species described from *Sonneratia alba* mangrove wood collected in the Seychelles and India (Hyde & Borse 1986a). It has immersed subglobose to pyriform ascomata that are black and carbonaceous, cylindrical asci and brown, septate ascospores with hyaline, globose refractive chamber or an appendage at each end. Septation is unusual in that ascospores are non-septate in the center but septate at both ends and not constricted at the septa. Additional collections have been made from mangroves in Hong Kong, Malaysia and Thailand (Jones *et al*. 2006, E.B.G. Jones unpubl. data).

(ii) *Saccardoella rhizophorae* Clade XIX. *Saccardoella* species have been regarded as having unitunicate asci and thus classified in the *Clypeosphaeriaceae* (Barr 1994). However, Mathiassen (1989) was of the opinion that the asci are bitunicate and this would appear to be supported by the current study. *Saccardoella* species are known from terrestrial, marine and freshwater habitats (Hyde 1992c, Tsui *et al*. 1998). However in all phylogenetic analyses to date this species does not group within any known family or order, and further studies are required to determine its phylogenetic relationship.

## 4. *Jahnulales*

### *Aliquandostipitaceae* (data not shown)

The family *Aliquandostipitaceae* was established for species in the genus *Aliquandostipite* based on the phylogenetic analyses of SSU nrDNA sequences (Inderbitzin *et al.* 2001). Subsequently Pang *et al*. (2002) introduced the new order *Jahnulales* into the *Dothideomycetes*, *Ascomycota*, based on phylogenetic analysis of SSU nrDNA sequences of *Aliquandostipite*, *Jahnula* and *Patescospora*. More recently, Campbell *et al*. (2007) studied the phylogenetic relationships of taxa in the *Jahnulales* inferred from SSU and LSU nrDNA sequences and recognised four groups : 1) a basal group with *Megalohypha aqua-dulces*; 2) a *Jahnula* group comprising the type species *J. aquatica*; 3) five *Aliquandostipite* species; and 4) four *Jahnula* species and the anamorphic genera *Brachiosphaera* and *Xylomyces*. They emended the ordinal description to include brown, wide hyphae (>10 µm) and greater variation of ascospore morphology.

Three marine fungi belong in the *Jahnulales*, the teleomorph *Manglicola guatemalensis* and the anamorphic species *Xylomyces chlamydosporus* and *X. rhizophorae* (Suetrong *et al*. 2010). *Manglicola guatemalensis* is a poorly known species with only three previous collections (Kohlmeyer & Kohlmeyer 1971, Hyde 1988, Jones *et al*. 2009, Suetrong *et al*. 2010). The type strain was collected from dead roots of *Rhizophora mangle* in Guatemala (Kohlmeyer & Kohlmeyer 1971). Subsequent collections have been made on intertidal prop roots of *Rhizophora apiculata* at Kpg. Danau, Brunei (Hyde 1988) and frond bases of *Nypa fruticans* (Jones *et al*. 2009). Common features *M. guatemalensis* shares with the *Jahnulales* include stipitate ascomata, bitunicate asci, reticulate pseudoparaphyses and 1-septate brown ascospores. *Manglicola guatemalensis* differs from other bitunicate ascomycetes by its large

ascomata, wide ostiole, large unequally 1-septate ascospores and mangrove habitat on *R. mangle* and the frond bases of *N. fruticans*.

Huhndorf (1994) referred *Manglicola* to the *Hypsostromataceae*, a family with no known relationship to any group in the *Dothideomycetes* (*Loculoascomycetes*) but "probably with affinities to the *Melanommatales*" (Mugambi & Huhndorf 2009; this volume). Characteristics that unite *Manglicola* and the *Hypsostromataceae* include superficial, large, elongate ascomata (stalked) with a soft-texture, trabeculate pseudoparaphyses, stipitate asci attached in a basal arrangement in the centrum and fusiform, septate ascospores (Huhndorf 1994).

## *Dothideomycetidae*

## 5. *Capnodiales,* Fig. 1

Fourteen genera, such as *Belizeana*, *Caryosporella, Coronopapilla*, *Lautospora*, *Loratospora*, *Pontoporeia* and *Thalassoascus*, assigned to the subclass *Dothideomycetidae*, have only marine species, and represent new lineages of fungi that may be associated with the *Capnodiales* (Jones *et al*. 2009). Importantly, few have been studied at the molecular level. Placement of the genera *Passeriniella* and *Pontoporeia* has already been discussed above.

### Clade XXV. *Mycosphaerellaceae*

*Mycosphaerella eurypotami*, a halotolerant terrestrial species found on *Juncus roemerianus*, was tentatively referred to the genus by Kohlmeyer *et al*. (1997b). In the current study it is a sister taxon to all *Mycosphaerella* species with moderate support. Jones *et al*. (2009) list three marine *Mycosphaerella* species (*M. salicorniae*, *M. staticiola*, *M. suaedae-australis*) found on salt marsh plants (*Armeria*, *Limonium*, *Salicornia* and *Suaeda*), while *M. pneumatophorae* is a common species on the pneumatophores of *Avicennia* species in Asia and the Carribean (Kohlmeyer & Kohlmeyer 1979, Schmit & Shearer 2003, E.B.G. Jones, pers. comm.). However recent molecular phylogenies containing a single culture did not support the placement of *M. pneumatophorae* in *Mycosphaerella* (Schoch *et al*. 2006); instead it was found on a poorly resolved branch within *Dothideomycetes*.

In our analysis, *Scirrhia annulata*, described from senescent leaves of *Juncus roemerianus* (Kohlmeyer *et al*. 1996), groups with various *Mycosphaerella* species with moderate support. Diagnostic features are the linear stromata, 1–3 mm long, generally superficial, multiloculate with ascomata in longitudinal rows, asci clavate with apical apparatus (several rings), ascospores 3-septate, brown, with a thin evanescent sheath, and measuring 46–60 x 9–11.5 µm.

### Clade XVIII. Unresolved taxa (Fig. 1)

(i) The taxonomic position of *Heleiosa barbatula* (Fig. 1) is unresolved as observed by its swapping position in different analyses (data not shown) and previously referred to the *Dothideales* and *Pleosporales incertae sedis*, respectively (Kohlmeyer *et al*. 1996, Jones *et al*. 2009). This species, collected on *Juncus roemerianus*, is rare and is not obligately marine. Characteristics include immersed ostiolate epapillate ascomata formed beneath a clypeus, with pseudoparaphyses, asci cylindrical with short pedicel, refractive apical apparatus and ascospores that are pale brown, ellipsoid, 1-septate with 10 or more cilia-like polar appendages at each end.

(ii) The genera *Caryosporella*, and *Lineolata* form a basal clade in all analyses with weak support, genera previously assigned to *Melanommataceae* and *Pleosporales incertae sedis,* respectively (Jones *et al*. 2009). Both occur on mangrove substrata and have been widely reported from different geographical locations (Schmit & Shearer 2003).

*Caryosporella* was thought to be related to *Caryospora,* with which it shares a number of common features (Kohlmeyer 1985). It is found on dead wood of intertidal roots and branches of mangrove trees and has large ascomata and 1-septate, dark-brown ascospores that are thickened at their apices.

*Lineolata* was initially described as a *Didymosphaeria* but transferred to this genus (Kohlmeyer & Volkmann-Kohlmeyer 1990) as it differs in the following respects: no clypeus, almost superficial ascomata, hamathecium with a gelatinous matrix, asci with an apical ring-like structure around the ocular chamber and ornamented brown ascospores. It remains enigmatically placed here, although three monophyletically placed isolates obtained from different geographic locations heighten our confidence in the provenance of these sequences.

## DISCUSSION

### Marine lineages of the *Dothideomycetes*

The study confirms the occurrence of several marine *Dothideomycetes* with well supported sequence data. The *Pleosporales* includes ten families and three unresolved clades with marine species, while the orders *Capnodiales*, *Jahnulales*, *Mytilinidiales*, and *Patellariales* are represented by few taxa. This is in common with their known diversity (?) in nature (Kohlmeyer & Kohlmeyer 1979, Jones *et al*. 2009). While many terrestrial genera have marine members, *e.g. Mycosphaerella*, *Passeriniella*, *Lophiostoma*, *Massarina*, *Trematosphaeria* and *Phaeosphaeria*, others have no known terrestrial counterparts. The uniqeness of these has necessitated the introduction of two new families in the *Pleosporales*, *Aigialaceae* (all marine genera: *Aigialus*, *Ascocratera*, *Rimora*) and *Morosphaeriaceae* (marine genera *Helicascus*, *Morosphaeria* and the freshwater species *Kirschsteiniothelia elaterascus*). The taxonomic position of other exclusively marine genera/species remains to be resolved *e.g.* the seagrass ascomycetes *Halotthia posidoniae*, *Pontoporeia biturbinata* (CladeXIV), and *Lineolata rhizophorae* (Clade XVIII) and *Biatriospora marina* (Clade XIV).

A number of new marine lineages have been highlighted as result of molecular studies including *Manglicola guatemalensis,* the first member of the *Jahnulales* reported from marine habitats (Suetrong *et al*. 2010). This is of particular interest as all other *Jahnulales* members are fresh water or peat swamp species and raises the question as to whether these marine fungi are derived from terrestrial and freshwater taxa that have migrated to the sea. This would support earlier phylogenetic analyses (Spatafora *et al*. 1998) that strongly suggest a terrestrial origin of another marine ascomycete family in the *Sordariomycetes*, the *Halosphaeriaceae*. A more recent data set (Schoch *et al.* 2009a; this volume) continues to support this hypothesis. The marine species *M. guatemalensis* occurs in estuarine mangrove habitats on the palm fronds of *Nypa fruticans* and *Rhizophora* wood and may well form a link between lignicolous freshwater taxa and species from estuarine to marine environments. Another *Jahnulales* species of interest is the anamorph *Xylomyces rhizophorae*, found on various marine and

mangrove substrata (Kohlmeyer & Volkmann-Kohlmeyer 1998, S. Sivichai, pers. comm.). Campbell *et al*. (2007) and Prihatini *et al*. (2008) have shown that *Xylomyces chlamydosporus* has a teleomorph in the *Jahnulales*.

A second marine lineage is the *Aigialaceae* comprising three genera: *Aigialus*, *Ascocratera*, and the new genus *Rimora*, a family within the *Pleosporales*. Morphologically they show few common characteristics but all are to be found in mangrove habitats.

Schoch *et al*. (2006) showed that *Verruculina enalia* is a member of the *Testudinaceae,* and another marine lineage in the *Dothideomycetes*. Previously referred to the *Didymosphaeriaceae* (Kohlmeyer & Volkmann-Kohlmeyer 1990), it forms a well supported basal clade to the *Pleosporales*. Continued molecular studies of unresolved taxa may yield further lineages of marine ascomycetes.

## Taxa for future phylogenetic study

Marine *Dothideomycetes* include a broad spectrum of genera and a wide variety has been sequenced for the current study. However, several remain to be investigated with DNA sequence data, especially the genera *Belizeana*, *Capillatospora* and *Thalassoascus* (*Dothideales incertae sedis*); *Lautospora* (*Dothideomycetidae incertae sedis*); *Bicrouania* (*Melanommataceae*?); *Lautitia* (*Phaeosphaeriaceae*?) and *Tirisporella* (*Pleosporales incertae sedis*). Most are only rarely collected, have yet to be isolated, are intertidal, or rarely totally submerged. Other more frequently collected taxa also require further analysis: *Quintaria lignatilis* (mangrove species), *Decaisnella formosa* (wood in association with sand) and *Byssothecium obiones* (on *Spartina* grass).

## Adaptation to the marine environment

Of the 64 genera (108 species) of marine *Dothideomycetes* nearly all are intertidal species found in mangrove habitats, with the exception of those that occur on marine algae, saltmarsh plants or seagrasses, *e.g. Thalassoascus, Lautitia, Pharcidia* (algae), *Bicrouania* (marsh plants)*, Halotthia*, *Pontoporeia* (seagrasses); *Caryospora australiensis*, *Decaisnella formosa* and *Platystomum scabridisporum* (wood associated with sand) (Abdel-Wahab & Jones 2000, 2003). Most of them would appear to be well adapted to intertidal estuarine habitats with active discharge of their ascospores. Although they lack the elaborate ascospore appendages found in the *Halosphaeriaceae* (Jones 1994, 1995) many have mucilaginous sheaths, often elaborated to form polar appendages (Yusoff *et al*. 1994, Read *et al*. 1997a, b, Alias *et al*. 2001, Au *et al*. 1999). Ascospores within the ascus are surrounded by a well-defined delimiting membrane which prevents the mucilaginous sheath from expanding, thus ensuring effective ascospore discharge (Read *et al*. 1994, Yusoff *et al*. 1994). Once ejected from the ascus the sheaths (and appendages) take up water, swell and help in the attachment of the spores to suitable substrata (Jones 1995).

Some species form ascospore appendages by fragmentation of a sheath *e.g. Capronia ciliomaris* (Au *et al*. 1999) and *Tirisporella beccariana* (Jones *et al*. 1996). A similar mechanism of appendage unfolding appears to occur in *Heleiosa barbatula* (Kohlmeyer *et al.* 1996). As with the ensheathed ascospores, the appendages do not dilate until they are dispersed into water.

Few marine anamorphic fungi have been reported in comparison to those found in freshwater habitats (Marvanová 1997, Belliveau & Bärlocher 2005, Cai *et al*. 2006). Currently some 94 marine anamorphs are known, but only a few have been linked to teleomorphs in the *Dothideomycetes*: *Amorosia littoralis* (Mantle *et al*. 2006), *Dendryphiella arenaria*, *D. salina* (Jones *et al*. 2008), *Xylomyces* spp. (Campbell *et al*. 2007, Prihatini *et al*. 2008), *Pseudorobillarda phragmitis* (Rungjindamai, pers. comm.), and *Robillarda rhizophorae* (Rungjindamai, pers. comm.). A strain of *Alternaria maritima* groups within the *Pleosporaceae* in the current study, while other marine anamorphic species *e.g. Stemphylium* spp. *Stagonospora* spp., may also be linked to teleomorphs in the *Dothideomycetes.*

Freshwater anamorphic fungi are uniquely adapted to their habitat with branched, sigmoid and tetraradiate conidia (Jones 2006, Campbell *et al*. 2007); many have teleomorphs in the *Dothideomycetes* (Webster & Descals 1979, Tsui & Berbee 2006, Tsui *et al*. 2006). In contrast few of the marine hyphomycetes appear to be adapted to their milieu, lacking any elaboration of their conidia (except *e.g. Varicosporina ramulosa* and *Dwayaangam junci*). This is particularly so for species with recorded teleomorphs in the *Dothideomycetes* (Jones *et al*. 2008).

## Specific habitats of marine *Dothideomycetes*

Marine *Dothideomycetes* are generally intertidal ascomycetes and more common in mangroves, with only a few documented from temperate climates.

(i) *Nypa fruticans:* Currently some 100 saprophytic fungi have been documented from *Nypa fruticans*, a brackish water palm that occurs from fully saline conditions to freshwater habitats. Common fungi on this palm include *Astrosphaeriella nypae*, *Astrosphaeriella striatispora*, *Helicascus nypae*, *Linocarpon appendiculatum* and *Tirisporella beccariana*. Many of the fungi occurring in *Nypa* are not found on other mangrove or marine substrata, for example, *Linocarpon* spp., *Astrosphaeriella* spp., *Oxydothis* spp. and *Fasciatispora lignicola.* Therefore one could ask, are these fungi host-specific or is their occurrence on *Nypa* determined by the salinity of the habitat? A significant number of fungi on *Nypa* are unique to the palm, *e.g. Helicascus nypae*, *Tirisporella beccariana* and *Carinispora nypae* while recently *Manglicola guatemalensis* has been found to be common on this palm in Thailand.

(ii) *Seagrasses*: The diversity of fungi in seagrasses has been a neglected field (Raghukumar 2008). Generally, diverse seagrass species support low diversity and density of saprophytic and endophytic fungi, as confirmed by many studies (Wilson 1998, Alva *et al*. 2002, Devarajan *et al.* 2002, Rodríguez 2008, Sakayaroj *et al*. 2010). The most common marine fungi associated with seagrasses include *Sordariomycetes*, *Corollospora maritima*, *Lindra thalassiae, Lulworthia* sp. and anamorphic fungi (Kohlmeyer & Kohlmeyer 1979, Newell & Fell 1980). Cuomo *et al.* (1982, 1985) reported that the marine *Dothideomycetes*, *Pontoporeia biturbinata*, and *Halotthia posidoniae* were commonly found on *Posidonia oceanica* and *Cymodocea nodosa* from Mediterranean coasts (Cuomo *et al.* 1982, 1985) and Cyprus (Jones *et al.* 2009). These two obligate marine *Dothideomycetes* appear to be host specific and are frequently found on rhizomes of seagrass (Kohlmeyer & Kohlmeyer 1979).

Many anamorphic dothideomycetous fungi have been found predominantly as endophytes associated with living seagrass tissues (Sakayaroj *et al.* 2010). They are mostly sterile mycelia and have only been identified by DNA sequence analysis (Sakayaroj *et al.* 2010). So far the diversity of marine fungi associated with seagrasses, compared with other substrata, is relatively low (Kohlmeyer & Kohlmeyer 1979). This is probably due to 1) growth

inhibiting substances present in seagrass, 2) possibly the frail leaves of seagrass break up before most of the ascomycetes are able to colonise or sporulate and finally 3) they are attacked by other competitors such as bacteria, protozoa, lower fungi, fast growing anamorphic and/or terrestrial fungi (Sakayaroj *et al*. 2010).

(iii) *Saltmarsh plants*: *Spartina* and *Juncus roemerianus*: The mycota of the saltmarsh plant *Juncus roemerianus*, endemic to the U.S. east coast and to the Gulf of Mexico, is unique among herbaceous plants and can only be vaguely compared to that of mangrove trees, which also host obligate marine as well as terrestrial species. The terete leaves of *J. roemerianus* remain standing for three years or more and the extreme conditions of the habitat are the reason for the unique fungal diversity (117 species, 17 families; Kohlmeyer & Volkmann-Kohlmeyer 2001). Bitunicates appear to be less abundant than other groups of fungi; they range from obligate marine taxa at the base to terrestrial but halotolerant species at the tip of the leaves.

*Spartina* species are common saltmarsh plants in temperate climates that support a wide range of fungi. Kohlmeyer & Volkmann-Kohlmeyer (2002) list 39 obligate and facultative marine fungi reported from *Spartina* species, of which 13 are bitunicate species. *Phaeosphaeria* species appear to be the most common bitunicate genus on this substratum.

(iv) *Mangroves*: Some 54 species of mangrove trees and 60 associates occur in the new and old world (Tomlinson 1986) with senescent wood, leaves and fruits offereing a unique habitat for fungi. It is interesting that maglicolous fungi are predominantly bitunicate species, while unitunicate ascomycetes are more prevalent in other marine habitats. Of the 108 described marine *Dothideomycetes*, 90 sequences are currently available enabling the taxonomic resolution of a number of genera and species; in particular of *Massarina* species which are frequently found on mangrove substrata.

## Future studies

Many habitats, substrata, geographical locations remain virgin territory for studies on marine fungi. For example, a recent investigation of the fungal diversity associated with the brown alga *Fucus serratus* found several unknown phylotypes within the *Dothideomycetes*, including some grouping with an anamorph species isolated from leaf litter (*Sporidesmium obclavatulum;* Shenoy *et al.* 2006) without obvious marine assocations (Zuccaro *et al*. 2008). Previously Zuccaro & Mitchell (2005) isolated fungi from living and cast fronds of the alga, with 33 % belonging in the *Dothideomycetes*. Many other niches such as endophytes from marine animals and mangroves await intense study (Pang *et al*. 2008, Schulz *et al*. 2008, Wang *et al*. 2008). Practical applications are also possible as marine endophytes from plants and animals have already yielded a wide range of new chemical structures (Jones 2008, Pan *et al*. 2008). Unknown fungi, including those belonging to the *Dothideomycetes*, have even been isolated from extreme marine environments, *e.g.* ocean sediments and deep sea hydrothermal ecosystems (Burgaud *et al*. 2009). Although it remains to be seen whether these fungi truly qualify as marine fungi the increase in fungal and dothideomycete phylotypes from these environments suggest additional sources of untapped diversity (Le Calvez *et al*. 2009).

In conclusion, marine bitunicate ascomycetes, (as other marine fungi) is a broadly defined ecological group that occupy a wide range of habitats within the maritime environment. Within this study facultative and halotolerant species from *Juncus roemerianus* were also included, as well as two genera on submerged seagrasses from European regions. The vast majority of fungi presented are predominantly tropical/subtropical mangrove species. When compared to the other diverse groups of marine fungi in the *Sordariomycetes* the prevalence of mangrove fungi in *Dothideomycetes* is even more noticeable. Does this ecological predominance reflect a radiation event of these fungi in the *Dothideomycetes*? Or is our sampling still biased towards specific geographies and ecologies? Only a renewed focus on the niches described above will provide us with the answer. It is our hope that a broader scope will provide enough resolution to begin to address ecological shifts in this fascinating group of fungi.

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## SUPPLEMENTARY INFORMATION

**Table 1.** The list of species used in this study.

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| ***Acrocordiopsis patilii*** | Mangrove wood | J. Sakayaroj | Thailand, Hat Khanom Mu Ko Thale Tai National Park | BCC 28166 | **GU479736** | **GU479772** | **GU479811** | – |
| ***Acrocordiopsis patilii*** | Mangrove wood | J. Sakayaroj. | Thailand, Hat Khanom Mu Ko Thale Tai National Park | BCC 28167 | **GU479737** | **GU479773** | **GU479812** | – |
| ***Aigialus grandis*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18419 | **GU479738** | **GU479774** | **GU479813** | **GU479838** |
| ***Aigialus grandis*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 20000 | **GU479739** | **GU479775** | **GU479814** | **GU479839** |
| ***Aigialus grandis*** | Mangrove wood | J. Kohlmeyer | Belize, Stewart Island | JK 5244A | **GU296131** | **GU301793** | **GU371762** | – |
| ***Aigialus grandis*** | Mangrove wood | J. Kohlmeyer | Bahamas, Mores Island | JK 4770 | **GU479740** | – | – | – |
| ***Aigialus grandis*** | Mangrove wood | E.B.G Jones | Malaysia, Morib | CY 2909 | AF441172 | – | – | – |
| ***Aigialus mangrovei*** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 33563 | **GU479741** | **GU479776** | **GU479815** | **GU479840** |
| ***Aigialus mangrovei*** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 33564 | **GU479742** | **GU479777** | **GU479816** | **GU479841** |
| ***Aigialus parvus*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18403 | **GU479743** | **GU479778** | **GU479817** | **GU479842** |
| ***Aigialus parvus*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 32558 | **GU479744** | **GU479779** | **GU479818** | **GU479843** |
| ***Aigialus parvus*** | Mangrove wood | E.B.G. Jones | Malaysia Morib | CY 5061 | AF441173 | – | – | – |
| ***Aigialus rhizophorae*** | Mangrove wood | S. Suetrong | Thailand, Mu Ko Chang National Park | BCC 33572 | **GU479745** | **GU479780** | **GU479819** | **GU479844** |
| ***Aigialus rhizophorae*** | Mangrove wood | S. Suetrong | Thailand, Mu Ko Chang National Park | BCC 33573 | **GU479746** | **GU479781** | **GU479820** | **GU479845** |
| *Allewia eureka* | | | | DAOM 195275 | DQ677994 | DQ678044 | DQ677938 | DQ677883 |
| *Alternaria alternata* | | | | CBS 916.96 | DQ678031 | DQ678082 | DQ677980 | DQ677927 |
| ***Alternaria maritima*** | Ubiquitous | | | CBS 126.60 | **GU456294** | **GU456317** | – | – |
| ***Amorosia littoralis*** | Littoral zone | P.G. Mantle | Bahamas, Crooked Island | NN 6654 | AM292056 | AM292055 | – | – |
| *Ascochyta pisi* | | | | CBS 126.54 | DQ678018 | DQ678070 | DQ677967 | DQ677913 |
| ***Ascocratera manglicola*** | | K. Tanaka | Japan, Okinawa | HHUF 30032 | **GU479748** | **GU479783** | **GU479822** | **GU479847** |
| ***Ascocratera manglicola*** | Mangrove wood | E.B.G. Jones | Thailand, Ranong Mangrove forest | BCC 09270 | **GU479747** | **GU479782** | **GU479821** | **GU479846** |
| ***Ascocratera manglicola*** | | J. Kohlmeyer | Belize, Tobacco Range | JK 5262C, CBS 120023 | **GU296136** | **GU301799** | **GU371763** | – |
| *Aureobasidium pullulans* | | | | CBS 584.75 | DQ471004 | DQ470956 | DQ470906 | DQ471075 |
| *Berkleasmium micronescium* | | | | BCC 8141 | DQ280268 | DQ280272 | – | – |
| *Berkleasmium nigroapicale* | | | | BCC 8220 | DQ280269 | DQ280273 | – | – |
| ***Biatriospora marina*** | Mangrove wood | E.B.G. Jones | Singapore, Singapore mangrove forest | CY 1228 | GQ925835 | GQ925848 | **GU479823** | **GU479848** |
| *Bimuria novae-zelandiae* | | | | CBS 107.79 | DQ677998 | DQ678051 | DQ677944 | DQ767637 |
| *Botryosphaeria dothidea* | | | | CBS 115476 | DQ677998 | DQ678051 | DQ677944 | DQ767637 |
| *Botryosphaeria ribis* | | | | CBS 115475 | DQ678000 | DQ678053 | DQ677947 | DQ677893 |
| *Botryosphaeria stevensii* | | | | CBS 431.82 | DQ678012 | DQ678064 | DQ677960 | DQ677907 |
| *Botryosphaeria tsugae* | | | | CBS 418.64 | AF271127 | DQ767655 | DQ767644 | DQ677914 |

**Table 1.** (Continued).

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| *Byssothecium circinnans* | | | | CBS 675.92 | AY016339 | AY016357 | DQ767646 | - |
| *Capnodium coffeae* | | | | CBS 147.52 | DQ247808 | DQ247800 | DQ247788 | DQ471089 |
| *Capnodium salicinum* | | | | CBS 131.34 | DQ677997 | DQ678050 | – | DQ677889 |
| ***Carinispora nypae*** | Mangrove wood (*Nypa fruticans*) | A. Loilong | Thailand,Tambon Bang Pao | BCC 36316 | **GU479749** | – | – | **GU479849** |
| ***Caryosporella rhizophorae*** | Mangrove wood | J. Kohlmeyer | Fiji, Suva | JK 5302A | **GU479750** | **GU479784** | – | – |
| *Cladosporium cladosporioides* | | | | CBS 170.54 | DQ678004 | DQ678057 | DQ677952 | DQ677898 |
| *Columnosphaeria fagi* | | | | CBS 171.93 | AY016342 | AY016359 | DQ677966 | – |
| *Davidiella tassiana* | | | | CBS 399.80 | DQ678022 | DQ678074 | DQ677971 | DQ677918 |
| ***Decaisnella formosa*** | | E.B.G. Jones | Australia, The Mornington Peninsula National Park | BCC 25617 | GQ925834 | GQ925847 | **GU479824** | **GU479850** |
| ***Decaisnella formosa*** | Wood, sand | E.B.G. Jones | Australia, The Mornington Peninsula National Park | BCC 25616 | GQ925833 | GQ925846 | **GU479825** | **GU479851** |
| ***Decorospora gaudefroyi*** | Salt marsh plants | | | CBS 322.63 | AF394542 | – | – | – |
| *Delitschia winteri* | | | | CBS 225.62 | DQ678026 | DQ678077 | DQ677975 | DQ677922 |
| *Delphinella strobiligena* | | | | CBS 735.71 | DQ471029 | DQ470977 | DQ677951 | DQ471100 |
| ***Dendryphiella arenaria*** | Algae, sand | J. Nicot | France, Gironde, Arcachon area | CBS 181.58 | DQ471022 | DQ470971 | DQ470924 | DQ677890 |
| ***Dendryphiella salina*** | *Spartina* sp. | E.B.G. Jones | U.K., England; Southampton, Langstone Harbour | CBS 142.60 | – | – | DQ435066 | DQ414251 |
| *Didymella cucurbitacearum* | | | | IMI 373225 | AY293779 | AY293792 | – | – |
| ***Didymella fucicola*** | Alga (*Fucus vesiculosus*) | J. Kohlmeyer | U.K., West Looe | JK 2932 | – | EF177852 | – | – |
| *Dothidea hippophaes* | | | | DAOM 231303 | U42475 | DQ678048 | DQ677942 | DQ677887 |
| *Dothidea insculpta* | | | | CBS 189.58 | DQ247810 | DQ247802 | AF107800 | DQ471081 |
| *Dothidea sambuci* | | | | DAOM 231303 | AY544722 | AY544681 | DQ522854 | DQ497606 |
| *Dothiora cannabinae* | | | | CBS 737.71 | DQ479933 | DQ470984 | DQ470936 | DQ471107 |
| *Elsinoë centrolobi* | | | | CBS 222.50 | DQ678041 | DQ678094 | – | DQ677934 |
| *Elsinoë phaseoli* | | | | CBS 165.31 | DQ678042 | DQ678095 | – | DQ677935 |
| *Elsinoë veneta* | | | | CBS 150.27 | DQ767651 | DQ767658 | – | DQ767641 |
| ***Falciformispora lignatilis*** | Mangrove wood (*Elaeis guineensis*) | U. Pinruan | Thailand, Ban Bang Sak | BCC 21118 | **GU371835** | **GU371827** | – | **GU371820** |
| ***Falciformispora lignatilis*** | Mangrove wood (*Elaeis guineensis*) | U. Pinruan | Thailand, Ban Bang Sak | BCC 21117 | **GU371834** | **GU371826** | – | **GU371819** |
| *Farlowiella carmichaeliana* | | | | CBS 206.36 | AY541482 | AY541492 | DQ677989 | DQ677931 |
| ***Floricola striata*** | *Juncus roemerianus* (Facultative) | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5678I | **GU296149** | **GU301813** | **GU371758** | **GU479852** |
| ***Floricola striata*** | *Juncus roemerianus* (Facultative) | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5603K | **GU479751** | **GU479785** | – | – |
| *Gloniopsis praelonga* | | | | CBS 112415 | FJ161134 | FJ161173 | FJ161113 | FJ161090 |
| *Gloniopsis subrugosa* | | | | CBS 123346 | FJ161170 | FJ161210 | FJ161131 | – |
| *Guignardia bidwellii* | | | | CBS 237.48 | DQ678034 | DQ678085 | DQ677983 | – |
| *Guignardia gaultheriae* | | | | CBS 444.70 | – | DQ678089 | DQ677987 | DQ677930 |
| ***Halomassarina* (*Massarina*) *thalassiae*** | Mangrove wood | J. Kohlmeyer | Fiji, Viti Levu, Suva | JK 5385B | – | **GU479804** | – | **GU479853** |
| ***Halomassarina* (*Massarina*) *thalassiae*** | Mangrove wood | J. Kohlmeyer. | Belize, Tobacco Range | JK 5262D | – | **GU301816** | – | **GU349011** |

**Table 1.** (Continued).

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| ***Halomassarina* (*Massarina*) *thalassiae*** | Mangrove wood | E.B.G. Jones | U.S.A., Florida | BCC 17055 | GQ925843 | GQ925850 | – | – |
| ***Halomassarina* (*Massarina*) *thalassiae*** | Mangrove wood | E.B.G. Jones | U.S.A., Florida | BCC 17054 | GQ925842 | GQ925849 | – | – |
| ***Halotthia posidoniae*** | Seagrasses (*Posidoniae oceanica*) | E.B.G. Jones | Cyprus | BBH 22481 | **GU479752** | **GU479786** | – | – |
| ***Heleiosa barbatula*** | *Juncus roemerianus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5548I | **GU479753** | **GU479787** | – | – |
| ***Helicascus kanaloanus*** | | | | A 237 | AF053729 | – | – | – |
| ***Helicascus nypae*** | Mangrove wood (*Nypa fruticans*) | A. Loilong | Thailand, Tambon Bang Pao | BCC 36751 | **GU479754** | **GU479788** | **GU479826** | **GU479854** |
| ***Helicascus nypae*** | Mangrove wood (*Nypa fruticans*) | A. Loilong | Thailand, Tambon Bang Pao | BCC 36752 | **GU479755** | **GU479789** | **GU479827** | **GU479855** |
| ***Helicascus nypae*** | Mangrove wood (*Nypa fruticans*) | E.B.G. Jones | Malaysia, Kuala Selangor | PP 6066 | AF441174 | – | – | – |
| *Helminthosporium solani* | | | | HSWS 04 | AF120253 | – | – | – |
| *Helminthosporium velutinum* | | | | ATCC 38969 | AF120254 | – | – | – |
| *Herpotrichia diffusa* | | | | CBS 250.62 | DQ678019 | DQ678071 | DQ677968 | DQ677915 |
| *Herpotrichia juniperi* | | | | CBS 200.31 | DDQ678029 | DQ678080 | DQ677978 | DQ677925 |
| *Hysterium andinense* | | | | CBS 123562 | FJ161159 | FJ161199 | FJ161125 | FJ161107 |
| *Hysterium angustatum* | | | | CBS 236.34 | – | FJ161180 | FJ161117 | FJ161096 |
| *Hysterium pulicare* | | | | CBS 123377 | FJ161161 | FJ161201 | FJ161127 | FJ161109 |
| *Hysterobrevium mori* | | | | CBS 123564 | FJ161158 | FJ161198 | – | FJ161106 |
| *Hysterobrevium smilacis* | | | | CBS 114601 | FJ161135 | FJ161174 | FJ161114 | FJ161091 |
| *Hysteropatella clavispora* | | | | CBS 247.34 | DQ678006 | AY541493 | DQ677955 | DQ677901 |
| *Hysteropatella elliptica* | | | | CBS 935.97 | EF495114 | DQ767657 | DQ767647 | DQ767640 |
| ***Julella avicenniae*** | Mangrove wood | E.B.G. Jones | Thailand, Mu Ko Chang National Park | BCC 18422 | **GU371831** | **GU371823** | **GU371787** | **GU371816** |
| ***Julella avicenniae*** | Mangrove wood | E.B.G. Jones | Thailand, Mu Ko Chang National Park | BCC 20173 | **GU371830** | **GU371822** | **GU371786** | **GU371815** |
| ***Julella avicenniae*** | Mangrove wood | J. Kohlmeyer | | JK 5326A | **GU479756** | **GU479790** | – | – |
| ***Julella avicenniae*** | Mangrove wood | E.B.G. Jones | Hong Kong Tingkok | CY 2462 | AF441175 | – | – | – |
| *Keissleriella cladophila* | | | | CBS 104.55 | **GU296155** | **GU301822** | **GU371735** | **GU349043** |
| ***Keissleriella rara*** | *Juncus roemerianus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | CBS 118429 | **GU479757** | **GU479791** | – | – |
| *Kirschsteiniothelia elaterascus* | | | | HKUCC 7769 & A22-5A | AF053727 | AY787934 | – | – |
| ***Kirschsteiniothelia maritima*** | Driftwood | J. Kohlmeyer, B. Kohlmeyer | U.S.A., Washington, Friday Harbor Laboratories | CBS 221.60 | – | **GU323203** | – | **GU349001** |
| ***Lentithecium* (*Massarina*) *phragmiticola*** | *Phragmites*, grass | C. Tsui | Hong KongTai, O Lantau Island | CBS 110446 | DQ813512 | DQ813510 | – | – |
| *Lentithecium arundinaceum* (*Massarina arundinacea*) | | | | CBS 619.86 | DQ813513 | DQ813509 | – | – |
| *Leptosphaeria biglobosa* | | | | CBS 303.51 | – | **GU301826** | – | **GU349010** |
| *Leptosphaeria doliolum* | | | | CBS 505.75 | U43447 | U43474 | – | – |
| *Leptosphaeria maculans* | | | | DAOM 2229267 | DQ470993 | DQ470946 | DQ471062 | DQ471062 |
| *Leptosphaerulina australis* | | | | CBS 939.69 | EU754068 | EU754167 | – | – |

**Table 1.** (Continued).

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| *Lewia infectoria* | | | | IMI 303186 | U43465 | U43482 | – | – |
| ***Lineolata rhizophorae*** | Mangrove wood | J. Kohlmeyer | U.S.A., Florida | CBS 641.66 | **GU479758** | **GU479792** | **GU479828** | – |
| ***Lineolata rhizophorae*** | Mangrove wood | J. Kohlmeyer | Australia, Queensland | CBS 118422 | – | **GU479805** | – | – |
| ***Lineolata rhizophorae*** | Mangrove wood | J. Kohlmeyer | Belize, Blue Ground Range | JK 5248A | – | **GU479806** | – | – |
| ***Lophiostoma* (*Platystomum*) *scabridisporum*** | Wood, sand | E.B.G. Jones | Australia, The Mornington Peninsula National Park | BCC 22836 | GQ925832 | GQ925845 | **GU479829** | **GU479856** |
| ***Lophiostoma* (*Platystomum*) *scabridisporum*** | Wood, sand | E.B.G. Jones | Australia, The Mornington Peninsula National Park | BCC 22835 | GQ925831 | GQ925844 | **GU479830** | **GU479857** |
| *Lophiostoma arundinis* | | | | CBS 621.86 | DQ782383 | DQ782384 | DQ782386 | DQ782387 |
| *Lophiostoma bipolarae* (*Massarina bipolaris*) | | | | HKUCC 1053 | AF164365 | – | – | – |
| *Lophiostoma crenatum* | | | | CBS 629.86 | DQ678017 | DQ678069 | DQ677965 | DQ677912 |
| *Lophiostoma fuckelii* | | | | CBS 113432 | – | EU552139 | – | – |
| *Lophiostoma fuckelii* | | | | CBS 101952 | – | DQ399531 | – | – |
| *Lophiostoma macrostomum* | | | | KT 709 | AB521732 | AB433274 | – | – |
| *Lophiostoma macrostomum* | | | | KT 635 | AB521731 | AB433273 | – | – |
| *Lophiostoma sagittiforme* | | | | HHUF 29754 | – | AB369267 | – | – |
| *Lophium mytilinum* | | | | CBS 269.34 | DQ678030 | DQ678081 | DQ677979 | DQ677926 |
| ***Loratospora aestuarii*** | *Juncus roemerianus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5535D | **GU296168** | **GU301838** | **GU371760** | |
| *Macrophomina phaseolina* | | | | CBS 277.33 | DQ678037 | DQ678088 | DQ677986 | DQ677929 |
| *Massaria platani* | | | | CBS 221.37 | DQ678013 | DQ678065 | DQ677961 | DQ677908 |
| *Massarina eburnea* | | | | CBS 473.64 | AF164367 | – | – | – |
| *Massarina eburnea* | | | | HKUCC 4054 | AF164366 | – | – | – |
| *Massarina igniaria* | | | | CBS 845.96 | DQ813511 | DQ810223 | – | – |
| ***Massarina ricifera*** | *Juncus roemerianus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5535F | **GU479759** | **GU479793** | – | – |
| ***Mauritiana rhizophorae*** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 28866 | **GU371832** | **GU371824** | **GU371796** | **GU371817** |
| ***Mauritiana rhizophorae*** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 28867 | **GU371833** | **GU371825** | **GU371797** | **GU371818** |
| *Melanomma pulvis-pyrius* | | | | CBS 109.77 | AF164369 | DQ384095 | – | – |
| *Melanomma radicans* | | | | ATCC 42522 | U43461 | U43479 | AY485625 | – |
| *Montagnula opulenta* | | | | CBS 168.34 | AF164370 | DQ678086 | DQ677984 | – |
| ***Morosphaeria* (*Massarina*) *ramunculicola*** | Mangrove wood | J. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5304B | **GU479760** | **GU479794** | **GU479831** | – |
| ***Morosphaeria* (*Massarina*) *ramunculicola*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18405 | GQ925839 | GQ925854 | – | – |
| ***Morosphaeria* (*Massarina*) *ramunculicola*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18404 | GQ925838 | GQ925853 | – | – |
| ***Morosphaeria* (*Massarina*) *ramunculicola*** | Mangrove wood | | | HKUCC 7649 | – | DQ528762 | – | – |

**Table 1.** (Continued).

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| ***Morosphaeria* (*Massarina*) *velataspora*** | Mangrove wood | E.B.G. Jones | U.S.A., Florida | BCC 17059 | GQ925841 | GQ925852 | – | – |
| ***Morosphaeria (Massarina) velataspora*** | Mangrove wood | E.B.G. Jones | U.S.A., Florida | BCC 17058 | GQ925840 | GQ925851 | – | – |
| ***Mycosphaerella eurypotami*** | *Juncus roemerianus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5586J | **GU479761** | **GU301852** | **GU371722** | **GU371722** |
| *Mycosphaerella fijiensis* | | | | OSC 100622 | DQ767652 | DQ678098 | DQ677993 | – |
| *Mycosphaerella graminicola* | | | | CBS 292.38 | DQ678033 | DQ678084 | DQ677982 | – |
| *Mycosphaerella punctiformis* | | | | CBS 113265 | DQ471017 | DQ470968 | DQ470920 | – |
| *Myrangium duriaei* | | | | CBS 260.36 | AY016347 | DQ678059 | DQ677954 | DQ677900 |
| *Myriangium hispanicum* | | | | CBS 247.33 | **GU296180** | **GU301854** | **GU371744** | **GU349055** |
| *Mytilinidimytilinellum* | | | | CBS 303.34 | FJ161144 | FJ161184 | FJ161119 | FJ161100 |
| *Neotestudina rosatii* | | | | CBS 690.82 | DQ384069 | DQ384107 | – | – |
| *Oedohysterium insidens* | | | | CBS 238.34 | FJ161142 | FJ161182 | FJ161118 | FJ161097 |
| *Oedohysterium sinense* | | | | EB 0333 | FJ161169 | FJ161209 | FJ161130 | – |
| *Opegrapha dolomitica* | | | | – | DQ883706 | – | DQ883714 | DQ883732 |
| *Ophiosphaerella herpotrichus* | | | | ATCC 12279 | U43453 | U43471 | – | – |
| *Ostreichnicurtisii* | | | | CBS 19834 | FJ161137 | FJ161176 | – | FJ161093 |
| *Ostreichnisassafras* | | | | CBS 322.34 | FJ161148 | FJ161188 | FJ161122 | – |
| ***Paraliomyces lentiferus*** | Mangrove wood | E.B.G. Jones | Hong Kong, North Lantau | CY 3525 | AF441176 | – | – | – |
| ***Passeriniella savoryellopsis*** | Mangrove wood | J. Kohlmeyer | Belize, Tobacco Range | JK 5167C | **GU479762** | **GU479795** | – | **GU479858** |
| *Patellaria atrata* | | | | CBS 958.97 | **GU296181** | **GU301855** | – | **GU349038** |
| ***Patellaria* cf. *atrata* 1** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 28877 | **GU371837** | **GU371829** | – | – |
| ***Patellaria* cf. *atrata* 2** | Mangrove wood | S. Suetrong | Thailand, Kung Krabaen Bay Royal development Study Center | BCC 28876 | **GU371836** | **GU371828** | – | - |
| *Phaeodothis winteri* | | | | CBS 182.58 | DQ678021 | DQ678073 | DQ677970 | DQ677917 |
| ***Phaeosphaeria albopunctata* (*Leptosphaeria albopunctata*)** | *Spartina alterniflora* | J. Kohlmeyer | U.S.A., North Carolina, Beaufort | CBS 254.64 | – | **GU45631** | – | – |
| *Phaeosphaeria avenaria* | | | | DAOM 226215 | AY544725 | AY544684 | DQ677941 | DQ677885 |
| *Phaeosphaeria eustoma* | | | | CBS 576.86 | DQ678011 | DQ678063 | DQ677959 | DQ677906 |
| ***Phaeosphaeria olivacea*** | *Juncus roemeriaus* | J. Kohlmeyer, B. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5540Q | – | **GU479807** | – | – |
| ***Phaeosphaeria spartinicola*** | *Spartina* sp. | J.Kohlmeyer | U.S.A., Maryland, Solomons | JK 5177A | – | **GU479808** | – | – |
| *Phoma herbarum* | | | | CBS 615.75 | EU754087 | EU754186 | – | – |
| *Platychora ulmi* | | | | CBS 361.52 | EF114726 | EF114702 | – | – |
| *Pleospora herbarum* | | | | CBS 191.86 | DQ247812 | DQ247804 | DQ247794 | DQ471090 |
| *Pleospora sedicola* | | | | CBS 109843 | – | AY849958 | – | – |
| *Pleosporaceae* sp. 1 | | | | OSC 100706 | – | **GU479809** | – | – |
| ***Pontoporeia biturbinata*** | Seagrasses | E.B.G. Jones | Cyprus | BBH 23338 | **GU479763** | **GU479796** | **GU479837** | – |
| *Preussia minima* | | | | CBS 524.50 | DQ678003 | DQ678056 | DQ677950 | DQ677897 |

**Table 1.** (Continued).

| Taxon | Substrate | Collector | Location | Source | SSU | LSU | *RPB2* | *TEF1* |
|---|---|---|---|---|---|---|---|---|
| *Preussia terricola* | | | | DAOM 230091 | AY544726 | AY544686 | DQ470895 | DQ471063 |
| *Pseudorobillarda phragmitis* | | | | CBS 842.84 | EU754103 | EU754202 | – | – |
| *Pseudorobillarda siamensis* | | | | BCC 12531 | FJ825365 | FJ825375 | – | – |
| *Pseudorobillarda texana* | | | | BCC 12535 | FJ825367 | FJ825377 | – | – |
| *Psiloglonium araucanum* | | | | CBS 112412 | FJ161133 | FJ161172 | FJ161112 | FJ161089 |
| *Psiloglonium clavisporum* | | | | CBS 123339 | FJ161157 | FJ167526 | FJ161124 | FJ161105 |
| *Psiloglonium simulans* | | | | CBS 206.34 | FJ161139 | FJ161178 | FJ161116 | FJ161094 |
| *Pyrenophora phaeocomes* | | | | DAOM 222769 | DQ499595 | DQ499596 | DQ497614 | DQ497607 |
| *Pyrenophora tritici-repentis* | | | | OSC 100066 | AY544716 | AY544672 | – | DQ677882 |
| ***Quintaria lignatilis*** | Mangrove wood | J. Kohlmeyer, B. Kohlmeyer | French Polynesia, Moorea | JK 5390A, CBS 117700 | **GU296188** | **GU301865** | **GU371761** | – |
| ***Quintaria lignatilis*** | Mangrove wood | E.B.G. Jones | U.S.A., Florida | BCC 17444 | **GU479764** | **GU479797** | **GU479832** | **GU479859** |
| *Quintaria submersa* | | | | CBS 115553 | – | **GU479810** | – | – |
| *Repetophragma ontariense* | | | | HKUCC 10830 | – | DQ408575 | DQ435077 | – |
| ***Rimora* (*Lophiostoma*) *mangrovei*** | Mangrove wood | J. Kohlmeyer | Belize, Blue Ground Range | JK 5246A | **GU296193** | **GU301868** | **GU371759** | – |
| ***Rimora* (*Lophiostoma*) *mangrovei*** | Mangrove wood | J. Kohlmeyer | India, Goa | JK 5437B | **GU479765** | **GU479798** | –- | – |
| *Roccella fuciformis* | | | | DUKE 15572 | AY584678 | AY584654 | DQ782866 | – |
| ***Saccardoella rhizophorae*** | Mangrove wood | J. Kohlmeyer, B. Kohlmeyer | Hawaii, Oahu | JK 5456A | **GU479766** | **GU479799** | – | **GU479860** |
| ***Salsuginea ramicola*** | Mangrove wood | K. Tanaka | Japan, Okinawa | KT 2597.1 | **GU479767** | **GU479800** | **GU479833** | **GU479861** |
| ***Salsuginea ramicola*** | | K. Tanaka | Japan, Okinawa | KT 2597.2 | **GU479768** | **GU479801** | **GU479834** | **GU479862** |
| ***Scirrhia annulata*** | *Juncus roemerianus* | S. Newell | U.S.A., Georgia, Sapelo Island | JK 5546G | **GU479769** | – | – | – |
| *Scorias spongiosa* | | | | CBS 325.33 | DQ678024 | DQ678075 | DQ677973 | DQ677920 |
| *Stylodothis puccinioides* | | | | CBS 193.58 | AY016353 | AY004342 | – | DQ677886 |
| *Sydowia polyspora* | | | | CBS 116.29 | DQ678005 | DQ678058 | DQ677953 | DQ677899 |
| ***Tremateia halophila*** | *Juncus roemeriaus* | J. Kohlmeyer | U.S.A., North Carolina, Carteret County | JK 5517J | **GU296201** | – | **GU371721** | – |
| *Trematosphaeria* (*Lophiostoma*) *heterospora* | | | | CBS 644.86 | AY016354 | AY016369 | DQ497615 | DQ471049 |
| *Trematosphaeria pertusa* | | | | CBS 122371 | FJ201993 | FJ201992 | – | – |
| *Trematosphaeria pertusa* | | | | CBS 122368 | FJ201991 | FJ201990 | – | – |
| *Ulospora bilgramii* | | | | CBS 110020 | DQ678025 | DQ678076 | DQ677974 | DQ677921 |
| ***Verruculina enalia*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18401 | **GU479770** | **GU479802** | **GU479835** | **GU479863** |
| ***Verruculina enalia*** | Mangrove wood | E.B.G. Jones | Malaysia, Morib | BCC 18402 | **GU479771** | **GU479803** | **GU479836** | **GU479864** |
| ***Verruculina enalia*** | Mangrove wood | J. Kohlmeyer, B. Kohlmeyer | Belize, Blue Ground Range | JK 5253A | DQ678028 | DQ678079 | DQ677977 | – |
| *Westerdykella* (*Eremodothis*) *angulata* | | | | CBS 610.74 | DQ384067 | DQ384105 | – | – |
| *Westerdykella cylindrica* | | | | CBS 454.72 | AY016355 | AY004343 | DQ470925 | DQ497610 |
| *Westerdykella dispersa* | | | | CBS 508.75 | U42488 | DQ468050 | – | – |
| *Wettsteinina lacustris* | | | | CBS 618.86 | DQ678023 | – | DQ677972 | DQ677919 |

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# Molecular taxonomy of bambusicolous fungi: *Tetraplosphaeriaceae*, a new pleosporalean family with *Tetraploa*-like anamorphs

K. Tanaka[1*], K. Hirayama[1], H. Yonezawa[1], S. Hatakeyama[1], Y. Harada[1], T. Sano[1], T. Shirouzu[2] and T. Hosoya[3]

[1]*Faculty of Agriculture & Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan;* [2]*Fungus/Mushroom Resource and Research Center, Tottori University, Minami 4-101, Koyama, Tottori, Tottori 680-8553 Japan;* [3]*National Museum of Nature and Science, Amakubo 4-1-1, Tsukuba, Ibaraki 305-0005, Japan*

**Correspondence*: Kazuaki Tanaka, k-tanaka@cc.hirosaki-u.ac.jp

**Abstract:** A new pleosporalean family *Tetraplosphaeriaceae* is established to accommodate five new genera; 1) *Tetraplosphaeria* with small ascomata and anamorphs belonging to *Tetraploa s. str.*, 2) *Triplosphaeria* characterised by hemispherical ascomata with rim-like side walls and anamorphs similar to *Tetraploa* but with three conidial setose appendages, 3) *Polyplosphaeria* with large ascomata surrounded by brown hyphae and anamorphs producing globose conidia with several setose appendages, 4) *Pseudotetraploa*, an anamorphic genus, having obpyriform conidia with pseudosepta and four to eight setose appendages, and 5) *Quadricrura*, an anamorphic genus, having globose conidia with one or two long setose appendages at the apex and four to five short setose appendages at the base. Fifteen new taxa in these genera mostly collected from bamboo are described and illustrated. They are linked by their *Tetraploa s. l.* anamorphs. To infer phylogenetic placement in the *Pleosporales*, analyses based on a combined dataset of small- and large-subunit nuclear ribosomal DNA (SSU+LSU nrDNA) was carried out. *Tetraplosphaeriaceae*, however, is basal to the main pleosporalean clade and therefore its relationship with other existing families was not completely resolved. To evaluate the validity of each taxon and to clarify the phylogenetic relationships within this family, further analyses using sequences from ITS-5.8S nrDNA (ITS), transcription elongation factor 1-α (TEF), and β-tubulin (BT), were also conducted. Monophyly of the family and that of each genus were strongly supported by analyses based on a combined dataset of the three regions (ITS+TEF+BT). Our results also suggest that *Tetraplosphaeria* (anamorph: *Tetraploa s. str.*) is an ancestral lineage within this family. Taxonomic placement of the bambusicolous fungi in *Astrosphaeriella*, *Kalmusia*, *Katumotoa*, *Massarina*, *Ophiosphaerella*, *Phaeosphaeria*, *Roussoella*, *Roussoellopsis*, and *Versicolorisporium*, are also discussed based on the SSU+LSU phylogeny.

**Key words:** Anamorphic fungi, *Bambusoideae*, bitunicate ascomycetes, *Didymella*, *Dothideomycetes*, evolution, *Lophiostoma*, teleomorph.
**Taxonomic novelties:** *Tetraplosphaeriaceae* Kaz. Tanaka & K. Hiray., fam. nov., *Tetraplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Tetraplosphaeria nagasakiensis* Kaz. Tanaka & K. Hiray., sp. nov., *Tetraplosphaeria sasicola* Kaz. Tanaka & K. Hiray., sp. nov., *Tetraplosphaeria tetraploa* (Scheuer) Kaz. Tanaka & K. Hiray., comb. nov., *Tetraplosphaeria yakushimensis* Kaz. Tanaka, K. Hiray. & Hosoya, sp. nov., *Triplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Triplosphaeria acuta* Kaz. Tanaka & K. Hiray., sp. nov., *Triplosphaeria cylindrica* Kaz. Tanaka & K. Hiray., nom. nov., *Triplosphaeria maxima* Kaz. Tanaka & K. Hiray., sp. nov., *Triplosphaeria yezoensis* (I. Hino & Katum.) Kaz. Tanaka, K. Hiray. & Shirouzu, comb. nov., *Polyplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov., *Polyplosphaeria fusca* Kaz. Tanaka & K. Hiray., sp. nov., *Pseudotetraploa* Kaz. Tanaka & K. Hiray., gen. nov., *Pseudotetraploa curviappendiculata* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Pseudotetraploa javanica* (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., comb. nov., *Pseudotetraploa longissima* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov., *Quadricrura* Kaz. Tanaka, K. Hiray. & Sat. Hatak., gen. nov., *Quadricrura bicornis* Kaz. Tanaka, K. Hiray. & H. Yonez., sp. nov., *Quadricrura meridionalis* Kaz. Tanaka & K. Hiray., sp. nov., *Quadricrura septentrionalis* Kaz. Tanaka, K. Hiray. & Sat. Hatak., sp. nov.

## INTRODUCTION

Bamboo is the vernacular or common term applied to small to large woody grasses ranging from 10 cm to 40 m in height. They are currently classified as a subfamily *Bambusoideae* within the extensive grass family *Poaceae* and comprise *ca*. 80–90 genera and 1 000–1 500 species. Indications are that major radiations of grasses including *Bambusoideae* occurred 40–50 million years ago in the Paleogene age. Bamboos are distributed all over the world except in Europe which has no native species, and are found at latitudes from 46 °N to 47 °S and from sea level to 4 000 m elevation. However, the major species richness is found in the Asian Pacific region (China: 626, India: 102, Japan: 84) and South America (Brazil: 134, Venezuela: 68, Colombia: 56) (Suzuki 1996, Scurlock *et al*. 2000, Das *et al*. 2008, Sungkaew *et al*. 2009). Approximately 1 500 commercial applications of bamboo — as fishing rods, flutes, paper, flooring materials, foods and energy feedstock — have been identified, and it is estimated that 2.5 billion people depend on or use bamboo materials valued at US$ 7 billion per annum (Scurlock *et al*. 2000, Bystriakova *et al*. 2003).

In addition to studies on economically important bambusicolous pathogenic fungi, such as *Ceratosphaeria phyllostachydis* and *Stereostratum corticioides* (Hyde *et al*. 2002b), a large number of studies on saprobic (Hyde *et al*. 2001, 2002c, Zhou & Hyde 2002) and endophytic fungi (Morakotkarn *et al.* 2007, 2008, Tanaka & Tanaka 2008, Tanaka *et al*. 2008) have also been conducted due to the diversity of fungal species on bamboo. According to Hyde *et al*. (2002b), more than 1 100 fungal species have been described or recorded worldwide from bamboo. In Japan, *ca*. 300 fungi are known from bamboo (Tanaka & Harada 2004), of which *ca*. 60 spp. belong to *Dothideomycetes* (Anonymous 2000). This number suggests that bamboo is a promising substrate for the study of *Dothideomycetes* diversity. Several *Dothideomycetes* with peculiar taxonomic features such as *Shiraia* (Amano 1983) and *Katumotoa* (Tanaka & Harada 2005b) have been reported from bamboo. However, phylogenetic information based on molecular data is poorly known for many bambusicolous fungi.

In our ongoing study of bambusicolous fungi in Japan (Shirouzu & Harada 2004, Tanaka & Harada 2004, 2005a, b, Tanaka *et al*. 2005, Hatakeyama *et al*. 2005, 2008, Sato *et al*. 2008), we

encountered many undescribed *Dothideomycetes* resembling the genus *Massarina*. These fungi produced *Tetraploa*-like anamorphs in culture. The teleomorph-anamorph connection between *Massarina* and *Tetraploa* has been elucidated based only on one example of *M. tetraploa* and *T. aristata* on *Carex* (Scheuer 1991), but the molecular phylogenetic position of this species remains uncertain at the familial/generic level.

*Massarina* is a taxonomically heterogenous genus in the order *Pleosporales*, because *Massarina s. l.* contains many phylogenetically unrelated elements. Attempts to revise the genus have been undertaken by several authors (Bose 1961, Barr 1992, Aptroot 1998). In particular, Aptroot (1998) carried out taxonomic re-assessment of 160 species that had been placed in this genus previously and amended the generic concept of *Masssarina* by accepting 43 species in the genus. Nevertheless, this study also pointed out that *Massarina* appears to be polyphyletic, because members of this genus have diverse anamorphs, like *Tetraploa*, *Periconia*, *Tumularia*, *Ceratophoma*, and others. Regarding this problem, Aptroot (1998) noted that the species accepted in *Massarina* may not form a monophyletic group; however, on the basis of morphological characteristics, no clear subdivision could be made. He further pointed out the need for examining the molecular and ultrastructural characteristics to gain a better understanding of the genus.

The current taxonomic concept of *Massarina* has been extensively amended based on its DNA sequence data (Liew *et al*. 2002, Belliveau & Bärlocher 2005, Kodsueb *et al*. 2007, Wang *et al*. 2007, Zhang *et al*. 2009b). Liew *et al*. (2002) revealed that five species of *Massarina* (*e.g. M. corticola*) possessing narrowly fusiform ascospores belong to the genus *Lophiostoma*, which is morphologically similar to *Massarina*, based on phylogenetic analyses of SSU and ITS sequences of nrDNA. They further suggested that other *Massarina* species with ascospores of similar morphology might have affinity with *Lophiostoma* (Liew *et al*. 2002). Following this suggestion, Hyde *et al*. (2002a) transferred 26 species of *Massarina* to *Lophiostoma* primarily based on their ascospore morphology. *Massarina tetraploa*, which produces the *Tetraploa* anamorph, was also transferred to *Lophiostoma* (Hyde *et al*. 2002a).

The phylogenetic position or the relationships of bambusicolous species with fungi from non-bamboo host plants have not been established. In this paper, phylogenetic analyses using 53 isolates of bambusicolous *Dothideomycetes* were carried out based on a combined dataset of small and large subunit nuclear ribosomal DNA (SSU+LSU), to infer their familial placement. These analyses include species placed in *Astrosphaeriella*, *Kalmusia*, *Katumotoa*, *Massarina*, *Ophiosphaerella*, *Phaeosphaeria*, *Roussoella*, *Roussoellopsis*, and *Versicolorisporium*. Special emphasis was paid to the taxonomy and phylogeny of *Massarina s. l.*, which possess *Tetraploa*-like hyphomycetous anamorphs. In order to assess their validity at familial, generic and specific levels, phylogeny of 29 isolates were analysed on the basis of their sequences from ITS-5.8S nrDNA (ITS), transcription elongation factor 1-α (TEF) and β-tubulin (BT), as well as SSU+LSU. We propose here a new family *Tetraplosphaeriaceae* to encompass five new genera, *Tetraplosphaeria*, *Triplosphaeria*, *Polyplosphaeria*, *Pseudotetraploa* and *Quadricrura*. Fifteen new taxa in these genera are also described and illustrated.

## MATERIALS AND METHODS

### Morphological studies and fungal isolates

Measurements of all structures were taken from material mounted in water. India ink was added to water mounts to detect the gelatinous sheath and ascospore appendages. To observe the internal conidial structure, 5 % sodium hypochlorite solution (NaClO) was used for bleaching of strongly melanised spores as described in Eriksson (1989). The ascospore septum position was noted using the decimal system (Shoemaker 1984, Raja *et al*. 2008). To observe details of ascomal anatomy, ascomata were boiled in water for a few minutes and sectioned with a freezing microtome (HM 400R; MICROM, Germany). Light microscopy observations were conducted using an Olympus microscope (BX51) equipped with Nomarski interference differential contrast objectives. Specimens cited in this paper are maintained at the herbaria of Hirosaki University (HHUF) and National Museum of Nature and Science (TNS), and some materials were borrowed from the herbaria of Yamaguchi University (YAM) and Karl-Franzens-Universität Graz (GZU).

Single ascospore cultures were obtained according to the methods of Tubaki (1978). Growth rate and colony characteristics were recorded from cultures grown on potato-dextrose agar (PDA, Difco) within 3 wk at 25 ºC in the dark. Colours were designated according to Kornerup & Wanscher (1978). Induction of anamorph/teleomorph formation was attempted by culturing the isolates on rice straw agar (RSA; Tanaka & Harada 2003a) and/or incubating small colony pieces in sterilised water (Scheuer 1991, Hatakeyama *et al*. 2005). Fungal cultures newly obtained in this study were deposited at the CBS-KNAW Fungal Biodiversity Centre (Centraalbureau voor Schimmelcultures; CBS), the Japan Collection of Microorganisms (JCM), the Ministry of Agriculture, Forestry, and Fisheries, Japan (MAFF), and the National Biological Resources Center, Japan (NBRC) (Table 1).

### DNA extraction and amplification

Mycelia were grown in malt extract broth (20 g malt extract, 1 000 mL distilled water). DNA from mycelia was extracted using the ISOPLANT Kit (Nippon Gene, Japan) according to the manufacturer's instructions. Partial SSU (*ca*. 1 000–1 300 bp of the 5' end) and LSU nrDNA (*ca*. 1 250 bp of the 5' end) regions were determined for 53 isolates mostly obtained from bamboo to reveal their familial or generic positions; and complete internally transcribed spacers (ITS) region of nrDNA (*ca*. 500 bp), the intron sequence of the TEF gene (*ca*. 300 bp), and exons 1 to 6 with the respective introns of the BT gene (*ca*. 600 bp) were sequenced for 31 isolates to confirm their generic or species validities (Table 1). These regions were amplified by the polymerase chain reaction (PCR) using the primer pairs NS1–NS4 (White *et al*. 1990) and LR0R–LR7 (Rehner & Samuels 1994) for SSU and LSU, respectively. Three primer sets, ITS1–ITS4 (White *et al*. 1990), EF1-728F–EF1-986R (Carbone & Kohn 1999), and T1–BT2B (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997) were used for the amplification of ITS, TEF and BT, respectively. Amplifications were conducted in 25 μL of PCR mixtures containing 1 μM of each primer, 0.125 U TaKaRa Ex *Taq* polymerase (TaKaRa Bio, Otsu, Japan), dNTP mixture (2.5 mM each stock), and Ex *Taq* reaction buffer (containing 2 mM $Mg^{2+}$). PCR was carried out as follows: initial denaturation at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 1 min; annealing for 1 min at 48.8 °C for SSU nrDNA,

46.2 °C for LSU nrDNA, 61.5 °C for ITS, 57.2 °C for TEF, and 60 °C for BT; an extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The size of PCR products were verified using 7.5 % poly-acrylamide gels stained with ethidium bromide, and then sequenced directly at SORGENT Co., Ltd. (Korea).

## Phylogenetic analyses

Preliminary multiple alignments of sequences were conducted using MAFFT v. 6 (Katoh *et al*. 2005; http://align.bmr.kyushu-u.ac.jp/mafft/software). Final alignments were manually adjusted using BioEdit v. 7.08 (Hall 1999). Alignment gaps and ambiguous positions were excluded from the analyses. Alignments used in this study were deposited in TreeBASE (S2505).

Two phylogenetic analyses, maximum-parsimony (MP) using a close-neighbour-interchange heuristic search with an initial tree by random addition sequence (100 replicates) and neighbour-joining (NJ) based on the Kimura 2-parameter substitution model, were carried out using MEGA v. 4 (Tamura *et al*. 2007). Characters were weighted equally and gaps were excluded. The bootstrap support (BS) values for nodes were computed from 1 000 replicates for both the MP and NJ analyses. In addition to these analyses, Bayesian analyses were done using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). MrModeltest v. 2.2 (Nylander 2004) in conjunction with PAUP 4.0b10 (Swofford 2003) was used to select substitution models for Bayesian analyses. On the basis of AIC (Akaike Information Criterion) of MrModeltest v. 2.2, a GTR+I+G model for the SSU+LSU nrDNA, ITS and BT, and a HKY+I+G model for TEF gene sequences were applied. Two runs with 10 chains of Markov chain Monte Carlo (MCMC) iterations were performed for 6 million and 1.2 million generations, keeping one tree every 100 generations, for a combined alignment of the SSU+LSU nrDNA sequences and the ITS+TEF+BT gene sequences, respectively. The first 5 million generations of the SSU + LSU and 200 000 generations of the ITS+TEF+BT were discarded as burn-in, and the remaining 20 002 trees were used to calculate 50 % majority rule trees and to determine the posterior probabilities (PP) for the individual branches.

# RESULTS

## Taxonomy

A new family, *Tetraplosphaeriaceae* typified by *Tetraplosphaeria*, is established in this paper. This family includes five new genera, 1) *Tetraplosphaeria* with small ascomata and anamorphs belonging to *Tetraploa s. str.*, 2) *Triplosphaeria* characterised by hemispherical ascomata with rim-like side walls and anamorphs similar to *Tetraploa* but with three conidial setose appendages, 3) *Polyplosphaeria* with large-sized ascomata surrounded by brown hyphae and anamorphs producing globose conidia with several setose appendages, 4) *Pseudotetraploa*, an anamorphic genus, having obpyriform conidia with pseudosepta and four to eight setose appendages, and 5) *Quadricrura*, an anamorphic genus, having globose conidia with one or two long apical setose appendages and four to five short basal setose appendages. Fifteen new taxa of these genera are described below.

***Tetraplosphaeriaceae*** Kaz. Tanaka & K. Hiray., **fam. nov.** MycoBank MB515253.

*Etymology*: In reference to the name of the type genus.

Ascomata immersa vel superficialia, globosa vel subglobosa. Rostrum breviter papillatum vel cylindricum, interdum nullum. Pseudoparaphyses septatae, ramificantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes vel late cylindricae, 1–3-septatae, hyalinae vel brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia brunnea, cum plus quam 3–8 appendicibus.

*Ascomata* scattered to gregarious, immersed to superficial, globose to subglobose, glabrous or with brown hyphae. *Beak* short-papillate to cylindrical or absent, central. *Ascomatal wall* composed of hyaline to brown cells, sometimes with rim-like structure at the sides and poorly developed at the base. *Pseudoparaphyses* cellular or trabeculae, septate, branched. *Asci* fissitunicate, basal to somewhat lateral, cylindrical to clavate, short-stalked, with 8 ascospores. *Ascospores* narrowly fusiform to broadly cylindrical, straight or slightly curved, 1–3-septate, hyaline to pale brown, smooth, surrounded by an entire mucilaginous sheath or narrow appendage-like sheath. *Anamorph Tetraploa*-like. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* composed of 3–8 columns or internal hyphal structure, brown, mostly verrucose at the base, with more than 3–8 setose appendages.

*Type genus*: *Tetraplosphaeria* Kaz. Tanaka & K. Hiray., gen. nov.

*Notes*: *Tetraplosphaeriaceae* fits well in the *Pleosporales* on morphological grounds, but there is no suitable family to accommodate it in this order. The most common diagnostic features of this family are *Massarina*-like teleomorphs with almost hyaline 1(–3)-septate ascospores and/or *Tetraploa*-like anamorphs with several setose appendages.

***Tetraplosphaeria*** Kaz. Tanaka & K. Hiray., **gen. nov.** MycoBank MB515254.

*Anamorph*: *Tetraploa* Berk. & Broome.

*Etymology*: In reference to the anamorphic state belonging to *Tetraploa*.

Ascomata immersa vel erumpentia, globosa vel subglobosa. Rostrum breviter papillatum vel cylindricum. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu stricto. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia breviter cylindricae vel obpyriformes, brunnea, cum 4 appendicibus.

*Ascomata* scattered to gregarious, immersed to erumpent, globose to subglobose, glabrous. *Beak* short-papillate to cylindrical, central, with periphyses. *Ascomatal wall* composed of nearly rectangular to polygonal thin-walled cells, sometimes poorly developed at the base. *Pseudoparaphyses* cellular, septate, branched. *Asci* fissitunicate, basal to somewhat lateral, cylindrical to clavate, short-stalked, with 8 ascospores. *Ascospores* narrowly fusiform, straight or slightly curved, with a septum and slightly constricted, hyaline, smooth, surrounded by a narrow mucilaginous appendage-like sheath. *Anamorph Tetraploa s. str*. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* composed of 4 columns, short-cylindrical, brown, verrucose at the base, euseptate, with 4 setose appendages at the apex.

**Table 1.** Cultures and Genbank accession number of bambusicolous fungi used in this study.

| Taxon | Host [a)] | Original no. | Herbarium no. | Strain no. | GenBank no. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SSU | LSU | ITS | BT | TEF |
| *Astrosphaeriella aggregata* | 9 | KT 767 | HHUF 28232 | MAFF 239485 | AB524449 | AB524590 | – | – | – |
| | 7 | KT 984 | HHUF 28233 | MAFF 239486 | AB524450 | AB524591 | – | – | – |
| *Astrosphaeriella stellata* | 7 | KT 998 | HHUF 28494 | MAFF 239487 | AB524451 | AB524592 | – | – | – |
| *Kalmusia scabrispora* | 7 | KT 1023 | HHUF 28608 | JCM 12851 = MAFF 239517 | AB524452 | AB524593 | – | – | – |
| | 7 | KT 2202 | HHUF 30013 | NBRC 106237 | AB524453 | AB524594 | – | – | – |
| *Katumotoa bambusicola* | 9 | KT 1517a | HHUF 28661 | JCM 13131 = MAFF 239641 | AB524454 | AB524595 | – | – | – |
| *Massarina arundinariae* | 7 | KT 856 | HHUF 27547 | MAFF 239461 | AB524455 | AB524596 | AB524786 | AB524848 | AB524817 |
| | 7 | KT 2200 | HHUF 30014 | NBRC 106238 | AB524456 | AB524597 | AB524787 | AB524849 | AB524818 |
| | 7 | KT 1034 | HHUF 30015 | NBRC 106239 | AB524457 | AB524598 | – | – | – |
| *Ophiosphaerella sasicola* | 9 | KT 1706 | HHUF 29443 | JCM 13134 = MAFF 239644 | AB524458 | AB524599 | – | – | – |
| *Phaeosphaeria brevispora* | 12 | KT 1466 | HHUF 28229 | MAFF 239276 | AB524459 | AB524600 | – | – | – |
| | 9 | KT 2313 | HHUF 30016 | NBRC 106240 | AB524460 | AB524601 | – | – | – |
| *Phaeosphaeria* sp. | 9 | KT 2564 | HHUF 30017 | NBRC 106255 | AB524461 | AB524602 | – | – | – |
| *Polyplosphaeria fusca* | 7 | KT 1043 | HHUF 29392 | JCM 13173 = MAFF 239683 | AB524462 | AB524603 | AB524788 | AB524850 | AB524819 |
| | 8 | KT 1616 | HHUF 29399 | JCM 13175 = MAFF 239685 | AB524463 | AB524604 | AB524789 | AB524851 | AB524820 |
| | 4 | KT 1640 | HHUF 29405 | JCM 13176 = MAFF 239686 | AB524464 | AB524605 | AB524790 | AB524852 | AB524821 |
| | 3 | KT 1686 | HHUF 29406 | JCM 13177 = MAFF 239687 | AB524465 | AB524606 | – | – | – |
| | 9 | KT 2124 | HHUF 30018 | CBS 125425 | AB524466 | AB524607 | AB524791 | AB524853 | AB524822 |
| *Pseudotetraploa curviappendiculata* | 9 | HC 4930 | HHUF 28582 | JCM 12852 = MAFF 239495 | AB524467 | AB524608 | AB524792 | AB524854 | AB524823 |
| | 9 | HC 4932 | HHUF 28590 | MAFF 239496 | AB524468 | AB524609 | AB524793 | AB524855 | AB524824 |
| | 9 | KT 2558 | HHUF 30019 | CBS 125426 = NBRC 106241 | AB524469 | AB524610 | AB524794 | AB524856 | AB524825 |
| *Pseudotetraploa javanica* | 8 | HC 4934 | HHUF 28596 | JCM 12854 = MAFF 239498 | AB524470 | AB524611 | AB524795 | AB524857 | AB524826 |
| *Pseudotetraploa longissima* | 8 | HC 4933 | HHUF 28580 | JCM 12853 = MAFF 239497 | AB524471 | AB524612 | AB524796 | AB524858 | AB524827 |
| *Quadricrura bicornis* | 5 | yone 153 | HHUF 30023 | CBS 125427 | AB524472 | AB524613 | AB524797 | AB524859 | AB524828 |
| *Quadricrura meridionalis* | 3 | KT 2607 | HHUF 30024 | CBS 125684 = NBRC 106242 | AB524473 | AB524614 | AB524798 | AB524860 | AB524829 |
| *Quadricrura septentrionalis* | 9 | HC 4983 | HHUF 28781 | CBS 125429 | AB524474 | AB524615 | AB524799 | AB524861 | AB524830 |
| | 9 | HC 4984 | HHUF 28782 | CBS 125430 | AB524475 | AB524616 | AB524800 | AB524862 | AB524831 |
| | 9 | KT 920 | HHUF 30020 | CBS 125428 | AB524476 | AB524617 | AB524801 | AB524863 | AB524832 |
| | 9 | yone 44 | HHUF 29747 | CBS 125431 | AB524477 | AB524618 | AB524802 | AB524864 | AB524833 |
| | 9 | yone 176 | HHUF 30021 | CBS 125432 = NBRC 106243 | AB524478 | AB524619 | AB524803 | AB524865 | AB524834 |
| | 9 | yone 179 | HHUF 30022 | CBS 125433 = NBRC 106244 | AB524479 | AB524620 | AB524804 | AB524866 | AB524835 |
| *Roussoella hysterioides* | 13 | KT 1651 | HHUF 29217 | JCM 13126 = MAFF 239636 | AB524480 | AB524621 | – | – | – |

**Table 1.** (Continued).

| Taxon | Host [a)] | Original no. | Herbarium no. | Strain no. | GenBank no. | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SSU | LSU | ITS | BT | TEF |
| *Roussoella hysterioides* | 9 | HH 26988 | HHUF 26988 | CBS 125434 | AB524481 | AB524622 | – | – | – |
| *Roussoella pustulans* | 9 | KT 1709 | HHUF 29229 | JCM 13127 = MAFF 239637 | AB524482 | AB524623 | – | – | – |
| *Roussoella* sp. | 9 | KT 2303 | HHUF 30025 | NBRC 106245 | AB524483 | AB524624 | – | – | – |
| *Roussoellopsis tosaensis* | 3 | KT 1659 | HHUF 29234 | JCM 13128 = MAFF 239638 | AB524484 | AB524625 | – | – | – |
| *Roussoellopsis* sp. | 9 | KT 1710 | HHUF 30026 | NBRC 106246 | AB524485 | AB524626 | – | – | – |
| *Tetraploa aristata* | 1 | - | CBS H-18781 | CBS 996.70 | AB524486 | AB524627 | AB524805 | AB524867 | AB524836 |
| *Tetraploa* sp. 1 | 3 | KT 1684 | HHUF 29625 | JCM 14424 | AB524487 | AB524628 | – | – | – |
| *Tetraploa* sp. 2 | 6 | KT 2578 | HHUF 30027 | NBRC 106251 | AB524488 | AB524629 | – | – | – |
| *Tetraplosphaeria nagasakiensis* | 3 | KT 1682 | HHUF 29378 | JCM 13168 = MAFF 239678 | AB524489 | AB524630 | AB524806 | AB524868 | AB524837 |
| *Tetraplosphaeria sasicola* | 11 | KT 563 | HHUF 27566 | JCM 13167 = MAFF 239677 | AB524490 | AB524631 | AB524807 | AB524869 | AB524838 |
| *Tetraplosphaeria yakushimensis* | 2 | KT 1906 | HHUF 29652 | CBS 125435 | AB524491 | AB524632 | AB524808 | AB524870 | AB524839 |
| *Trisplophaeria acuta* | 10 | KT 1170 | HHUF 29387 | JCM 13171 = MAFF 239681 | AB524492 | AB524633 | AB524809 | AB524871 | AB524840 |
| *Triplosphaeria cylindrica* | 9 | KT 1256 | HHUF 29381 | JCM 13169 = MAFF 239679 | AB524493 | AB524634 | – | – | – |
| | 9 | KT 1800 | HHUF 29626 | JCM 14425 | AB524494 | AB524635 | AB524810 | AB524872 | AB524841 |
| | 9 | KT 2550 | HHUF 30028 | NBRC 106247 | AB524495 | AB524636 | AB524811 | AB524873 | AB524842 |
| *Triplosphaeria maxima* | 9 | KT 870 | HHUF 29390 | JCM 13172 = MAFF 239682 | AB524496 | AB524637 | AB524812 | AB524874 | AB524843 |
| *Triplosphaeria yezoensis* | 9 | KT 1715 | HHUF 30029 | CBS 125436 | AB524497 | AB524638 | AB524813 | AB524875 | AB524844 |
| | 12 | KT 1732 | HHUF 30030 | CBS 125437 | AB524498 | AB524639 | AB524814 | AB524876 | AB524845 |
| *Triplosphaeria* sp. | 9 | HC 4665 | HHUF 27481 | NBRC 106248 | AB524499 | AB524640 | AB524815 | AB524877 | AB524846 |
| *Triplosphaeria* sp. | 9 | KT 2546 | HHUF 30031 | NBRC 106249 | AB524500 | AB524641 | AB524816 | AB524878 | AB524847 |
| *Versicolorisporium triseptatum* | 8 | SH 130 | HHUF 28815 | JCM 14775 | AB524501 | AB330081 | – | – | – |

[a)] 1. *Alpinia formosa*; 2. *Arundo donax*; 3. bamboo; 4. *Chimonobambusa marmorea*; 5. conifer; 6. gramineae; 7. *Phyllostachys bambusoides*; 8. *Pleioblastus chino*; 9. *Sasa kurilensis*; 10. *Sasa nipponica*; 11. *Sasa senanensis*; 12. *Sasa* sp.; 13. *Sasa veitchii*

*Type species*: *Tetraplosphaeria sasicola* Kaz. Tanaka & K. Hiray., sp. nov.

*Notes*: A new genus *Tetraplosphaeria* is erected to accommodate four pleosporalean species having *Massarina*/*Lophiostoma*-like teleomorphs and anamorphs belonging to *Tetraploa s. str*. These species do not have clypeate stromata around the ascomatal beak similar to the type species of *Massarina* (*M. eburnea*; Hyde 1995). Some species in *Tetraplosphaeria* have a well-developed beak similar to *Lophiostoma* species, but they do not have slit-like ostioles which is a characteristic feature of *Lophiostoma* (Holm & Holm 1988, Tanaka & Harada 2003a, Tanaka & Hosoya 2008).

***Tetraplosphaeria nagasakiensis*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515259. Fig. 1.

*Anamorph*: *Tetraploa aristata s. l*.

*Etymology*: In reference to the collection site.

Ascomata 260–330 × 290–350 µm, immersa vel erumpentia, globosa vel subglobosa. Rostrum 75–150 × 85–110 µm, ostiolatum. Paries ascomatis 17–30 µm crassus ad latus, ex cellulis 5–6-stratis 5–13 x 2.5–5 µm compositus. Pseudoparaphyses 1–3 µm latae, septatae, ramificantes et anastomosantes. Asci (82–)86–105(–110) × 10.5–13.5 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae (27–)29–35(–37) × 3.5–6 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu stricto. Conidia in vitro (28–)32.5–42(–43) × 20–33 µm, brunnea, cum 4 appendicibus; appendices (70–)95–225(–263) µm longae, 3–13-septatae.

*Ascomata* 260–330 µm high, 290–350 µm diam, scattered to gregarious, immersed in sheath or erumpent from bare culm, globose to subglobose, with sparse brown hyphae at sides. *Beak* 75–150 µm long, 85–110 µm diam, central, papillate to cylindrical, composed of dark brown, thick-walled cells, with numerous periphyses. *Ascomatal wall* at sides 17–30 µm thick, composed of 5–6 layers of rectanglar to polygonal brown cells of 5–13 × 2.5–5 µm, at the base 5–7.5 µm thick, composed of globose to polygonal cells of 2.5–7.5 µm diam. *Pseudoparaphyses* numerous, 1–3 µm thick, branched and anastomosed, with slime coating. *Asci* (82–)86–105(–110) × 10.5–13.5 µm (av. 95.4 × 11.9 µm, *n* = 50), fissitunicate, numerous, basal to somewhat lateral, clavate to cylindrical, short-stalked (*ca.* 10–20 µm long), 8-spored. *Ascospores* (27–)29–35(–37) × 3.5–6 µm (av. 32 × 4.4 µm, *n* = 100), L/W 5.9–8.5 (av. 7.3, *n* = 100), narrowly fusiform with acute ends, slightly curved, with a septum nearly median (0.48–0.52; av. 0.50, *n* = 100), hyaline, smooth, with bipolar elongated sheath of 1–3 µm long at both ends (but in india ink, an entire sheath 13–18 µm thick at sides is observed).

*Culture characteristics*: Colonies on PDA attaining a diam of 1.1–1.2 cm, dull-green (28E4; Kornerup & Wanscher 1978); reverse almost black; no pigment produced. On RSA both teleomorphic and anamorphic states are produced. Ascospores are slightly smaller than those on the host, measuring 29–32 × 5 µm. *Anamorph* is *Tetraploa aristata s. l*. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, clearly verruculose, (28–)32.5–42(–43) × 20–33 µm (av. 37.4 × 27.2 µm, *n* = 30), L/W 1.2–1.7 (av. 1.4, *n* = 30), 5–6-celled, composed of 4 columns and 4 setose appendages. Appendages (70–)95–225(–263) µm long (av. 161.2 µm, *n* = 100), 2–3 µm wide at the apex, 5.5–8 µm at the base, with 3–13-septa at 10 to 25 µm intervals.

*Specimen examined*: **Japan,** Nagasaki, Nagayo, Nagasaki Siebold University, on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, HHUF 29378 **holotype** designated here, living culture *KTC 1682* (= JCM 13168 = MAFF 239678).

*Notes*: This species is most similar to *Tetraplosphaeria yakushimensis* in having ascospores overlapping in size, but *T. nagasakiensis* differs from the latter in the dimension of conidia and the length of conidial appendages. The *Tetraploa* state of *T. nagasakiensis* shares some features with *Tetraploa aristata* (Berkeley & Broome 1850, Ellis 1949), but has larger conidia (av. 37.4 × 27.2 µm vs. 31.8 × 20.6 µm) and considerably longer conidial appendages (av. 161.2 µm vs. 36 µm).

***Tetraplosphaeria sasicola*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515260. Fig. 2.

*Anamorph*: *Tetraploa ellisii s. l*.

*Etymology*: In reference to the host plant of collection.

Ascomata 150–200 × 230–290 µm, immersa vel erumpentia, globosa vel subglobosa. Rostrum 30–40 × 50–55 µm, ostiolatum. Paries ascomatis 12–20 µm crassus ad latus, ex cellulis 3–6-stratis 7–13 × 2–5 µm compositus. Pseudoparaphyses 1.5–2.5 µm latae, septatae, ramificantes et anastomosantes. Asci (61–)65–89(–100) × 9–11(–13) µm, fissitunicati, clavati vel cylindrici, octospori. Ascosporae 22.5–31.5(–34) × 3–5 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu stricto. Conidia in vitro (32–)35–50(–52.5) × 20–30 µm, brunnea, cum 4 appendicibus; appendices (88–)113–190(–200) µm longae, 9–15-septatae.

*Ascomata* 150–200 µm high, 230–290 µm diam, scattered, immersed below the epidermis, later erumpent, globose to subglobose with a flattened base, glabrous. *Beak* short-papillate, 30–40 µm high, 50–55 µm diam, central, composed of globose to polygonal dark brown thick-walled cells of 3–6 µm diam, with sparse short periphyses. *Ascomatal wall* 12–20 µm thick at sides, composed of 3–6 layers of rectangular to polygonal hyaline to pale brown cells of 7–13 × 2–5 µm; at the base much thinner, of compressed small hyaline cells. *Pseudoparaphyses* cellular, numerous, 1.5–2.5 µm thick, septate, branched. *Asci* (61–)65–89(–100) × 9–11(–13) µm (av. 76.6 × 9.9 µm, *n* = 50), fissitunicate, numerous, basal to somewhat lateral, clavate to cylindrical, short-stalked (*ca.* 5–15 µm long), with 8 ascospores triseriate to biseriate above and uniseriate below. *Ascospores* 22.5–31.5(–34) × 3–5 µm (av. 26.8 × 3.7 µm, *n* = 80), L/W 6.4–8.3 (av. 7.2, *n* = 80), narrowly fusiform, straight or slightly curved, with a submedian (0.49–0.54; av. 0.51, *n* = 51) septum and slightly constricted, hyaline, smooth, surrounded by a narrow mucilaginous sheath, 2–6 µm long at the apex, 1–3 µm long at the base, slightly wider at sides of septum. Senescent spores pale brown.

*Culture characteristics*: Colonies on PDA attaining 3.5–4 cm diam, velvety in appearance, grey (7C1), with entire margin; reverse pompeian-red (9C7), and coral (9B7) pigment produced. On RSA, *Tetraploa* state similar to *T. ellisii* is formed on the surface of rice straw within 2 mo. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, slightly verruculose, (32–)35–50(–52.5) × 20–30 µm (av. 41.2 × 26 µm, *n* = 43), L/W 1.3–1.9 (av. 1.6, *n* = 42), composed of 4 columns. The columns 12.5–15(–19.5) µm diam, 5–6-celled. Setose appendages 4, brown, (88–)113–190(–200) µm long (av. 142.9 µm, *n* = 21), 2–4 µm wide at the apex, with 9–15-septa at 10 to 20 µm intervals. After the conidial state is formed, the ascomatal state is soon found. *Asci* 66–107 × 8–11 µm (av. 84.5 × 9.9 µm, *n* = 36). *Ascospores* similar in appearance to those on the host, but slightly larger, measuring 25–35 × 3.5–5.5 µm (av. 29.8 × 4.5 µm, *n* = 75), L/W 5.9–7.5 (av. 6.6, *n* = 75), with a mid-septum submedian (0.50–0.53; av. 0.51, *n* = 55).

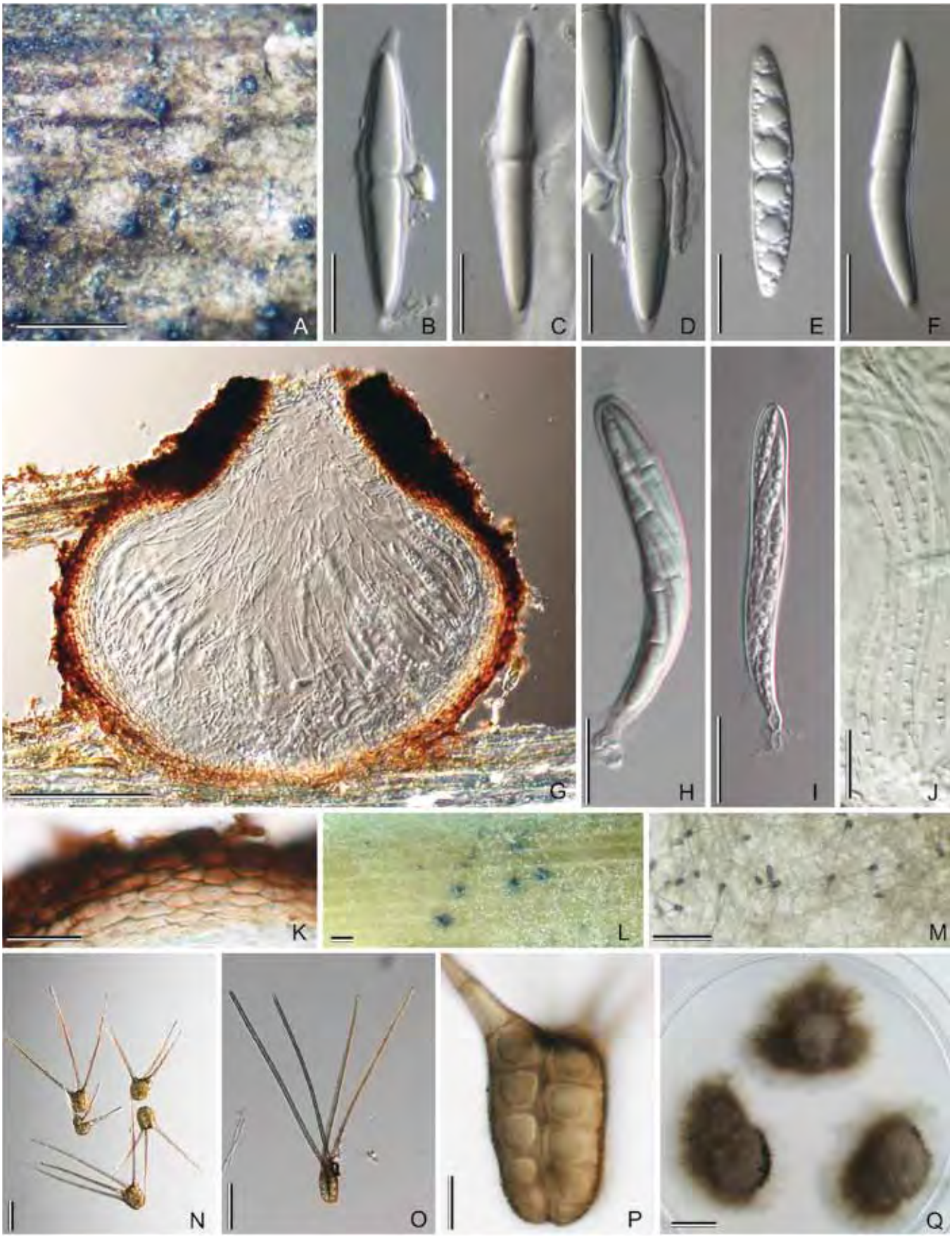


**Fig. 1.** *Tetraplosphaeria nagasakiensis*. A. Ascomata on host surface. B–F. Ascospores; G. Ascoma in longitudinal section; H–I. Asci; J. Pseudoparaphyses; K. Ascomal wall; L. Ascomata on rice straw agar; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, P = 10 µm; G, L = 100 µm; H–K = 20 µm; M = 200 µm; N–O = 50 µm; Q = 1 cm. A–D, G–H, K from HHUF 29378 holotype; E–F, I–J, L–Q from culture KT 1682.

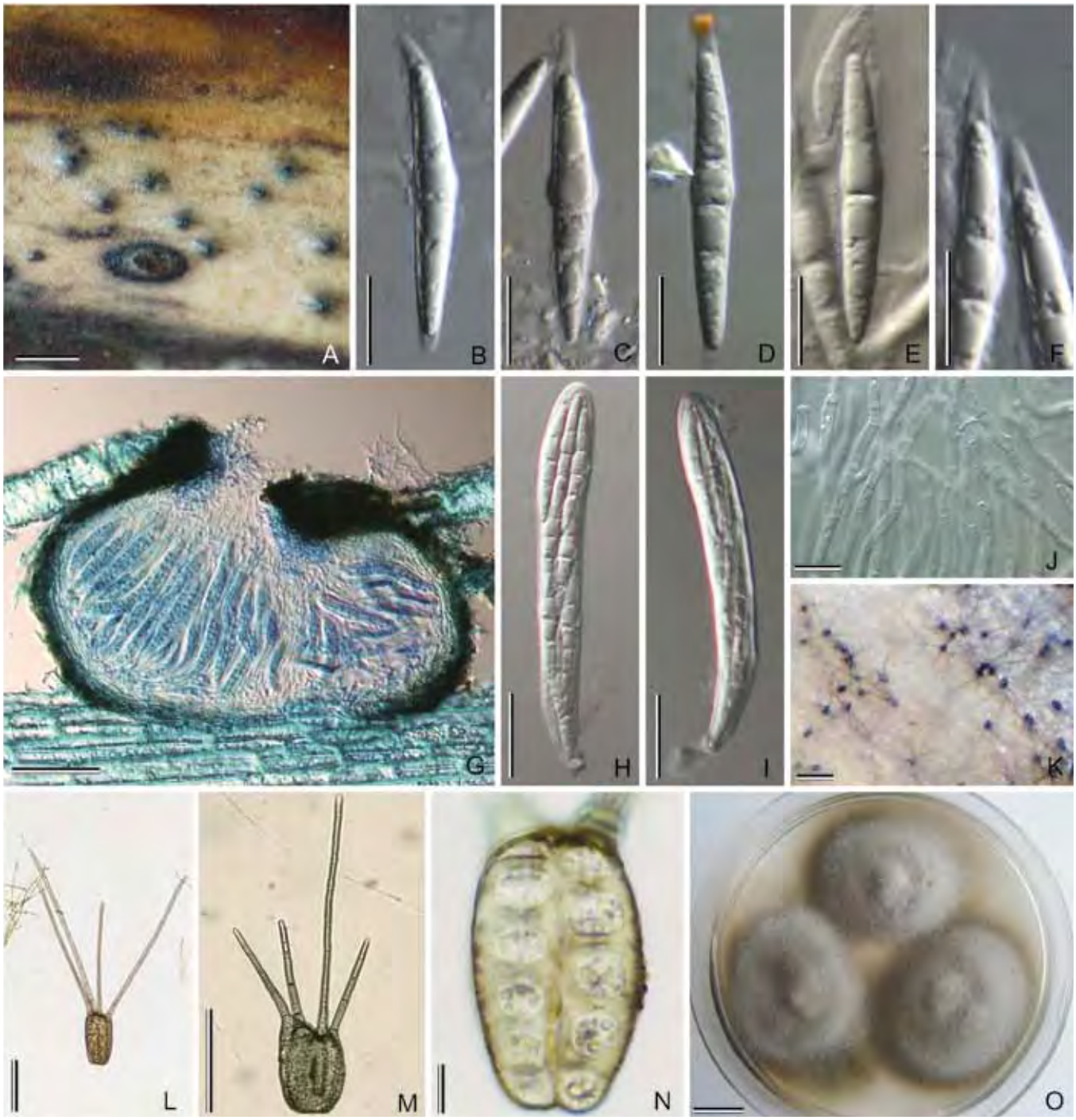


**Fig. 2.** *Tetraplosphaeria sasicola*. A. Ascomata on host surface; B–F. Ascospores; G. Ascoma in longitudinal section; H–I. Asci; J. Pseudoparaphyses; K. Conidia on rice straw agar; L–M. Conidia; N. Conidial body; O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, J, N = 10 µm; G, L–M = 50 µm; H–I = 20 µm; K = 100 µm; O = 1 cm. A–J from HHUF 27566 holotype; K–O from culture KT 563.

*Specimen examined*: **Japan,** Hokkaido, Yoichi, Sawamachi (140°46'E, 43°11'N), on culms of *Sasa senanensis*, 7 July 2001, K. Tanaka, HHUF 27566 **holotype** designated here, living culture *KTC 563* (= JCM 13167 = MAFF 239677).

*Notes*: This species is characterised by the smallest asci and ascospores. The conidial morphology of this species resembles that of *Tetraploa ellisii*, but the latter species has more slender conidia (30–51 × 15–26 µm, L/W 1.9; Ellis 1949).

***Tetraplosphaeria tetraploa*** (Scheuer) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515261. Fig. 3.

*Basionym*: *Massarina tetraploa* Scheuer, Mycol. Res. 95: 126. 1991.

≡ *Lophiostoma tetraploa* (Scheuer) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Diversity Res. Ser. (Hong Kong) 7: 108. 2002.

*Anamorph*: *Tetraploa aristata s. l.*

*Ascomata* 180–200 µm high, 150–280 µm diam, scattered, immersed, globose to somewhat pyriform, glabrous to sometimes covered with sparse brown hyphae at sides. *Beak* 50–80(–100) µm long, 50–75 µm diam, central, papillate to short cylindrical, composed of subglobose to polygonal cells, with hyaline periphyses. *Ascomatal wall* uniformly 6–12 µm thick, composed of 3–4 layers of

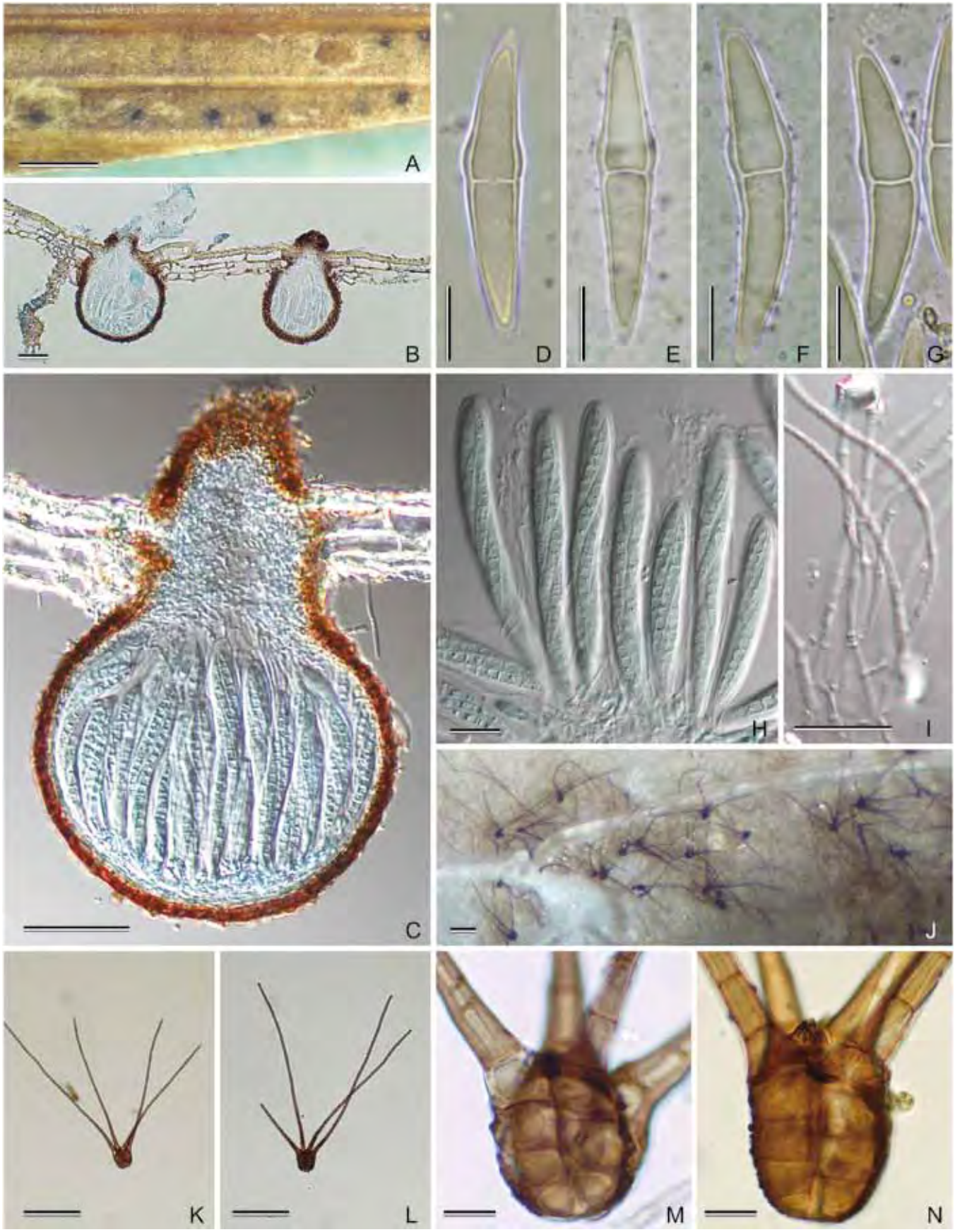


**Fig. 3.** *Tetraplosphaeria tetraploa*. A. Ascomata on host surface; B–C. Ascomata in longitudinal section; D–G. Ascospores; H. Asci; I. Pseudoparaphyses; J. Conidia on malt extract agar; K–L. Conidia; M–N. Conidial bodies. Scale bars: A = 500 µm; B–C = 50 µm; D–G, M–N = 10 µm; H–I = 20 µm; J–L = 100 µm. A–I from GZU 36-91 holotype of *Massarina tetraploa*; J–N from GZU 32-91 (dried culture specimen of *Tetraploa* state).

polygonal brown cells (3.5–12.5 × 2.5–5 µm). *Pseudoparaphyses* cellular, 1.5–2.5 µm wide, branched and anastomosed, with septa at 8 to 15 µm intervals. *Asci* (90–)95–128(–140) × 13–16(–19) µm (av. 109.4 × 14.2 µm, *n* = 50), numerous, basal, fissitunicate, cylindrical, with a short stipe of 5–15 µm long, with 8 biseriate ascospores. *Ascospores* (29–)32–41.5(–43) × 4–6(–7) µm (av. 37 × 5.2 µm, *n* = 50), L/W 6.4–8.1 (av. 7.1, *n* = 50), narrowly fusiform with acute ends, slightly curved, with a septum supramedian (0.44–0.49; av. 0.47, *n* = 45) and constricted, hyaline, smooth, with a sheath; sheath entire, narrow, 2–4 µm long at both ends, 1–1.5 µm thick at upper of the septum.

*Culture characteristics*: Not examined. According to Scheuer (1991) this fungus produces *Tetraploa aristata* as anamorph. The anamorph on the dried culture specimen (GZU 32-91) examined in this study is as follows: *Conidia* 30–33 × 23–25 µm (av. 30.8 × 23.3 µm, *n* = 6), L/W = 1.3, solitaly, short cylindrical, pale brown, verrucose, consist of 4 columns of 10–13 µm wide, 4-celled. Appendages 263–350 µm long (av. 295.8 µm, *n* = 6), 10–13 µm thick at the base, 2–3 µm at the apex, 17–22-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, straight.

*Specimens examined*: **U.K.,** England, Exeter, Exminster marshes, on leaves of *Carex acutiformis*, 13 Nov. 1988, Ch. Scheuer, GZU 36-91 **holotype** of *Massarina tetraploa*; Dried culture specimen of conidial state grown on malt extract agar (derived from ex-type culture), GZU 32-91.

*Notes*: This species was originally described as a species of *Massarina* (Scheuer 1991), but later was transferred to the genus *Lophiostoma* (Hyde *et al.* 2002a). The original strain isolated by Scheuer (1991) from the holotype of *Massarina tetraploa* is no longer preserved (Scheuer, pers. comm.). There is one strain that is deposited as *M. tetraploa* in CBS (CBS 101683), but it is considered as a misidentified material because it produced a *Phaeosphaeria*-like teleomorph having 39–49 × 5.5–6.5 µm, yellowish, 3-septate ascospores on RSA. Therefore, *M. tetraploa* was not included in phylogenetic analyses in this study. However, morphological evidence obtained from the holotype and the dried culture specimen (anamorphic state) of *M. tetraploa* clearly indicates that it belongs to *Tetraplosphaeria*. This species can be distinguished from other species of this genus by the large-sized asci and ascospores. The anamorph of this species has been reported as *Tetraploa aristata* (Scheuer 1991), but the presence of several *T. aristata*-like anamorphs with sequence differences revealed in this study suggest that redefinition of *T. aristata* along with molecular evidence would be required for this anamorphic species.

***Tetraplosphaeria yakushimensis*** Kaz. Tanaka, K. Hiray. & Hosoya, **sp. nov.** MycoBank MB515262. Fig. 4.

*Anamorph*: *Tetraploa aristata s. l*.

*Etymology*: In reference to the collection site.

Ascomata 135–180 × 150–250 µm, immersa, subglobosa. Rostrum 50 × 55–65 µm, ostiolatum. Paries ascomatis 15–20 µm crassus ad latus, ex cellulis 4–6-stratis 5–15 × 2.5–4 µm compositus. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci 85–110 × 10.5–13 µm, fissitunicati, clavati vel cylindrici, octospori. Ascosporae 26.5–36.5 × 4–6 µm, anguste fusiformes, 1-septatae, hyalinae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu stricto. Conidia in vitro 25–37(–40) × 20–30 µm, brunnea, cum 4 appendicibus; appendices (52–)62–142(–150) µm longae, 3–8-septatae.

*Ascomata* 135–180 µm high, 150–250 µm diam, scattered, immersed below the epidermis, subglobose, glabrous. *Beak* short-papillate, *ca*. 50 µm high, 55–65 µm diam, central, *Ascomatal wall* 15–20 µm thick at sides, composed of 4–6 layers of rectangular to polygonal hyaline to pale brown cells of 5–15 × 2.5–4 µm; at the base 10–20 µm thick. *Pseudoparaphyses* cellular, numerous, 1.5–4 µm wide, septate, branched, anastomosed. *Asci* 85–110 × 10.5–13 µm (av. 99.3 × 11.8 µm, *n* = 20), fissitunicate, numerous, basal and lateral, clavate to cylindrical, short-stalked (*ca*. 5–10 µm long), with 8 ascospores biseriate above and uniseriate below. *Ascospores* 26.5–36.5 × 4–6 µm (av. 30.6 × 4.5 µm, *n* = 31), L/W 5.1–8.3 (av. 6.9, *n* = 31), narrowly fusiform, straight or slightly curved, with a nearly median (0.47–0.51; av. 0.49, *n* = 30) septum and slightly constricted, hyaline, smooth, with a mucilaginous sheath. Sheath 3–10 µm long at the ends, 1–2 µm wide at the sides.

*Culture characteristics*: On RSA, both teleomorphic and anamorphic states are produced. *Ascospores* are similar to those on the host, measuring 29–37 × 4–5.5 µm (av. 32 × 4.7 µm, *n* = 20). *Anamorph* is *Tetraploa aristata s. l. Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* produced directly on the mycelium, solitary, short cylindrical, brown, verruculose, 25–37(–40) × 20–30 µm (av. 31.4 × 24.9 µm, *n* = 50), L/W 1.1–1.5 (av. 1.3, *n* = 50), 4-celled, composed of 4 columns and 4 setose appendages. Appendages (52–)62–142(–150) µm long (av. 96.2 µm, *n* = 60), 2–3 µm wide at the apex, 7–8 µm at the base, with 3–8-septa at 8 to 16 µm intervals.

*Specimen examined*: **Japan,** Kagoshima, Isl. Yakushima, near the mouth of Kurio-river, on culms of *Arundo donax*, 20 Oct. 2005, K. Tanaka & T. Hosoya, HHUF29652 **holotype** designated here (isotype TNS-F-12442), living culture *KTC 1906* (= CBS 125435).

*Notes*: In terms of ascus and ascospore morphology, this species is quite close to *Tetraplosphaeria nagasakiensis*, but it is distinct from the latter in its conidial morphology. *Tetraplosphaeria yakushimensis* and *Tetraploa aristata* (CBS 996.70), both collected from non-bamboo species (*Arundo donax* and *Alpinia formosa*, respectively), clustered together (see phylogenetic section). Morphological comparison of these two strains could not be made, because the strain CBS 996.70 did not sporulate in any of the culture methods used. Sequence differences between the strains (*e.g.* 24/459 nucleotides in ITS) also suggest that they are different species.

***Tetraploa* sp. 1** (*T. aristata s. l.*). Fig. 5A–D.
*Teleomorph*: unknown.

*Conidia* short cylindrical, brown, verruculose, 26–31.5(–35) × 17.5–24 µm (av. 29.4 × 20.8 µm, *n* = 20), L/W 1.2–1.9, 3–5-celled, with 4 setose appendages of 100–175 µm long (av. 136.7 µm, *n* = 20).

*Specimen examined*: **Japan,** Kagoshima, Nagayo, Nagasaki Siebold University (129°52.4'E, 32°48.2'N), on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, HHUF 29625, living culture *KTC 1684* (= JCM 14424).

*Notes*: This species is most similar to anamorphs of *Tetraplosphaeria tetraploa* and *Tetraplosphaeria yakushimensis*, but has more slender conidia. The conidial morphologies of these three species match well with the description of *Tetraploa aristata* provided by Ellis (1949), but they may not be conspecific as discussed later.


**Fig. 4.** *Tetraplosphaeria yakushimensis*. A. Ascomata on host surface; B. Ascoma in longitudinal section; C–F. Ascospores; G–H, Asci; I. Ascus with an ocular chamber; J. Pseudoparaphyses; K. Conidia on agar piece immersed in water; L. Conidia; M. Conidia with verruculose ornamentation; N. Conidial body; O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–E, I–J, M–N = 10 µm; F, L = 50 µm; G–H = 20 µm; K = 100 µm; O = 1 cm. A–J from HHUF 29652 holotype; K–O from culture KT 1906.

***Tetraploa* sp. 2** (*T. ellisii s. l.*). Fig. 5E–H.
*Teleomorph*: unknown.

*Conidia* short cylindrical, broader at the base, brown, verruculose, 38–50 × 22–33 µm (av. 43.1 × 27.9 µm, *n* = 20), L/W 1.3–1.8, 4–5-celled, with 4 setose appendages of 142–330 µm long (av. 232 µm, *n* = 30).

*Specimen examined*: **Japan,** Okinawa, Isl. Iriomote, near Oomijya river, on culms of gramineae, 22 Nov. 2008, K. Tanaka & K. Hirayama, HHUF 30027, living culture *KTC 2578* (= NBRC 106251).

*Notes*: This fungus has relatively large-sized conidia as compared with those of other *Tetraploa* species examined in this study. It is close to *Tetraploa ellisii* that was reported by Ellis (1949) and the anamorph of *Tetraplosphaeria sasicola* in terms of conidial dimension, but differs from the latter in having longer appendages.

***Triplosphaeria*** Kaz. Tanaka & K. Hiray., **gen. nov.** MycoBank MB515255.

*Anamorph*: Undescribed *Tetraploa*-like state having conidia with three setose appendages.

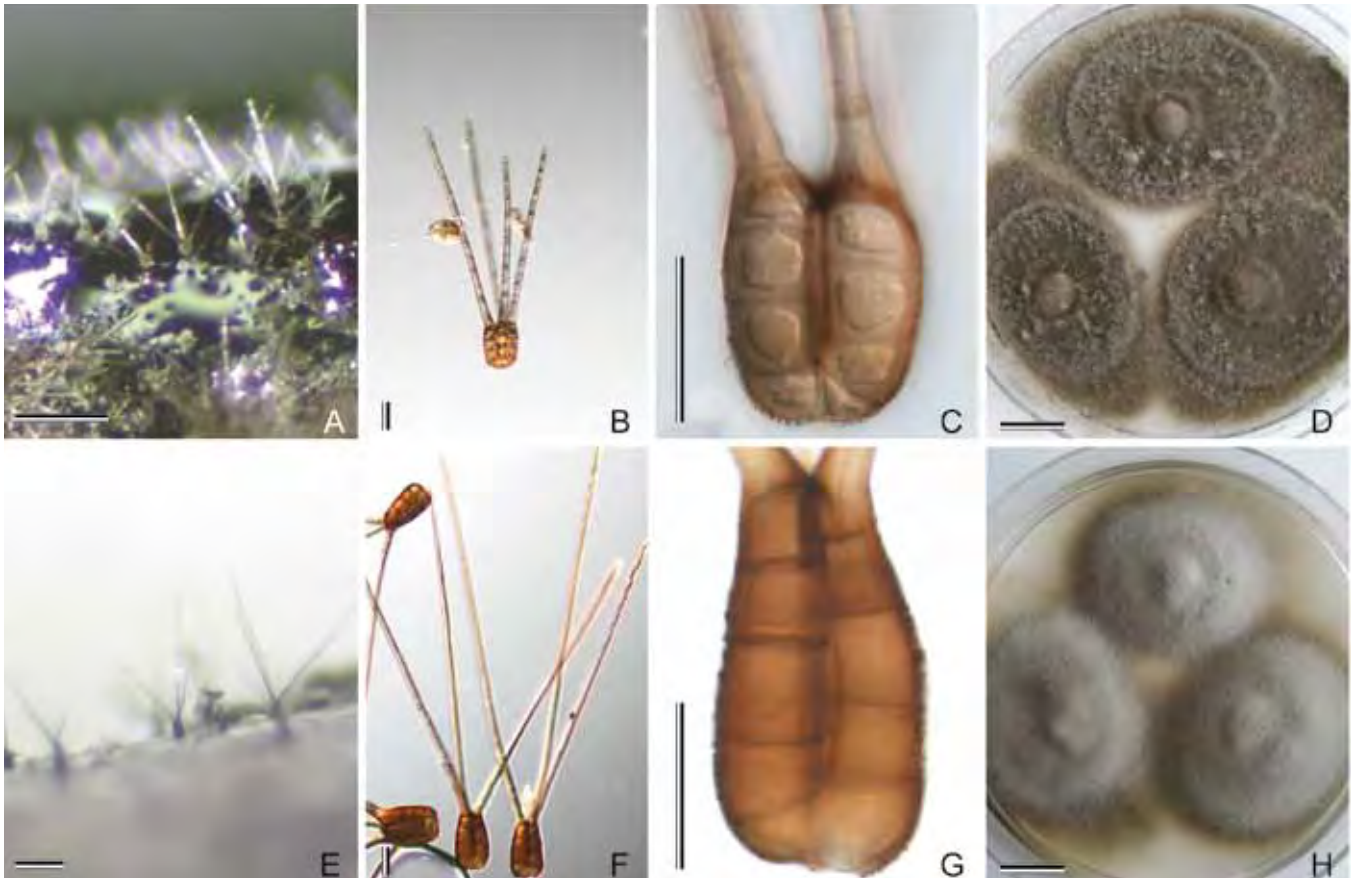


**Fig. 5.** *Tetraploa* spp. A–D. *Tetraploa* sp. 1 (culture KT 1684); E–H. *Tetraploa* sp. 2 (HHUF 30027); A, E. Conidia on agar piece immersed in water; B, F. Conidia; C, G. Conidial bodies; D, H. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A, E = 200 µm; B–C, F–G = 20 µm; D, H = 1 cm.

*Etymology*: In reference to the anamorphic state of *Tetraploa*-like conidia with three setose appendages.

Ascomata immersa, subglobosa. Rostrum nullum vel breve. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, cylindrici vel clavati, octospori. Ascosporae anguste fusiformes vel late fusiformis, 1-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia ovata vel obpyriformis, brunnea, cum 3 appendicibus.

*Ascomata* scattered to gregarious, immersed below the epidermis, subglobose, with single locule, glabrous. *Beak* none to short, with hyaline sparse periphyses. *Ascomatal wall* rim-like at sides, composed of vertically orientated rectangular to cylindrical hyaline hyphoid cells, flattened and poorly developed at the base. *Pseudoparaphyses* narrowly cellular, numerous, branched and anastomosed, septate. *Asci* fissitunicate, basal and lateral, cylindrical to clavate, rounded at the apex, short-stalked, with 8 ascospores. *Ascospores* narrowly fusiform to broadly fusiform with rounded ends, 1-septate, constricted at the septum, hyaline, smooth, with an entire sheath. *Anamorph Tetraploa*-like with 3 setose appendages. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* composed of 3 columns with pseudosepta, ovoid to obpyriform, brown, almost smooth, verrucose at the base, with 3 setose appendages at the apex.

*Type species*: *Triplosphaeria maxima* Kaz. Tanaka & K. Hiray., sp. nov.

*Notes*: A new genus *Triplosphaeria* is introduced here to place *Massarina*-like ascomycetes with *Tetraploa*-like anamorphs having three setose appendages. The ascomata of *Triplosphaeria* species are hemispherical with a flattened base and have rim-like regions composed of vertically oriented hyphoid cells at the side in longitudinal section. Morphology of anamorphs is superficially similar to that of *Tetraploa*, but conidia are composed of three columns and three setose appendages.

***Triplosphaeria acuta*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515263. Fig. 6.

*Etymology*: In reference to the fusiform ascospores with acute ends.

Ascomata 135–230 × 540–750 µm, immersa, subglobosa. Rostrum nullum vel breve, ostiolatum. Paries ascomatis 85–180 µm crassus ad latus, ex cellulis 5–10 × 3.5–7.5 µm compositus. Pseudoparaphyses 1–2 µm latae, ramificantes et anastomosantes, septatae. Asci (62–)73–106 × 11–15 µm, fissitunicati, cylindrici vel clavati, octospori. Ascosporae 25–35 × 4–6(–7) µm, anguste fusiformes, 1-septatae, hyalinae, strato mucoso 6–18 µm lato circumdatae. Anamorphosis *Tetraploa* sensu lato. Conidia in vitro (25–)31–50(–65) × 14–22 µm, brunnea, cum 3 appendicibus; appendices (37–)44–120(–130) µm longae, 3–8-septatae.

*Ascomata* 135–230 µm high, 540–750 µm diam (including the rim), with single locule of 230–400 µm diam, scattered to gregarious, immersed below the epidermis, subglobose, glabrous. *Beak* none or short, with hyaline sparse periphyses, ostiolate, filled with tips of pseudoparaphyses. *Ascomatal wall* at sides, 85–180 µm wide and rim-like, composed of vertically orientated rectangular to subglobose hyaline hyphoid cells of 5–10 × 3.5–7.5 µm; near the epidermis, 25–38 µm thick, composed of polygonal to subglobose brown thick-walled cells of 3.5–10 µm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–2 µm wide, guttulate, branched and anastomosed, septate, with slime coating. *Asci* (62–)73–106 × 11–15 µm (av. 86.1 × 12.6 µm, *n* =

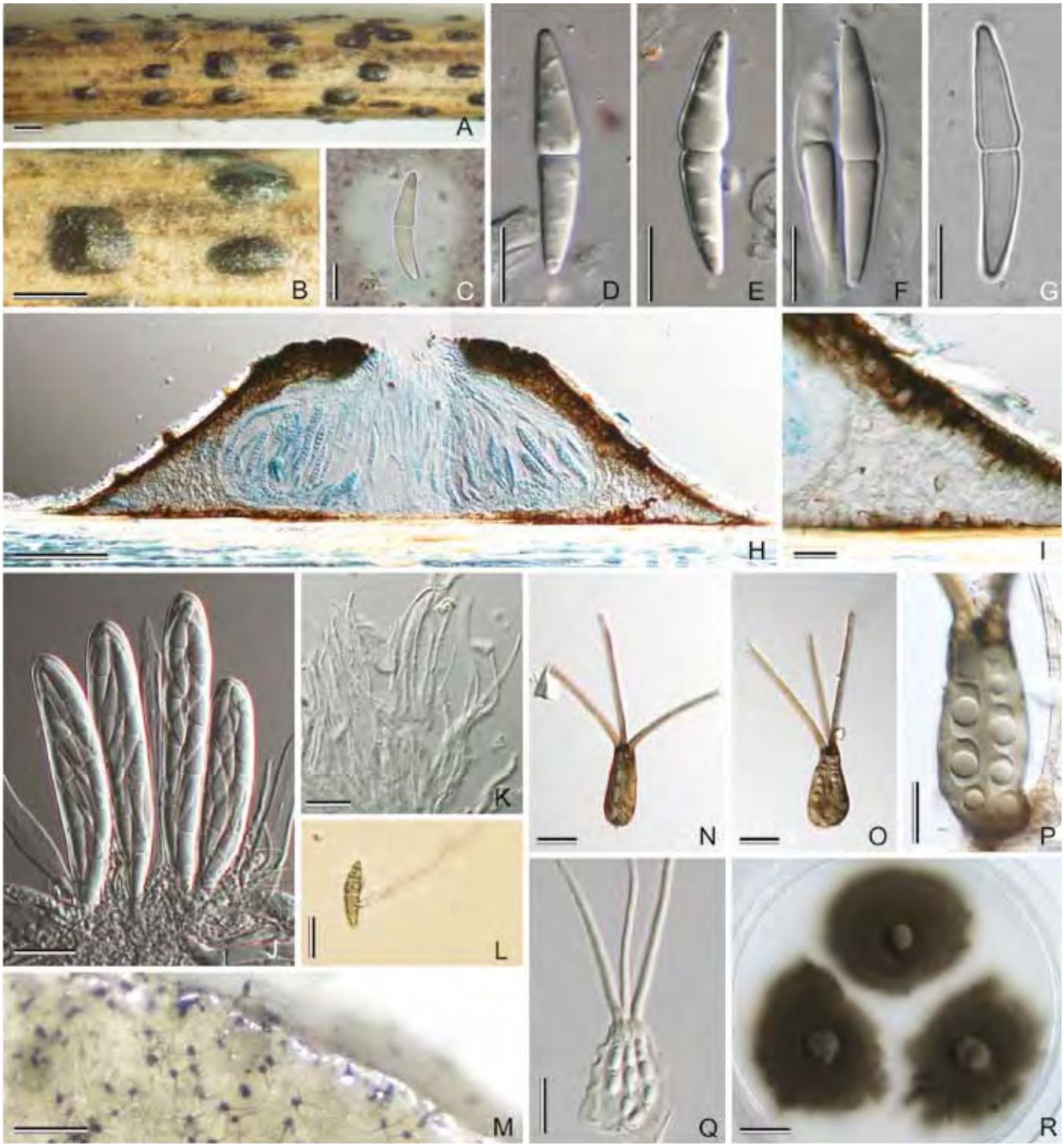


**Fig. 6.** *Triplosphaeria acuta*. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomal wall at side; J. Asci; K. Pseudoparaphyses; L. Germinating ascospore; M. Conidia on agar piece immersed in water; N–O. Conidia; P. Conidial body; Q. Breached conidia composed of three columns; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 500 µm; C–G, P = 10 µm; H, M = 100 µm; I–L, N–O, Q = 20 µm; R = 1 cm. A–L from HHUF 29387 holotype; M–R from culture KT 1170.

50), fissitunicate, numerous, basal and somewhat lateral, cylindrical to clavate, rounded at the apex, short-stalked (5–15 µm long), with 8 biseriate ascospores. *Ascospores* 25–35 × 4–6(–7) µm (av. 29.6 × 5.5 µm, *n* = 126), L/W 4.8–6.2 (av. 5.5, *n* = 126), narrowly fusiform with acute ends, mostly curved, with a septum usually submedian (0.49–0.53; av. 0.51, *n* = 113) and constricted, hyaline, smooth, with an inconspicuous entire sheath of 6–18 µm wide.

*Culture characteristics*: Colonies on PDA attaining 3–3.1 cm diam, velvety in appearance, dark green (30F4) with greyish green (25D6) entire margin (2 mm); reverse similar; no pigment produced. On RSA, *Tetraploa*-like anamorph having 3 appendages is found. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* consist of one conidial body and 3 or rarely 4 appendages, solitary. Conidial body (25–)31–50(–65) × 14–22 µm (av. 40.9 × 17.2 µm, *n* = 92), L/W = 1.8–3.3 (av. 2.4, *n* = 92), 3–4-pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Setose appendages (37–) 44–120(–130) µm long (av. 90.3 µm, *n* = 70), 3–5 µm thick at the base, 2–3 µm at the apex, 3–8-septate, pale brown at the base and almost hyaline apex, smooth, unbranched, straight.

*Specimens examined*: **Japan,** Hokkaido, Akkeshi, Ariake, Small stream (144°52.0'E, 43°01.2'N), on submerged culms of bamboo (*Sasa nipponica*?), 3 June 2003, K. Tanaka & S. Hatakeyama, HHUF 29387 **holotype** designated here, living culture *KTC 1170* (= JCM 13171 = MAFF 239681); Hokkaido, Akkeshi, Ootakita, Sattebetsu-river (144°49.0'E, 43°08.1'N), on submerged culms of bamboo (*Sasa nipponica*?), 3 June 2003, K. Tanaka & S. Hatakeyama, KT 1218 = HHUF 29388.

*Note*: This species is quite similar to *Triplosphaeria yezoensis* in its overall morphology, but has more slender ascospores with acute ends (L/W 5.5 vs. 4.4).

***Triplosphaeria cylindrica*** Kaz. Tanaka & K. Hiray., **nom. nov.** MycoBank MB515264. Fig. 7.

≡ *Massarina yezoensis* I. Hino & Katum., in Hino, Icon. Fung. Bambus. Jpn.: 188. 1961.

*Ascomata* 110–190 µm high, 450–1180 µm diam (including the rim), with single locule of 220–350 µm diam, scattered, immersed below the epidermis, subglobose, glabrous. *Beak* none to short, with hyaline, sparse periphyses, filled with tips of pseudoparaphyses. *Ascomatal wall* at sides, 100–350 µm wide and rim-like, composed of vertically orientated rectangular to cylindrical hyaline hyphoid cells of 5–15 × 2.5–5 µm; about 20 µm thick near the epidermis, composed of polygonal brown thick-walled cells of 3–10 µm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–3 µm wide, guttulate, branched and anastomosed, septate. *Asci* (70–)80–126 × 14.5–21(–23.5) µm (av. 98.2 × 17.9 µm, *n* = 82), fissitunicate, numerous, basal and lateral, cylindrical to clavate, rounded at the apex, short-stalked (4–25 µm long), with 8 biseriate ascospores. *Ascospores* (22–)25–31(–33) × 6–10 µm (av. 28.2 × 8 µm, *n* = 153), L/W 3.0–4.4 (av. 3.5, *n* = 153), broadly fusiform to cylindrical with rounded ends, with a septum submedian (0.50–0.56; av. 0.53, *n* = 143) and strongly constricted, hyaline, smooth, with an entire sheath of 7–20 µm thick.

*Culture characteristics*: Colonies on PDA attaining 3.1 cm diam, velvety in appearance, brownish grey (6E2) with whitish entire margin of 2 mm; reverse similar to surface; no pigment produced. On RSA, a *Tetraploa*-like anamorph with 3 setose appendages is formed. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* consist of one conidial body and 3 long appendages, solitary. Conidial body 29.5–40 × 14–23.5 µm (av. 36.1 × 19.4 µm, *n* = 20), L/W = 1.4–2.3 (av. 1.9, *n* = 20), 2–4-pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Setose appendages 33–120 µm long (av. 73.4 µm, *n* = 26), 4–4.5 µm thick at the base, 2–3 µm at the apex, 3–9-septate, pale brown at the base and almost hyaline apex, smooth, unbranched, straight.

*Specimens examined*: **Japan**, Hokkaido, Oiwaki, on culms of *Sasa kurilensis*, 16 Sept. 1956, I. Hino, YAM 21797 **holotype** of *Massarina yezoensis*; Aomori, Souma, Jinba-dake (1049m a.s.l.), 14 June 2003, Y. Harada, HHUF 29381, living culture *KTC 1256* (= JCM 13169 = MAFF 239679); Aomori, Mt. Iwaki, 9 July 2005, K. Tanaka, HHUF 29626, living culture *KTC 1800* (= JCM 14425); Hokkaido, Isl. Rishiri, Afutoromanai trail, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30028, living culture *KTC 2550* (= NBRC 106247).

*Notes*: This species was originally described as *Massarina yezoensis* (Hino 1961), but is transferred to *Triplosphaeria* because of its hemispherical ascomata with a flattened base and rim-like side wall. The most distinctive feature of this species is the relatively wider ascospores (L/W 3.5) with rounded ends. A new name is introduced for this species because the epithet "yezoensis" has been applied for *Triplosphaeria yezoensis* [= *Didymella yezoensis* (Hino & Katumoto 1958)] in this study.

***Triplosphaeria maxima*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515265. Fig. 8.

*Etymology*: In reference to the large-sized ascospores.

Ascomata 250–300 × 900–1000 µm, immersa, globosa vel subglobosa. Rostrum nullum vel breve, ostiolatum. Paries ascomatis 170–270 µm crassus ad latus, ex cellulis 5–13 × 3.5–8 µm compositus. Pseudoparaphyses 1–2.5 µm latae, ramificantes et anastomosantes, septatae. Asci 95–133 × 14.5–21 µm, fissitunicati, clavati vel cylindrici, octospori. Ascosporae (32.5–)34–45(–48.5) × (6–)7–9(–10) µm, anguste fusiformes, 1-septatae, hyalinae, strato mucoso 3–7 µm lato circumdatae. Anamorphosis *Tetraploa* sensu lato. Conidia in vitro 41–55 × 17–23(–27.5) µm, brunnea, cum 3 appendicibus; appendices 12–66 µm longae, 1–6-septatae.

*Ascomata* 250–300 µm high, 900–1000 µm diam (including the rim), with single locule of 420–530 µm diam, scattered to sometimes clustered, immersed below the epidermis, globose to subglobose, glabrous. *Beak* none or short, with hyaline sparse periphyses-like hyphae, filled with pseudoparaphyses tips. *Ascomatal wall* at sides 170–270 µm wide and rim-like, composed of vertically orientated rectangular to polygonal 5–13 × 3.5–8 µm hyaline cells; near the epidermis composed of polygonal to subglobose brown cells of 3–10 µm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–2.5 µm wide, guttulate, branched and anastomosed, with thin septa at 7 to 20 µm intervals. *Asci* 95–133 × 14.5–21 µm (av. 113 × 18 µm, *n* = 50), fissitunicate, numerous, basal and somewhat lateral, clavate to cylindrical, rounded at the apex, short-stalked (7–25 µm long), with (4–)8 biseriate ascospores. *Ascospores* (32.5–)34–45(–48.5) × (6–)7–9(–10) µm (av. 38.9 × 7.9 µm, *n* = 120), L/W 4.2–5.9 (av. 5.0, *n* = 120), narrowly fusiform with acute ends, straight or slightly curved, 1-septate, submedian (0.50–0.54; av. 0.52, *n* = 94), constricted at the septum, hyaline, up to 4 guttules in each cell or without guttules, smooth, with an inconspicuous sheath of 3–7 µm wide.

*Culture characteristics*: Colonies on PDA attaining 2.6–2.8 cm diam, velvety in appearance, olive (2E4), with whitish entire margin of 2 mm; reverse dark green (29F6); no pigment produced. On RSA, a *Tetraploa*-like anamorph with 3 setose appendages is formed. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* consist of a conidial body and 3 setose appendages, solitary. Conidial body 41–55 × 17–23(–27.5) µm (av. 48.3 × 19.3 µm, *n* = 61), L/W = 2.2–3.2 (av. 2.5, *n* = 61), 5–6-pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Appendages 12–66 µm long (av. 27.4 µm, *n* = 65), 3–4 µm wide, 1–6-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, slightly curved.

*Specimen examined*: **Japan,** Aomori, Nishimeya, Ookawa, on culms of *Sasa kurikensis*, 23 July 2002, S. Hatakeyama, HHUF29330 **holotype** designated here, living culture *KTC 870* (= JCM 13172 = MAFF 239682).

*Note*: This fungus is clearly distinguishable from other species of *Triplosphaeria* by its largest asci and ascospores.

***Triplosphaeria yezoensis*** (I. Hino & Katum.) Kaz. Tanaka, K. Hiray. & Shirouzu, **comb. nov.** MycoBank MB515266. Fig. 9.

*Basionym*: *Didymella yezoensis* I. Hino & Katum., Bull. Fac. Agr. Yamaguchi Univ. 9: 902. 1958.

*Ascomata* 140–160 µm high, 450–550 µm diam (including the rim), with single locule of 240–330 µm diam, scattered to sometimes 2–3


**Fig. 7.** *Triplosphaeria cylindrica*. A. Ascomata on host surface; B–E. Ascospores; F. Ascoma in longitudinal section; G. Ascomal wall at side; H. Pseudoparaphyses; I–J. Asci; K. Fissitunicate ascus with endoascus extending from ectoascus; L. Ascospores in India ink; M–N. Developing conidia; O. Conidial body; P–Q. Conidia; R. Breached conidium composed of three columns; S. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–E, O, R = 10 µm; F = 50 µm; G–L, N, P–Q = 20 µm; M = 100 µm; S = 1 cm. A–B, F, J from YAM 21797 holotype of *Massarina yezoensis*; C–D, I from HHUF 29626; E, G–H, K–L from HHUF 29381; M–R from culture KT 1256; S from culture KT 1800.

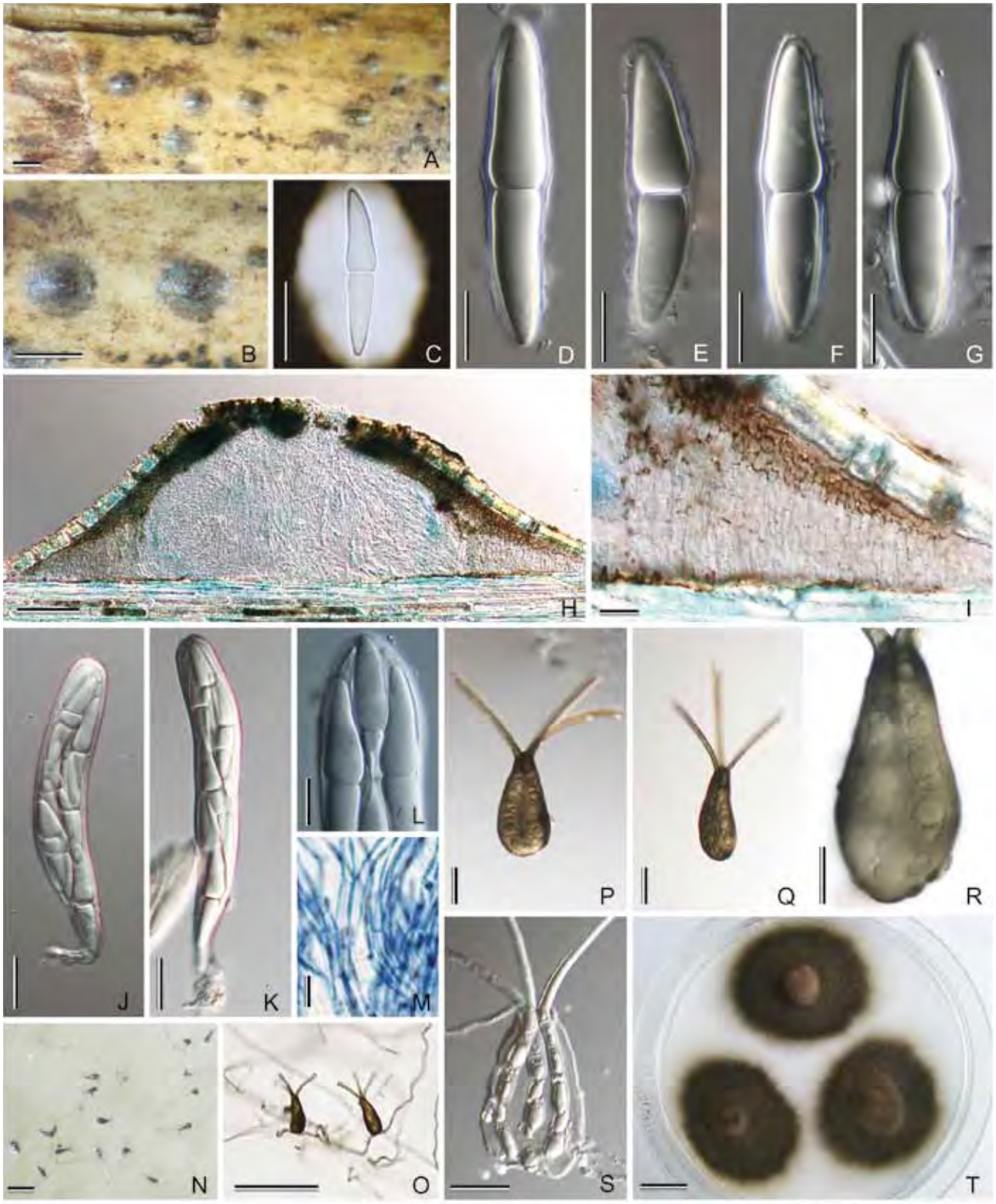


**Fig. 8.** *Triplosphaeria maxima*. A–B. Ascomata on host surface; C. Ascospore in India ink; D–G. Ascospores; H. Ascoma in longitudinal section; I. Ascomal wall at side; J–K. Asci; L. Apex of ascus; M. Pseudoparaphyses; N. Conidia on agar piece immersed in water; O. Developing conidia; P–Q. Conidia; R. Conidial body; S. Breached conidium composed of three columns; T. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 500 µm; C, I–K, P–Q, S = 20 µm; D–G, L–M, R = 10 µm; H, N–O = 100 µm; T = 1 cm. A–M from HHUF 29390 holotype; N–T from culture KT 870.


**Fig. 9.** *Triplosphaeria yezoensis*. A. Ascomata on host surface; B–F. Ascospores; G. Ascoma in longitudinal section; H. Ascospore in India ink; I. Apex of ascus; J. Pseudoparaphyses; K–M. Asci; N. Conidia on rice straw agar; O–P. Conidia; Q. Conidial body; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 500 µm; B–F, H–J, Q = 10 µm; G = 50 µm; K–M, O–P = 20 µm; N = 100 µm; R = 1 cm. A–B, G, I–K from YAM 21758 holotype of *Didymella yezoensis*; C–D, H, L from HHUF 30029; E–F, M from HHUF 30030; N–O from culture KT 1732; P–R from culture KT 1715.

grouped, immersed below the epidermis, subglobose, glabrous. *Beak* none or short, with hyaline sparse periphyses. *Ascomatal wall* at sides, 100–130 µm wide and rim-like, composed of vertically orientated, rectangular to subglobose, hyaline to pale brown, hyphoid cells of 5–15 × 5–7.5 µm; near the epidermis composed of polygonal to subglobose brown thick-walled cells of 2.5–7.5 µm diam; at the base flattened and poorly developed. *Pseudoparaphyses* narrowly cellular, numerous, 1–2 µm wide, guttulate, branched and anastomosed, septate, with slime coating. *Asci* (60–)72–119(–141) × 12–18.5 µm (av. 93.3 × 15.3 µm, *n* = 86), fissitunicate, numerous, basal and somewhat lateral, cylindrical to clavate, rounded at the apex, short-stalked (5–24 µm long), with 8 biseriate ascospores. *Ascospores* (22.5–)26–32(–35) × 5–8 µm (av. 29.1 × 6.6 µm, *n* = 109), L/W 3.6–5.3 (av. 4.4, *n* = 109), narrowly fusiform with acute ends, mostly curved, with a septum usually submedian (0.50–0.55; av. 0.52, *n* = 109) and constricted, hyaline, smooth, with an inconspicuous entire sheath of 2–8 µm thick.

*Culture characteristics*: On RSA, a *Tetraploa*-like anamorph with 3 setose appendages is formed. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* consist of a conidial body and 3 setose appendages, solitary. Conidial body 30–40(–45) × (13–)15–22 µm (av. 34.4 × 18 µm, *n* = 30), L/W = 1.7–2.2 (av. 1.9, *n* = 30), 3–4-pseudoseptate, pale brown, smooth, narrowly ovate or ovate. Appendages (34–)40–75(–87) µm long (av. 51.6 µm, *n* = 40), 2.5–3 µm at the apex, 3–4.5 µm wide at the base, 2–9-septate, pale brown at the base and almost hyaline at the apex, smooth, unbranched, slightly curved.


**Fig. 10.** *Triplosphaeria* sp. A. Conidia on host surface; B. Conidia on agar piece immersed in water; C–E. Conidia; F. Conidial body; G. Germinating conidium; H. Breached conidium composed of three columns; I. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–F, H = 20 µm; G = 50 µm; I = 1 cm. A, D from HHUF 27481; B, F from culture KT 2546; C, E, G–H from HHUF 30031; I from culture HC 4665.

*Specimens examined*: **Japan**, Hokkaido, Asahikawa, Kagura, on culms of *Sasa palmata*, 20 Sept. 1956, I. Hino, YAM 21758 **holotype** of *Didymella yezoensis*; Hokkaido, Yoichi, Sawamachi (140°46'E, 43°11'N), 6 June 2004, K. Tanaka, HHUF 30029, living culture *KTC 1715* (= CBS 125436); Nagano, Sugadaira, Tsukuba Univ., on culms of *Sasa* sp., 28 June 2004, T. Shirouzu, HHUF 30030, living culture *KTC 1732* (= CBS 125437).

*Notes*: Hino & Katumoto (1958) described this fungus as a species of *Didymella*, but the general characteristics of this fungus do not fit within the current concept of *Didymella* (Gruyter *et al*. 2009, Woudenberg *et al*. 2009). Due to the presence of hemispherical ascomata having rim-like side wall, and the morphology of the conidial state, it is transferred to the genus *Triplosphaeria*. This species is close to *Triplosphaeria acuta*, but differs from the latter in having relatively broader ascospores (L/W 5.5 vs. 4.4 µm) and slightly smaller conidia (av. 34.4 × 18 µm vs. 40.9 × 17.2 µm).

***Triplosphaeria* sp.** (undescribed anamorphic state of *Triplosphaeria* sp.) Fig. 10.

*Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* (26–)31.5–46 × 14–23 µm (av. 38.4 × 18 µm, *n* = 61), L/W 1.7–2.8 (av. 2.2, *n* = 61), brown, 3–5-pseudoseptate, with 3 setose appendages. Appendages 36–90 µm long (av. 54 µm, *n* = 86), 2–8-septate.

*Culture characteristics*: *Conidia* produced on RSA are considerably larger than those on the host, 52–85 × 17–31 µm (av. 67.3 × 23.6 µm, *n* = 13), L/W 1.9–3.7 (av. 2.9, *n* = 13), 6–8-pseudoseptate, having 3 appendages of 51–120(–160) µm long (av. 78.5 µm, *n* = 14) with 4–12 septa.

*Specimens examined*: **Japan**, Aomori, Nakatsugaru, Nishimeya, Oosawa tril, on culms of *Sasa kurilensis*, 22 July 2002, S. Hatakeyama, HHUF 27481, living culture *HC 4665* (= NBRC 106248); Hokkaido, Isl. Rishiri, Kutugata trail, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30031, living culture *KTC 2546* (= NBRC 106249).

*Notes*: The conidia of *Triplosphaeria* sp. on the host plant (av. 38.4 × 18 µm) are similar to those of *Triplosphaeria maxima* produced under culture conditions (av. 48.3 × 19.3 µm), but *Triplosphaeria* sp. forms quite larger conidia in culture (av. 67.3 × 23.6 µm). The teleomorph of this fungus is unknown, but it obviously belongs to *Triplosphaeria* based on the anamorph morphology and molecular evidence. A new anamorph genus is needed to describe this species formally. However, we retain this species as *Triplosphaeria* sp. until further information is available, *e.g.* the possibility of collecting a teleomorph for this species.

***Polyplosphaeria*** Kaz. Tanaka & K. Hiray., **gen. nov.** MycoBank MB515256.

*Anamorph*: Undescribed *Tetraploa*-like state producing conidia with three to eight setose appendages.

*Etymology*: In reference to the anamorphic state producing conidia with many setose appendages.

Ascomata erumpentia vel superficialia, globosa. Rostrum aliquantum papillatum. Pseudoparaphyses septatae, ramificantes et anastomosantes. Asci fissitunicati, clavati, octospori. Ascosporae anguste fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia globosa vel subglobosa, brunnea, cum 3–8 appendicibus.

*Ascomata* scattered to clustered, erumpent to superficial, globose, black to sometimes reddish-brown, with brown short hyphae at sides, mostly associated with reddish pigment. *Beak* slightly papillate, central, with hyaline periphyses. *Ascomatal wall* composed of rectangular to polygonal brown cells, sometimes poorly developed at the base. *Pseudoparaphyses* trabecular, numerous, tortuous, septate, branched and anastomosed, associated with gelatinous material. *Asci* fissitunicate, clavate, short-stalked, with 8 biseriate ascospores. *Ascospores* narrowly fusiform, slightly curved,

1(–3)-septate, constricted at the primary septum, hyaline to pale olive-brown, with an entire sheath. *Anamorphs Tetraploa*-like with 3 to 8 setose appendages. *Conidiophores* absent. *Conidiogenous cells* monoblastic, *Conidia* globose to subglobose, with thin peel-like outer wall of conidia, composed of numerous internal hyphae at the inside, brown, almost smooth, verrucose at the base. Appendages brown, straight.

*Type species*: *Polyplosphaeria fusca* Kaz. Tanaka & K. Hiray., sp. nov.

*Notes*: The characteristics of this new genus include globose ascomata surrounded by numerous brown hyphae, reddish pigment on the host surface around ascomata, clavate asci with fissitunicate dehiscence, and narrowly fusiform ascospores provided with an entire sheath. The anamorphic state of *Polyplosphaeria* produces almost globose conidia composed of numerous internal hyphae, thin peel-like outer wall, and three to eight setose appendages. These appearances of conidia are slightly similar to those of *Piricauda* (*e.g. P. cochinensis* and *P. longispora*), but *Piricauda* has been defined primarily based on monotretic conidiogenous cells and its muriform conidia (Mercado Sierra *et al*. 2005).

***Polyplosphaeria fusca*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515267. Fig. 11.

*Etymology*: From the Latin *fuscus*, in reference to the coloured ascospores.

Ascomata 180–420 × 300–680 µm, erumpentia vel superficialia, globosa. Rostrum 50–90 × 75 µm, ostiolatum. Paries ascomatis 20–50 µm crassus ad latus, ex cellulis 4–7-stratis 2.5–12.5 × 2.5–5 µm compositus. Pseudoparaphyses 1–2 µm latae, septatae, ramificantes et anastomosantes. Asci (84–) 92.5–135 × 17–23 µm, fissitunicati, clavati, octospori. Ascosporae 36.5–49(–57) × 7–10 µm, anguste fusiformes, 1(–3)-septatae, hyalinae vel pallide brunneae, cum vagina gelatinosa obtectae. Anamorphosis *Tetraploa* sensu lato. Conidia in vitro 43–100(–125) µm diam, globosa vel subglobosa, brunnea, cum 3–8 appendicibus; appendices 92–200(–235) µm longae, 4–10-septatae.

*Ascomata* 180–420 µm high, 300–680 µm diam, scattered to clustered, erumpent to superficial, globose, black to sometimes reddish-brown, with short brown hyphae at sides, mostly associated with reddish pigment. *Beak* 50–90 µm long, 75 µm diam, slightly papillate, central, with hyaline periphyses, composed of subglobose to polygonal slightly thickened cells of 2–5 µm diam. *Ascomatal wall* at sides 20–50 µm thick, composed of 4–7 layers of (irregular to paralell rows) rectangular to polygonal brown cells of 2.5–12.5 × 2.5–5 µm diam, sometimes poorly developed at the base. *Pseudoparaphyses* trabecular, numerous, tortuous, 1–2 µm wide, septate, branched and anastomosed, associated with gelatinous material. *Asci* (84–) 92.5–135 × 17–23 µm (av. 107.9 × 20.1 µm, *n* = 32), fissitunicate, clavate, short-stalked (10–30 µm long), with 8 biseriate ascospores. *Ascospores* 36.5–49(–57) × 7–10 µm (av. 43.8 × 8.4 µm, *n* = 111), L/W 4.5–5.8 (av. 5.2, *n* = 111), narrowly fusiform, slightly curved, with a submedian primary septum (0.49–0.53; av. 0.51, *n* = 106), constricted at the primary septum, 1(–3)-septate, hyaline to pale olive-brown, with a sheath up to 12 µm wide. At germination ascospores become 3- to 5-septate and produce germ tubes from both end cells.

*Culture characteristics*: Colonies on PDA attaining 1.9–2 cm diam, velvety in appearance, dark green (28F8), with whitish entire margin of 2 mm; reverse raw-sienna (6D7); mellon (5A6) pigment produced. On RSA, a *Tetraploa*-like anamorph with 3 to 8 setose appendages and a teleomorph are observed. *Conidiophores* absent. *Conidiogenous cells* monoblastic. *Conidia* 43–100(–125) µm diam (av. 71.2 µm, *n* = 58), globose to subglobose, brown, almost smooth, verrucose at the base. Appendages 92–200(–235) µm long (av. 147.6 µm, *n* = 56), 7–10 µm wide at the base, 2–3.5 µm wide at the apex, with 4–10 septa at 15 to 28 µm intervals. The teleomorph is similar to that found on the host, but the asci and ascospores in culture are slightly larger. Asci 120–155 × 17.5–23 µm (av. 135.4 × 20 µm, *n* = 56). *Ascospores* 39–54(–57) × 8.5–10.5 µm (av. 47.7 × 9.6 µm, *n* = 70), L/W 4.3–5.7 (av. 5.0, *n* = 70), with a submedian primary septum (0.50–0.53; av. 0.52 *n* = 69), 1–3-septate.

*Specimens examined*: **Japan,** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al*., HHUF 29399 **holotype** designated here, living culture *KTC 1616* (= JCM 13175 = MAFF 239685); Tochigi, Kanuma, Simosawa (139°42.2'E, 36°34.4'N), on culms of *Phyllostachys bambusoides*, 20 Mar. 2003, N. Asama, HHUF 29392, living culture *KTC 1043* (= JCM 13173 = MAFF 239683); Shizuoka, Syuntou, Nagaizumi, Minami-isshiki, Fuji bamboo garden (138°53.1'N, 35°09.3'N), on culms of *Chimonobambusa marmorea*, 8 Mar. 2004, K. Tanaka & Y. Harada, HHUF 29405, living culture *KTC 1640* (= JCM 13176 = MAFF 239686); Nagasaki, Nagayo, Nagasaki Siebold University (129°52.4'E, 32°48.2'N), on culms of bamboo, 30 May 2004, K. Tanaka & S. Hatakeyama, HHUF 29406, living culture *KTC 1686* (= JCM 13177 = MAFF 239687); Aomori, Souma, Ainai trail, on culms of *Sasa kurilensis*, 29 July 2006, K. Tanaka *et al*., HHUF 30018, living culture *KTC 2124* (= CBS 125425).

*Notes*: This species has a broad host preference within *Bambusoideae* because it has been associated with four bamboo genera in two subtribes; *Arundinariinae* (*Pleioblastus* and *Sasa*) and *Shibataeinae* (*Chimonobambusa* and *Phyllostachys*). As discussed later, two distinct clades, KT1043+1640 and KT1616+2124, were found for this species in the tree. Ascomata in these specimens are "almost superficial without associated pigmentation" and "immersed to erumpent with reddish pigments", respectively. Possibly, they may reflect the differences between the bamboo hosts, *Arundinariinae* and *Shibataeinae*. Additional material will be helpful to evaluate the taxonomic significance of these variations.

***Pseudotetraploa*** Kaz. Tanaka & K. Hiray., **gen. nov.** MycoBank MB515257.

*Teleomorph*: Unknown.

*Etymology*: In reference to the *Tetraploa*-like conidial morphology.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia obpyriformes vel anguste obpyriformes, brunnea vel atro brunnea, cum 4 (raro 6 vel 8) appendicibus.

*Mycelium* superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* composed of 4 to 8 columns, obpyriform to long obpyriform, brown to dark brown, almost smooth, verrucose at the base, pseudoseptate, with setose appendages at the apical part. Appendages mostly 4, rarely 6 to 8, curved or straight.

*Type species*: *Pseudotetraploa curviappendiculata* (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., comb. nov.

*Notes*: An anamorphic genus *Pseudotetraploa* is established for species with conidia similar to those of *Tetraploa*. The conidial body of *Pseudotetraploa* is obpyriform to long obpyriform rather than short cylindrical, and has pseudosepta rather than eusepta. In general, setose appendages of *Pseudotetraploa* are short and


**Fig. 11.** *Polyplosphaeria fusca*. A. Ascomata on host surface; B–C. Ascomata in longitudinal section; D–G. Ascospores; H. Ascospore in India ink; I. Germinating ascospore; J–K. Asci; L. Ascomal wall; M. Pseudoparaphyses; N. Conidia on rice straw agar; O–P. Conidia; Q. Conidial body with peel-like wall; R. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 1 000 µm; B, N = 200 µm; C–F, M = 10 µm; G–H, J–L, Q = 20 µm; I, O–P = 50 µm; R = 1 cm. A–C, H–J, L from HHUF 29399 holotype; D, N, P, R from culture KT 1616; E from HHUF 29405; F–G, K, M from HHUF 30018; O from culture KT 1043; Q from culture KT 2124.

curved, as compared with those of *Tetraploa* (long and straight). There are several hyphomycetes with conidia resembling those of *Pseudotetraploa*, such as *Ceratosporella* (Kuthubutheen & Nawawi 1991), *Paratetraploa* (Wong *et al*. 2002), *Triposporium* (Rifai 1972), and *Tretospeira* (Pirozynski 1972, Ho *et al*. 2000), but they have macro- or semimacronematous conidiophores. *Kodonospora* (Ando 1993) shares some features with *Pseudotetraploa*, but this genus does not have well-developed appendages. The following three species previously described as *Tetraploa* (Hatakeyama *et al*. 2005) are transferred to *Pseudotetraploa*.


**Fig. 12.** *Pseudotetraploa* spp. A–E. *P. curviappendiculata*; F–J. *P. longissima*; K–O. *P. javanica*; A, F, K. Conidia; B, G, L. Conidial bodies; C, H, M. Weakly breached conidia; D, I, N. Strongly breached conidia (D, N. with four columns, I. with six columns); E, J, O. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A, F, K = 50 µm; B–D, G–I, L–N = 20 µm; E, J, O = 1 cm. A–D from HHUF 28582 holotype; E from culture HC 4930; F–I from HHUF 28580 holotype; J form culture HC 4933; K–N from HHUF 28596; O from culture HC 4934.

***Pseudotetraploa curviappendiculata*** (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515268. Fig. 12A–E.

*Basionym*: *Tetraploa curviappendiculata* Sat. Hatak., Kaz. Tanaka & Y. Harada, Mycoscience 46: 196. 2005.

*Specimens examined*: **Japan,** Aomori, Hirosaki, Mt. Kudoji (140°25'E, 40°31'N), on culms of *Sasa kurilensis*, 9 May 2003, Y. Harada, HHUF 28582 **holotype**, living culture *HC 4930* (= JCM 12852 = MAFF 239495); Aomori, Hirosaki, Matsukitai (140°29'E, 40°33'N), on culms of *Sasa kurilensis*, 7 Dec. 2003, K. Tanaka & N. Asama, HHUF 28590, living culture *HC 4932* (= MAFF 239496); Hokkaido, Isl. Rishiri, Shinrin-park, on culms of *Sasa kurilensis*, 25 July 2008, K. Tanaka & K. Hirayama, HHUF 30019, living culture *KTC 2558* (= CBS 125426 = NBRC 106241).

***Pseudotetraploa longissima*** (Sat. Hatak., Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515270. Fig. 12F–J.

*Basionym*: *Tetraploa longissima* Sat. Hatak., Kaz. Tanaka & Y. Harada, Mycoscience 46: 198. 2005.

*Specimen examined*: **Japan.** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al.*, HHUF 28580 **holotype**, living culture *HC 4933* (= JCM 12853 = MAFF 239497).

***Pseudotetraploa javanica*** (Rifai, Zainuddin & Cholil) Kaz. Tanaka & K. Hiray., **comb. nov.** MycoBank MB515269. Fig. 12K–O.

*Basionym*: *Tetraploa javanica* Rifai, Zainuddin & Cholil, Reinwardtia 10: 420. 1988.

*Specimen examined*: **Japan,** Aomori, Sannohe, Gonohe, Asamizu (141°18.0'E, 40°28.1'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al.*, HHUF 28596, living culture *HC 4934* (= JCM 12854 = MAFF 239498).

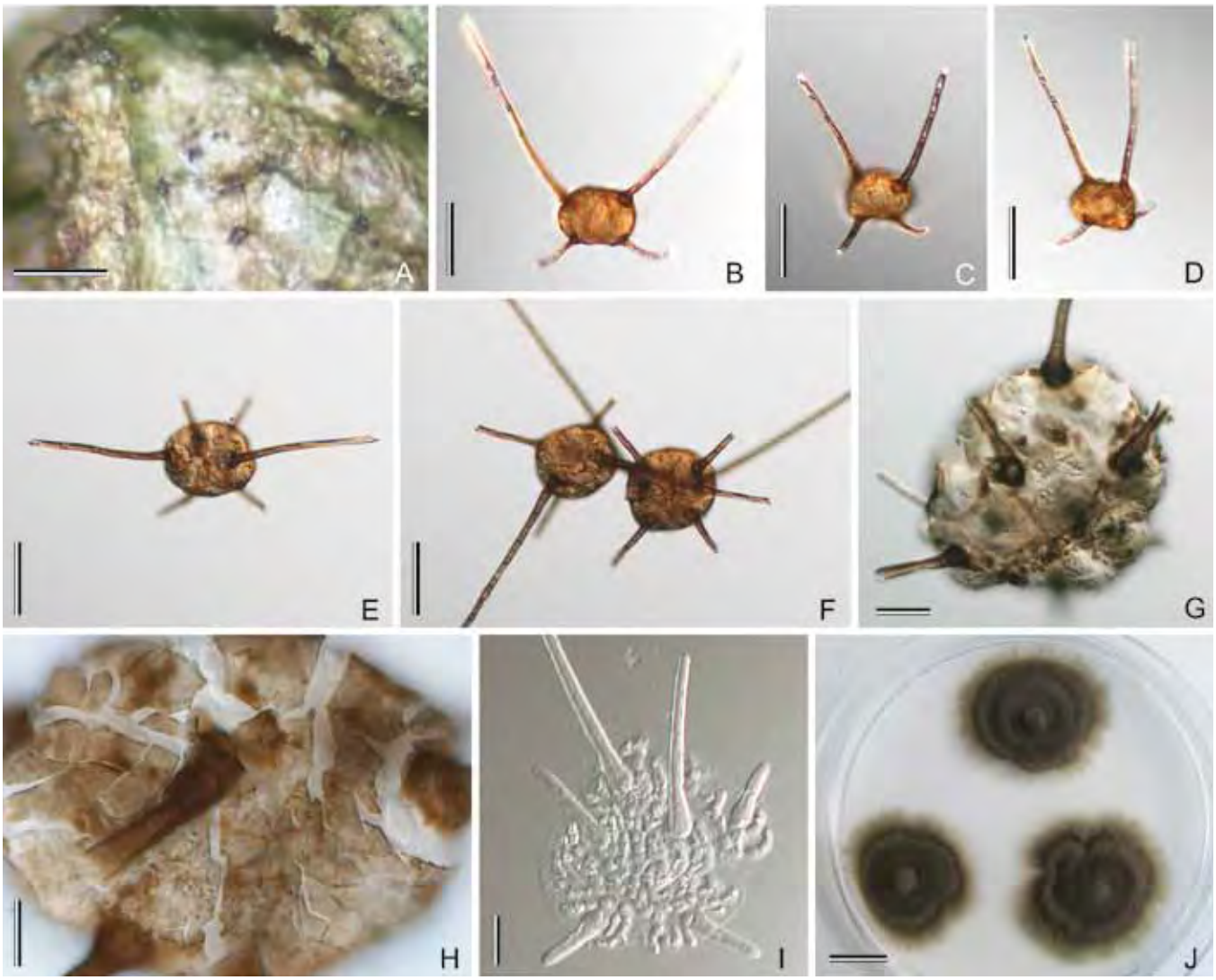


**Fig. 13.** *Quadricrura bicornis*. A. Conidia on host surface; B–F. Conidia; G. Base of conidium with warty surface; H. Conidial body with peel-like wall; I. Breached conidium with internal hyphal structure; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A = 200 µm; B–H = 10 µm; I = 20 µm; J = 1 cm. A–F, H–I from HHUF 30023 holotype; G, J from culture (yone 153).

***Quadricrura*** Kaz. Tanaka, K. Hiray. & Sat. Hatak., **gen. nov.** MycoBank MB515258.

*Teleomorph*: Unknown.

*Etymology*: From Latin *quadri* meaning four and *crura* meaning leg, in reference to the conidial morphology with four leg-like short appendages.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia globosa vel subglobosa, brunnea vel atro brunnea, cum 1 vel 2 longiappendicibus et 4 vel 5 breviappendicibus.

*Mycelium* superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* globose to subglobose, with thin peel on the outer wall of conidia, composed of numerous internal hyphae at the inside, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages of two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendages usually single or 2, arising from apical part of conidia; short appendages mostly 4 to 5, arising from basal side part of conidia.

*Type species*: *Quadricrura septentrionalis* Kaz. Tanaka, K. Hiray. & Sat. Hatak.

*Notes*: This new genus is characterised by globose to subglobose conidia that are composed of internal hyphae and thin peel-like outer wall similar to the *Polyplosphaeria* anamorph. The presence of internal hyphae in conidia is known in the genus *Piricaudilium* (Holubová-Jechová 1988). Likewise, the peel-like outer wall of conidia is found in the genus *Megacapitula* (Chen & Tzean 1993). *Quadricrura*, however, differs from these genera in the morphology of setose appendages of conidia; one or two long appendages arising from the apical part and mostly four to five short appendages around the basal sides. *Bioconiosporium* (Ellis 1976, Narayan & Kamal 1986) and *Pseudopetrakia* (Ellis 1976) have conidia resembling those of *Quadricrura* to some degree, but produce setose appendages only on the apex of muriform conidia.

***Quadricrura bicornis*** Kaz. Tanaka, K. Hiray. & H. Yonez., **sp. nov.** MycoBank MB515271. Fig. 13.

*Etymology*: From Latin *bi* meaning two and *cornis* meaning horned, referring to the two long setose appendages of conidia.

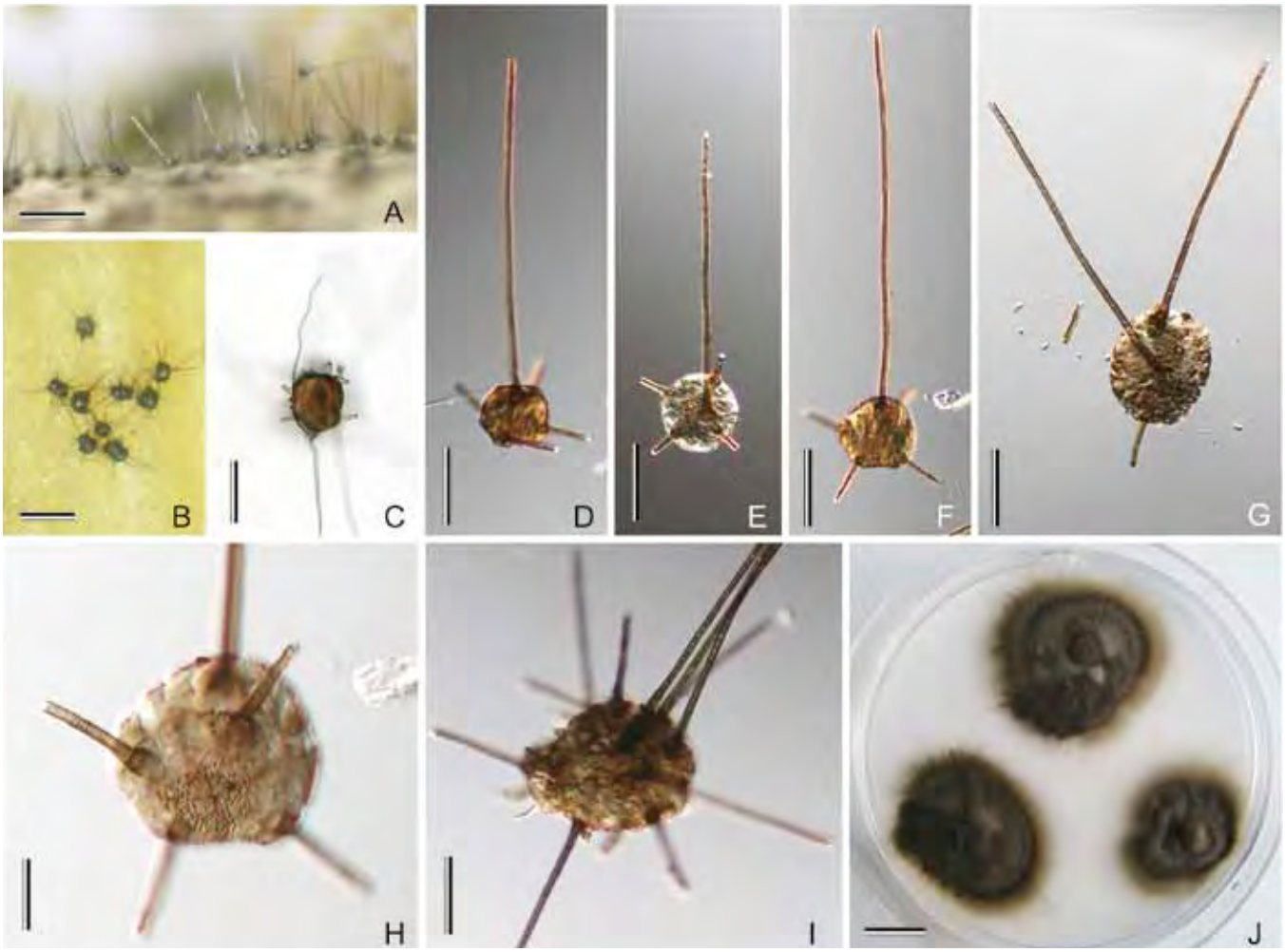


**Fig. 14.** *Quadricrura meridionalis*. A. Conidia on host surface; B. Conidia on rice straw agar; C. Germinating conidium; D–G. Conidia; H. Base of conidium with warty surface; I. Conidial body; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–G, I = 50 µm; H = 20 µm; J = 1 cm. A, C–H from HHUF 30024 holotype; B, I–J from culture KT 2607.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 32.5–60 × 40–65 µm, subglobosa, brunnea vel atro brunnea, cum appendicibus; longiappendices 2, 65–175(–200) µm longae, 10–13-septatae; breviappendices 4, 17.5–45.5 µm longae, 0–2-septatae.

*Mycelium* superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* 32.5–60 × 40–65 µm (av. 40.6 × 48.8 µm, *n* = 32), subglobose, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages of two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendages 2, 65–175(–200) µm long (av. 130.6 µm, *n* = 36), 10–13 µm wide at the base, 4–5 µm wide at the apex, 4–8-septate, arising from apical part of conidia; short appendages usually 4, 17.5–45.5 µm long (av. 30.6 µm, *n* = 39), 7–11.5 µm wide at the base, 4–5 µm wide at the apex, 0–2-septate, arising excentric from the conidial base.

*Culture characteristics*: The conidial state in culture conidition is similar to that on the host, but the conidia are slightly larger (50–77.5 × 60–80 µm).

*Specimens examined*: **Japan,** Aomori, Shirakami, Chisan-dam, on leaf litter of a conifer, 21 July 2007, H. Yonezawa & K. Tanaka, HHUF 30023 **holotype** designated here, living culture *yone 153* (= CBS 125427); Aomori, Shirakami, Chisan-dam, on culms of *Sasa kurilensis*, 21 July 2007, H. Yonezawa & K. Tanaka, yone154 = HHUF 30035.

*Notes*: One of the most striking features of *Q. bicornis* is the presence of two pairs of long appendages at the conidial apex. The holotype of this fungus was collected from leaf litter of a conifer, but it is uncertain whether the conifer is a natural host of *Q. bicornis*. An additional specimen of this fungus on *Sasa kurilensis* (HHUF 30035) was also identified as *Q. bicornis* based on morphology, although there is no isolate and molecular evidence from this specimen. These two specimens were collected from the same locality, and the holotype was found around the base of a thicket of *Sasa kurilensis*.

***Quadricrura meridionalis*** Kaz. Tanaka & K. Hiray., **sp. nov.** MycoBank MB515273. Fig. 14.

*Etymology*: In reference to the southern distribution of the taxon.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 36–43.5(–56.5) × 41–75 µm, subglobosa, brunnea vel atro brunnea, cum appendicibus; longiappendices 1 vel 2, 170–295 µm longae, 10–16-septatae; breviappendices 4 vel 5, 15–37.5 µm longae, 0–2-septatae.

*Mycelium* superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable from creeping hyphae. *Conidia* 36–43.5(–56.5) × 41–75 µm (av. 48.8 × 57.5 µm, *n* = 22), subglobose, solitary, brown to dark brown, verrucose at the base, with setose

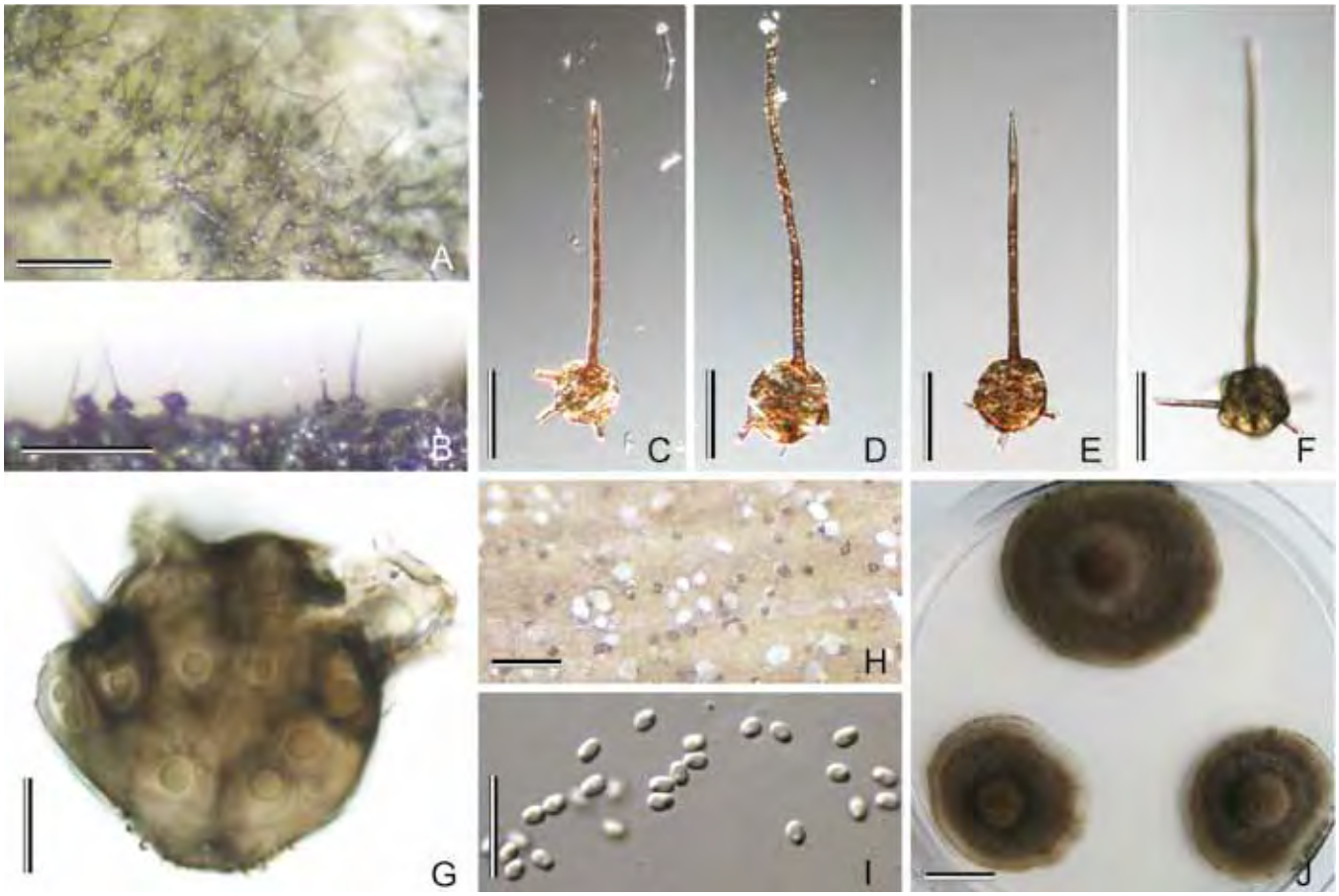


**Fig. 15.** *Quadricrura septentrionalis*. A. Conidia on host surface; B. Conidia on agar piece immersed in water; C–F. Conidia; G. Conidial body composed of internal hyphoid structure; H. Spermogonia on rice straw agar; I. Spermatia; J. Colonies on PDA after 45 d at 25 °C in the dark. Scale bars: A–B = 200 µm; C–F = 50 µm; G, I = 10 µm; H = 500 µm; J = 1 cm. A, C from HHUF 30021; B, F–J from culture HC 4984; D–E from HHUF 28782 holotype.

appendages. Appendages of two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendages usually single, rarely 2, 170–295 µm long (av. 236.3 µm, *n* = 15), 10–12 µm wide at the base, 3–4 µm wide at the apex, with 10 to 16 septa at 7.5 to 30 µm intervals, arising from the apical part of conidia; short appendages usually 4, rarely 5, 15–37.5 µm long (av. 24.9 µm, *n* = 27), 6–7 µm wide at the base, 3–4 µm wide at the apex, 0–2-septate, arising excentric from the conidial base.

*Culture characteristics*: On RSA, sporulation is observed on the surface of rice straw, but the conidial morphology is considerably different as compared with those on the host. The conidial body is larger, measuring 90–100 × 95–112 µm, and with 3–6 long appendages.

*Specimen examined*: **Japan,** Okinawa, Isl. Yonaguni, Irinda trail, on culms of bamboo, 23 Nov. 2008, K. Tanaka & K. Hirayama, HHUF 30024 **holotype** designated here, living culture *KTC 2607* (NBRC 106242 = CBS 125684).

*Note*: It bears a slight resemblance to *Q. septentrionalis*, but can be separated on the basis of larger and subglobose conidia (av. 48.8 × 57.5 µm vs. 37.4 µm diam).

***Quadricrura septentrionalis*** Kaz. Tanaka, K. Hiray. & Sat. Hatak., **sp. nov.** MycoBank MB515272. Fig. 15.

*Etymology*: In reference to the northern distribution of the taxon.

Mycelia superficialia. Conidiophora absentia. Cellulae conidiogenae monoblasticae. Conidia 30–45(–52.5) µm, globosa, brunnea vel atro brunnea, cum appendicibus; longiappendices unica, 115–210 µm longae, 6–12-septatae; breviappendices 4, 10–20 µm longae, 0–1-septatae.

*Mycelium* superficial. *Conidiophores* absent. *Conidiogenous cells* monoblastic, indistinguishable form creeping hyphae. *Conidia* 30–45(–52.5) µm (av. 37.4 µm, *n* = 50) diam, globose, solitary, brown to dark brown, verrucose at the base, with setose appendages. Appendages two forms, unbranched, smooth, brown at the base and almost hyaline at the apex: long appendage single, 115–210 µm long (av. 159.7 µm, *n* = 50), 5–6 µm wide at the base, 3–4 µm wide at the apex, 6–12-septate, arising from apical part of conidia; short appendages usually 4, 10–20 µm long (av. 14.7 µm, *n* = 50), 3–4 µm wide at the base and apex, 0–1-septate, arising excentric from the conidial base.

*Culture characteristics*: Colonies on PDA attaining a diam of 2.9–3.2 cm, velvety in appearance, metal-grey (5E2) with 2 mm whitish entire margin; reverse clay (5D5); no pigment produced. On RSA, an anamorphic state is formed on the surface of rice straw. *Conidia* from culture are similar to those on natural specimen, but conidial body is slightly smaller (25–42.5 µm diam) and long appendage is longer (135–240 µm). In the culture HC 4984, a spermatial state is also produced; *Spermogonia* 80–150 µm, globose, black; *Spermatia* 2–2.5 × 1.5 µm, subglobose, hyaline.

*Specimens examined*: **Japan,** Aomori, Hirosaki, Serisawa-park, on culms of *Sasa kurilensis*, 3 May 2003, K. Tanaka & N. Asama, HHUF 28782 **holotype** designated

here, living culture *HC 4984* (= CBS 125430); Aomori, Shimokita, Hotokegaura, on culms of *S. kurilensis*, 20 Oct. 2002, N. Asama, HHUF 30020, living culture *KTC 920* (= CBS 125428); Aomori, Hirosaki, Serisawa-park, on culms of *S. kurilensis*, 7 Dec. 2002, K. Tanaka & N. Asama, HHUF 28781, living culture *HC 4983* (= CBS 125429); Aomori, Zatoishi, Ogamisawa, on culms of *S. kurilensis*, 8 July 2006, K. Tanaka, HHUF 29747, living culture *yone 44 = HC 5254* (= CBS 125431); Hokkaido, Isl. Rishiri, Kutsugata tail, on culms of *S. kurilensis*, 28 July 2007, K. Tanaka & G. Sato, HHUF 30021, living culture *yone 176* (= CBS 125432 = NBRC 106243); Hokkaido, Isl. Rishiri, Oniwaki trail, on culms of *S. kurilensis*, 29 July 2007, K. Tanaka & G. Sato, living culture *yone 179* (= CBS 125433 = NBRC 106244); Aomori, Hirosaki, Serisawa-park, on culms of *S. kurilensis*, 29 Nov. 2003, K. Tanaka & N. Asama, SH 91 = HHUF 28788; Iwate, Nishine, Mt. Iwate, on culms of *S. kurilensis*, 19 Oct. 2003, K. Tanaka, SH 89 = HHUF 28786; Aomori, Hirosaki, Zatoishi, on culms of *S. kurilensis*, 8 Nov. 2003, K. Tanaka & T. Shirouzu, SH 35 = HHUF 28787; Hokkaido, Yoichi, Sawamachi (140º46'E, 43º11'N), on culms of *S. kurilensis*, 6 June 2004, K. Tanaka, SH 193 = HHUF 28790; Hokkaido, Sapporo, Maruyama (141º18.4'E, 43º02.4'N), on culms of *S. kurilensis*, 6 June 2004, K. Tanaka, SH 195 = HHUF 28792; Hokkaido, Sapporo, Botanical garden of Hokkaido Univ. (141º20.4'E, 43º03.4'N), on culms of *Sasamorpha borealis* var. *borealis*, 6 June 2004, K. Tanaka, SH 194 = HHUF 28791; Hokkaido, Notsuke, Bekkai, Notsukefuren park (145º14'E, 43º31'N), on culms of *Sasa niopponica*, 8 Sept. 2003, K. Tanaka & S. Hatakeyama, SH 118 = HHUF 28783; Hokkaido, Akkeshi, Ootakita, Sattebetu-river (144°49.0'E, 43°08.1'N), on bamboo culms, 7 Sept. 2003, K. Tanaka & S. Hatakeyama, SH 88 = HHUF 28784; Hokkaido, Kamikawa, Shintoku, Shinnai, Karikachi mountain pass (142°46.1'E, 43°07.6'N, 644m a.s.l.), on bamboo culms, 9 Sept. 2003, K. Tanaka & S. Hatakeyama, SH 92 = HHUF 28785; Aomori, Towada, Denbouzi (141º16.1'E, 40º34.2'N), on culms of *Pleioblastus chino*, 2 Dec. 2003, K. Tanaka *et al*., SH 87 = HHUF 28789.

*Note*: *Quadricrura septentrionalis* is frequently collected from various bamboos, particularly *Sasa kurilensis*, and might be widely distributed in northern Japan.

## Phylogenetic analyses

*SSU+LSU*: Approximately 990–1 350 bp of SSU and 1 260–1 290 bp of LSU nrDNA sequences were determined for 53 isolates of bamboo fungi. A combined dataset of SSU (893 bp) and LSU (985 bp) sequences were generated after excluding insertions of several species which correspond to positions 471–832 of *Roussoellopsis tosaensis* (GenBank AB524484) and positions 1 247–1 591 of *Neottiosporina paspali* (GenBank EU754073) in the SSU sequences. The combined dataset was aligned with sequences of 39 species belonging to *Dothideomycetes* (mainly *Pleosporales*) obtained from GenBank. *Botryosphaeria dothidea*, *Spencermartinsia viticola* (both belonging to *Botryosphaeriales*) and *Dothidea insculpta* (*Dothideales*) were used as the outgroup taxa. Of the 1 878 characters, 442 (23.5 %) were variable, of which 349 (18.6 %) were parsimony informative. An MP analysis yielded 31 equally most parsimonious trees with a tree length (TL) of 1 503 steps [consistency index (CI), retention index (RI) of 0.403 and 0.777, respectively]. A consensus tree was constructed from the 31 MP trees (Fig. 16). The trees obtained from NJ and Bayesian analysis had a similar topology to that of the MP tree on the whole, although the monophyly of *Triplosphaeria* was rejected in the Bayesian analysis. Bambusicolous fungi represented by 53 isolates comprising 32 species in 14 genera are scattered in nine clades. The new family *Tetraplosphaeriaceae* formed a monophyletic clade moderately or strongly supported by NJBS value (86 %) or Bayesian PP (1.00), but the monophyly was not well supported in MP analysis (54 %). *Tetraplosphaeriaceae* was positioned as a sister group to a clade composed of mainly pleosporalean families, such as *Lophiostomataceae*, *Massarinaceae*, *Phaeosphaeriaceae*, *Pleomassariaceae* and *Pleosporaceae*, but these relationships were not supported in the MP analysis (< 50 %) and not found in the Bayesian analysis. In the NJ analysis, *Tetraplosphaeriaceae* clustered with the *Massarina arundinariae-Testudinaceae* clade.

*ITS+TEF+BT*: From 31 isolates of *Tetraplosphaeriaceae* species including the outgroup taxon (*Massarina arundinariae*), sequences of *ca*. 482–503 bp, 293–333 bp, 570–662 bp were obtained for the ITS, TEF and BT regions. The final alignment of the ITS region after eliminating gaps and ambiguous sites was composed of 459 bp. These included 131 variable sites (28.5 %) and 106 parsimony informative sites (23.1 %). The NJ tree using this alignment rejected the monophyly of *Quadricrura* and *Triplosphaeria*. In this analysis, the other three genera, *Polyplosphaeria*, *Pseudotetraploa* and *Tetraplosphaeria*, were supported with moderate or strong BS values (71–100 %; Fig. 17A). The data matrix of TEF comprised of 281 aligned characters with 157 variable positions (55.9 %) and 141 parsimony-informative positions (50.2 %). Although the NJ tree generated from this dataset indicated that the four genera, *Polyplosphaeria*, *Pseudotetraploa*, *Quadricrura* and *Triplosphaeria*, form monophyletic clades, respectively (79–100 %), *Tetraplosphaeria* was separated into two clades (Fig. 17B). A dataset from BT sequences included 553 sites after truncating both ends and excluding ambiguous regions. Of these, 248 (44.8 %) and 228 (41.2 %) were variable and parsimony informative, respectively. The NJ tree based on this alignment showed five genera each in *Tetraplosphaeriaceae* as monophyletic clades. However, the BS value of *Quadricrura* was relatively low (67 %) and relationships between the genera were poorly resolved from the BT tree alone (Fig. 17C).

In addition to the individual datasets of ITS, TEF and BT, a combined alignment of these regions (1 293 bp) was used for further analyses. The phylogenetic tree obtained from the Bayesian analysis is shown in Fig. 18. It was generally similar to the results form the individual analyses (Fig. 17) in terms of the arrangement of each genus. Other trees generated from MP and NJ analyses had essentially similar topologies, but monophyly of *Tetraplosphaeria* was rejected in the MP tree. Each genus was supported by strong statistical values of more than 96 % BS or 1.00 PP, except for the *Tetraplosphaeria* clade. *Quadricrura* and *Polyplosphaeria* together formed a well-supported single clade (1.00 PP and > 87 % BS), which was a sister group to *Triplosphaeria*, and the relationships of these three genera received strong support (1.00 PP and > 99 % BS). *Pseudotetraploa* was a sister taxon of the *Quadricrura-Polyplosphaeria-Triplosphaeria* clade. *Tetraplosphaeria* occurred at the most basal position in this family.

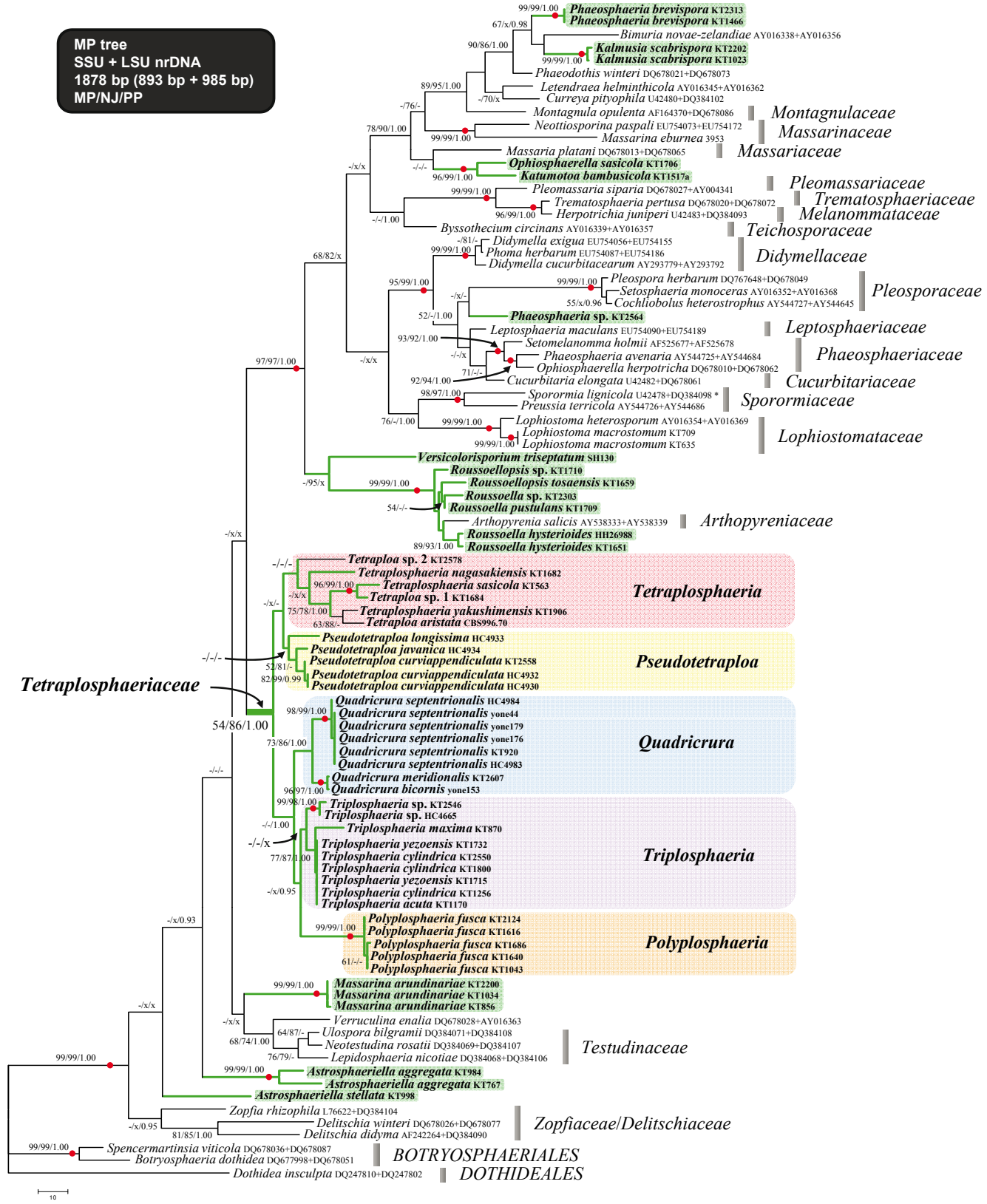


**Fig. 16.** Consensus tree of the 31 equally most parsimonious trees based on a combined dataset of SSU (893 bp) and LSU (985 bp) nrDNA sequences. MP and NJ bootstrap support greater than 50 % and Bayesian posterior probabilities above 0.90 are indicated at the nodes as MPBS/NJBS/PP. Hyphen ("-") indicates a value lower than 50 % (BS) or 0.90 (PP), and a node not present in an analysis is shown with "x". A small red circle is used for a clade with high statistical support (more than 90 % BS and 1.00 PP). The green branches represent lineages of bambusicolous fungi. TL = 1 503, CI = 0.403, RI = 0.777. Either two GenBank numbers (SSU+LSU) or the original isolate numbers are noted after the species names. An asterisk ("*") indicates sequences obtained from two different strains of the same species. The tree was rooted to *Botryosphaeria dothidea*, *Spencermartinsia viticola* and *Dothidea insculpta*. Species of bambusicolous fungi are indicated in **bold**.

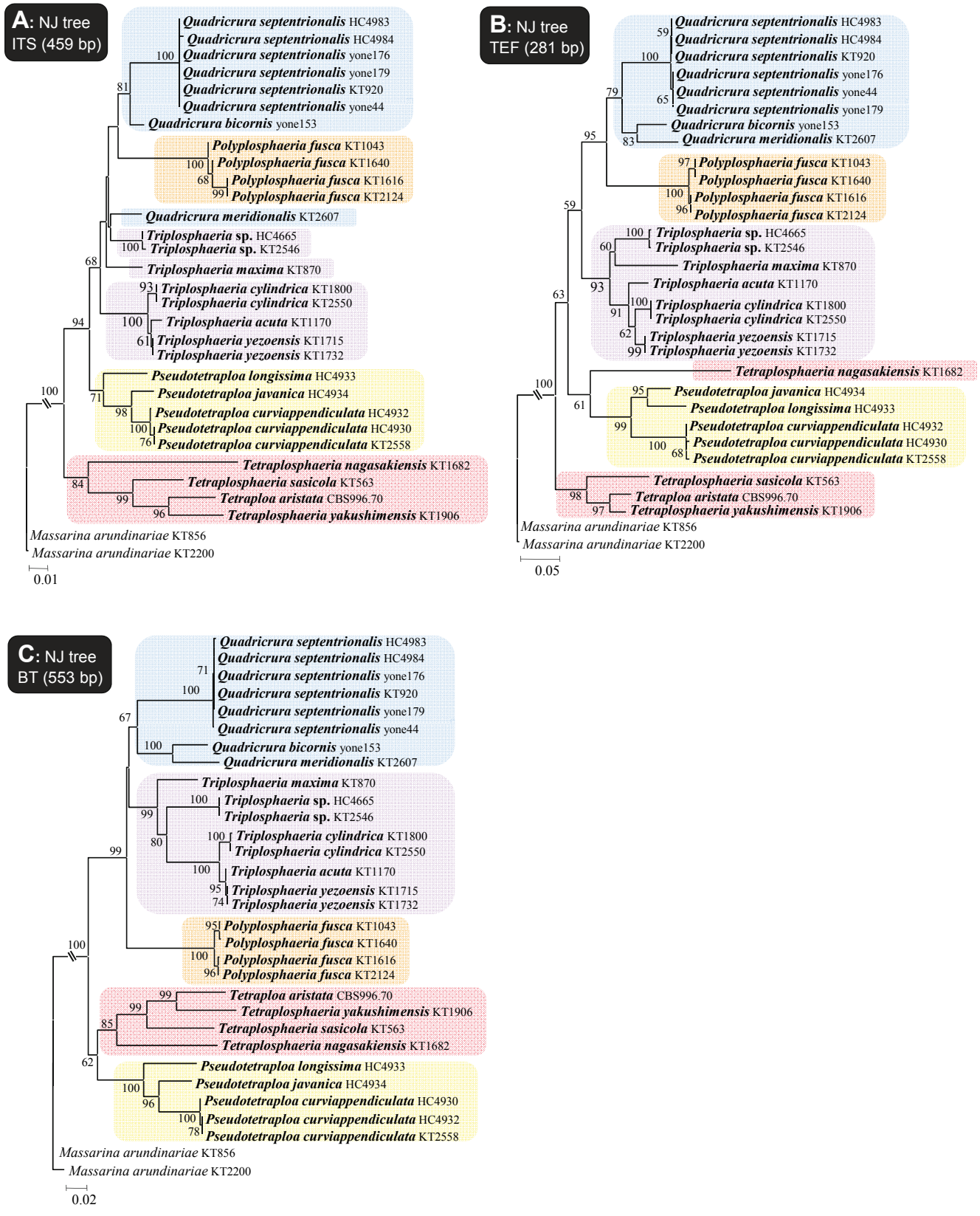


**Fig. 17.** Neighbour-joining trees of the *Tetraplosphaeriaceae* based on the sequences from ITS (A: 459 bp), TEF (B: 281 bp), and BT (C: 553 bp). Bootstrap support greater than 50 % are shown at the nodes. An original isolate number is noted after the species name. The tree is rooted to *Massarina arundinariae*.

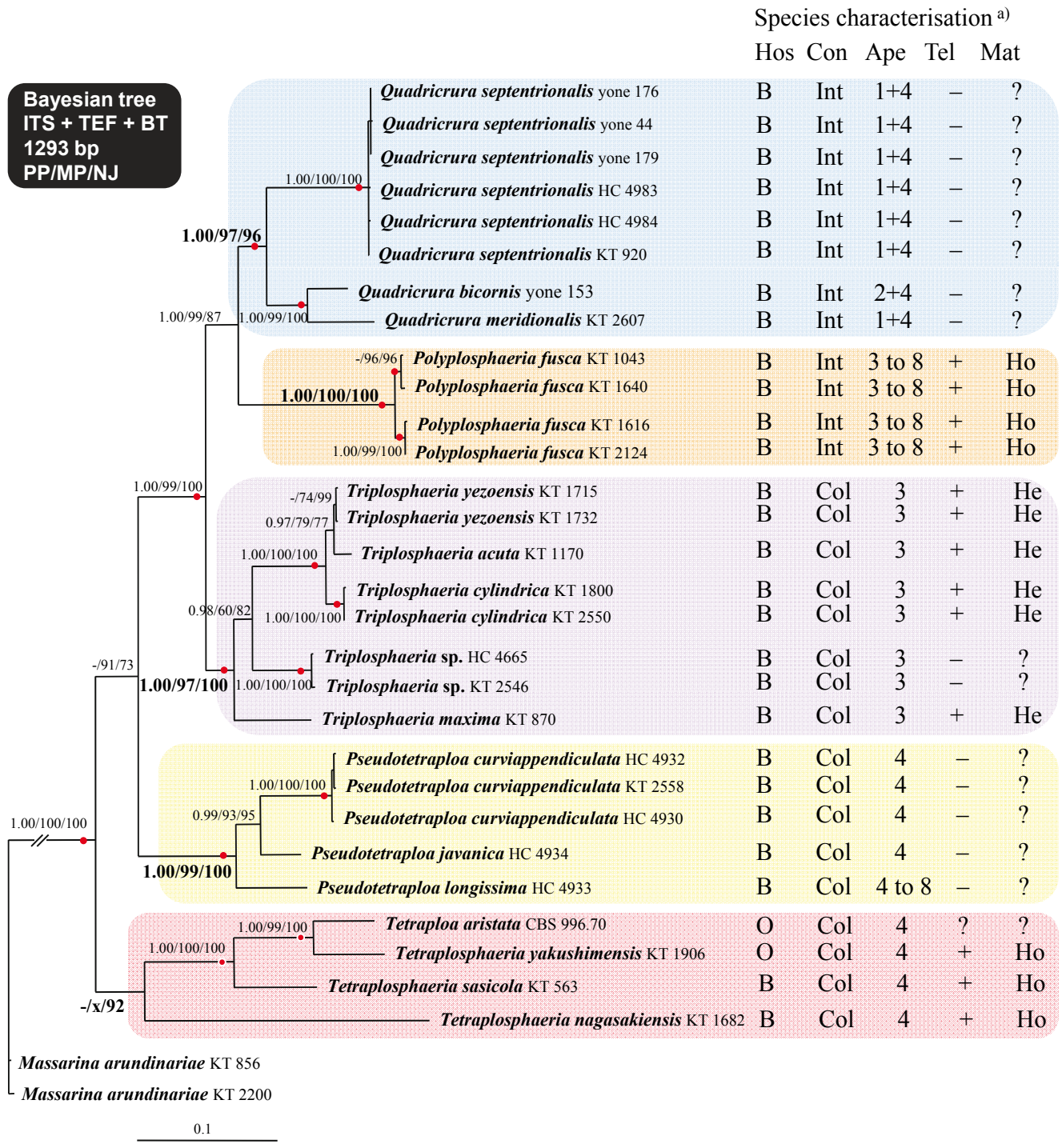


**Fig. 18.** Phylogeny of *Tetraplosphaeriaceae* from Bayesian analysis based on a combined dataset (1 293 bp) of ITS, TEF, and BT. Bayesian posterior probabilities above 0.90 and MP and NJ bootstrap values greater than 50 % are indicated at the nodes as PP/MPBS/NJBS. Hyphen ("-") indicates values lower than 0.90 (PP) or 50 % (BS), and a node not present in an analysis is shown with "x". A small red circle is used for a clade with high statistical support (more than 1.00 PP and 90 % BS). An original isolate number is noted after the species name. The tree was rooted to *Massarina arundinariae*. [a)] Abbreviations for species characterisation: *Hos* = host, B: bamboo, O: other plant; *Con* = conidial structure, int: with internal hyphae, col: with columns; *Ape* = number of conidial appendages, 1+4 or 2+4 indicates number of apical appendages + basal appendages; *Tel* = teleomorph formation, +: present, -: absent, ?: unknown; *Mat* = mating type, Ho: homothallic, He: heterothallic, ?: unknown.

## DISCUSSION

### Phylogenetic position of selected bambusicolous fungi

In this study, phylogenetic analyses of bambusicolous fungi were carried out based on SSU+LSU sequences. Fifty-three isolates from bamboo comprising 32 species in 14 genera were found to cluster in nine clades. Notes on phylogenetic placements of species in the following nine genera except for members in *Tetraplosphaeriaceae* are described below.

*Astrosphaeriella* (Fig. 19A–B): This genus is characterised by the cone-shaped, large ascomata composed of carbonaceous firm peridium, with starlike flanges of ruptured host tissue around the base (Fig. 19A); the numerous trabeculate pseudoparaphyses in gel matrix; the bitunicate cylindrical-clavate asci; and the narrowly fusiform ascospores (Barr 1990, Hyde & Fröhlich 1998, Fröhlich & Hyde 2000). Currently, 47 taxa are accepted in *Astrosphaeriella* (Wang *et al*. 2004, Jagadeesh Ram *et al*. 2005, Tanaka & Harada 2005a, Chen & Huang 2006), and most of them are recorded on bamboo. *Astrosphaeriella* has been provisionally placed in *Melanommataceae*, *Pleosporales* (Lumbsch & Huhndorf 2007), although the molecular phylogeny of this genus has not been revealed to date. The result from our study (Fig. 16) suggests that *Astrosphaeriella* is not a member of *Melanommataceae*, because *Astrosphaeriella stellata*, the type of the genus, deviated from *Herpotrichia juniperi*, a representative species of the *Melanommataceae* (Zhang *et al*. 2008), and was located at the basal position of *Pleosporales*. Monophyly of *Astrosphaeriella* was not supported. Tanaka & Harada (2005a) transferred *Melanopsamma aggregata* (Hino & Katumoto 1955) to *Astrosphaeriella* according to the broad generic concept of *Astrosphaeriella* proposed by Hyde *et al.* (2000) to accept *Massarina*-like species having a slit-like ostiole at the ascomata. However, this classification was not supported by our results, because *A. aggregata* with a slit-like ostiole (Fig. 19B) did not form a clade with *A. stellata* (Fig. 16). Chen & Hsieh (2004) recognised three elements in this genus: 1) typical *Astrosphaeriella* species (*e.g. A. stellata*), 2) *Trematosphaeria*-like species with striate ascospores (*e.g. A. africana*), and 3) *Massarina*-like species with immersed ascomata (*e.g. A. bakeriana*); they proposed a strict generic concept excluding *Massarina*-like species. The phylogeny obtained from our study support their opinion.

*Kalmusia* (Fig. 19C–D): One species of the genus, *Kalmusia scabrispora* (Tanaka *et al.* 2005), was used for phylogenetic analyses. This fungus was originally described as a species of *Leptosphaeria* by Teng (1934) and was later transferred to *Massariosphaeria* by Shoemaker & Babcock (1989). The phylogenetic tree based on the SSU+LSU nrDNA sequences in this study did not accept these two classifications, although we could analyse only SSU sequences for the type species of the latter genus (*M. phaeospora*). It is uncertain whether the species belong to *Kalmusia* from a molecular perspective, because there are no sequence data available for other *Kalmusia* species. The genus *Kalmusia*, typified by *K. ebuli*, has been assigned to the *Montagnulaceae* (Barr 2001), and the clypeate ascomata (Fig. 19C) and asci with a long stipe (Fig. 19D) of *K. scabrispora* fit well in the family.

*Katumotoa* (Fig. 19E–F): The monotypic genus *Katumotoa*, based on *K. bambusicola*, is characterised by apiosporous ascospores provided with bipolar enlarged sheath (Fig. 19E–F). Based on morphological features of the species, such as immersed perithecioid ascomata, thin ascomatal wall composed of small pseudoparenchymatous cells, cellular pseudoparaphyses, and fissitunicate asci, *Katumotoa* has been tentatively assigned to *Phaeosphaeriaceae* (Tanaka & Harada 2005b). However, *Katumotoa* did not group within *Phaeosphaeria*, and formed a clade with O*phiosphaerella sasicola*, another bambusicolous fungus (Figs 16, 19I). This clade was sister to *Massaria platani* (*Massariaceae*) but the affinity of these taxa was insufficiently supported (<50 % BS).

*Massarina* (Fig. 19G–H): Several species in this genus (*e.g. M. alpina*, *M. pustulata*, *M. bambusina*) have been recorded from bamboo (Eriksson & Yue 1998, Tanaka & Harada 2003b), but there is no sequence data for most of them. In this study, *M. arundinariae*, which has been accepted as *Massarina* (Aptroot 1998) but was later transferred to *Lophiostoma* (Hyde *et al.* 2002a), was used for the analyses. All phylogenetic analyses revealed that placement of this taxon in either *Massarina* or *Lophiostoma* was not suitable (Fig. 16). The species grouped with the *Verruculina-Testudinaceae* clade, and they were isolated from a core member of *Pleosporales* in the MP tree or were positioned as a sister group of *Tetraplosphaeriaceae* in the NJ tree. In the analyses of *Pleosporales* using sequences from nrDNA, *TEF1* and *RPB2* in this volume (Zhang *et al.* 2009a), this species is treated as a *Lophiotrema*. Phylogenetic re-evaluation of the generic placement of other *Massarina* species from bamboo would be required, because recent molecular studies on the genus suggest a considerable polyphyly of *Massarina s. l.* (Kodsueb *et al.* 2007, Zhang *et al.* 2009b).

*Ophiosphaerella* (Fig. 19I): *Ophiosphaerella sasicola* deviated from the *Phaeosphaeriaceae* clade including *Ophiosphaerella* or *Phaeosphaeria*, genera that previously accommodated the species (Nagasawa & Otani 1977, Shoemaker & Babcock 1989). The multi-septated scolecospores (Fig. 19I) found in *O. sasicola* might suggest an affinity with species of *Cochliobolus* (*Pleosporaceae*), but this relationship was not supported (Fig. 16). *Ophiosphaerella sasicola* formed a monophyletic clade with *K. bambusicola* supported by strong statistical values (>96 % BS, 1.00 PP; Fig. 16), although there is no morphological similarity between the taxa. Most probably, a new genus should be established to accommodate this species.

*Phaeosphaeria* (Fig. 19J–K): Two species of *Phaeosphaeria* on bamboo, *P. brevispora* and *Phaeosphaeria* sp., were examined in our analyses, but they did not locate to *Phaeosphaeria* or *Phaeosphaeriaceae*. The separation of *P. brevispora* from the *Phaeosphaeria* clade might be due to morphological heterogeneity of this species among the genus, such as gregarious ascomata with clypei and clavate asci with a relatively long stipe (Fig. 19J; Tanaka & Harada 2004). These morphological features of the species are similar to those of *Kalmusia scabrispora* (Fig. 19C–D), although the relationships between the taxa were not supported according to the molecular phylogeny in this study. While *Phaeosphaeria* sp. [Fig. 19K; the same species reported by Tanaka & Harada (2004) as *Phaeosphaeria* sp.] shares several characters with *Phaeosphaeria* on various monocots (Shoemaker & Babcock 1989). This might indicate that fungal species on bamboo are a peculiar lineage and do not belong to existing genera from other host plants, even though they have morphological similarities with the genera. Molecular phylogenetic studies of other *Phaeosphaeria* species described from bamboo (*e.g. P. bambusae*) should be conducted to confirm this expectation.

*Roussoella* (Fig. 19L–M): *Roussoella* is characterised by gregarious, clypeate ascomata, trabeculate pseudoparaphyses embedded in a gel matrix, bitunicate asci without obvious


**Fig. 19.** Selected bambusicolous fungi; A. *Astrosphaeriella stellata* (HHUF 28494); B. *Astrosphaeriella aggregata* (HHUF 28232); C–D. *Kalmusia scabrispora* (HHUF 28608); E–F. *Katumotoa bambusicola* (culture KT 1517a); G–H. *Massarina arundinariae* (HHUF 30014); I. *Ophiosphaerella sasicola* (HHUF 29443); J. *Phaeosphaeria brevispora* (HHUF 30016); K. *Phaeosphaeria* sp. (HHUF 30017); L–M. *Roussoella hysterioides* (L from HHUF 29217; M from culture KT 1651); N–P. *Roussoellopsis tosaensis* (N–O from HHUF 29234; P from culture KT 1659); Q. *Versicolorisporium triseptatum* (HHUF 28815); A–B. Ascomata on host surface; C. Ascomata in longitudinal section; D, G, I–J, N. Asci; E–F, H, K–L, O. Ascospores; M, P–Q. Conidia. Scale bars: A–B = 500 µm; C = 200 µm; D–Q = 20 µm.

fissitunicate dehiscence, and brown, 1-septate ascospores with distinctive wall ornamentation (Fig. 19L; Hyde *et al*. 1999). This genus has traditionally been considered as a member of *Amphisphaeriaceae* (*Xylariales*) because of the misinterpretation of the asci as unitunicate with IKI ± apical rings (Aptroot 1995a), and the presence of heterogenous element in the genus, now treated as *Arecophila* (Hyde 1996). The genus, typified by *R. hysterioides*, is currently placed in *Didymosphaeriaceae* (Ju *et al*. 1996, Lumbsch & Huhndorf 2007), although the validity of this classification has not been assessed in previous phylogenetic studies (Kang *et al.* 1998, Verkley *et al.* 2004). *Roussoella* include more than 11 species (Hyde 1997, Hyde *et al.* 1999, Zhou *et al*. 2003) and most of them are known from bamboo. Four isolates of *Roussoella* used in our analyses did not cluster with members of *Didymosphaeriaceae*, such as *Didymosphaeria futilis* in the LSU tree (data not shown) or *Verruculina enalia*, and formed a strongly supported clade (99 % BS, 1.00 PP) with *Roussoellopsis* and *Arthopyrenia salicis* (Fig. 16). This result might suggest that *Roussoella* belongs to *Arthopyreniaceae*, but this relationship is not fully resolved because of the morphological differences between both taxa. Many of the characters found in *Arthopyreniaceae*, *e.g.* lichenised or non-lichenised nature, hemispherical ascomata with wall sometimes staining green by KOH, cellular pseudoparaphyses, fissitunicate asci, and mostly hyaline ascospores (Eriksson 1981, Cannon & Kirk 2007), are significantly different from those of *Roussoella*. Our results further suggest that *Roussoella* is not a monophyletic genus, but additional evidence would be necessary before taxonomic revisions of the genus can be proposed.

*Roussoellopsis* (Fig. 19N–P): Ascomata of this genus are extremely similar to those found in *Roussoella*, but *Roussoellopsis* species have clavate asci and large-sized (*ca.* 28–66 × 10–17 µm) fusiform ascospores strongly constricted at the submedian septum (Fig. 19N–O; Hino 1961, Hino & Katumoto 1965). All three species in *Roussoellopsis* have been considered to belong to *Astrosphaeriella* or *Roussoella* on the basis of their original descriptions (Aptroot 1995b). However, two isolates of *Roussoellopsis* appeared in the basal lineage of the main families in *Pleosporales* and far away from *Astrosphaeriella* clade in this

study (Fig. 16). The transfer of *Roussoellopsis* to an older genus *Roussoella* appears to be reasonable from the topology, but careful consideration must be given to the treatment. In this study, it was revealed for the first time that *Roussoellopsis tosaensis* has a *Melanconiopsis* or *Neomelanconium*-like anamorph producing annellidic conidiogenous cells, and almost globose, black, 1-celled, thick-walled conidia (*ca*. 21–30 µm diam) surrounded by an entire gelatinous material (Fig. 19P). Differences found in anamorphs between *Roussoellopsis* and *Rousoella* having a *Cytoplea* state (Fig. 19M; Hyde *et al*. 1996) indicate that they are not congeneric.

*Versicolorisporium* (Fig. 19Q): It has been reported that this genus has a phylogenetic relatedness with *Arthopyrenia* based on the similarity of LSU sequences (Hatakeyama *et al*. 2008). In the result from our study using the SSU+LSU dataset, *Versicolorisporium* clustered as a sister taxon with the clade of *Roussoella-Roussoellopsis-Arthopyrenia* (Fig. 16), although these relationships were supported only from the NJ analysis. Besides, the versicolous, 3-septate conidia of *Versicolorisporium* (Fig. 19Q) are quite different from those found in anamorphs of *Roussoella* or *Roussoellopsis*. Phylogenetic inference of this anamorphic genus could not be elucidated at this time, but it is probable that *Versicolorisporium* does not belong to the main existing families in *Pleosporales*.

## Monophyly of *Tetraploa* and *T. aristata*

The anamorphic genus *Tetraploa* is a well-known dematiaceous hyphomycete. *Tetraploa* species mostly occur throughout the year on leaves or stems of monocotyledons including bamboo, and also on various dicotyledons (Ellis 1949). Sixteen taxa have been accepted in the genus until now (Ellis 1949, Sharma 1978, Arambarri *et al*. 1987, Rifai *et al*. 1988, Révay 1993, Matsushima & Matsushima 1996, Hatakeyama *et al*. 2005, Pratibha & Bhat 2008, Zhao *et al*. 2009). There have been no doubt regarding the monophyly of *Tetraploa* characterised by conidia that consist of a main body and four setose appendages and that are formed from a conidiogenous cell indistinguishable from creeping hyphae (Hatakeyama *et al*. 2005). However, our analyses revealed that the genus is composed of at least two lineages, *i.e. Tetraploa s. str.* and *Pseudotetraploa* (Figs 16–18). Several species previously described as *Tetraploa* might have phylogenetic affinities with *Pseudotetraploa* or might represent an additional lineage retaining a close relationship with *Tetraploa*. For example, *T. opacta* most likely belongs to *Pseudotetraploa* based on the original description and illustration of the species (Zhao *et al*. 2009). *Tetraploa abortiva* (Arambarri *et al*. 1987) and *T. setifera* (Révay 1993, Markovskaja 2007) should probably be separated from *Tetraploa s. str.* owing to their unusual features such as conidial body composed of three columns or hyaline appendages. Results from our analyses indicate that the genus *Tetraploa* should be restricted to species with conidial features similar to that of *T. aristata* and *T. ellisii*.

Interestingly, monophyly of *T. aristata,* the type species of the genus (Berkeley & Broome 1850), was also rejected in this study. *Tetraploa aristata*, the most well-known species in this genus, has been considered to have a wide geographical distribution (Ellis 1949). It has been recorded on more than 120 plant species (Farr & Rossman 2009), in particular on senescent culms of *Gramineae* (*e.g. Pennisetum*, *Phragmites*, *Miscanthus*) and *Cyperaceae* (*e.g. Schoenoplectus*) as a major saprophytic fungus (Wong & Hyde 2001). Moreover, there are several reports of the species as "facultative aquatic hyphomycete" (Kirk 1969, Descals & Moralejo 2001) or "terrestrial-aquatic hyphomycete" (Ando 1992, Goh & Hyde 1996), as an air-borne fungus (Sreeramulu & Ramalingam 1962, Tseng & Chen 1982, Green *et al*. 2006), and sometimes as a human pathogen causing keratomycosis or phaeohyphomycotic cysts (Markham *et al*. 1990). Traditionally, *T. aristata* has been believed to be a single species having high ecological diversity. However, the circumscription of *T. aristata* would be problematic because four isolates identified morphologically as *T. aristata* or *Tetraploa* cf. *aristata* (KT 1682, 1684, 1906, and CBS 996.70) showed low sequence similarities with each other (Fig. 16). Probably, this species-complex can likely be separated into several species based on minute morphological differences, *e.g.* dimension and degree of ornamentation of conidial body and length of setose appendages. Therefore, morphological re-assessment of *T. aristata s. l.* (Ellis 1949) based on the type specimen of *T. aristata* (Berkeley & Broome 1850) would be required. Among the 16 species in *Tetraploa*, only one species, *T. aristata*, is known to have a *Massarina* teleomorph of pleosporalean ascomycete (Scheuer 1991), but the identification of this anamorphic state should be re-evaluated in the future.

## Generic placement of ascomycetes having *Tetraploa* anamorphs

Although the teleomorphic fungus of "*T. aristata*" found on *Carex* by Scheuer (1991) has been assigned to the genera *Massarina* (Scheuer 1991, Aptroot 1998) or *Lophiostoma* (Hyde *et al*. 2002a), our analyses revealed that these generic placements are inappropriate. These two genera are placed in *Massarinaceae* and *Lohiostomataceae*, respectively (Lumbsch & Huhndorf 2007). *Massarinaceae* seems to be poorly defined family in view of morphological aspects, but the type species of *Massarina* (*M. eburnea*) has phylogenetic relationships with *Aquaticheirospora* (Kodsueb *et al*. 2007), *Helminthosporium* (Oliver *et al*. 2000), *Saccharicola* (Eriksson & Hawksworth 2003). On the other hand, *Lophiostoma* characterised by the slit-like ostiole of ascomata is a well-defined genus because several taxa including the type species of this genus formed a family *Lophiostomataceae* as a sister group of *Sporormiaceae* (Fig. 16; see also Tanaka & Hosoya 2008). Because six isolates of *Tetraploa s. str.* with or without teleomorphs did not cluster with *Massarinaceae* or *Lophiostomataceae* (Fig. 16), a new genus, *Tetraplosphaeria,* was introduced for this lineage producing *Tetraploa* anamorphs as a common feature. In the protologue of *M. tetraploa* (anam.: *T. aristata*), Scheuer (1991) noted the morphological affinities of this species with several genera, such as *Massarina*, *Keissleriella*, *Lophiostoma*, *Lophiotrema*, and *Massariosphaeria*. All of them belong to a core group of *Pleosporales*, a clade with strong support values (97 % BS, 1.00 PP), ranging from *Phaeosphaeria brevispora* to *Roussoella hysterioides* in Fig. 16. *Tetraplosphaeria* having *Tetraploa* anamorphs *s. str.* formed a single clade with four other genera (*Triplosphaeria*, *Polyplosphaeria*, *Pseudotetraploa* and *Quadricrura*) having *Tetraploa*-like anamorphs, and this new lineage (*Tetraplosphaeriaceae*) deviated from a core group of *Pleosporales*, although it has characteristic features of the order, *i.e. Pleospora*-type centrum (Luttrell 1973). The five genera in *Tetraplosphaeriaceae* are clearly separated based on their anamorphs (Figs 16–18). All these results suggest that morphology of anamorphs is a good predictor of phylogenetic relationships at the familial and genus levels, rather than their teleomorphs. Similar observations about the significance of anamorphic characters have been reported for *Pleosporaceae* in *Dothideomycetes* (Kodsueb *et al*. 2006) and for *Chaetosphaeriaceae* in *Sordariomycetes* (Réblová

2000, 2006, Réblová & Seifert 2007). However, the usefulness of anamorphic morphologies for species identification might be limited as in the case of *Tetraploa aristata s. l*. Similarly, *Triplosphaeria* species have relatively few morphological differences in their anamorphs, but significant differences in their teleomorphs, especially in their ascospores.

## Relationshiphs between genera in *Tetraplosphaeriaceae*

*Tetraplosphaeriaceae* was introduced to accommodate five new genera producing conidia with setose appendages. The monophyly of this family based on the SSU+LSU analyses was supported by NJ (86 % BS) and Bayesian (1.00 PP) trees, but not by the MP tree (54 % BS). Furthermore, the relationships of *Tetraplosphaeriaceae* with other existing families were poorly resolved, since the topologies were incongruent according to the different analyses. Further phylogenetic evidence from an additional dataset, such as sequences from the second largest RNA polymerase II subunit (*RPB2*) would provide useful information to understand the phylogenetic relatedness of the new family among the pleosporalean fungi (Schoch *et al*. 2006, Wang *et al*. 2007). To clarify intergeneric relationships of five genera in *Tetraplosphaeriaceae*, analyses using ITS, TEF, BT, and a combined dataset of these sequences were also conducted in this study. The branching patterns and monophyletic status of the five genera were slightly different according to each individual dataset and the intergeneric relationship could not be resolved in these analyses (Fig. 17A–C), but most likely and reliable phylogenies were obtained from analyses of the combined dataset (ITS+TEF+BT; Fig. 18). The result suggests that *Tetraplosphaeria* with anamorphs *Tetraploa s. str.* is an ancestral lineage within this family. Species in *Tetraplosphaeria* appear to have wide host selectivity, while species in the other four genera derived from this basal genus are restricted to bamboo as their host plants. *Pseudotetraploa* is the second basal lineage in this family and was strongly supported (1.00 PP and >99 % BS). *Pseudotetraploa* species produce conidia resembling those of *Tetraploa* in overall morphology, but conidia are composed of more than four columns with pseudosepta. In this genus, a teleomorph has not been found for any of the known species. *Triplosphaeria* species produce conidia with pseudosepta similar to those of *Pseudotetraploa* but with a reduced number of conidial columns and setose appendages. Most species in *Triplosphaeria* are likely to be heterothallic, because they form ascomata-like structures from single ascospore isolates but mature teleomorphs have never been observed under culture conditions. The monophyly of *Polyplosphaeria* and *Quadricrura*, the most terminal lineages in this family, are also supported by their resemblance in their anamorphs. They have globose conidia composed of internal hyphal structure and more than four setose appendages, unlike the basal three genera having cylindrical conidia with several columns. Probably, the ability of teleomorph formation has been lost at least three times within this family, and anamorphs appear to have contributed greatly to their evolution.

It is interesting that there are several microfossil records of *Tetraploa* from the Palaeocene to the Holocene era (Saxena & Sarkar 1986, Kumaran *et al*. 2001, Antoine *et al*. 2006, Worobiec *et al*. 2009). The oldest record of *Tetraploa*-like fossil from Devonian deposits has been reported as an acritarch genus *Frasnacritetrus* (Taugourdeau 1968), but this is regarded as a contamination by a recent *Tetraploa* (Worobiec *et al*. 2009). On account of the presence of *Tetraploa* fossils from the late Miocene accompanied by pollen grains of a bamboo (*Graminidites bambusoides*) and abundant freshwater phytoplanktons, it has been considered that the *Tetraploa* species could grow on *G. bambusoides*, a presumable origin of bamboo, in swamp forests (Worobiec *et al*. 2009). A more complete fossil of *Tetraploa* as well as the other four genera in *Tetraplosphaeriaceae* would contribute to a better understanding of the evolutionary relationships within this family.

## Outlook for further research

*Tetraplosphaeriaceae* was established for *Massarina*-like ascomycetes with conidial state similar to *Tetraploa*, morphologically most strongly supported by the common character of their anamorphs. Although application of an anamorphic phenotype for fungal classification is currently insufficient, our results suggest that anamorphs are good indicators of phylogenetic relationship at interfamilial or intergeneric levels. There are several anamorphic genera, *e.g. Bioconiosporium* (Ellis 1976, Narayan & Kamal 1986), *Piricauda* (Mercado Sierra *et al*. 2005) and *Piricaudilium* (Holubová-Jechová 1988), having conidia similar to those of *Tetraplosphaeriaceae*. Their morphological resemblance, however, is possibly the result of convergence. The characteristic morphologies of *Tetraploa*, *i.e.* "tetraradiate" or "staurosporous" conidia and conidiogenous cells without conspicuous conidiophores, have been interpreted as a means of adaptation to small amounts of terrestrial water films. Namely, they need to possess water around the appendaged conidium for as long as possible to increase the possibility of germination, and they need to produce their conidia quickly and directly from conidiogenous cells without formation of conidiophores due to limitations of water resources on terrestrial host plants (Bandoni 1972, Ando 1992, Goh & Hyde 1996). There are many examples about the convergent evolution of anamorphic morphology resulting in adaptation to aquatic environments (Belliveau & Bärlocher 2005, Campbell *et al*. 2006, Tsui & Berbee 2006, Tsui *et al*. 2006). Therefore, molecular phylogenetic studies would be required to clarify the affinities between aforementioned dematiaceous hyphomycetes and *Tetraplosphaeriaceae*.

Bamboo is broadly divided into two tribes, *Bambuseae* (woody bamboos) and *Olyreae* (herbaceous bamboos). The former is a major group, which includes 67 genera in nine subtribes (Das *et al*. 2008). In this study, woody bamboos belonging to only four genera in two subtribes, *Arundinariinae* (*Pleioblastus* and *Sasa*) and *Shibataeinae* (*Chimonobambusa* and *Phyllostachys*), were examined as host plants of bambusicolous fungi in Japan. Nevertheless, many novel fungal taxa were obtained from a limited area. It can be expected that there exists much more diverse *Dothideomycetes* on herbaceous bamboos and on the seven other subtribes of woody bamboos. In particular, we believe, a lineage referred to as "Neotropical woody bamboos" should receive more attention for taxonomic investigation of fungi. This bamboo group consisting of three subtribes, *Arthrostylidiinae*, *Chusqueinae* and *Guaduinae*, is distributed in Central and South America (Sungkaew *et al*. 2009), but our knowledge of bambusicolous fungi from these regions is still limited. Even though fundamental taxonomic studies are well advanced on this group, phylogenetic decisions based on molecular evidence would be required because bambusicolous fungi have the tendency to constitute an independent clade, deviating from existing families or genera on other host plants, even though they have morphological similarities with those known fungal groups, as was indicated in this study.

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