

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

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Abstract: Five loci, nucSSU, nuLSU 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 occurred 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 (Cámara *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 hamathelial 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 paraphyses, 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 *Mytiliniaceae* (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*, *Tetraplospira* 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.* cleistothecoid, 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 *fRPB2*-5F and *fRPB2*-7cR primers were used for the amplification of the partial RNA polymerase second largest subunit (*RPB2*) (Liu *et al.* 1999). The *EF1*-Fa and *EF1*-Ra 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 MgCl₂, 0.8 units *Taq* polymerase and 5–10 ng gDNA. The PCR thermal cycle programme for partial LSU nu-rDNA amplification 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 (Katoch *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 jackknife 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 % Jackknife (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*, *Ascochyrella*, *Bipolaris*, *Ceratophoma*, *Coniothyrium*, *Corynespora*, *Curvularia*, *Cytoplea*, *Drechslera*, *Exserohilum*, *Hendersonia*, *Leptophoma*, *Metabotryon*, *Microsphaeropsis*, *Myxocyclus*, *Nigrolentilocus*, *Nimbya*, *Phoma*, *Pithomyces*, *Pleurophomopsis*, *Prosthemium*, *Pseudospiropes*, *Pyrenochaeta*, *Scolecosporella*, *Scolicosporium*, *Shearia*, *Sphaerellopsis*, *Stagonospora*, *Steganosporium*, *Stemphylium* and *Tiarospora* (www.cbs.knaw).

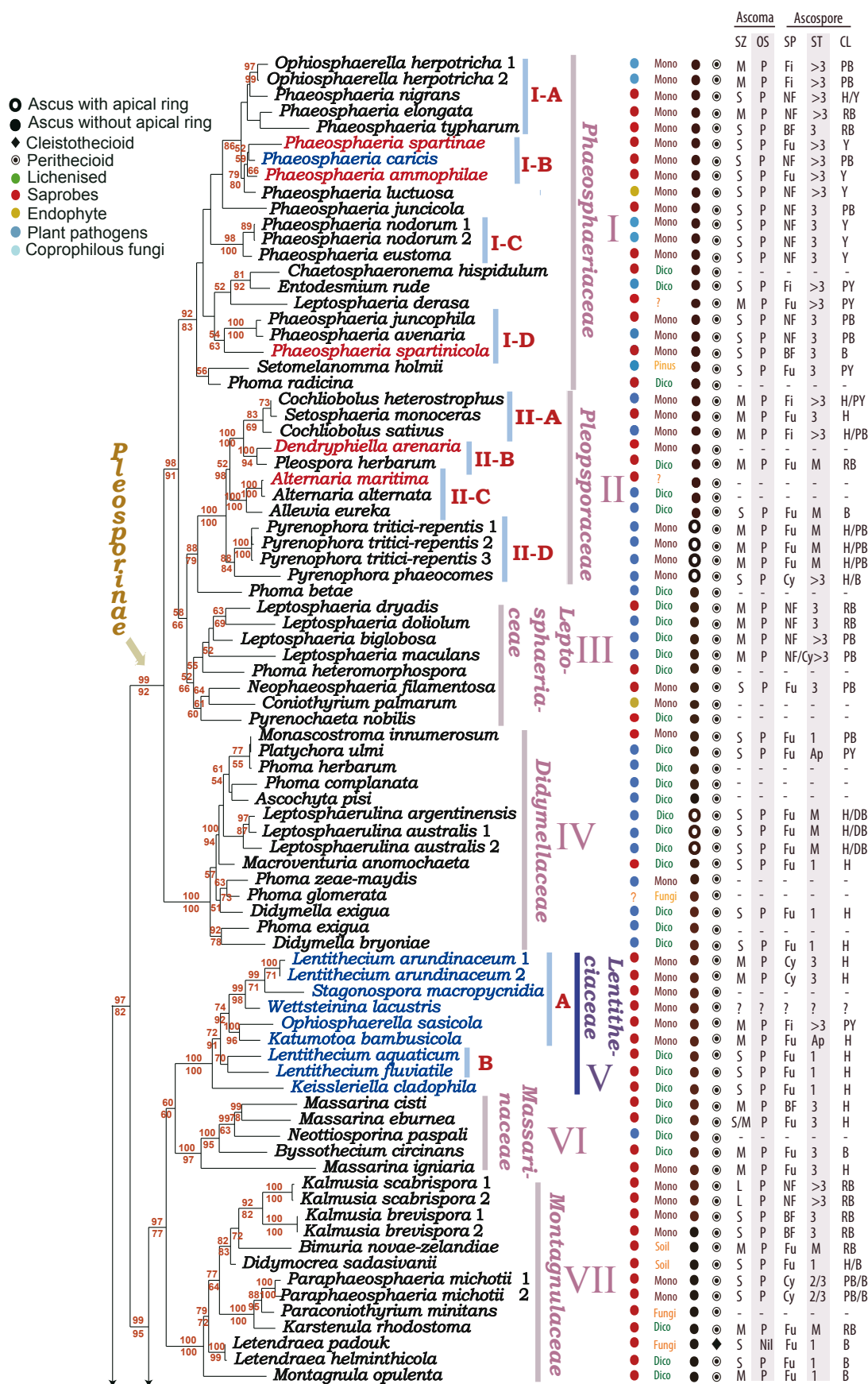


Fig 1. RAxML tree with bootstrap values after 1000 pseudo repetitions on the nodes. The values below the nodes are percentages of 500 jackknife resamplings. Pleosporalean leaves highlighted in red and bold are marine or maritime taxa, in blue and bold are freshwater taxa, and others are terrestrial ones. 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 – slit-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.

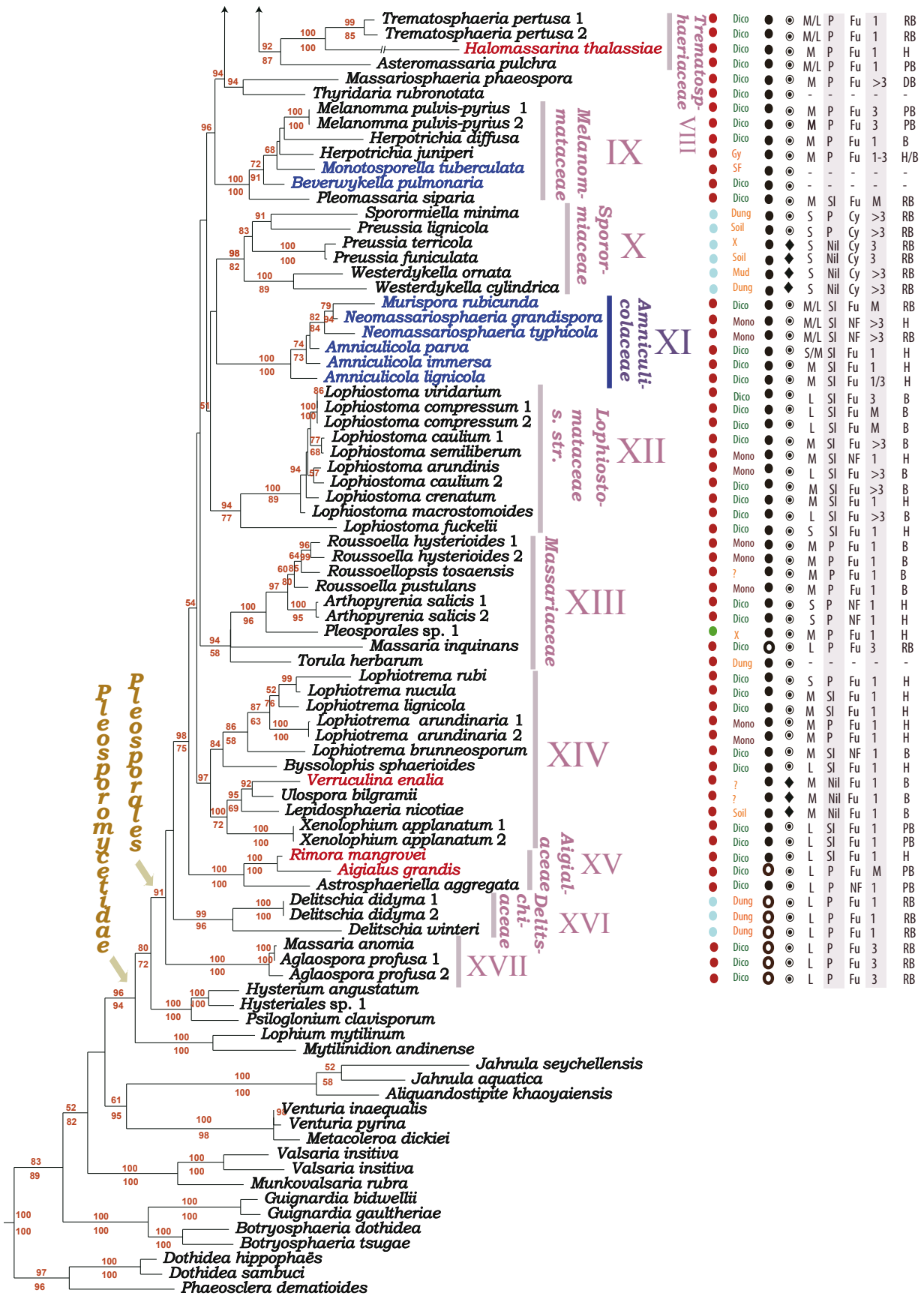


Fig 1. (Continued).

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, Krus *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*, *Ascochyttella*, *Bipolaris*, *Coniothyrium*, *Curvularia*, *Drechslera*, *Exserohilum*, *Leptophoma*, *Metabotryon*, *Nimbya*, *Phoma*, *Pithomyces*, *Scolecosporella*, *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 dera*, *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*, *Eudarlucia*, *Heptameria*, *Leptosphaeria*, *Loculohypoxylon*, *Metameris*, *Microthelia*, *Nodulosphaeria*, *Ophiobolus*, *Paraphaeosphaeria*, *Rhopoglyphus*, *Scirrhodopsis* 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, Leuchtman 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 *Plenodomus* clustered in the *Didymellaceae* and *Leptosphaeriaceae* respectively. Although *Chaetosphaeronema* was associated with *Ophiobolus* (Petraik 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*, *Plectrophomella* 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 deliquescent 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*, *Phaeosphaeriaceae* 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 ascospores (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 ascospores 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. Ascospores immersa, lenticular, solitaria vel disseminata, nigra. Asci bitunicati, fissitunicati, clavati vel oblongati-cylindrici, pedicellati. Ascospores cylindrica vel fusiforme vel filiforme, uniseptatae vel aliquando 3-septatae cum supra-maturae, parce multiseptatae, hyalinae vel fulvum.

Freshwater or terrestrial habitat. Saprobiic. *Ascospores* 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 ascospores 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 ascospores, 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 ascospores 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. Saprobiic. *Ascospores* 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*, *Letendreaa* 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*, ? *Letendreaa*, ? *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 ascospores 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, Kruijs *et al.* 2006). Phylogenetic analysis based on ITS-nLSU rDNA, mtSSU rDNA and β -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* (Kruijs & 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*) (Kruijs & Wedin 2009). Currently, after modifying their concept, three genera, *i.e.* *Sporormia*, *Preussia* and *Westerdykella* are accepted under *Sporormiaceae* (Kruijs & Wedin 2009).

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

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

Currently accepted genera: ? *Sporormia* (including *Sporormiopsis*), *Preussia* (including *Sporormiella* and *Spororminula*) and *Westerdykella* (including *Eremodothis* and *Pycnidiothra*) (Kruijs & 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 ascospores 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. Saprobiticus. Ascospores globosa vel subglobosa vel lenticular, nigra, solitaria, immersa vel partim immersa vel superficialia. Apex productum. Peridium exilis. Trabeculae, hyalinae, gelatina circumdatae. Asci, 8-spore, cylindrico vel clavati, fissitunicati, brevis pedicellati. Ascospores, fusiforme vel peranguste fusiforme, uniseptatae vel multiseptatae vel muriforme, hyalinae vel pallide brunneae vel rubrobrunneae, tunica gelatinosa praeditae. Substratum malvaceo purpureum.

Freshwater habitat. Saprobitic. *Ascospores* 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. *Ascospores* 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. Saprobiticus. Ascospores immersa vel partim immersa vel superficialia. Peridium exilis. Trabeculae, hyalinae, gelatina circumdatae. Asci, 8-spore, clavati vel late clavati, fissitunicati, brevis pedicellati. Ascospores, fusiforme, muriforme, brunneae, tunica gelatinosa praeditae. Substratum malvaceo purpureum.

Freshwater habitat. Saprobitic. *Ascospores* 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. *Ascospores* 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. Ascospores, peranguste fusiforme, multiseptatae, hyalinae vel rufobrunneus, tunica gelatinosa praeditae. Substratum plerumque purpureus.

Aquatic. Saprobiic. 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. Saprobiic. Ascomata perithecioid, medium to large-sized, solitary or scattered, immersed to erumpent or rarely superficial with protruding, compressed papilla and slit-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*, ? *Rousoella*.

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 cleistothecoid 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 cleistothecoid 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 raphithamni* Keissl., Nat. Hist. Juan. Fernandez Easter Lsl. 2: 480. 1927.

= *Mycosphaerella raphithamni* (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, conspicuous 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).

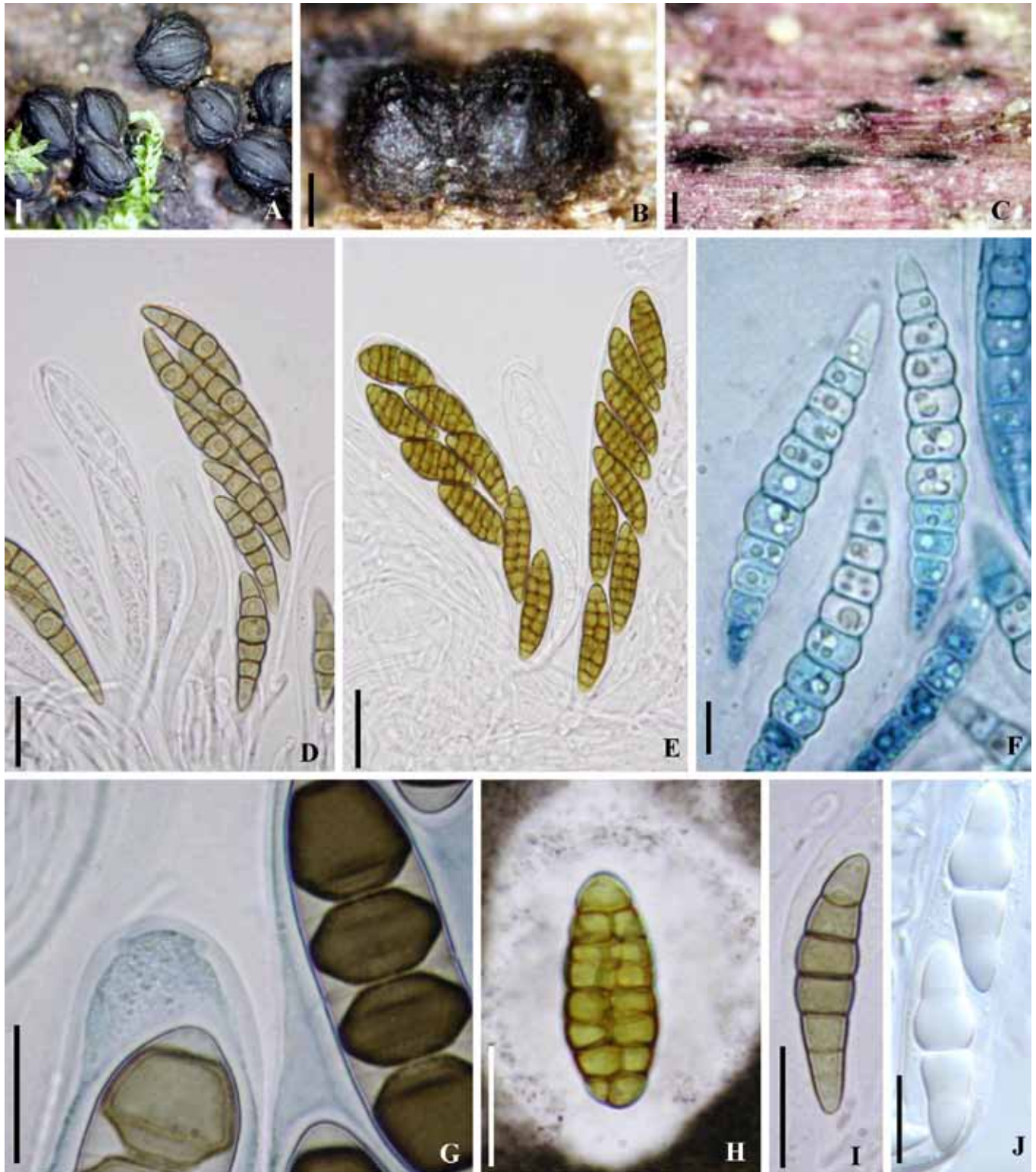


Fig. 2. A. *Xenolophium applanatum*. Ascomata on the host surface. Note the slit-like ostiole. B. *Trematosphaeria pertusa*. Ascomata on the host surface. Note the pore-like ostiole. C, E, H. *Murispora rubicunda*. C. Ascomata on the host surface. Note the purple woody substrate. E. Clavate 8-spored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

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 ascus shape can be of phylogenetic significance to varying degrees. The purple staining nature of the substrate found in some *Ammniculicolaceae* 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 *Ammniculicolaceae* (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*, *Aglaspora profusa* is also an early diverging lineage. Members of both *Delitschiaceae* and *Aglaspora* 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 *Aglaspora*, 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 unsuccessful. 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 interpretation 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
<i>Aigialaceae</i>	<i>Aigialus grandis</i>	JK 5244A	GU296131	GU301793		GU371762	
	<i>Astrosphaeriella aggregata</i>	MAFF 239486	AB524450	AB524591	AF242264	AB539092	AB539105
	<i>Rimora mangrovei</i> (as <i>Lophiostoma mangrovei</i>)	JK 5246A	GU296193	GU301868		GU371759	
<i>Amniculicolaceae</i>	<i>Amniculicola immersa</i>	CBS 123083	GU456295	FJ795498		GU456358	GU456273
	<i>Amniculicola lignicola</i>	CBS 123094	EF493861	EF493863		EF493862	GU456278
	<i>Amniculicola parva</i>	CBS 123092	GU296134	FJ795497			GU349065
	<i>Neomassariosphaeria grandispora</i>	CBS 613.86	GU296172	GU301842	GU357747	GU371725	GU349036
	<i>Neomassariosphaeria typhicola</i>	CBS 123126	GU296174	FJ795504		GU371795	
	<i>Murispora rubicunda</i>	IFRD 2017	GU456308	FJ795507			GU456289
<i>Delitschiaceae</i>	<i>Delitschia didyma</i> 1	UME 31411		DQ384090			
	<i>Delitschia didyma</i> 2 (duplicate)	UME 31411	AF242264	DQ384090		DQ677975	DQ677922
	<i>Delitschia winteri</i>	CBS 225.62	DQ678026	DQ678077		DQ677975	DQ677922
<i>Didymellaceae</i>	<i>Ascochyta pisi</i>	CBS 126.54	DQ678018	DQ678070		DQ677967	DQ677913
	<i>Didymella exigua</i>	CBS 183.55	GU296147		GU357800	GU371764	
	<i>Didymella bryoniae</i>	CBS 133.96		GU456335		GU371767	
	<i>Leptosphaerulina argentinensis</i>	CBS 569.94		AY849947	GU357759		GU349008
	<i>Leptosphaerulina australis</i> 1	CBS 311.51-T		FJ795500		GU456357	GU456272
	<i>Leptosphaerulina australis</i> 2	CBS 317.83	GU296160	GU301830		GU371790	GU349070
	<i>Macroventuria anomochaeta</i>	CBS 525.71	AY787936	GU456315		GU456346	GU456262
	<i>Monascostroma innumerosum</i>	CBS 345.50	GU296179	GU301850			GU349033
	<i>Phoma complanata</i>	CBS 268.92	EU754081	EU754180		GU371778	GU349078
	<i>Phoma exigua</i>	CBS 431.74	EU754084	EU754183		GU371780	GU349080
	<i>Phoma glomerata</i>	CBS 528.66	EU754085	EU754184		GU371781	GU349081
	<i>Phoma herbarum</i>	CBS 276.37	DQ678014	DQ678066	GU357792	DQ677962	DQ677909
	<i>Phoma zaeae-maydis</i>	CBS 588.69	EU754093	EU754192		GU371782	GU349082
	<i>Platychora ulmi</i>	CBS 361.52	EF114726	EF114702			
<i>Lentitheciaceae</i>	<i>Katumotoa bambusicola</i>	JCM 13131, MAFF 239641	AB524454	AB524595		AB539095	AB539108
	<i>Keissleriella cladophila</i>	CBS 104.55	GU296155	GU301822		GU371735	GU349043
	<i>Lentithecium aquaticum</i>	CBS 123099	FJ795477	FJ795434		FJ795455	GU349068
	<i>Lentithecium arundinaceum</i> 1	CBS 123131	GU456298	GU456320			GU456281
	<i>Lentithecium arundinaceum</i> 2	CBS 619.86	DQ813513	DQ813509		FJ795473	
	<i>Lentithecium fluviatile</i> (as <i>Massarina fluviatile</i>)	CBS 122367	FJ795493	FJ795451			GU456290
	<i>Ophiosphaerella sasicola</i>	JCM 13134, MAFF 239644	AB524458	AB524599		AB539098	AB539111
	<i>Stagonospora macropycnidia</i>	OSC 100965	GU296198	GU301873			GU349026
	<i>Wettsteinina lacustris</i>	CBS 618.86	DQ678023			DQ677972	DQ677919
<i>Leptosphaeriaceae</i>	<i>Coniothyrium palmarum</i>	CBS 400.71	DQ678008	DQ676653		DQ677956	DQ677903
	<i>Leptosphaeria biglobosa</i>	CBS 303.51		GU301826			GU349010
	<i>Leptosphaeria doliolum</i>	CBS 505.75	GU296159	FJ795499			GU349069
	<i>Leptosphaeria dryadis</i>	CBS 643.86		GU301828		GU371733	GU349009
	<i>Leptosphaeria maculans</i>	DAOM 229267	DQ470993	DQ470946	DQ471136	DQ470894	DQ471062
	<i>Neophaeosphaeria filamentosa</i>	CBS 102202	GQ387516	GQ387577		GU371773	GU349084

Table 1. (Continued).

Classification	Species name	Culture/voucher ¹	SSU	LSU	RPB1	RPB2	TEF1
	<i>Phoma heteromorphospora</i>	CBS 115.96	EU754089	EU754188		GU371775	GU349077
	<i>Pyrenochaeta nobilis</i>	CBS 407.76		DQ678096		DQ677991	DQ677936
<i>Lophiostomataceae</i> s. str.	<i>Lophiostoma arundinis</i>	CBS 621.86	DQ782383	DQ782384		DQ782386	DQ782387
	<i>Lophiostoma caulium</i> 1	CBS 623.86	FJ795479	FJ795436		FJ795456	
	<i>Lophiostoma caulium</i> 2	CBS 624.86		GU301832			GU349007
	<i>Lophiostoma compressum</i> 1	IFRD 2014	FJ795480	FJ795437		FJ795457	
	<i>Lophiostoma compressum</i> 2	IFRDCC2081	FJ795486	GU456321		GU456349	GU456264
	<i>Lophiostoma crenatum</i>	CBS 629.86	DQ678017	DQ678069		DQ677965	DQ677912
	<i>Lophiostoma fuckelii</i>	CBS 101952	FJ795496	DQ399531		FJ795472	
	<i>Lophiostoma macrostomoides</i>	CBS 123097	FJ795482	FJ795439		FJ795458	GU456277
	<i>Lophiostoma semiliberum</i>	CBS 626.86	FJ795484	FJ795441		FJ795460	
	<i>Lophiostoma viridarium</i>	IFRDCC2090	FJ795486	FJ795443		FJ795468	
<i>Massariaceae</i>	<i>Arthopyrenia salicis</i> 1	CBS 368.94	AY538333	AY538339	GU371814		
	<i>Arthopyrenia salicis</i> 2	1994Coppins		AY607730	AY607742		
	<i>Massaria inquinans</i>	CBS 122369	GU456300	GU456322			GU456282
	<i>Pleosporales</i> sp. 1 (as <i>Thelenella luridella</i>)	CBS 101277	GU456309			GU456361	
	<i>Roussoella hysteroioides</i> 1	JCM 13126, MAFF 239636	AB524480	AB524621		AB539101	AB539114
	<i>Roussoella hysteroioides</i> 2	CBS 125434	AB524481	AB524622		AB539102	AB539115
	<i>Roussoella pustulans</i>	JCM 13127, MAFF 239637	AB524482	AB524623		AB539103	AB539116
	<i>Roussoellopsis tosaensis</i>	NBRC 106245		AB524625		AB539104	AB539117
	<i>Torula herbarum</i>	CBS 379.58				GU456362	
<i>Massarinaceae</i>	<i>Byssothecium circinans</i>	CBS 675.92	AY016339	AY016357		DQ767646	
	<i>Massarina cisti</i>	CBS 266.62	FJ795490	FJ795447		FJ795464	
	<i>Massarina eburnea</i>	CBS 473.64	AF164367	FJ795449	GU357755	FJ795466	GU349040
	<i>Massarina igniaria</i>	CBS 845.96	FJ795494	FJ795452		FJ795469	
	<i>Neottiosporina paspali</i>	CBS 331.37	EU754073	EU754172		GU371779	GU349079
<i>Melanomataceae</i>	<i>Beverwykella pulmonaria</i>	CBS 283.53		GU301804		GU371768	
	<i>Herpotrichia diffusa</i>	CBS 250.62	DQ678019	DQ678071		DQ677968	DQ677915
	<i>Herpotrichia juniperi</i>	CBS 200.31	DQ678029	DQ678080		DQ677978	DQ677925
	<i>Melanomma pulvis-pyrius</i> 1	CBS 109.77	FJ201987	FJ201986		GU456359	GU456274
	<i>Melanomma pulvis-pyrius</i> 2	CBS 124080	GU456302	GU456323		GU456350	GU456265
	<i>Monotosporella tuberculata</i>	CBS 256.84		GU301851			GU349006
	<i>Pleomassaria siparia</i>	CBS 279.74	DQ678027	DQ678078		DQ677976	AY544726
<i>Sporormiaceae</i>	<i>Preussia funiculata</i>	CBS 659.74	GU296187	GU301864		GU371799	GU349032
	<i>Preussia lignicola</i> (as <i>Sporormia lignicola</i>)	CBS 264.69	GU296197	GU301872		GU371765	GU349027
	<i>Preussia terricola</i>	DAOM 230091	AY544726	AY544686	DQ471137	DQ470895	DQ471063
	<i>Sporormiella minima</i>	CBS 524.50	DQ678003	DQ678056		DQ677950	DQ677897
	<i>Westerdykella cylindrica</i>	CBS 454.72	AY016355	AY004343	DQ471168	DQ470925	DQ497610
	<i>Westerdykella ornata</i>	CBS 379.55	GU296208	GU301880		GU371803	GU349021
<i>Montagnulaceae</i>	<i>Bimuria novae-zelandiae</i>	CBS 107.79	AY016338	AY016356	DQ471159	DQ470917	DQ471087
	<i>Didymocrea sadasivanii</i>	CBS 438.65	DQ384066	DQ384103			
	<i>Kalmusia brevispora</i> 1	NBRC 106240	AB524459	AB524600		AB539100	AB539113
	<i>Kalmusia brevispora</i> 2	MAFF 239276	AB524460	AB524601		AB539099	AB539112
	<i>Kalmusia scabriscpora</i> 1	NBRC 106237	AB524453	AB524594		AB539094	AB539107

Table 1. (Continued).

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

Table 1. (Continued).

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

Table 1. (Continued).

Classification	Species name	Culture/voucher¹	SSU	LSU	RPB1	RPB2	TEF1
<i>Venturiaceae</i> (outgroup)	<i>Venturia pyrina</i>	ATCC 38995		EF114714			
	<i>Venturia inaequalis</i>	CBS 476.61		GU456336			GU456288
	<i>Metacoleroa dickei</i>	medipc		EF114695			
<i>Arthoniomycetes</i> (outgroup)	<i>Opegrapha dolomitica</i>	DUKE 0047528	DQ883706		DQ883717	DQ883714	DQ883732
	<i>Opegrapha varia</i>	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, Kunming, 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.