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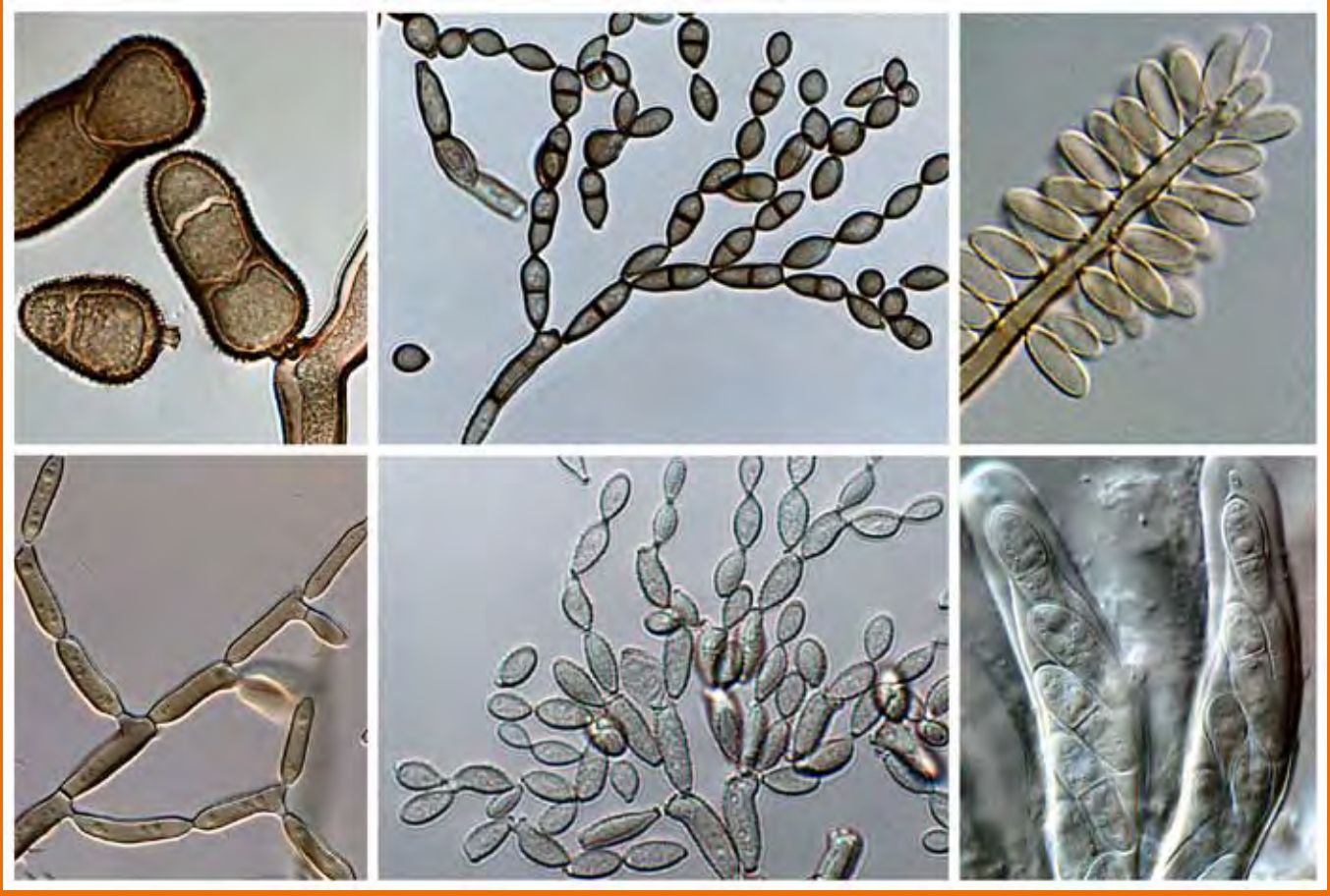
The genus *Cladosporium* and similar dematiaceous hyphomycetes

Pedro W. Crous, Uwe Braun, Konstanze Schubert
and Johannes Z. Groenewald



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Cover: Top: page 137, Fig. 30C *Cladosporium pseudiridis* (CBS 116463), conidiophores and conidia; page 40, Fig. 4D *Toxicocladosporium irritans* (type material), macroconidiophores; page 70, Fig. 11C *Ramichloridium musae* (CBS 365.36), sympodially proliferating conidiogenous cells, resulting in a long conidium-bearing rachis; Bottom: page 19, Fig. 8F *Penidiella columbiana* (type material), conidiophores with chains of disarticulating conidia; page 120, Fig. 12C *Cladosporium bruhnei* (CPC 12211), conidial chains; page 126, Fig. 18L *Davidiella tassiana* (CPC 12181), asci in culture.

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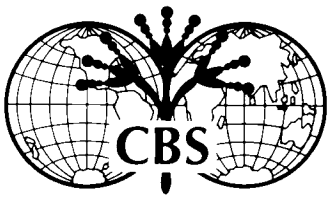

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PREFACE

DEDICATION

This volume of the *Studies in Mycology* is dedicated to the memory of Gerardus Albertus de Vries (1919–2005), who spent his scientific career as mycologist at the CBS, where he was appointed as medical mycologist.

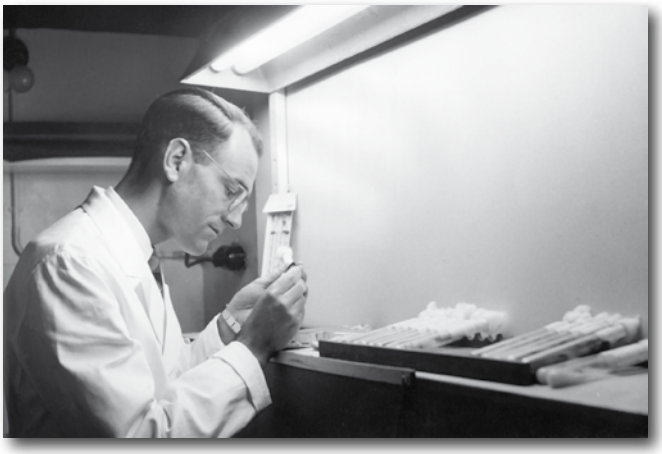

Fig. 1. Gerard de Vries studying *Cladosporium* spp. on different cultural growth media.

On the 10th of July 1952, Gerard de Vries graduated from the University of Utrecht, where he completed his Ph.D. on the topic "*Contribution to the knowledge of the genus Cladosporium Link ex Fr.*" under the guidance of the then director of the CBS, Prof. dr Johanna Westerdijk. At this time, his thesis (de Vries 1952) represented a benchmark and synthesis of our knowledge and understanding of *Cladosporium* spp. studied in culture (Fig. 1).

Gerard de Vries learned the basics of mycology by working during the 1930's with mushroom taxonomy under the guidance of Abraham van Luyk. Via van Luyk he was also introduced to the Dutch Mycological Society, who organised excursions, also around Baarn, which is where the de Vries family lived. For the rest of his career, Gerard would retain his love for studying and collecting mushrooms. In 1948 he was employed at CBS under Johanna Westerdijk, and given the task of establishing and heading a new division called Medical Mycology. This he did, right up to his retirement in 1984. For several years de Vries played an important role in this discipline, and attended numerous medical congresses and workshops, and also published extensively on the topic. De Vries loved the outdoors and traveling, and combined this with his other passion, which was ornithology (van der Aa 2005). In 1952 he graduated from the University of Utrecht, producing a revision of major species in the genus *Cladosporium* of importance to the medical, industrial and plant pathology disciplines. His book soon became highly popular, and with a strong demand for additional copies, resulting in it being reprinted by J. Cramer. His contribution to *Cladosporium* was also recently acknowledged with the introduction of the cladosporium-like heat-tolerant genus, *Devriesia* by Seifert *et al*. (2004). Additional books published by de Vries dealt with mushrooms for amateur mycologists (1955) and a treatment of *Hypogaea* and truffels published in 1971 as part 3 of the "Fungi of the Netherlands" (van der Aa 2005).

Gerard de Vries was a well tempered, softly spoken man, who avoided conflicts, except when it dealt with scientific issues. He never married, and died a bachelor, surrounded by a circle of loyal friends and fellow mycologists dating back to the "Baarn-era" of CBS. To the very end the CBS received a yearly Christmas card, which was always a water colour painting depicting some fascinating birds that he happened to be studying at the time. The *Cladosporium* notebooks, annotations and live cultures are still at the CBS. It is thus with great joy that we dedicate this volume to Gerard de Vries, and build on his *Cladosporium* legacy, most of which is still sporulating, and will be available for scientific debate for generations to come.

The genus *Cladosporium* and similar dematiaceous hyphomycetes

INTRODUCTION

Species of *Cladosporium* are common and widespread, and interact with humans in every phase of life, from growing behind your bed or bedroom cupboard and producing allergens, or growing on the bathroom ceiling, to the fruit decay happening in the fruit basket in the kitchen, to colonising the debris lying outside your house, and even the plant diseases observed on some of the shrubs, trees, or flowers cultivated in your garden. However, scientists generally shy away from trying to identify these similar looking organisms, and therefore the main aims of this volume were to:

1) Establish standardised conditions and protocols for studying cladosporioid species and their teleomorphs in culture;
2) Determine how they can morphologically be distinguished in culture, and highlight important diagnostic features;
3) Circumscribe the genus, and delineate it from morphologically similar dematiaceous hyphomycetes;
4) Determine which DNA gene loci are informative to accurately distinguish species of *Cladosporium*, and initiate a database of *Cladosporium* sequences that can in future be used to set up an online polyphasic identification key.

Although *Cladosporium* is one of the largest and most heterogeneous genera of hyphomycetes, currently comprising more than 772 names (Dugan *et al*. 2004), only a mere fraction of these species are known from culture, and thus the real number of taxa that exist remains unknown. Species of *Cladosporium* are commonly encountered on plant and other kinds of debris, frequently colonising lesions of plant pathogenic fungi, and are also isolated from air, soil, food, paint, textiles and other organic matters (Ellis 1971, 1976; Schubert 2005a), they are also common endophytes (Brown *et al*. 1998, El-Morsy 2000) as well as phylloplane fungi (Islam & Hasin 2000, de Jager *et al*. 2001, Inacio *et al*. 2002, Stohr & Dighton 2004, Levetin & Dorsey 2006). Some species of *Cladosporium* have a medical relevance in clinical laboratories, and also cause allergic lung mycoses (de Hoog *et al*. 2000). In spite of its obvious importance, species of *Cladosporium* are still poorly understood.

Taxonomy of the anamorph

The first binominal introduced for this group of fungi was that of *Dematium herbarum* Pers. (Persoon 1794) (Fig. 2). *Cladosporium herbarum* (Pers.) Link was subsequently selected to serve as

lectotype for the genus by Clements & Shear (1931), a proposal which was accepted by de Vries (1952), Hughes (1958), and others (Prasil & de Hoog 1988). In subsequent years the number of taxa described in the genus grew rapidly, though the generic concept was rather vague. As a consequence, numerous morphologically similar dematiaceous hyphomycetes with catenulate conidia were incorrectly assigned to *Cladosporium*, making this one of the largest genera of hyphomycetes.

In an attempt to circumscribe some of the more well-known taxa, de Vries (1952) published a revision of nine *Cladosporium* species *in vivo* and *in vitro*, and 13 additional taxa in an appendix. Ellis (1971, 1976) described and illustrated 43 species, while Morgan-Jones and McKemy dealt with selected species in the series "Studies in the genus *Cladosporium s. lat.*" (Morgan-Jones & McKemy 1990, McKemy & Morgan-Jones 1990, 1991a–c). Other significant works that also treated *Cladosporium* species include Ho *et al*. (1999), and Zhang *et al.* (2003), though these authors still followed a wider generic concept. David (1997) followed the taxonomy of de Vries (1952), who first considered *Heterosporium* as a synonym of *Cladosporium*, and introduced the combination *Cladosporium* subgen. *Heterosporium*. Subsequent to this publication, further monographic studies on the genus *Cladosporium s. lat*. were initiated by Braun and co-workers (Braun *et al*. 2003, 2006, Dugan *et al*. 2004, Schubert & Braun 2004, 2005a, b, 2006, 2007, Schubert 2005a, b, Heuchert *et al*. 2005).

Treatments of human pathogenic *Cladosporium* species (Masclaux *et al*. 1995, Untereiner 1997, Gerrits van den Ende & de Hoog 1999, Untereiner & Naveau 1999, Untereiner *et al*. 1999; de Hoog *et al*. 2000), concluded that they represent species belonging to the *Herpotrichiellaceae* (*Capronia* Sacc./*Cladophialophora* Borelli). Saprobic species, which appear morphologically similar, were found to belong to the *Venturiaceae* (*Caproventuria* U. Braun/ *Pseudocladosporium* U. Braun; Braun *et al.* 2003, Schubert *et al.* 2003, Beck *et al.* 2005) (see Crous *et al*. 2007 – this volume). Further genera that were separated from *Cladosporium* include *Sorocybe resinae* (Fr.) Fr. [≡ *Cladosporium resinae* (Lindau) G.A. de Vries, teleomorph: *Amorphotheca resinae* Parbery; Partridge & Morgan-Jones 2002] (see Seifert *et al*. 2007 – this volume), *Devriesia* Seifert & N.L. Nickerson, erected for heat tolerant species (Seifert *et al.* 2004), *Cladoriella* Crous, erected for saprobic species (Crous *et al*. 2006b), *Metulocladosporiella* Crous, Schroers, Groenewald, U. Braun & K. Schub., erected for the causal agent of banana speckle disease (Crous *et al*. 2006a), *Digitopodium* U. Braun, Heuchert & K. Schub. and *Parapericoniella* U. Braun, Heuchert & K. Schub., representing two genera of hyperparasitic hyphomycetes (Heuchert *et al*. 2005).

Fig. 2. Type specimen of *Dematium herbarum* Pers. (1794), preserved in the National Herbarium of the Netherlands in Leiden.

Taxonomy of the teleomorph

Teleomorphs of *Cladosporium* have traditionally been described in *Mycosphaerella* Johanson. The first indication that this may not be the case was the rDNA ITS sequence data presented by Crous *et al*. (2001), which revealed cladosporium-like taxa to cluster basal to *Mycosphaerella s. str.* This finding was further strengthened by adding 18S rDNA data, which clearly distinguished the *Cladosporium* clade from *Mycosphaerella* (Braun *et al.* 2003). These results lead to the erection of the genus *Davidiella* Crous & U. Braun for teleomorphs of *Cladosporium*, though it was largely established based on its unique anamorphs, rather than distinct teleomorph features. In a revision of the genus *Mycosphaerella*, Aptroot (2006) provided the first clear morphological characteristics to distinguish *Davidiella* from *Mycosphaerella*, referring to their sole-shaped ascospores, and angular lumina that are to be seen in *Davidiella* ascospores. Further phylogenetic evidence for the distinction was found by Schoch *et al*. (2006), which led to the erection of the family *Davidiellaceae* (*Capnodiales*). Detailed cultural studies of *Davidiella* teleomorphs, however, were still lacking (see Schubert *et al*. 2007 – this volume).

What is *Cladosporium*?

David (1997) provided the first modern concept of *Cladosporium* by conducting comprehensive scanning electron microscopic (SEM) examinations of the scar and hilum structure in *Cladosporium* and *Heterosporium*, thereby confirming the observations of Roquebert (1981). He introduced the term "coronate" for the *Cladosporium* scar type, which is characterised by having a central convex part (dome), surrounded by a raised periclinal rim (Fig. 3), and proved that these anamorphs are linked to teleomorphs now placed in *Davidiella* (see David 1997, fig. 12).

This new concept of *Cladosporium s. str*. and *Davidiella* (David 1997, Braun *et al*. 2003, Aptroot 2006), supported by morphological and molecular data, rendered it possible to initiate a comprehensive revision of the genus. The first step was the preparation of a general, annotated check-list of *Cladosporium* names (Dugan *et al*. 2004), followed by revisions of fungicolous (Heuchert *et al*. 2005) and foliicolous species of *Cladosporium s. lat*. (Schubert 2005b, Braun *et al.* 2006, Schubert & Braun 2004, 2005a, b, 2006, 2007). The present study is the first to integrate these concepts on cladosporioid species in culture, in an attempt to further elucidate species of *Cladosporium*, and delineate the genus from other, morphologically similar dematiaceous genera that have traditionally been confused with *Cladosporium s. str*.

How natural should anamorph genera be?

Article 59 of the International Code of Botanical Nomenclature was introduced to enable mycologists to name the asexual states of fungi that they encountered, and for which no teleomorph association was known. It was and remains a completely artificial system, complicated further by the evolution of the same anamorph morphology in different families, and even orders. In 1995 Gams discussed "How natural should anamorph genera be", concluding that paraphyletic genera should be an acceptable option, and that anamorphs cannot reflect natural relationships. In a special volume dedicated at integrating molecular data and morphology, Seifert *et al*. (2000) proposed using anamorph names as adjectives, e.g., acremonium-like, when they clustered in different clades, or were linked to different teleomorphs than the type species of the genus

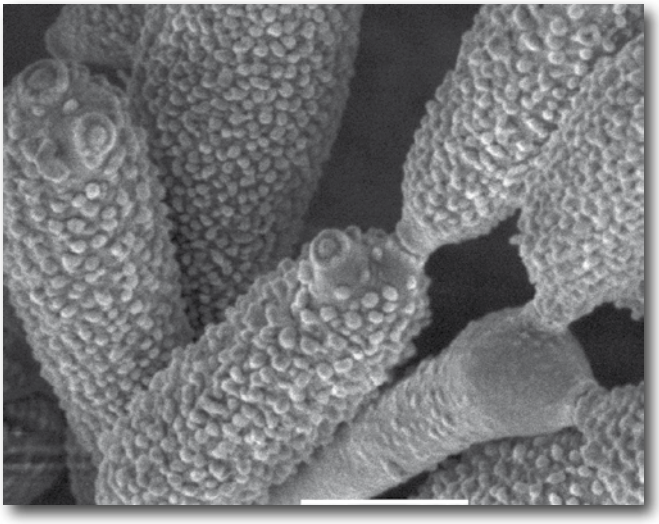

Fig. 3. Coronate scar structure of *Cladosporium herbaroides*, visible by means of Scanning Electron Microscopy. Scale bar = 5 μm (Photo: Jan Dijksterhuis).

Acremonium. In a phylogenetic study of the *Herpotrichiellaceae*, Haase *et al*. (1999) proposed to accept anamorphs as poly- and paraphyletic within the order *Chaetothyriales*, as their taxonomy was unsupported by phylogeny, and Cook *et al*. (1997) as well as Braun *et al.* (2002) followed this methodology in naming anamorphs of the *Erysiphaceae* (*Erysiphales*). After much debate, we have chosen to use the same approach in this volume, and will refrain from introducing different anamorph genera for the same phenotype clustering within different clades of the same order. It is hoped that this approach will stop the unnecessary proliferation of names, until we can move to a single nomenclature for ascomycetous fungi.

Anamorphs are just form taxa, established for the sole purpose of enabling mycologists to name asexual states that occur in the absence of their teleomorphs. Anamorph genera are simply phenotypic concepts that lack phylogenetic relevance within the order.

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The Editors *1 July 2007*

Propositions for this volume:

"Evolution gives rise to lineages, which we try to recognise as genera and species"

"The most interesting fungi are those isolated by accident"

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Mycosphaerella is polyphyletic

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Abstract: *Mycosphaerella,* one of the largest genera of ascomycetes, encompasses several thousand species and has anamorphs residing in more than 30 form genera. Although previous phylogenetic studies based on the ITS rDNA locus supported the monophyly of the genus, DNA sequence data derived from the LSU gene distinguish several clades and families in what has hitherto been considered to represent the *Mycosphaerellaceae*. Several important leaf spotting and extremotolerant species need to be disposed to the genus *Teratosphaeria*, for which a new family, the *Teratosphaeriaceae*, is introduced. Other distinct clades represent the *Schizothyriaceae*, *Davidiellaceae*, *Capnodiaceae*, and the *Mycosphaerellaceae*. Within the two major clades, namely *Teratosphaeriaceae* and *Mycosphaerellaceae*, most anamorph genera are polyphyletic, and new anamorph concepts need to be derived to cope with dual nomenclature within the *Mycosphaerella* complex.

Taxonomic novelties: *Batcheloromyces eucalypti* (Alcorn) Crous & U. Braun, comb. nov., *Catenulostroma* Crous & U. Braun, gen. nov., *Catenulostroma abietis* (Butin & Pehl) Crous & U. Braun, comb. nov., *Catenulostroma chromoblastomycosum* Crous & U. Braun, sp. nov., *Catenulostroma elginense* (Joanne E. Taylor & Crous) Crous & U. Braun, comb. nov., *Catenulostroma excentricum* (B. Sutton & Ganap.) Crous & U. Braun, comb. nov., *Catenulostroma germanicum* Crous & U. Braun, sp. nov., *Catenulostroma macowanii* (Sacc.) Crous & U. Braun, comb. nov., *Catenulostroma microsporum* (Joanne E. Taylor & Crous) Crous & U. Braun, comb. nov., *Catenulostroma protearum* (Crous & M.E. Palm) Crous & U. Braun, comb. nov., *Penidiella* Crous & U. Braun, gen. nov., *Penidiella columbiana* Crous & U. Braun, sp. nov., *Penidiella cubensis* (R.F. Castañeda) U. Braun, Crous & R.F. Castañeda, comb. nov., *Penidiella nectandrae* Crous, U. Braun & R.F. Castañeda, nom. nov., *Penidiella rigidophora* Crous, R.F. Castañeda & U. Braun, sp. nov., *Penidiella strumelloidea* (Milko & Dunaev) Crous & U. Braun, comb. nov., *Penidiella venezuelensis* Crous & U. Braun, sp. nov., *Readeriella blakelyi* (Crous & Summerell) Crous & U. Braun, comb. nov., *Readeriella brunneotingens* Crous & Summerell, sp. nov., *Readeriella considenianae* (Crous & Summerell) Crous & U. Braun, comb. nov., *Readeriella destructans* (M.J. Wingf. & Crous) Crous & U. Braun, comb. nov., *Readeriella dimorpha* (Crous & Carnegie) Crous & U. Braun, comb. nov., *Readeriella epicoccoides* (Cooke & Massee) Crous & U. Braun, comb. nov., *Readeriella gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Readeriella molleriana* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Readeriella nubilosa* (Ganap. & Corbin) Crous & U. Braun, comb. nov., *Readeriella pulcherrima* (Gadgil & M. Dick) Crous & U. Braun, comb. nov., *Readeriella stellenboschiana* (Crous) Crous & U. Braun, comb. nov., *Readeriella toledana* (Crous & Bills) Crous & U. Braun, comb. nov., *Readeriella zuluensis* (M.J. Wingf., Crous & T.A. Cout.) Crous & U. Braun, comb. nov., *Teratosphaeria africana* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria alistairii* (Crous) Crous & U. Braun, comb. nov., *Teratosphaeria associata* (Crous & Carnegie) Crous & U. Braun, comb. nov., *Teratosphaeria bellula* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria cryptica* (Cooke) Crous & U. Braun, comb. nov., *Teratosphaeria dentritica* (Crous & Summerell) Crous & U. Braun, comb. nov., *Teratosphaeria excentrica* (Crous & Carnegie) Crous & U. Braun, comb. nov., *Teratosphaeria fimbriata* (Crous & Summerell) Crous & U. Braun, comb. nov., *Teratosphaeria flexuosa* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria gamsii* (Crous) Crous & U. Braun, comb. nov., *Teratosphaeria jonkershoekensis* (P.S. van Wyk, Marasas & Knox-Dav.) Crous & U. Braun, comb. nov., *Teratosphaeria maxii* (Crous) Crous & U. Braun, comb. nov., *Teratosphaeria mexicana* (Crous) Crous & U. Braun, comb. nov., *Teratosphaeria molleriana* (Thüm.) Crous & U. Braun, comb. nov., *Teratosphaeria nubilosa* (Cooke) Crous & U. Braun, comb. nov., *Teratosphaeria ohnowa* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria parkiiaffinis* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria parva* (R.F. Park & Keane) Crous & U. Braun, comb. nov., *Teratosphaeria perpendicularis* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria plurituburalis* (Crous & Mansilla) Crous & U. Braun, comb. nov., *Teratosphaeria pseudafricana* (Crous & T.A. Cout.) Crous & U. Braun, comb. nov., *Teratosphaeria pseudocryptica* (Crous) Crous & U. Braun, comb. nov., *Teratosphaeria pseudosuberosa* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria quasicercospora* (Crous & T.A. Cout.) Crous & U. Braun, comb. nov., *Teratosphaeria readeriellophora* (Crous & Mansilla) Crous & U. Braun, comb. nov., *Teratosphaeria secundaria* (Crous & Alfenas) Crous & U. Braun, comb. nov., *Teratosphaeria stramenticola* (Crous & Alfenas) Crous & U. Braun, comb. nov., *Teratosphaeria suberosa* (Crous, F.A. Ferreira, Alfenas & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria suttonii* (Crous & M.J. Wingf.) Crous & U. Braun, comb. nov., *Teratosphaeria toledana* (Crous & Bills) Crous & U. Braun, comb. nov., *Teratosphaeriaceae* Crous & U. Braun, fam. nov.
Key words: *Ascomycetes*, *Batcheloromyces*, *Colletogloeopsis*, *Readeriella, Teratosphaeria, Trimmatostroma,* DNA sequence comparisons, systematics.

INTRODUCTION

The genus *Mycosphaerella* Johanson as presently circumscribed contains close to 3 000 species (Aptroot 2006), excluding its anamorphs, which represent thousands of additional species (Crous *et al.* 2000, 2001, 2004a, b, 2006a, b, 2007b, Crous & Braun 2003). Crous (1998) predicted that *Mycosphaerella* would eventually be split according to its anamorph genera, and Crous *et al*. (2000) recognised six sections, as originally defined by Barr (1972). This was followed by a set of papers (Crous *et al*. 2001, Goodwin *et al*. 2001), where it was concluded, based on ITS DNA sequence data, that *Mycosphaerella* was monophyletic. A revision of the various coelomycete and hyphomycete anamorph concepts led Crous & Braun (2003) to propose a system whereby the asexual morphs could be allocated to various form genera affiliated with *Mycosphaerella* holomorphs.

In a recent study that formed part of the US "Assembling the Fungal Tree of Life" project, Schoch *et al*. (2006) were able to show that the *Mycosphaerellaceae* represents a family within *Capnodiales*. Furthermore, some variation was also depicted within the family, which supported similar findings in other recent papers employing LSU sequence data, such as Hunter *et al*. (2006), and Batzer *et al*. (2007). To further elucidate the phylogenetic variation observed within the *Mycosphaerellaceae* in these studies, a subset of isolates was selected for the present study, representing the various species recognised as morphologically distinct from *Mycosphaerella s. str.*

The genus *Mycosphaerella* has in recent years been linked to approximately 30 anamorph genera (Crous & Braun 2003, Crous *et al*. 2007b). Many of these anamorph genera resulted from a reassessment of cercosporoid forms. Chupp (1954) was of the opinion that they all represented species of the genus *Cercospora* Fresen., although he clearly recognised differences in their morphology. In a series of papers by Deighton, as well as others such as Sutton, Braun and Crous, the genus *Cercospora* was delimited based on its type species, *Cercospora penicillata* (Ces.) Fresen., while taxa formerly included in the genus by Chupp (1954) but differing in conidiophore arrangement, conidiogenesis, pigmentation, conidial catenulation, septation, and scar/hilum structure were allocated to other genera. Similar studies in which the type species were recollected and subjected to DNA sequence

Table 1. Isolates for which new sequences were generated.

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank Accession number
Batcheloromyces eucalypti		CBS 313.76; CPC 3632	*Eucalyptus tessellaris*	Australia	J.L. Alcorn	EU019245
Batcheloromyces leucadendri		CBS 110892; CPC 1837	*Leucadendron* sp.	South Africa	L. Swart	EU019246
Batcheloromyces proteae		CBS 110696; CPC 1518	*Protea cynaroides*	South Africa	L. Viljoen	EU019247
Capnobotryella renispora		CBS 214.90*; CBS 176.88; IAM 13014; JCM 6932	*Capnobotrys neessii*	Japan	J. Sugiyama	EU019248
Catenulostroma abietis		CBS 290.90	Man, skin lesion	Netherlands	R.G.F. Wintermans	EU019249
Catenulostroma castellanii		CBS 105.75*; ATCC 24788	Man, *tinea nigra*	Venezuela	—	EU019250
Catenulostroma chromoblastomycosum		CBS 597.97	Man, chromoblastomycosis	Zaire	V. de Brouwere	EU019251
Catenulostroma elginense		CBS 111030; CPC 1958	*Protea grandiceps*	South Africa	J.E. Taylor	EU019252
Catenulostroma germanicum		CBS 539.88	Stone	Germany	—	EU019253
Catenulostroma macowanii		CBS 110756; CPC 1872	*Protea nitida*	South Africa	J.E. Taylor	EU019254
Catenulostroma microsporum	*Teratosphaeria microspora*	CBS 110890; CPC 1832	*Protea cynaroides*	South Africa	L. Swart	EU019255
Catenulostroma sp.	*Teratosphaeria pseudosuberosa*	CBS 118911; CPC 12085	*Eucalyptus* sp.	Uruguay	M.J. Wingfield	EU019256
Cercosporella centaureicola		CBS 120253	*Centaurea solstitiales*	Greece	D. Berner	EU019257
Cibiessia dimorphospora		CBS 120034; CPC 12636	*Eucalyptus nitens*	Australia	—	EU019258
Cibiessia minutispora		CPC 13071*	*Eucalyptus henryii*	Australia	A.J. Carnegie	EU019259
Cibiessia nontingens	*Teratosphaeria* sp.	CBS 120725*; CPC 13217	*Eucalyptus tereticornis*	Australia	B. Summerell	EU019260
Cladosporium bruhnei	*Davidiella allicina*	CBS 115683; ATCC 66670; CPC 5101	CCA-treated Douglas-fire pole	U.S.A., New York	C.J. Wang	EU019261
Cladosporium cladosporioides		CBS 109.21; ATCC 11277; ATCC 200940; IFO 6368; IMI 049625	Sooty mould on *Hedera helix*	U.K.	—	EU019262
Cladosporium sphaerospermum		CBS 188.54; ATCC 11290; IMI 049638	—	—	—	EU019263
Cladosporium uredinicola		ATCC 46649	Hyperparasite on *Cronartium fusiforme* f. sp. *quercum*	U.S.A., Alabama	—	EU019264
Coccodinium bartschii		CBS 121708; CPC 13861–13863	Sooty mould on unidentified tree	Canada	K.A. Seifert	EU019265
Dissoconium aciculare		CBS 342.82*; CPC 1534	*Erysiphe*, on *Medicago lupulina*	Germany	T. Hijwegen	EU019266
Dissoconium commune	*"Mycosphaerella" communis*	CBS 114238*; CPC 10440	*Eucalyptus globulus*	Spain	J.P.M. Vazquez	EU019267
Dissoconium dekkeri	*"Mycosphaerella" lateralis*	CBS 567.89*; CPC 1535	*Juniperus chinensis*	Netherlands	T. Hijwegen	EU019268
Fumagospora capnodioides	*Capnodium salicinum*	CBS 131.34	Sooty mould on *Bursaria spinosa*	Indonesia	—	EU019269
Hortaea werneckii		CBS 107.67*	Man, *tinea nigra*	Portugal	—	EU019270
Nothostrasseria dendritica	*Teratosphaeria dendritica*	CPC 12820	*Eucalyptus nitens*	Australia	A.J. Carnegie	EU019271
"Passalora" zambiae		CBS 112970*; CPC 1228	*Eucalyptus globulus*	Zambia	T. Coutinho	EU019272
		CBS 112971*; CMW 14782; CPC 1227	*Eucalyptus globulus*	Zambia	T. Coutinho	EU019273
Penidiella columbiana		CBS 486.80	*Paepalanthus columbianus*	Colombia	W. Gams	EU019274

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank Accession number
Penidiella nectandrae		CBS 734.87*; ATCC 200932; INIFAT 87/45	*Nectandra coriacea*	Cuba	R.F. Castañeda & G. Arnold	EU019275
Penidiella rigidophora		CBS 314.95*	Leaf litter of *Smilax* sp.	Cuba	R.F. Castañeda	EU019276
Penidiella strumelloidea		CBS 114484*; VKM F-2534	*Carex* leaf, from stagnant water	Russia	S. Ozerskaya	EU019277
Penidiella venezuelensis		CBS 106.75*	Man, *tinea nigra*	Venezuela	D. Borelli	EU019278
Phaeotheca triangularis		CBS 471.90*	Wet surface of humidifier of airconditioning	Belgium	H. Beguin	EU019279
Phaeothecoidea eucalypti		CPC 13010	*Corymbia henryii*	Australia	B. Summerell	EU019280
		CPC 12918*	*Eucalyptus botryoides*	Australia	B. Summerell	EU019281
Pleurophoma sp.	*Teratosphaeria fibrillosa*	CPC 1876	*Protea nitida*	South Africa	J.E. Taylor	EU019282
Pseudotaeniolina globosa		CBS 109889*	Rock	Italy	C. Urzi	EU019283
Ramularia pratensis var. *pratensis*		CPC 11294	*Rumex crispus*	Korea	H.D. Shin	EU019284
Ramularia sp.		CBS 324.87	On *Mycosphaerella* sp., leaf spot on *Brassica* sp.	Netherlands	—	EU019285
Readeriella brunneotingens		CPC 13303	*Eucalyptus tereticornis*	Australia	P.W. Crous	EU019286
Readeriella destructans		CBS 111369*; CPC 1366	*Eucalyptus grandis*	Indonesia	M.J. Wingfield	EU019287
Readeriella epicoccoides	*Teratosphaeria suttonii*	CPC 12352	*Eucalyptus* sp.	U.S.A.,Hawaii	W. Gams	EU019288
Readeriella eucalypti		CPC 11186	*Eucalyptus globulus*	Spain	M.J. Wingfield	EU019289
Readeriella gauchensis		CBS 120303*; CMW 17331	*Eucalyptus grandis*	Uruguay	M.J. Wingfield	EU019290
Readeriella mirabilis		CBS 116293; CPC 10506	*Eucalyptus fastigata*	New Zealand	W. Gams	EU019291
Readeriella molleriana	*Teratosphaeria molleriana*	CBS 111164*; CMW 4940; CPC 1214	*Eucalyptus globulus*	Portugal	M.J. Wingfield	EU019292
Readeriella ovata complex		CPC 18	*Eucalyptus cladocalyx*	South Africa	P.W. Crous	EU019293
		CBS 111149; CPC 23	*Eucalyptus cladocalyx*	South Africa	P.W. Crous	EU019294
Readeriella stellenboschiana		CBS 116428; CPC 10886	*Eucalyptus* sp.	South Africa	P.W. Crous	EU019295
Readeriella zuluensis		CBS 120301*; CMW 17321	*Eucalyptus grandis*	South Africa	M.J. Wingfield	EU019296
Septoria tritici	*Mycosphaerella graminicola*	CBS 100335; IPO 69001.61	*Triticum aestivum*	—	G.H.J. Kema	EU019297
		CBS 110744; CPC 658	*Triticum* sp.	South Africa	P.W. Crous	EU019298
Trimmatostroma betulinum		CBS 282.74	*Betula verrucosa*	Netherlands	W.M. Loerakker	EU019299
Trimmatostroma salicis		CPC 13571	*Salix alba*	Germany	U. Braun	EU019300
	Teratosphaeria bellula	CBS 111700; CPC 1821	*Protea eximia*	South Africa	J.E. Taylor	EU019301
	Teratosphaeria mexicana	CPC 12349	*Eucalyptus* sp.	U.S.A.,Hawaii	W. Gams	EU019302
	Teratosphaeria nubilosa	CBS 114419; CPC 10497	*Eucalyptus globulus*	New Zealand	—	EU019303
		CBS 116005*; CMW 3282; CPC 937	*Eucalyptus globulus*	Australia	A. Carnegie	EU019304

Table 1. (Continued).

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank Accession number
	Teratosphaeria ohnowa	CBS 112896*; CMW 4937; CPC 1004	*Eucalyptus grandis*	South Africa	M.J. Wingfield	EU019305
	Teratosphaeria secundaria	CBS 115608; CPC 504	*Eucalyptus grandis*	Brazil	A.C. Alfenas	EU019306
	Teratosphaeria sp.	CBS 208.94; CPC 727	*Eucalyptus grandis*	Indonesia	A.C. Alfenas	EU019307

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CMW: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa; IAM: Institute of Applied Microbiology, University of Tokyo, Institute of molecular and cellular bioscience, Tokyo, Japan; IFO: Institute For Fermentation, Osaka, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; INIFAT: Alexander Humboldt Institute for Basic Research in Tropical Agriculture, Ciudad de La Habana, Cuba; JCM: Japan Collection Of Microorganisms, RIKEN BioResource Center, Japan; VKM: All-Russian Collection of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia.

*Ex-type cultures.

analysis were undertaken to characterise *Mycosphaerella* (Verkley *et al*. 2004), and anamorph genera such as *Pseudocercospora* Speg., *Stigmina* Sacc., *Phaeoisariopsis* Ferraris (Crous *et al*. 2006a), *Ramulispora* Miura (Crous *et al.* 2003), *Batcheloromyces* Marasas, P.S. van Wyk & Knox-Dav. (Taylor *et al*. 2003), *Phaeophleospora* Rangel and *Dothistroma* Hulbary (Crous *et al*. 2000, 2001, Barnes *et al*. 2004).

To assess the phylogeny of the species selected for the present study, DNA sequences were generated of the 28S rRNA (LSU) gene. In a further attempt to address monophyletic groups within this complex, these data were integrated with their morphological characteristics. To further resolve pleomorphism among the species studied, isolates were examined on a range of cultural media to induce possible synanamorphs.

MATERIALS AND METHODS

Isolates

Chosen isolates represent various species previously observed to be morphologically distinct from *Mycosphaerella s. str.* (Crous 1998, Crous *et al*. 2004a, b, 2006a, b, 2007b). In a few cases, specifically *Teratosphaeria fibrillosa* Syd. & P. Syd. and *Coccodinium bartschii* A. Massal., fresh material had to be collected from South Africa and Canada, respectively. Excised tissue pieces bearing ascomata were soaked in water for approximately 2 h, after which they were placed in the bottom of Petri dish lids, with the top half of the dish containing 2 % malt extract agar (MEA) (Gams *et al*. 2007). Ascospore germination patterns were examined after 24 h, and single-ascospore and conidial cultures established as described by Crous (1998). Colonies were sub-cultured onto synthetic nutrient-poor agar (SNA), potato-dextrose agar (PDA), oatmeal agar (OA), MEA (Gams *et al.* 2007), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

DNA phylogeny

Fungal colonies were established on agar plates, and genomic DNA was isolated following the CTAB-based protocol described in Gams *et al*. (2007). The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al*. 1990), LR0R (Rehner & Samuels 1994), LR3R (www.biology.duke.edu/fungi/mycolab/primers.htm), and LR16 (Moncalvo *et al*. 1993), were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The ITS1, ITS2 and 5.8S rRNA gene (ITS) were only sequenced for isolates of which these data were not available. The ITS data were not included in the analyses but deposited in GenBank where applicable. The PCR conditions, sequence alignment and subsequent phylogenetic analyses using parsimony, distance and Bayesian analyses followed the methods of Crous *et al*. (2006c). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Taxonomy

Wherever possible, 30 measurements (× 1 000 magnification)

were made of structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Ascospores were frequently also mounted in water to observe mucoid appendages and sheaths. Colony colours (surface and reverse) were assessed after 1–2 mo on MEA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org).

RESULTS

DNA phylogeny

Amplification products of approximately 1 700 bases were obtained for the isolates listed in Table 1. The LSU region of the sequences was used to obtain additional sequences from GenBank which were added to the alignment. The manually adjusted alignment contained 97 sequences (including the two outgroup sequences) and 844 characters including alignment gaps. Of the 844 characters used in the phylogenetic analysis, 308 were parsimony-informative, 105 were variable and parsimony-uninformative, and 431 were constant.

The parsimony analysis of the LSU region yielded 1 135 equally most parsimonious trees (TL = 1 502 steps; CI = 0.446; RI = 0.787; RC = 0.351), one of which is shown in Fig. 1. Three orders are represented by the ingroup isolates, namely *Chaetothyriales* (100 % bootstrap support), *Helotiales* (100 % bootstrap support) and *Capnodiales* (100 % bootstrap support). These are discussed in detail in the Taxonomy and Discussion sections. A new collection of *Coccodinium bartschii* A. Massal clusters (100 % bootstrap support) with members of the *Herpotrichiellaceae* (*Chaetothyriales*), whereas the type species of the genus *Trimmatostroma* Corda, namely *T. salicis* Corda, as well as *T. betulinum* (Corda) S. Hughes, are allied (99 % bootstrap support) with the *Dermateaceae* (*Helotiales*). The *Capnodiales* encompasses members of the *Capnodiaceae*, *Trichosphaeriaceae*, *Davidiellaceae*, *Schizothyriaceae* and taxa traditionally placed in the *Mycosphaerellaceae*, which is divided here into the *Teratosphaeriaceae*, (65 % bootstrap support), and the *Mycosphaerellaceae* (76 % bootstrap support), which contains several subclades. Also included in the *Capnodiales* are *Devriesia staurophora* (W.B. Kendr.) Seifert & N.L. Nick., *Staninwardia suttonii* Crous & Summerell and *Capnobotryella renispora* Sugiy. as sister taxa to *Teratosphaeriaceae s. str*. Neighbour-joining analysis using three substitution models on the sequence data yielded trees supporting the same topologies, but differed from the parsimony tree presented with regard to the order of the families and orders at the deeper nodes, e.g., the *Helotiales* and *Chaetothyriales* are swapped around, as are the *Capnodiaceae* and the *Trichosphaeriaceae* / *Davidiellaceae* (data not shown). Using neighbour-joining analyses, the *Mycosphaerellaceae s. str*. clade obtained 71 %, 70 % and 70 % bootstrap support respectively with the uncorrected "p", Kimura 2-parameter and HKY85 substitution models wherease the *Teratosphaeriaceae* clade obtained 74 %, 79 % and 78 % bootstrap support respectively with the same models. The *Schizothyriaceae* clade appeared basal in the *Capnodiales*, irrespective of which substitution model was used.

Bayesian analysis was conducted on the same aligned LSU dataset using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies. The Markov Chain Monte Carlo (MCMC) analysis of 4 chains started from a random tree topology and lasted 23 881 500 generations. Trees were saved each 100 generations, resulting in 238 815 saved trees. Burn-in was set at 22 000 000 generations after which the likelihood values were stationary, leaving 18 815 trees from which the consensus tree (Fig. 2) and posterior probabilities (PP's) were calculated. The average standard deviation of split frequencies was 0.011508 at the end of the run. The same overall topology as that observed using parsimony was obtained, with the exception of the inclusion of *Staninwardia suttonii* in the *Mycosphaerellaceae* (PP value of 0.74) and not in the *Teratosphaeriaceae*. The *Mycosphaerellaceae s. str.* clade, as well as the *Teratosphaeriaceae* clade, obtained a PP value of 1.00.

Taxonomy

Based on the dataset generated in this study, several well-supported genera could be distinguished in the *Mycosphaerella* complex (Figs 1–2), for which we have identified morphological characters. These genera, and a selection of their species, are treated below.

Key to *Mycosphaerella*, and *Mycosphaerella*-like genera treated

1. Ascomata thyrothecial; anamorph *Zygosporium* ..... ***Schizothyrium***
1. Ascomata pseudothecial 2

2. Ascospores with irregular, angular lumens typical of *Davidiella*; anamorph *Cladosporium s. str.* ..... ***Davidiella***
2. Ascospores guttulate or not, lacking angular lumens; anamorph other than *Cladosporium* 3

3. Ascomata frequently linked by superficial stroma; hamathecial tissue, ascospore sheath, multi-layered endotunica, prominent periphysoids, and ascospores turning brown in asci frequently observed ..... ***Teratosphaeria***
3. Ascomata not linked by superficial stroma; hamathecial tissue, ascospore sheath, multi-layered endotunica, prominent periphysoids, ascospores turning brown in asci not observed 4

4. Conidiophores solitary, pale brown, giving rise to primary and secondary, actively discharged conidia; anamorph *Dissoconium* ..... teleomorph *Mycosphaerella*-like
4. Conidiomata variable from solitary conidiophores to sporodochia, fascicles to pycnidia, but conidia not actively discharged ..... ***Mycosphaerella*** *s. str.*

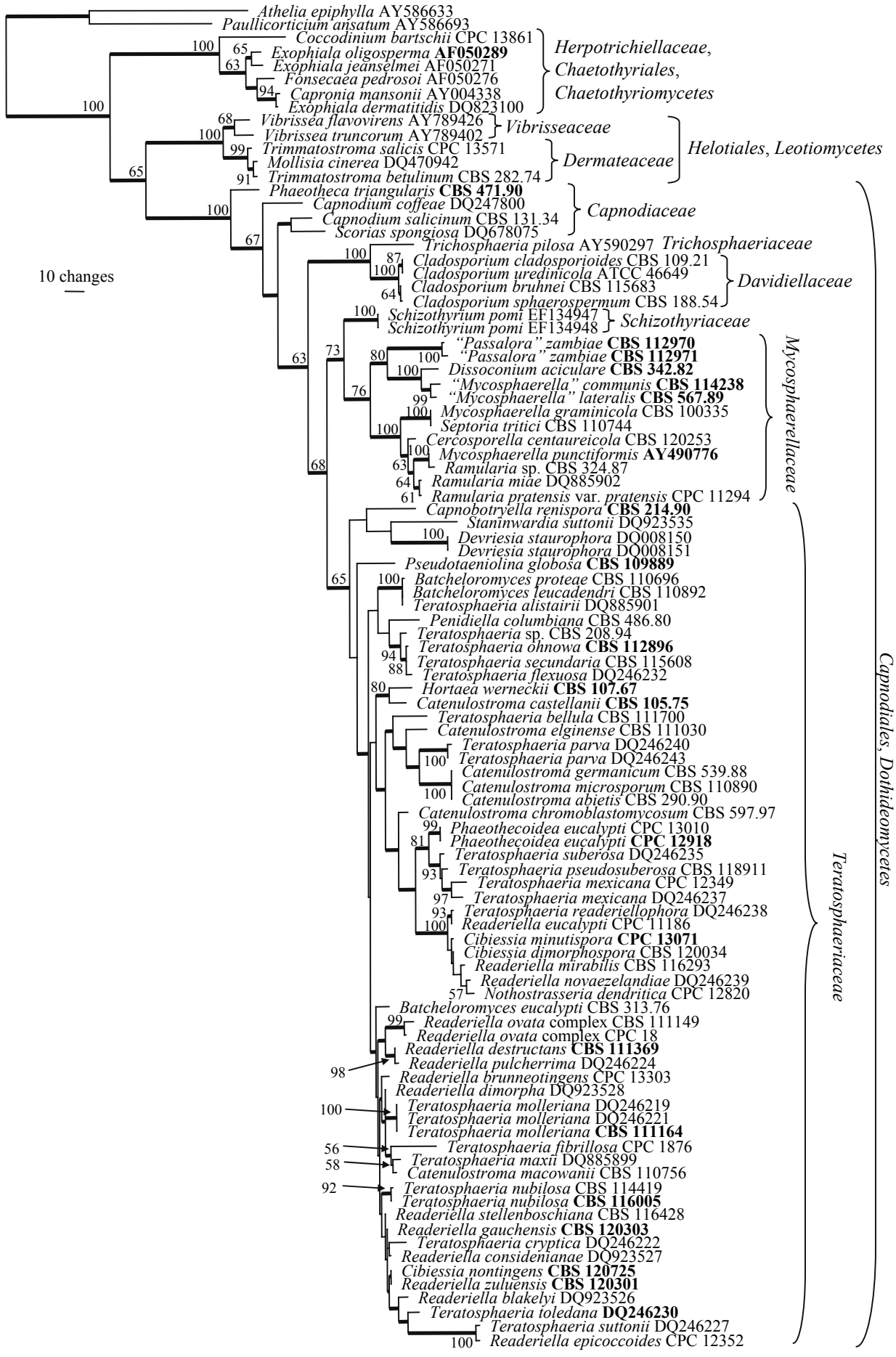

Fig. 1. One of 1 135 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

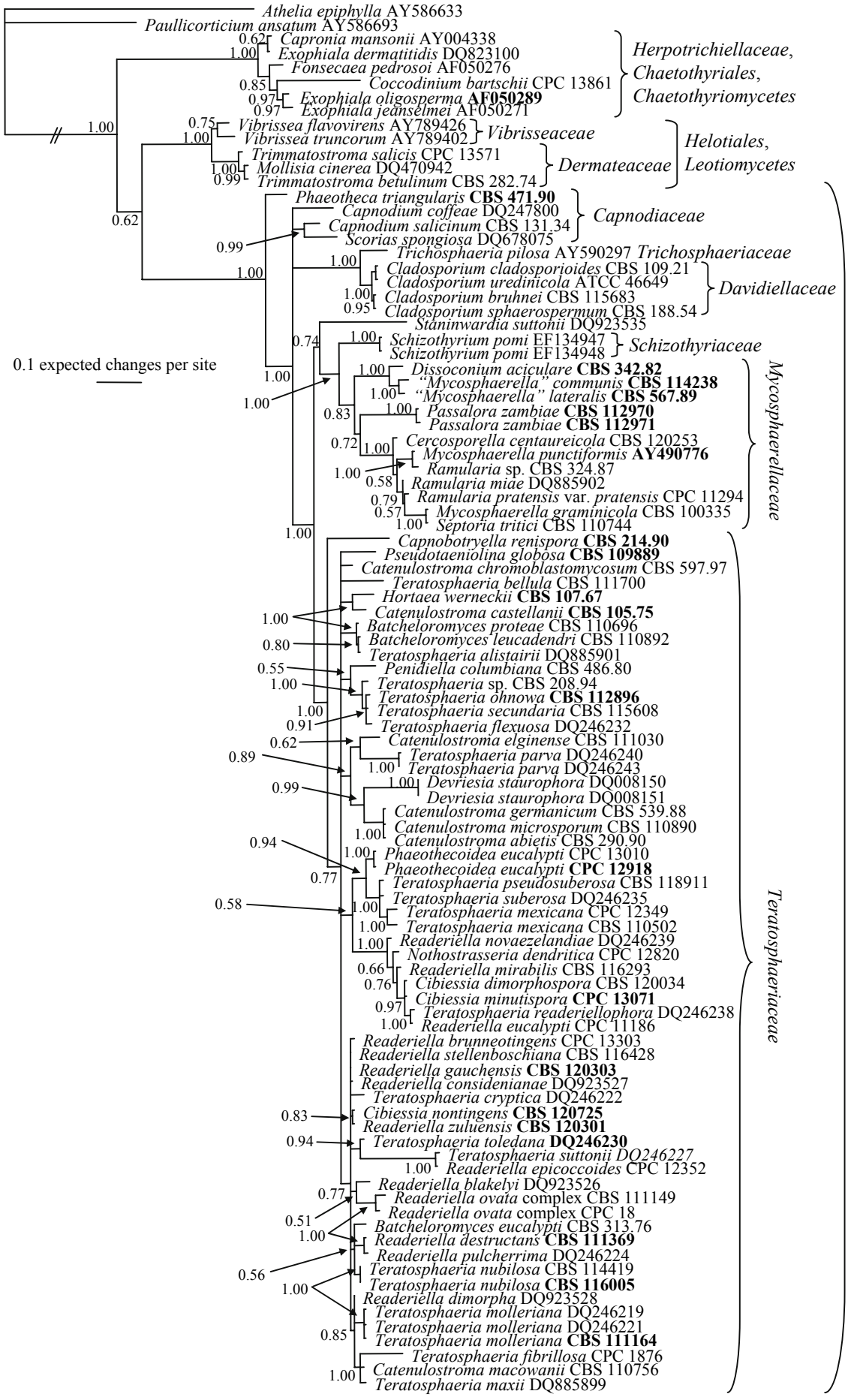

Fig. 2. Consensus phylogram (50 % majority rule) of 18 815 trees resulting from a Bayesian analysis of the LSU sequence alignment using MRBAYES v. 3.1.2. Bayesian posterior probabilities are indicated at the nodes. Ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

Treatment of phylogenetic clades

Davidiellaceae clade

Davidiella Crous & U. Braun, Mycol. Progr. 2: 8. 2003.

Type species: *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8. 2003.
Basionym: *Sphaerella tassiana* De Not., Sferiacei Italici 1: 87. 1863.

Description: Schubert *et al*. (2007 – this volume).

Anamorph: *Cladosporium* Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816.

Type species: *Cladosporium herbarum* (Pers. : Fr.) Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816.
Basionym: *Dematium herbarum* Pers., Ann. Bot. (Usteri), 11 Stück: 32. 1794: Fr., Syst. Mycol. 3: 370. 1832.

Description: Schubert *et al*. (2007 – this volume).

Notes: The genus *Davidiella* (*Davidiellaceae*) was recently introduced for teleomorphs of *Cladosporium s. str.* (Braun *et al*. 2003). The genus *Cladosporium* is well-established, and contains around 772 names (Dugan *et al*. 2004), while *Davidiella* presently has 33 names (www.MycoBank.org), of which only around five have acknowledged *Cladosporium* states.

Teratosphaeriaceae clade

Teratosphaeria Syd. & P. Syd., Ann. Mycol. 10: 39. 1912.

Type species: *Teratosphaeria fibrillosa* Syd. & P. Syd., Ann. Mycol. 10: 40. 1912. Fig. 3.

Description: Crous *et al.* (2004a; figs 182–185).

Notes: Although similar in morphology, the genus *Teratosphaeria* was separated from *Mycosphaerella* based on its ascomatal arrangement, and periphysate ostioles (Müller & Oehrens 1982). It was later synonymised under *Mycosphaerella* by Taylor *et al*. (2003), who showed that the type species clustered within *Mycosphaerella* based on ITS DNA sequence data. The LSU sequence data generated in the present study, has clearly shown that *Mycosphaerella* is polyphyletic, thus contradicting earlier reports of monophyly by Crous *et al.* (2000) and Goodwin *et al*. (2001), which were based on ITS data.

A re-examination of *T. fibrillosa*, the type species of *Teratosphaeria*, revealed several morphological features that characterise the majority of the taxa clustering in the clade, though several characters have been lost in some of the small-spored species. These characters are discussed below:

1. *Teratosphaeria fibrillosa* has a superficial stroma linking ascomata together, almost appearing like a spider's web on the leaf surface. Although this feature is not seen in other taxa in this clade, some species, such as *M. suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf. and *M. pseudosuberosa* Crous & M.J. Wingf. have a superficial stroma, into which the ascomata are inbedded (Crous 1998, Crous *et al*. 2006b).

2. Ascospores of *Teratosphaeria* become brown and verruculose while still in their asci. This feature is commonly observed in species such as *M. jonkershoekensis* P.S. van Wyk, Marasas & Knox-Dav., *M. alistairii* Crous, *M. mexicana* Crous, *M. maxii* Crous and *M. excentricum* Crous & Carnegie (Crous 1998, Crous & Groenewald 2006a, b, Crous *et al.* 2007b).

3. A few ascomata of *T. fibrillosa* were found to have some pseudoparaphysoidal remnants (cells to distinguish pseudoparaphyses), though they mostly disappear with age. This feature is rather uncommon, though pseudoparaphyses were observed in ascomata of *M. eucalypti* (Wakef.) Hansf.

4. Ascospores of *Teratosphaeria* were found to be covered in a mucous sheath, which is commonly observed in other taxa in this clade, such as *M. bellula* Crous & M.J. Wingf., *M. pseudocryptica* Crous*, M. suberosa, M. pseudosuberosa*, *M. associata* Crous & Carnegie*, M. dendritica* Crous & Summerell and *M. fimbriata* Crous & Summerell (Crous *et al*. 2004b, 2006b, 2007b). Re-examination of fresh collections also revealed ascospores of *M. cryptica* (Cooke) Hansf. and *M. nubilosa* (Cooke) Hansf. to have a weakly definable sheath. Germinating ascospores of species in this clade all exhibit a prominent mucoid sheath.

5. Asci of *T. fibrillosa* were observed to have a multi-layered endotunica, which, although not common, can be seen in species such as *M. excentrica, M. maxii, M. alistairii, M. pseudosuberosa, M. fimbriata* (Crous *et al.* 2006b, 2007b, Crous & Groenewald 2006a, b), and also *M. nubilosa*.

6. Finally, ascomata of *T. fibrillosa* and *T. proteae-arboreae* P.S. van Wyk, Marasas & Knox-Dav. have well-developed ostiolar periphyses, which are also present in species such as *M. suberosa*, *M. pseudosuberosa, M. maxii* and *T. microspora* Joanne E. Taylor & Crous (Crous 1998, Crous *et al.* 2004a, b, 2006b). Morphologically thus, the *Teratosphaeria* clade is distinguishable from *Mycosphaerella s. str*., though these differences are less pronounced in some of the smaller-spored species. Based on these distinct morphological features, as well as its phylogenetic position within the *Capnodiales*, a new family is herewith proposed to accommodate species of *Teratosphaeria*:

Teratosphaeriaceae Crous & U. Braun, **fam. nov.** MycoBank MB504464.

Ascomata pseudotheciales, superficiales vel immersa, saepe in stromate ex cellulis brunneis pseudoparenchymatibus disposita, globulares, uniloculares, papillata, apice ostiolato, periphysata, saepe cum periphysoidibus; tunica multistratosa, ex cellulis brunneis angularibus composita, strato interiore ex cellulis applanatis hyalinis; saepe cum pseudoparaphysibus subcylindricis, ramosis, septatis, anastomosibus. Asci fasciculati, octospori, bitunicati, saepe cum endotunica multistratosa. Ascosporae ellipsoideae-fusiformes vel obovoideae, 1-septatae, hyalinae, deinde pallide brunneae et verruculosae, saepe mucosae.

Ascomata pseudothecial, superficial to immersed, frequently situated in a stroma of brown pseudoparenchymatal cells, globose, unilocular, papillate, ostiolate, canal periphysate, with periphysoids frequently present; wall consisting of several layers of brown *textura angularis*; inner layer of flattened, hyaline cells. *Pseudoparaphyses* frequently present, subcylindrical, branched, septate, anastomosing. *Asci* fasciculate, 8-spored, bitunicate, frequently with multi-layered endotunica. *Ascospores* ellipsoid-fusoid to obovoid, 1-septate, hyaline, but becoming pale brown and verruculose, frequently covered in mucoid sheath.

Typus: *Teratosphaeria* Syd. & P. Syd., Ann. Mycol. 10: 39. 1912.

Teratosphaeria africana (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504466.

Fig. 3. *Teratosphaeria fibrillosa* (epitype material). A. Leaf spots. B. Subepidermal ascomata linked by means of stromatic tissue. C. Paraphyses among asci. D. Periphysoids. E. Ascospores becoming brown in asci. F–G. Multi-layered endotunica. H–K. Ascospores, becoming brown and verruculose. L–M. Germinating ascospores. Scale bars = 10 µm.

Basionym: *Mycosphaerella africana* Crous & M.J. Wingf., Mycologia 88: 450. 1996.

Teratosphaeria associata (Crous & Carnegie) Crous & U. Braun, **comb. nov.** MycoBank MB504467.
Basionym: *Mycosphaerella associata* Crous & Carnegie, Fungal Diversity 26: 159. 2007.

Teratosphaeria alistairii (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504468.
Basionym: *Mycosphaerella alistairii* Crous, in Crous & Groenewald, Fungal Planet, No. 4. 2006.
Anamorph: Batcheloromyces sp.

Teratosphaeria bellula (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504469.
Basionym: *Mycosphaerella bellula* Crous & M.J. Wingf., Mycotaxon 46: 20. 1993.

Teratosphaeria cryptica (Cooke) Crous & U. Braun, **comb. nov.** MycoBank MB504470.
Basionym: *Sphaerella cryptica* Cooke, Grevillea 20: 5. 1891.
≡ *Mycosphaerella cryptica* (Cooke) Hansf., Proc. Linn. Soc. New South Wales 81: 35. 1956.
Anamorph: ***Readeriella nubilosa*** (Ganap. & Corbin) Crous & U. Braun, **comb. nov.** MycoBank MB504471.
Basionym: *Colletogloeum nubilosum* Ganap. & Corbin, Trans. Brit. Mycol. Soc. 72: 237. 1979.
≡ *Colletogloeopsis nubilosum* (Ganap. & Corbin) Crous & M.J. Wingf., Canad. J. Bot. 75: 668. 1997.

Teratosphaeria dendritica (Crous & Summerell) Crous & U. Braun, **comb. nov.** MycoBank MB504472.
Basionym: *Mycosphaerella dendritica* Crous & Summerell, Fungal Diversity 26: 161. 2007.
Anamorph: ***Nothostrasseria dendritica*** (Hansf.) Nag Raj, Canad. J. Bot. 61: 25. 1983.
Basionym: Spilomyces dendriticus Hansf., Proc. Linn. Soc. New South Wales 81: 32. 1956.

Teratosphaeria excentrica (Crous & Carnegie) Crous & U. Braun, **comb. nov.** MycoBank MB504473.
Basionym: *Mycosphaerella excentrica* Crous & Carnegie, Fungal Diversity 26: 164. 2007.
Anamorph: ***Catenulostroma excentricum*** (B. Sutton & Ganap.) Crous & U. Braun, **comb. nov.** MycoBank MB504475.
Basionym: *Trimmatostroma excentricum* B. Sutton & Ganap., New Zealand J. Bot. 16: 529. 1978.

Teratosphaeria fibrillosa Syd. & P. Syd., Ann. Mycol. 10: 40. 1912.
≡ *Mycosphaerella fibrillosa* (Syd. & P. Syd.) Joanne E. Taylor & Crous, Mycol. Res. 107: 657. 2003.

Specimens examined: **South Africa**, Western Cape Province, Bains Kloof near Wellington, on living leaves of *Protea grandiflora*, 26 Feb. 1911, E.M. Doidge, **holotype** PREM; Stellenbosch, Jonkershoek valley, S33° 59' 44.7", E18° 58' 50.6", 1 Apr. 2007, on leaves of *Protea* sp., P.W. Crous & L. Mostert, **epitype designated here** CBS H-19913, culture ex-epitype CBS 121707 = CPC 13960.

Teratosphaeria fimbriata (Crous & Summerell) Crous & U. Braun, **comb. nov.** MycoBank MB504476.
Basionym: *Mycosphaerella fimbriata* Crous & Summerell, Fungal Diversity 26: 166. 2007.

Teratosphaeria flexuosa (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504477.
Basionym: *Mycosphaerella flexuosa* Crous & M.J. Wingf., Mycol. Mem. 21: 58. 1998.

Teratosphaeria gamsii (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504478.
Basionym: *Mycosphaerella gamsii* Crous, Stud. Mycol. 55: 113. 2006.

Teratosphaeria jonkershoekensis (P.S. van Wyk, Marasas & Knox-Dav.) Crous & U. Braun, **comb. nov.** MycoBank MB504479.
Basionym: *Mycosphaerella jonkershoekensis* P.S. van Wyk, Marasas & Knox-Dav., J. S. African Bot. 41: 234. 1975.

Teratosphaeria maxii (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504480.
Basionym: *Mycosphaerella maxii* Crous, in Crous & Groenewald, Fungal Planet No. 6. 2006.

Teratosphaeria mexicana (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504481.
Basionym: *Mycosphaerella mexicana* Crous, Mycol. Mem. 21: 81. 1998.

Teratosphaeria microspora Joanne E. Taylor & Crous, Mycol. Res. 104: 631. 2000.
≡ *Mycosphaerella microspora* (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous, Mycol. Res. 107: 657. 2003.
Anamorph: ***Catenulostroma microsporum*** (Joanne E. Taylor & Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504482.
Basionym: *Trimmatostroma microsporum* Joanne E. Taylor & Crous, Mycol. Res. 104: 631. 2000.

Teratosphaeria molleriana (Thüm.) Crous & U. Braun, **comb. nov.** MycoBank MB504483.
Basionym: *Sphaerella molleriana* Thüm., Revista Inst. Sci. Lit. Coimbra 28: 31. 1881.
≡ *Mycosphaerella molleriana* (Thüm) Lindau, Nat. Pfanzenfam. 1: 424. 1897.
= *Mycosphaerella vespa* Carnegie & Keane, Mycol. Res. 102: 1275. 1998.
= *Mycosphaerella ambiphylla* A. Maxwell, Mycol. Res. 107: 354. 2003.
Anamorph: ***Readeriella molleriana*** (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504484.
Basionym: *Colletogloeopsis molleriana* Crous & M.J. Wingf., Canad. J. Bot. 75: 670. 1997.

Teratosphaeria nubilosa (Cooke) Crous & U. Braun, **comb. nov.** MycoBank MB504485.
Basionym: *Sphaerella nubilosa* Cooke, Grevillea 19: 61. 1892.
≡ *Mycosphaerella nubilosa* (Cooke) Hansf., Proc. Linn. Soc. New South Wales 81: 36. 1965.
= *Mycosphaerella juvenis* Crous & M.J. Wingf., Mycologia 88: 453. 1996.

Teratosphaeria ohnowa (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504486.
Basionym: *Mycosphaerella ohnowa* Crous & M.J. Wingf., Stud. Mycol. 50: 206. 2004.

Teratosphaeria parkiiaffinis (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504487.
Basionym: *Mycosphaerella parkiiaffinis* Crous & M.J. Wingf., Fungal Diversity 26: 168. 2007.

Teratosphaeria parva (R.F. Park & Keane) Crous & U. Braun, **comb. nov.** MycoBank MB504488.
Basionym: *Mycosphaerella parva* R.F. Park & Keane, Trans. Brit. Mycol. Soc. 79: 99. 1982.
= *Mycosphaerella grandis* Carnegie & Keane, Mycol. Res. 98: 414. 1994.

Teratosphaeria perpendicularis (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504489.
Basionym: *Mycosphaerella perpendicularis* Crous & M.J. Wingf., Stud. Mycol. 55: 113. 2006.

Teratosphaeria pluritubularis (Crous & Mansilla) Crous & U. Braun, **comb. nov.** MycoBank MB504490.
Basionym: *Mycosphaerella pluritubularis* Crous & Mansilla, Stud. Mycol. 55: 114. 2006.

Teratosphaeria pseudafricana (Crous & T.A. Cout.) Crous & U. Braun, **comb. nov.** MycoBank MB504491.
Basionym: *Mycosphaerella pseudafricana* Crous & T.A. Cout., Stud. Mycol. 55: 115. 2006.

Teratosphaeria pseudocryptica (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504492.
Basionym: *Mycosphaerella pseudocryptica* Crous, Stud. Mycol. 55: 116. 2006.
Anamorph: ***Readeriella*** sp.

Teratosphaeria pseudosuberosa (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504493.
Basionym: *Mycosphaerella pseudosuberosa* Crous & M.J. Wingf., Stud. Mycol. 55: 118. 2006.
Anamorph: ***Catenulostroma*** sp.

Teratosphaeria quasicercospora (Crous & T.A. Cout.) Crous & U. Braun, **comb. nov.** MycoBank MB504494.
Basionym: *Mycosphaerella quasicercospora* Crous & T.A. Cout., Stud. Mycol. 55: 119. 2006.

Teratosphaeria readeriellophora (Crous & Mansilla) Crous & U. Braun, **comb. nov.** MycoBank MB504495.
Basionym: *Mycosphaerella readeriellophora* Crous & Mansilla, Stud. Mycol. 50: 207. 2004.
Anamorph: ***Readeriella readeriellophora*** Crous & Mansilla, Stud. Mycol. 50: 207. 2004. Fig. 18.

Teratosphaeria secundaria (Crous & Alfenas) Crous & U. Braun, **comb. nov.** MycoBank MB504496.
Basionym: *Mycosphaerella secundaria* Crous & Alfenas, Stud. Mycol. 55: 122. 2006.

Teratosphaeria stramenticola (Crous & Alfenas) Crous & U. Braun, **comb. nov.** MycoBank MB504497.
Basionym: *Mycosphaerella stramenticola* Crous & Alfenas, Stud. Mycol. 55: 123. 2006.

Teratosphaeria suberosa (Crous, F.A. Ferreira, Alfenas & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504498.
Basionym: *Mycosphaerella suberosa* Crous, F.A. Ferreira, Alfenas & M.J. Wingf., Mycologia 85: 707. 1993.

Teratosphaeria suttonii (Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504499.
Basionym: *Mycosphaerella suttonii* Crous & M.J. Wingf. (*suttoniae*), Canad. J. Bot. 75: 783. 1997.
Anamorph: ***Readeriella epicoccoides*** (Cooke & Massee) Crous & U. Braun, **comb. nov.** MycoBank MB504500.
Basionym: *Cercospora epicoccoides* Cooke & Massee apud Cooke, Grevillea 19: 91. 1891.
≡ *Phaeophleospora epicoccoides* (Cooke & Massee) Crous, F.A. Ferreira & B. Sutton, S. African J. Bot. 63: 113. 1997.
≡ *Kirramyces epicoccoides* (Cooke & Massee) J. Walker, B. Sutton & Pascoe, Mycol. Res. 96: 919. 1992.
= *Hendersonia grandispora* McAlp., Proc. Linn. Soc. New South Wales 28: 99. 1903.
= *Phaeoseptoria eucalypti* Hansf., Proc. Linn. Soc. New South Wales 82: 225. 1957.
= *Phaeoseptoria luzonensis* T. Kobayashi, Trans. Mycol. Soc. Japan 19: 377. 1978.
Synanamorph: ***Pseudocercospora*** sp.

Teratosphaeria toledana (Crous & Bills) Crous & U. Braun, **comb. nov.** MycoBank MB504501.
Basionym: *Mycosphaerella toledana* Crous & Bills, Stud. Mycol. 50: 208. 2004.
Anamorph: ***Readeriella toledana*** (Crous & Bills) Crous & U. Braun, **comb. nov.** MycoBank MB504502.
Basionym: *Phaeophleospora toledana* Crous & Bills, Stud. Mycol. 50: 208. 2004.

Key to treated anamorph genera of *Teratosphaeria* (*Teratosphaeriaceae*)

1. Hyphae submerged to superficial, disarticulating into arthroconidia 2
1. Hyphae not disarticulating into arthroconidia 3

2. Mature, brown hyphae disarticulating into thick-walled, spherical, smooth to verruculose 0(–2) transversely septate, brown conidia ..... ***Pseudotaeniolina*** (= *Friedmanniomyces*)
2. Hyphae superficial, brown to green-brown, smooth, disarticulating to form pale brown, cylindrical, 0–3-septate conidia with subtruncate ends, frequently with a *Readeriella* synanamorph ..... ***Cibiessia***

3. Hyphal ends forming endoconidia; hyphae pale to medium brown, verruculose, end cells dividing into several brown, verruculose, thick-walled, ellipsoid to obovoid endoconidia ..... ***Phaeothecoidea***
3. Endoconidia absent 4

4. Conidiogenous cells integrated in hyphae; well-developed conidiomata or long, solitary, macronematous, terminally penicillate conidiophores absent 5
4. Conidiomata well-developed or with long, solitary, terminally penicillate conidiophores 7

5. Conidia in chains, holoblastic, pseudocladosporium-like in morphology, but scars and hila not excessively thickened, nor refractive, producing chlamydospores in culture; species are mostly heat resistant ..... ***Devriesia***
5. Conidia solitary on indistict to well defined phialides on hyphae 6

6. Conidiogenous cells integrated in the distal ends of hyphae; conidia thick-walled, brown, smooth, 1-septate ..... ***Capnobotryella***

6. Conidiophores short and frequently reduced to conidiogenous cells that proliferate percurrently via wide necks, giving rise to hyaline, 0(–2)-septate, broadly ellipsoidal conidia ..... ***Hortaea***

7. Conidia brown, with hyaline basal appendages; conidiomata pycnidial, conidiogenous cells phialidic, but also percurrent, subhyaline ..... ***Nothostrasseria***
7. Conidia brown, but basal appendages lacking, amero- to scolecospores 8

8. Conidiomata pycnidial to acervular 9
8. Conidiomata not enclosed by host tissue, fasciculate to sporodochial or solitary, hyphomycetous 10

9. Conidia solitary, dry, without mucilaginous sheath ..... ***Readeriella***
9. Conidia catenulate, with persistant mucilaginous sheath ..... ***Staninwardia***

10. Conidiophores usually solitary, rarely densely fasciculate to synnematous (*in vivo*), penicillate, with a branched, apical conidiogenous apparatus giving rise to ramoconidia and branched chains of secondary conidia; scars not to slightly thickened and darkened-refractive ..... ***Penidiella***
10. Conidiophores not penicillate, without a branched conidiogenous apparatus, *in vivo* fasciculate to sporodochial 11

11. Biotrophic; fruiting composed of sporodochia and radiating layers of hyphae arising from the stromata, conidiophores arising from superficial sporodochia and radiating hyphae, conidiogenous cells unilocal, with conspicuous annellations, conidia solitary or in fragile disarticulating chains, aseptate or transversely 1–3-septate, usually with distinct frills, secession rhexolytic ..... ***Batcheloromyces***
11. Biotrophic, leaf-inhabiting, with distinct, subepidermal to erumpent, well-developed sporodochia, or saxicolous, saprobic, sometimes causing opportunistic human infections; radiating layers of hyphae arising from sporodochia; conidiogenous cells without annellations; conidia in true simple or branched basipetal chains, transversely 1- to pluriseptate or with longitudinal and oblique septa (dictyosporous), occasionally distoseptate ..... ***Catenulostroma***

To explain the arguments behind the selection and synonymies of some of these anamorphic genera, they are briefly discussed below:

Acidomyces Baker *et al*., Appl. Environ. Microbiol. 70: 6270. 2004. (nom. inval.)

Type species: *Acidomyces richmondensis* Baker *et al*., Appl. Environ. Microbiol. 70: 6270. 2004. (nom. inval.)

Notes: The genus presently clusters among isolates in the *Teratosphaeria* clade based on sequences deposited in GenBank. *Acidomyces* lacks a Latin description and holotype specimen, and is thus invalidly described. The genus, which was distinguished from other taxa based on its DNA phylogeny (*Dothideomycetes*), forms filamentous hyphae with disarticulating cells. It is unclear how it differs from *Friedmanniomyces* Onofri and *Pseudotaeniolina* J.L. Crane & Schokn.

Batcheloromyces Marasas, P.S. van Wyk & Knox-Dav., J. S. African Bot. 41: 41. 1975.

Type species: *Batcheloromyces proteae* Marasas, P.S. van Wyk & Knox-Dav., J. S. African Bot. 41: 43. 1975.

Description: Crous *et al.* (2004a; figs 4–26).

Notes: *Batcheloromyces* is presently circumscribed as a genus that forms emergent hyphae, giving rise to superficial sporodochial plates, forming brown, verrucose, erect conidiophores that proliferate holoblastically, with ragged percurrent proliferations that become visible with age. Conidia are produced singly or in fragile, disarticulating chains, are brown, thick-walled, 0–3 transversely euseptate (though at times they appear as distoseptate). The genus *Batcheloromyces* has in recent years been confused with *Stigmina* (Sutton & Pascoe 1989) on the basis that some collections showed conidiophores to give rise to solitary conidia only, though conidial catenulation was clearly illustrated by Taylor *et al*. (1999). In culture colonies tend to sporulate in a slimy mass (on OA), though a synanamorph can be seen (in *B. leucadendri*, Fig. 4) to sporulate via holoblastic conidiogenesis on hyphal tips of the aerial mycelium, forming elongate-globose to ellipsoid, muriformly septate, thick-walled conidia, that occur in clusters.

The finding that *Stigmina s. str*. [based on *S. platani* (Fuckel) Sacc., the type species] is a generic synonym of *Pseudocercospora* Speg. (Crous *et al*. 2006a), and that the type species of *Trimmatostroma* (*T. salicis*, Fig. 5) belongs to the *Helotiales* (Fig. 1), raises the question of where to place stigmina- and trimmatostroma-like anamorphs that reside in the *Teratosphaeria* clade. Although the stigmina-like species can be accommodated in *Batcheloromyces* (see Sutton & Pascoe 1989), a new genus is required for *Teratosphaeria* anamorphs that have a trimmatostroma-like morphology. The recognition of *Batcheloromyces* and the introduction of a new anamorph genus for trimmatostroma-like anamorphs of *Teratosphaeria* are also morphologically justified. *Batcheloromyces* is easily distinguishable from *Stigmina s. str.* by its special structure of the fruiting body, composed of sporodochia and radiating layers of hyphae arising from the sporodochia and the conidia often formed in delicate disarticulating chains. Trimmatostroma-like anamorphs of *Teratosphaeria* are morphologically also sufficently distinct from *Trimmatostroma s. str.* (see notes under *Catenulostroma* Crous & U. Braun) as well as *Batcheloromyces* (see key above).

Batcheloromyces eucalypti (Alcorn) Crous & U. Braun, **comb. nov.** MycoBank MB504503.
Basionym: *Stigmina eucalypti* Alcorn, Trans. Brit. Mycol. Soc. 60: 151. 1973.

Capnobotryella Sugiy., in Sugiyama, *Pleomorphic Fungi: The Diversity and its Taxonomic Implications* (Tokyo): 148. 1987.

Type species: *Capnobotryella renispora* Sugiy., in Sugiyama, *Pleomorphic Fungi: The Diversity and its Taxonomic Implications* (Tokyo): 148. 1987.

Description: Sugiyama & Amano (1987, figs 7.5–7.8).

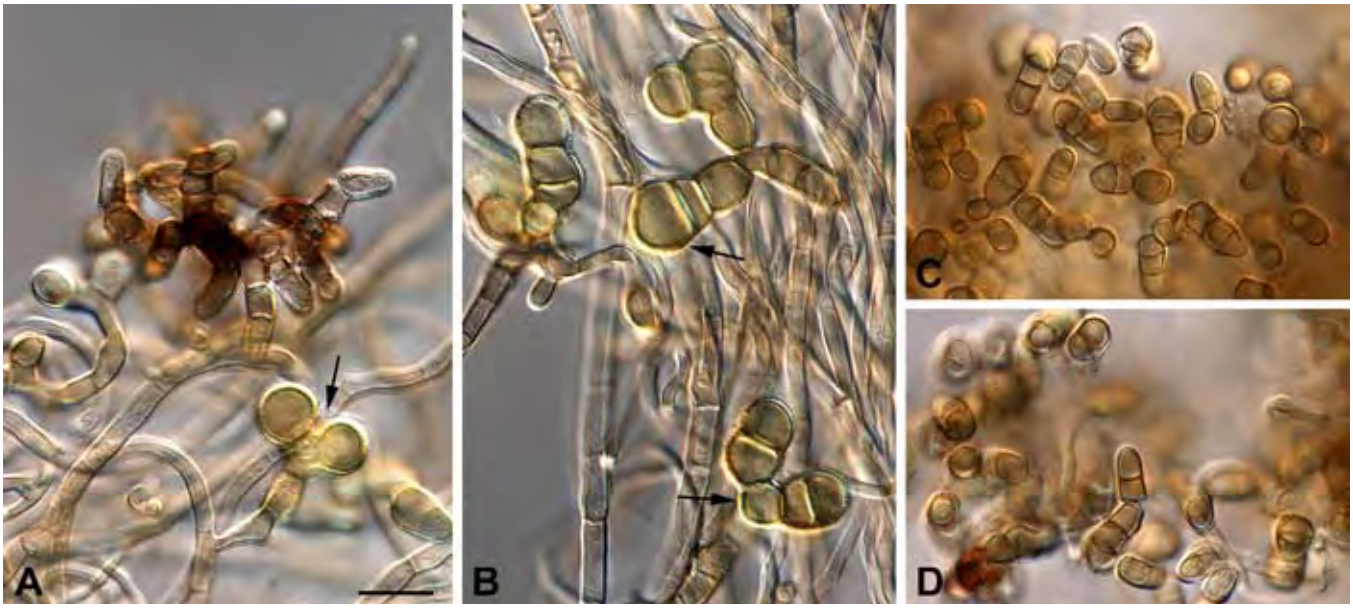

Fig. 4. *Batcheloromyces leucadendri in vitro*. A–B. *Batcheloromyces* state with synanamorph (arrows). C–D. Conidia occurring solitary or in short chains. Scale bar = 10 µm.

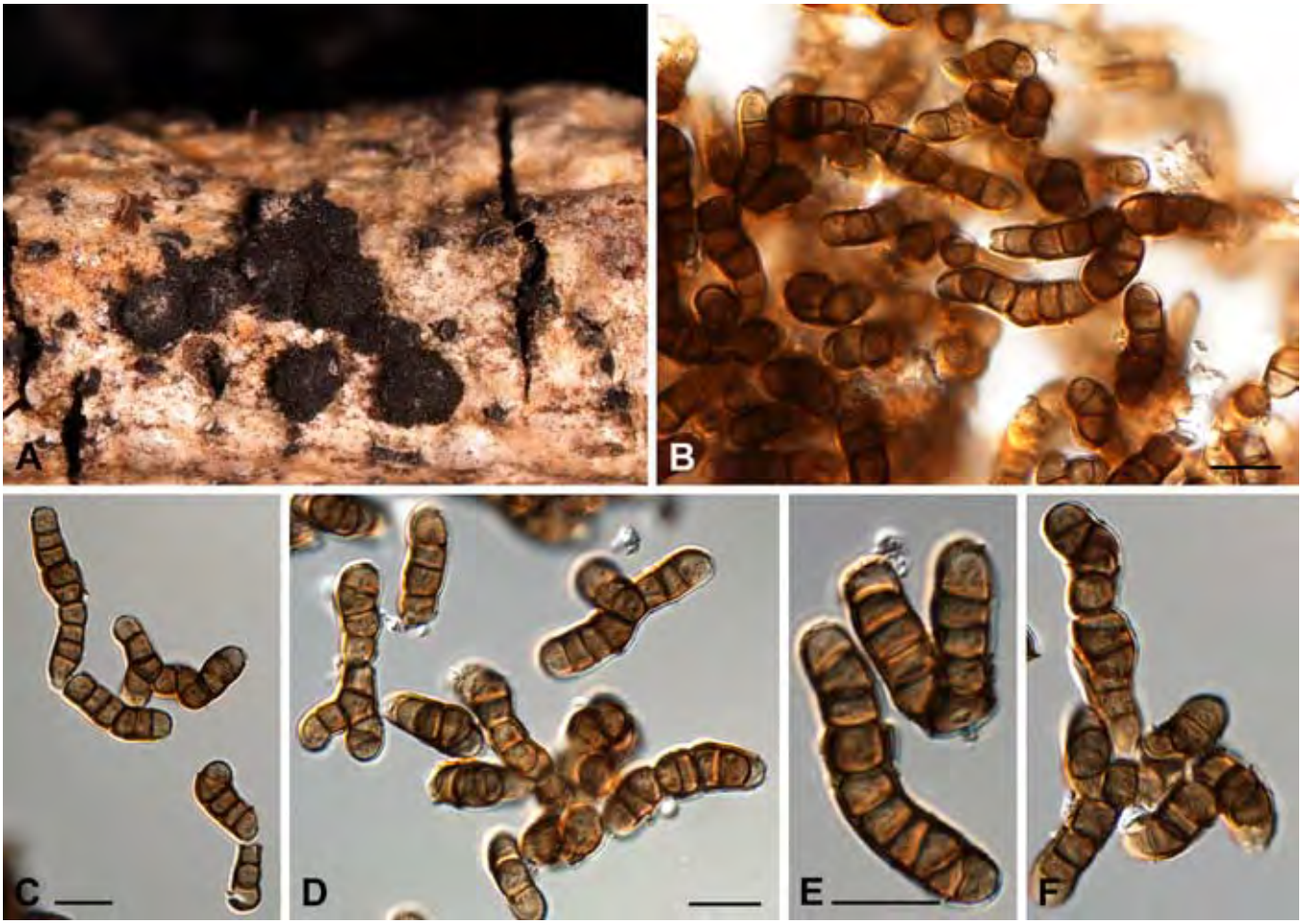

Fig. 5. *Trimmatostroma salicis*. A. Sporodochia on twig. B–E. Chains of disarticulating conidia. Scale bars = 10 µm.

Notes: The genus forms brown, septate, thick-walled hyphae, with ellipsoidal, 0–1-septate conidia forming directly on the hyphae, via minute phialides. Hambleton *et al*. (2003) also noted the occurrence of endoconidiation.

Catenulostroma Crous & U. Braun, **gen. nov.** MycoBank MB504474.

Etymology: Named after its catenulate conidia, and stromata giving rise to sporodochia.

Hyphomycetes. Differt a Trimmatostromate habitu phytoparasitico, maculis formantibus, conidiophoris saepe fasciculatis, per stoma emergentibus vel habitu saxiphilo-saprophytico, interdum sejunctis ex mycosibus humanis.

Habit plant pathogenic, leaf-spotting or saxicolous-saprobic, occasionally isolated from opportunistic human mycoses. *Mycelium* internal and external; hyphae dark brown, septate, branched. *Conidiomata in vivo* vary from acervuli to sporodochia or fascicles of conidiophores arising from well-developed or reduced, pseudoparenchymatal stromata. *Setae* and *hyphopodia* absent. *Conidiophores* arising from hyphae or stromata, solitary, fasciculate to sporodochial, in biotrophic, plant pathogenic species emerging through stomata, little differentiated, semimacronematous, branched or not, continuous to septate, brown, smooth to verruculose. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, holoblastic-thalloblastic, meristematic, unilocal, delimitation of conidium by a single septum with retrogressive delimitation of next conidium giving an unconnected chain of conidia, brown, smooth to verruculose, conidiogenous scars (conidiogenous loci) inconspicuous, truncate, neither thickened nor darkened. *Conidia* solitary or usually forming simple to branched basipetal chains of transversely to muriformly eu- or distoseptate, 1– to multiseptate, brown, smooth, verruculose to verrucose conidia, conidial secession schizolytic.

Type species: *Catenulostroma protearum* (Crous & M.E. Palm) Crous & U. Braun, comb. nov.

Description: Crous & Palm (1999), Crous *et al*. (2004a; figs 364–365).

Notes: *Catenulostroma* contains several plant pathogenic species previously placed in *Trimmatostroma*, a morphologically similar but, based on its type species, phylogenetically distinct genus belonging to *Helotiales* (Fig. 1). *Trimmatostroma s. str.* is well-distinguished from most *Catenulostroma* species by being saprobic, living on twigs and branches of woody plants, or occasionally isolated from leaf litter, i.e., they are not associated with leaf spots. The conidiomata of *Trimmatostroma* species are subepidermal, acervular-sporodochial with a well defined wall of *textura angularis*, little differentiated, micronematous conidiophores giving rise to long chains of conidia that disarticulate at the surface to form a grey-black to brown powdery mass. The generic affinity of other species assigned to *Trimmatostroma*, e.g. those having a lichenicolous habit, is unresolved.

Trimmatostroma abietis Butin & Pehl (Butin *et al*. 1996) clusters together with the plant pathogenic *Catenulostroma* species, but differs from these species in having a more complex ecology. *Trimmatostroma abietis* is usually foliicolous on living or necrotic conifer needles on which characteristic acervuli to sporodochia with densely arranged, fasciculate fertile hyphae are formed, comparable to the fasciculate conidiomata of the plant pathogenic species of *Catenulostroma* (Butin *et al*. 1996: 205, fig. 1). Although not discussed by Butin *et al*. (1996), *T. abietis* needs to be compared to *T. abietina* Doherty, which was orginally described from *Abies balsamea* needles collected in Guelph, Canada (Doherty 1900). Morphologically the two species appear to be synonymous, except for reference to muriformly septate conidia, which is a feature not seen *in vivo* in the type of *T. abietis*. Furthermore, as this is clearly a species complex, this matter can only be resolved once fresh Canadian material has been collected to serve as epitype for *T. abietina*.

Isolates from stone, agreeing with *T. abietis* in cultural, morphological and physiological characteristics, have frequently been found (Wollenzien *et al*. 1995, Butin *et al*. 1996, Gorbushina *et al*. 1996, Kogej *et al*. 2006, Krumbein *et al*. 1996). Furthermore, isolates from humans (ex skin lesions and ex chronic osteomycelitis of human patients) and *Ilex* leaves are known (Butin *et al*. 1996). De Hoog *et al*. (1999) included strains of *T. abietis* from stone, man and *Ilex* leaves in molecular sequence analyses and demonstrated their genetical identity based on 5.8S rDNA and ITS2 data, but strains from conifer needles were not included. Furthermore, we consider *T. abietis*, as presently defined, to represent a species complex, with Dutch isolates from *Pinus* again appearing distinct from German *Abies* isolates, suggesting that different conifer genera could harbour different *Catenulostroma* species. Isolates from stone form stromatic, durable microcolonies, which are able to grow under extreme xerophilic environmental conditions. Cultural growth resembles that of other meristematic black yeasts (Butin *et al*. 1996, Kogej *et al*. 2006). Another fungus isolated from stone in Germany is *in vitro* morphologically close to *C. abietis*, but differs in forming conidia with oblique septa. Furthermore, a human pathogenic isolate from Africa clusters together with other *Catenulostroma* species. The habit and origin of this human pathogenic fungus in nature and its potential morphology on "natural" substrates, which typically deviates strongly from the growth *in vitro*, are still unknown. However, *C. abietis*, usually growing as a foliicolous and saxicolous fungus, has already shown the potential ability of *Catenulostroma* species to cause opportunistic human infections.

Key to *Catenulostroma* species

1. Conidia formed in basipetal chains, smooth, 4-celled, consisting of two basal cells with truncate lateral sides, each giving rise to a secondary globose apical cell, that can extend and develop additional septa, appearing as two lateral arms ..... ***C. excentricum***
1. Conidia variable in shape, but without two basal cells giving rise to two lateral arms 2

2. Conidia smooth or almost so, at most very faintly rough-walled; usually foliicolous on conifer needles or saxicolous, forming stromatic, xerophilic durable microcolonies on stone, occasionally causing opportunistic human infections 3
2. Conidia distinctly verruculose to verrucose; plant pathogenic, forming leaf spots 5

3. Conidia (8–)20–35(–60) × 4–5(–7) µm, 1–10-septate ..... ***C. chromoblastomycosum***
3. Conidia much shorter, 8–20 µm long, 0–5-septate 4

4. Conidia 0–5 times transversely septate, mostly two-celled; usually foliicolous on conifer needles or saxicolous ..... ***C. abietis***
4. Conidia 2–4 times transversely septate and often with 1–2 oblique septa; isolated from stone ..... ***C. germanicum***

5. Conidia rather broad, usually wider than 10 µm 6

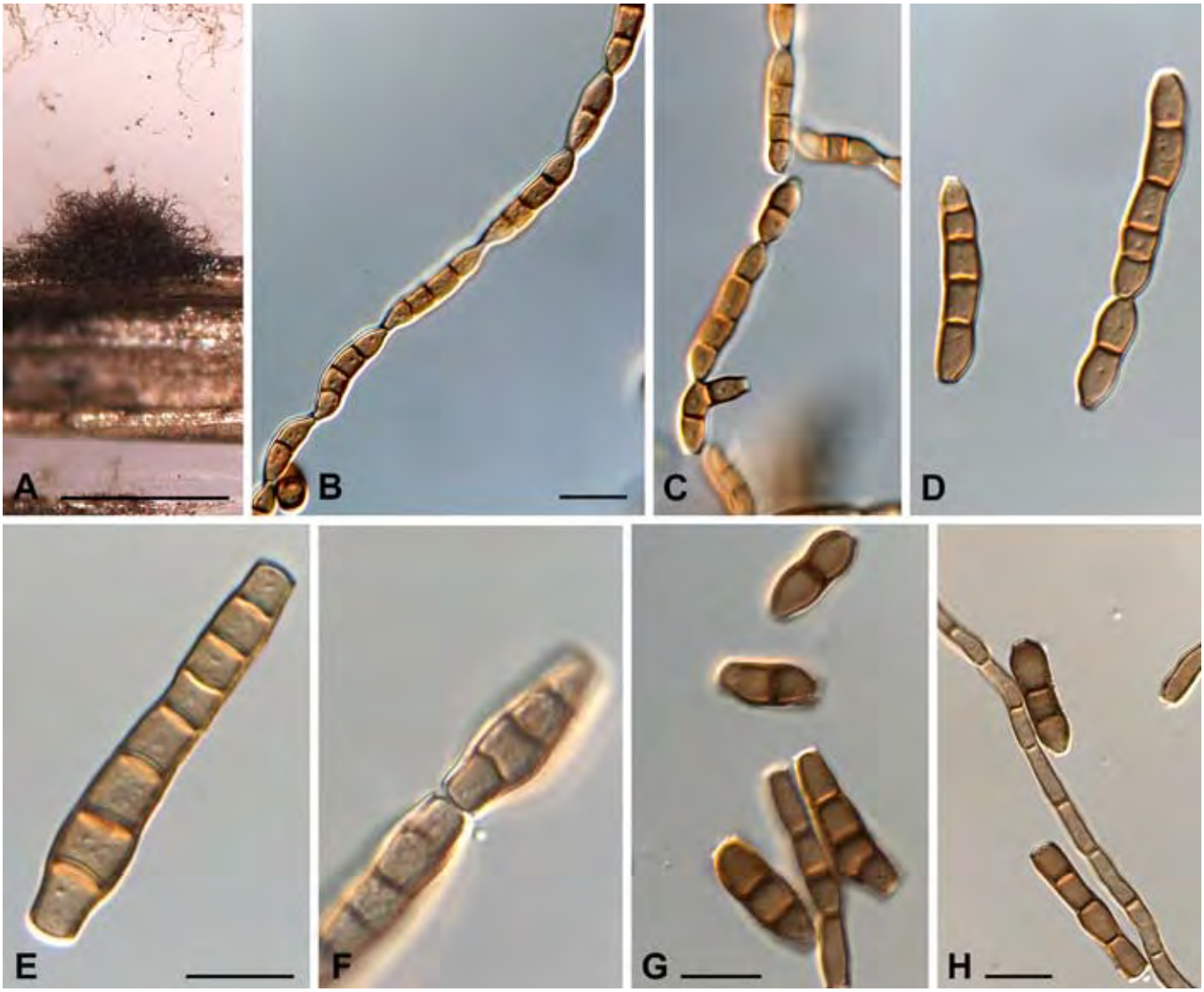

Fig. 6. *Catenulostroma chromoblastomycosum* (type material). A. Sporodochium on pine needle *in vitro*. B–H. Chains of disarticulating conidia. Scale bars: A = 350, B, E, G, H = 10 µm.

5. Conidia narrower, width below 10 µm 7

6. Conidia distoseptate, rather long, (12–)25–35(–45) × (7–)10–15(–25) µm; conidiomata large, up to 250 µm diam, on *Protea anceps* ..... ***C. protearum***
6. Conidia euseptate, shorter, (9–)16–20(–36) × (10–)14–18(–27) µm; sporodochia 90–100 × 40–80 µm; on *Protea grandiceps* ..... ***C. elginense***

7. Conidia 1- to multiseptate, (10–)15–17(–23) × (5–)6.5–7(–9) µm; on various *Proteaceae* ..... ***C. macowanii***
7. Conidia *in vivo* predominantly 1-septate, (8–)13–15(–21) × (3.5–)5.5–6(–8) µm; on *Protea cynaroides* ..... ***C. microsporum* (*Teratosphaeria microspora*)**

Catenulostroma abietis (Butin & Pehl) Crous & U. Braun, **comb. nov.** MycoBank MB504504.
Basionym: *Trimmatostroma abietis* Butin & Pehl, Antonie van Leeuwenhoek 69: 204. 1996.

Notes: *Catenulostroma abietis* needs to be compared to *Trimmatostroma abietina* Doherty (*Abies balsamea* needles Canada), which is either an older name for this species, or a closely related taxon. Presently *T. abietina* is not known from culture, and needs to be recollected.

Catenulostroma chromoblastomycosum Crous & U. Braun, **sp. nov.** MycoBank MB504505. Fig. 6.

Etymology: Named after the disease symptoms observed due to opportunistic human infection.

Differt a C. abieti et C. germanico conidiis longioribus, (8–)20–35(–60) × 4–5(–7) µm, 1–10-septatis.

Description based on cultures sporulating on WA supplemented with sterile pine needles. *Mycelium* consisting of branched, septate, smooth to finely verruculose, medium to dark brown, thick-walled, 3–4 µm wide hyphae. *Conidiomata* brown, superficial,

Fig. 7. *Catenulostroma germanicum* (type material). A–D. Chains of disarticulating conidia *in vitro*. Scale bars = 10 µm.

sporodochial, up to 350 µm diam. *Conidiophores* reduced to inconspicuous conidiogenous loci on hyphae, 2–4 µm wide, neither darkened nor thickened or refractive. *Conidia* occurring in branched chains, that tend to remain attached to each other, subcylindrical with subtruncate ends, straight to slightly curved, (8–)20–35(–60) × 4–5(–7) µm, 1–10-septate, medium brown, smooth to finely verruculose.

Cultural characteristics: Colonies on PDA erumpent, spreading, slow growing, with sparse to moderate aerial mycelium and smooth, irregular, submerged margins; greenish black (surface).

Specimen examined: **Zaire**, Pawa, isolated from man with chromoblastomycosis, Mar. 1997, V. de Brouwere, **holotype** CBS H-19935, culture ex-type CBS 597.97.

Notes: *Catenulostroma chromoblastomycosum* was originally identified as an isolate of *Stenella araguata* Syd. The latter fungus is morphologically distinct, however, having much shorter and narrower conidia, formed in acropetal chains, as well as quite different conidiogenous loci and conidial hila which are small, thickened and darkened.

Catenulostroma elginense (Joanne E. Taylor & Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504506.
Basionym: *Trimmatostroma elginense* Joanne E. Taylor & Crous, Mycol. Res. 104: 633. 2000.

Catenulostroma excentricum, see ***Teratosphaeria excentrica***.

Catenulostroma germanicum Crous & U. Braun, **sp. nov.** MycoBank MB504507. Fig. 7.

Etymology: Named after the geographic location of its type strain in Germany.

Differt a C. abieti conidiis 1–2 oblique septatis.

Mycelium consisting of branched, septate, smooth, pale to medium brown, 2–4 µm wide hyphae, giving rise to conidial chains. *Conidiophores* integrated, subcylindrical, branched or not, septate, little differentiated, micronematous, 3–5 µm wide, 3- to multiseptate, medium brown, thick-walled; conidiogenous cells integrated, terminal, inconspicuous, unilocal, conidiogenous loci

inconspicuous. *Conidia* in simple or branched basipetal chains, subcylindrical, straight to flexuous, (8–)10–15(–20) × 4–5(–6) µm, 2–4 transversely septate or with 1–2 oblique septa, medium to dark brown, thick-walled, smooth.

Cultural characteristics: Colonies on OA erumpent, spreading, with even, smooth margins and sparse to moderate aerial mycelium; olivaceous-grey, with iron-grey margins (surface). Colonies reaching 12 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Germany** (former West-Germany), isolated from stone, Oct. 1988, J. Kuroczkin, **holotype** CBS H-19936, culture ex-type CBS 539.88.

Notes: *Catenulostroma germanicum* was originally deposited as *Taeniolina scripta* (P. Karst.) P.M. Kirk. It is clearly distinct, however, as the latter fungus forms intricate, branched, brown conidia (Kirk 1981), unlike those of *C. germanicum*. Phylogenetically *C. germanicum* forms part of the *C. abietis* species complex.

Catenulostroma macowanii (Sacc.) Crous & U. Braun, **comb. nov.** MycoBank MB504508.
Basionym: *Coniothecium macowanii* Sacc., Syll. Fung. 4: 512. 1886.

≡ *Coniothecium punctiforme* G. Winter, Hedwigia 24: 33. 1885, non *C. punctiforme* Corda, *Icones Fungorum* (Prague) 1: 2. 1837.
≡ *Trimmatostroma macowanii* (Sacc.) M.B. Ellis, *More Dematiacous Hyphomycetes*: 29. 1976.

Catenulostroma microsporum, see ***Teratosphaeria microspora***.

Catenulostroma protearum (Crous & M.E. Palm) Crous & U. Braun, **comb. nov.** MycoBank MB504509.
Basionym: *Trimmatostroma protearum* Crous & M.E. Palm, Mycol. Res. 103: 1303. 1999.

Cibiessia Crous, Fungal Diversity 26: 151. 2007.

Type species: Cibiessia dimorphospora Crous & C. Mohammed, Fungal Diversity 26: 151. 2007.

Description: Crous *et al.* (2007b; figs 3–5).

Notes: The genus *Cibiessia* was introduced to accommodate species with chains of disarticulating conidia (arthroconidia). Some species have been shown to have a *Readeriella* synanamorph.

Devriesia Seifert & N.L. Nick., Can. J. Bot. 82: 919. 2004.

Type species: *Devriesia staurophora* (W.B. Kendr.) Seifert & N.L. Nick., Canad. J. Bot. 82: 919. 2004.

Description: Seifert *et al*. (2004; figs 2–42).

Notes: The genus is characterised by producing chains of pale brown, subcylindrical to fusiform, 0–1-septate conidia with somewhat thickened, darkened hila, forming chlamydospores in culture, and being heat resistant. Morphologically they resemble taxa placed in *Pseudocladosporium* U. Braun (= *Fusicladium* Bonord.; *Venturiaceae*), though phylogenetically *Devriesia* is not allied to this family.

Hortaea Nishim. & Miyaji, Jap. J. Med. Mycol. 26: 145. 1984.

Type species: *Hortaea werneckii* (Horta) Nishim. & Miyaji, Jap. J. Med. Mycol. 26: 145. 1984.

Description: de Hoog *et al*. (2000, illust. p. 721).

Notes: The genus forms brown, septate, thick-walled hyphae, with ellipsoidal, 0–1-septate (becoming muriformly septate), hyaline to pale brown conidia forming directly on the hyphae, via phialides with percurrent proliferation. Isolates of *H. werneckii* are restricted to tropical or subtropical areas, where they occur as halophilic saprobes, frequently being associated with *tinea nigra* of humans (de Hoog *et al*. 2000). The generic distinction with *Capnobotryella* is less clear, except that the latter tends to have darker, thick-walled conidia, and reduced, less prominent phialides.

Penidiella Crous & U. Braun, **gen. nov.** MycoBank MB504463.

Etymology: Named after its penicillate conidiophores.

Differt a Periconiellae conidiophoris apice penicillato ex cellulis conidiogenis et ramoconidiis compositis, cellulis conidiogenis saepe 1–3(–4) locis conidiogenis, terminalibus vel subterminalibus, subdenticulatis, non vel subincrassatis, non vel leviter fuscatis-refractivis, ramoconidiis praesentibus, saepe numerosis, conidiis ramicatenatis.

Mycelium consisting of branched, septate, smooth to verruculose, subhyaline to pale brown hyphae. *Conidiophores* macronematous, occasionally also with some micronematous conidiophores; macronematous conidiophores arising from superficial mycelium or stromata, solitary, fasciculate or in synnemata, erect, brown, thin- to thick-walled, smooth to finely verruculose; terminally penicillate, branched terminal part consisting of a conidiogenous apparatus composed of a series of conidiogenous cells and/or ramoconidia. *Conidiogenous cells* integrated, terminal, intercalary or pleurogenous, unbranched, pale to medium brown, smooth to finely verruculose, tapering to a flattened or rounded apical region or tips slightly inflated, polyblastic, sympodial, giving rise to a single or several sets of ramoconidia on different levels; with relatively few conidiogenous loci, 1–3(–4), terminal or subterminal, subdenticulate, denticle-like loci usually conical, terminally truncate, usually unthickened or at most very slightly thickened, not to slightly darkened or somewhat refractive. *Conidia* in branched acropetal chains. *Ramoconidia* 0–1-septate, pale to medium brown, smooth to verruculose, thin-walled, ellipsoidal, obovoid, fusiform, subcylindrical to obclavate; conidia subcylindrical, fusiform to ellipsoid-ovoid, 0–1-septate, pale olivaceous to brown, smooth to verruculose, thin-walled, catenate; hila truncate, unthickened or almost so, barely to somewhat darkened-refractive.

Type species: *Penidiella columbiana* Crous & U. Braun, sp. nov.

Notes: Three ramichloridium-like genera cluster within *Capnodiales*, namely *Periconiella* Sacc. [type: *P. velutina* (G. Winter) Sacc.], *Ramichloridium* Stahel ex de Hoog [type: *R. apiculatum* (J.H. Mill., Giddens & A.A. Foster) de Hoog] and *Penidiella* [type: *P. columbiana* Crous & U. Braun]. All three genera have brown, macronematous conidiophores with similar conidial scars. Within this complex, *Ramichloridium* is distinct in having a prominent rachis giving rise to solitary conidia. *Periconiella* and *Penidiella* are branched in the apical part of their conidiophores, and lack a rachis. In *Periconiella* conidia are solitary or formed in short, mostly simple chains, ramoconidia are lacking. The apical conidiogenous apparatus is composed of conidiogenous cells or branches with integrated, usually terminal conidiogenous cells, which are persistent. The conidiogenous cells are subcylindrical to somewhat clavate, usually not distinctly attenuated towards the tip, and have several, often numerous loci, aggregated or spread over the whole cell, terminal to usually lateral, flat, non-protuberant, not denticle-like, usually distinctly thickened and darkened, at least at the rim. In contrast, *Penidiella* has a quite distinct branching

system, consisting of a single terminal conidiogenous cell giving rise to several ramoconidia that form secondary ramoconidia, etc., or the branched apparatus is composed of several terminal and sometimes lateral conidiogenous cells giving rise to sequences of ramoconidia (conidiogenous cells and ramoconidia are often barely distinguishable, with conidiogenous cells disarticulating, becoming ramoconidia). The branched apparatus may be loose to dense, metula-like. The conidiogenous cells have only few, usually 1–3 (–4), terminal or subterminal subdenticulate loci, and ramoconidia are prominent and numerous, giving rise to branched chains of secondary conidia with flat-tipped hila. Some species of *Penidiella* with compact, metula-like branched apices are morphologically close to *Metulocladosporiella* Crous, Schroers, J.Z. Groenew., U. Braun & K. Schub. (Crous *et al*. 2006d). This genus encompasses two species of banana diseases belonging to *Herpotrichiellaceae* (*Chaetothyriales*), characterised by having conidiophore bases with rhizoid hyphal appendages and abundant micronematous conidiophores. *Penidiella* species with less pronounced penicillate apices, e.g. *P. strumelloidea* (Milko & Dunaev) Crous & U. Braun, are comparable with species of the genus *Pleurotheciopsis* B. Sutton (see Ellis 1976). The latter genus is distinct in having unbranched, often percurrently proliferating conidiophores, lacking ramoconidia and colourless conidia formed in simple chains.

Cladosporium helicosporum R.F. Castañeda & W.B. Kendr. (Castañeda *et al*. 1997) is another penidiella-like fungus with terminally branched conidiophores, subdenticulate conidiogenous loci and conidia in long acropetal chains, but its affinity to *Penidiella* has still to be proven.

Key to *Penidiella* species

1. Conidiophores *in vivo* in well-developed, dense fascicles and distinct synnemata arising from a basal stroma; on fallen leaves of *Ficus* sp., Cuba ..... ***P. cubensis***
1. Conidiophores solitary, at most loosely aggregated 2

2. Conidiophores with a terminal conidiogenous cell, often somewhat swollen, giving rise to several ramoconidia (on one level) that form chains of straight to distinctly curved conidia; isolated from leaf of *Carex* sp., Russia ..... ***P. strumelloidea***
2. Penicillate apex of the conidiophores composed of a system of true branchlets, conidiogenous cells and ramoconidia or at least a sequence of ramoconidia on several levels; conidia usually straight 3

3. Mycelium verruculose; long filiform conidiophores ending with a subdenticulate cell giving rise to sets of penicillate conidiogenous cells or ramoconidia which are barely distinguishable and turn into each other; ramoconidia and conidia consistently narrow, (1.5–)2(–2.5) µm wide, and aseptate, ramoconidia sometimes heterochromous; on living leaves of *Nectandra coriacea*, Cuba ..... ***P. nectandrae***
3. Mycelium more or less smooth; penicillate apex at least partly with true branchlets; conidia wider, 2–5 µm, at least partly septate, uniformly pigmented 4

4. Hyphae, conidiophores and conidia frequently distinctly constricted at the septa; penicillate apex of the conidiophores sparingly developed, branchlets more or less divergent; isolated from leaf litter of *Smilax* sp., Cuba ..... ***P. rigidophora***
4. Hyphae and conidia without distinct constrictions at the septa; penicillate apex of the conidiophores usually well-developed, with abundant branchings 5

5. Conidiophores short, up to 120 × 3–4 µm, frequently with intercalary conidiogenous cell, swollen at the conidiogenous portion just below the upper septum which render the conidiophores subnodulose to distinctly nodulose, apex ± loosely penicillate; conidia (4–)5–7(–8) µm long; occasionally with micronematous conidiophores; isolated from man with *tinea nigra*, Venezuela ..... ***P. venezuelensis***
5. Conidiophores much longer, up to 800 µm, 7–9 µm wide at the base, not distinctly nodulose, penicillate apex loose to often more compact, tight, metula-like; conidia longer, 7–25 × 2–5 µm; micronematous conidiophores lacking; isolated from dead leaf of *Paepalanthus columbianus*, Colombia ..... ***P. columbiana***

Penidiella columbiana Crous & U. Braun, **sp. nov.** MycoBank MB504510. Figs 8–9.

Etymology: Named after its country of origin, Colombia.

Mycelium ex hyphis ramosis, septatis, levibus, pallide brunneis, 2–3 µm latis compositum. Conidiophora ex hyphis superficialibus oriunda, penicillata, erecta, brunnea, crassitunicata, minute verruculosa, ad 800 µm longa, ad basim 7–9 µm lata, ad apicem pluriramosa, ex ramibus diversibus et cellulis conidiogenis composita, ramibus primariis (–2) subcylindraceis, 1–7-septatis, 50–120 × 4–6 µm; ramibus secundariis (–2) subcylindraceis, 1–5-septatis, 40–60 × 4–6 µm; ramibus tertiariis et subsequentibus 1–4-septatis, 10–30 × 3–5 µm. Cellulae conidiogenae terminales vel laterales, non ramosae, 5–15 × 3–5 µm, modice brunneae, minute verruculosae, apicem versus attenuatae, truncatae vel rotundatae, polyblasticae, sympodiales, cicatrices conidiales incrassatae, sed leviter fuscatae et non refractivae. Ramoconidia 0–1-septata, modice brunnea, levia, ellipsoidea, obclavata vel obovoidea, cum 1–3 hilis terminalibus, 10–20 × 3–5 µm; conidia subcylindrica vel ellipsoidea, 0–1-septata, pallide brunnea, catenata (–10), hila truncata, non incrassata, vix vel leviter fuscata.

Mycelium consisting of branched, septate, smooth, pale brown, 2–3 µm wide hyphae. *Conidiophores* arising from superficial mycelium, terminally penicillate, erect, brown, wall up to 1 µm wide, almost smooth to finely verruculose, up to 800 µm tall, 7–9 µm wide at the base; conidiogenous region consisting of a series of branches composed of true branchlets, conidiogenous cells and ramoconidia, branched portion usually rather compact, even metula-like, but also looser, with divergent branches; primary branches (–2), subcylindrical, 1–7-septate, 50–120 × 4–6 µm; secondary branches (–2), subcylindrical, 1–5-septate, 40–60 × 4–6 µm; tertiary and additional branches 1–4-septate, 10–30 × 3–5 µm. *Conidiogenous cells* terminal, intercalary or lateral, unbranched, 5–15 × 3–5 µm, medium brown, finely verruculose, tapering to a flattened or rounded (frequently swollen) apical region, scars thickened, but only somewhat darkened, not refractive. *Ramoconidia* 0–1-septate,

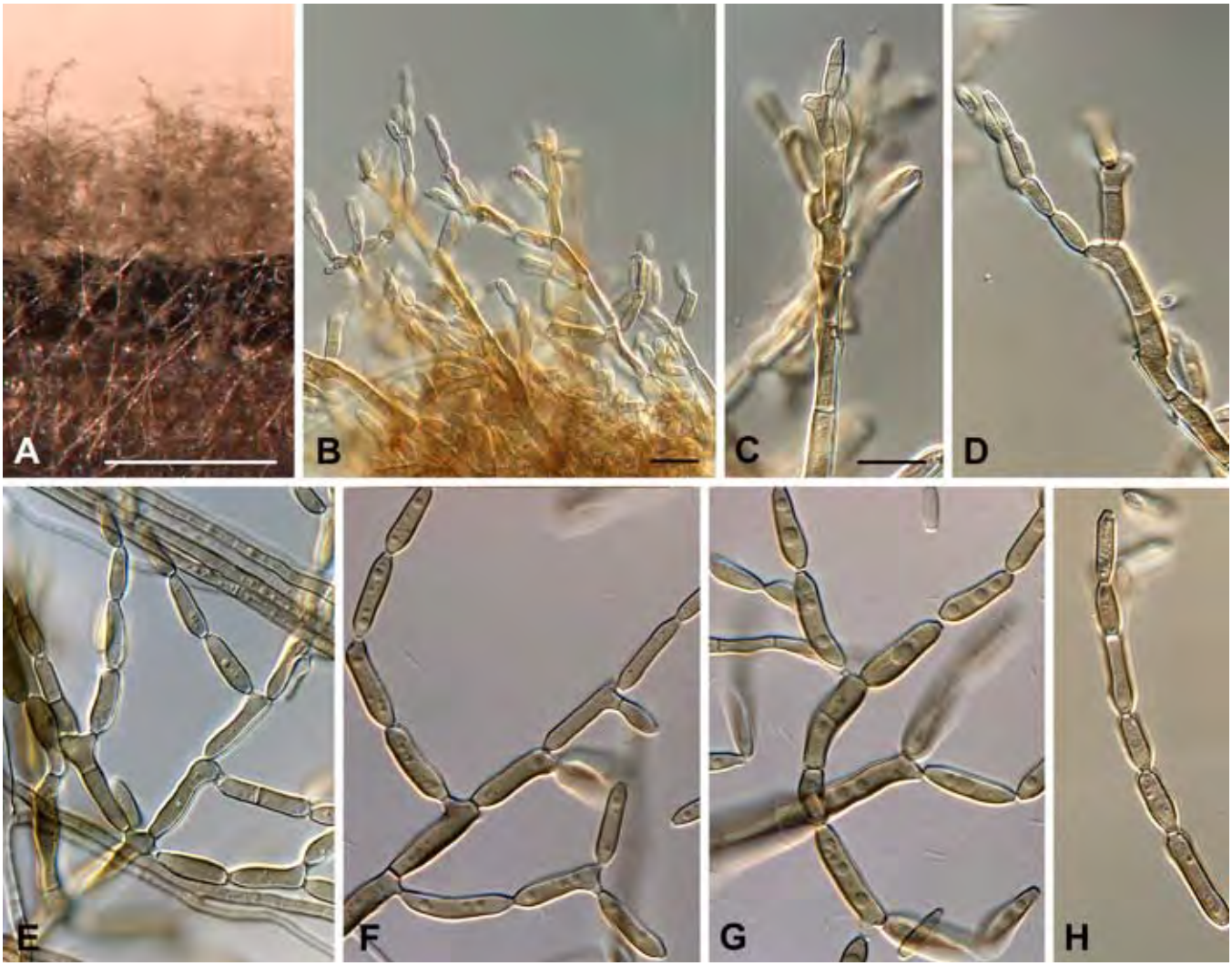

Fig. 8. *Penidiella columbiana* (type material). A. Conidiophores on pine needle *in vitro*. B–H. Conidiophores with chains of disarticulating conidia. Scale bars: A = 450, B–C = 10 µm.

medium brown, smooth, wall ≤ 1 µm wide, ellipsoidal to obclavate or obovoid, with 1–3 apical hila, 10–25 × 3–5 µm, ramoconidia with broadly truncate base, not or barely attenuated, up to 4 µm wide, or at least somewhat attenuated at the base, hila 1.5–3 µm wide. *Conidia* subcylindrical to ellipsoid, 0(–1)-septate, pale brown, in chains of up to 10, 7–15 × 2–3 µm, hila truncate, unthickened, barely to somewhat darkened, 1–2 µm wide.

Cultural characteristics: Colonies on PDA erumpent, spreading, with moderate aerial mycelium and smooth, even, submerged margins; olivaceous-grey in central part, iron-grey in outer region (surface); colonies fertile.

Specimen examined: **Colombia**, Páramo de Guasca, 3400 m alt., isolated from dead leaf of *Paepalanthus columbianus* (*Eriocaulaceae*), Aug. 1980, W. Gams, holotype CBS H-19937, culture ex-type CBS 486.80.

Notes: This isolate was originally identified as belonging to the *Stenella araguata* species complex. The latter name has been somewhat confused in the literature, and has been incorrectly applied to isolates associated with opportunistic human infections (de Hoog *et al*. 2000). The "*araguata*" species complex is treated elsewhere in the volume (see Crous *et al*. 2007a – this volume).

Penidiella cubensis (R.F. Castañeda) U. Braun, Crous & R.F. Castañeda, **comb. nov.** MycoBank MB504511. Fig. 10.
Basionym: *Cladosporium cubense* R.F. Castañeda, *Fungi Cubenses II* (*La Habana*): 4. 1987.

In vivo: *Colonies* on fallen leaves, amphigenous, effuse, pilose, brown. *Mycelium* usually external, superficial, but also internal, composed of branched, septate, brown, thin-walled, smooth to rough-walled hyphae, 2–3 µm wide. *Stromata* present, 40–80 µm diam, brown, immersed. *Conidiophores* densely fasciculate or in distinct synnemata, arising from stromata, erect, synnemata up to about 1000 µm long and (10–)20–40(–50) µm wide, individual threads filiform, pluriseptate throughout, brown, thin-walled (≤ 0.5 µm), smooth or almost so to distinctly verruculose, apically penicillate. *Conidiogenous cells* integrated, terminal and intercalary, 10–30 µm long, subcylindrical, terminal conidiogenous cells often slightly enlarged at the tip, with (1–)2–3(–4) terminal or subterminal subdenticulate conidiogenous loci, short conically truncate, 1–2 µm diam, unthickened or almost so, but often slightly refractive or darkened-refractive, intercalary conidiogenous cells usually with a single lateral locus just below the upper septum, conidiogenous cells giving rise to a single set of primary ramoconidia, or a sequence of ramoconidia at different levels. *Ramoconidia* cylindrical to ellipsoid-fusoid, 8–18(–25) × 2–3 µm, aseptate, pale olivaceous, olivaceous-brown to brown, thin-walled, smooth or almost so to

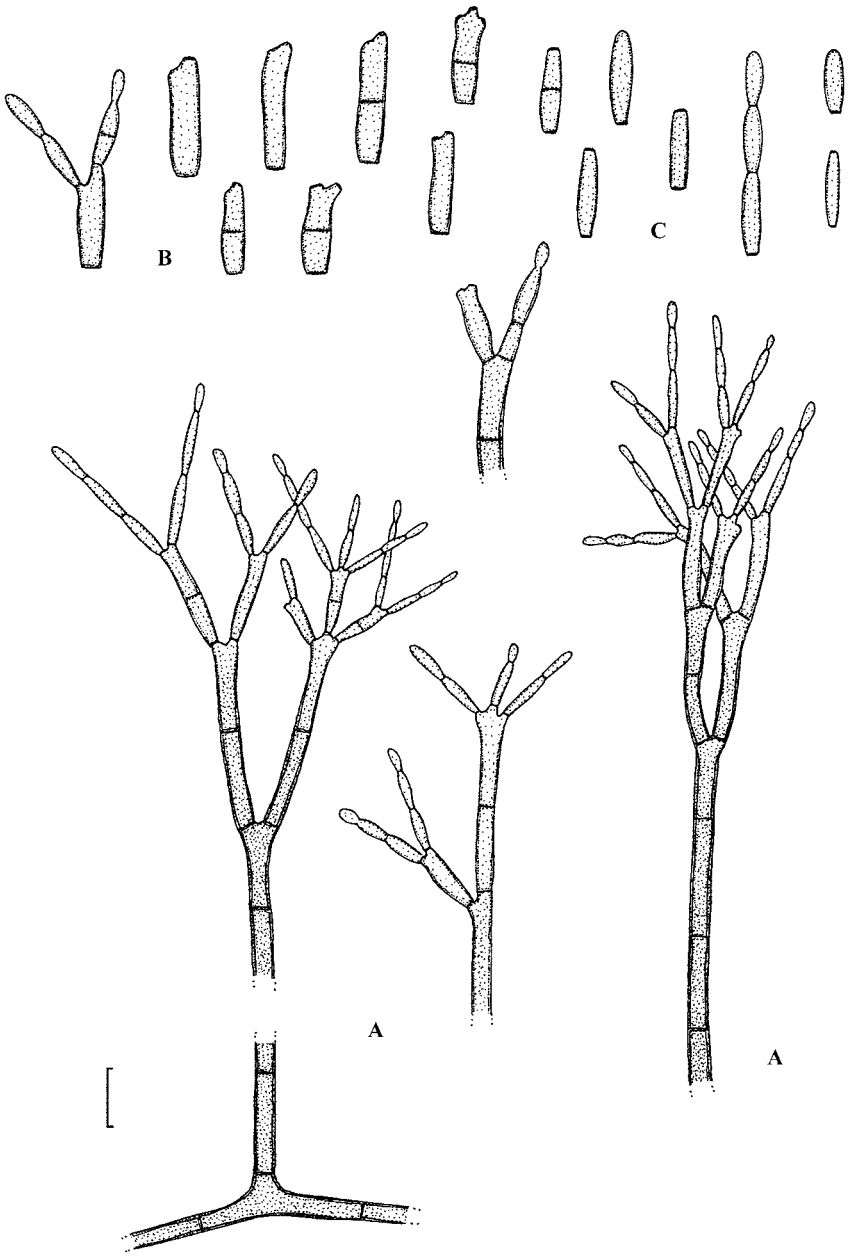

Fig. 9. *Penidiella columbiana* (type material). A. Conidiophores. B. Ramoconidia. C. Secondary conidia. Scale bar = 10 µm. U. Braun *del*.

faintly verruculose, ramoconidia with broadly truncate base, barely narrowed, or ramoconidia more or less attenuated at the base, hila 1–2 µm wide, unthickened or almost so, but often slightly refractive or darkened-refractive. *Conidia* in long acropetal chains, narrowly ellipsoid-ovoid, fusiform, 5–12(–15) × (1–)1.5–3 µm, aseptate, pale olivaceous to brownish, thin-walled, smooth to faintly rough-walled, ends attenuated, hila 1–1.5 µm wide, unthickened, not darkened, at most somewhat refractive.

Specimen examined: **Cuba**, Guantánamo, Maisí, on fallen leaves of *Ficus* sp., 24 Apr. 1986, M. Camino, **holotype** INIFAT C86/134 (HAL 2019 F, ex holotype).

Notes: *Cladosporium cubense* was not available in culture and molecular sequence data are not available, but type material could be re-examined and revealed that this species is quite distinct from *Cladosporium s. str.*, but agreeing with the concept of the genus *Penidiella*. *Penidiella cubensis* differs from all other species of this genus in having densely fasciculate conidiophores to synnematous conidiomata, arising from stromata.

Penidiella nectandrae Crous, U. Braun & R.F. Castañeda, **nom. nov.** MycoBank MB504512. Fig. 11.

Basionym: *Cladosporium ferrugineum* R.F. Castañeda, *Fungi Cubenses II* (*La Habana*): 4. 1987, homonym, non *C. ferrugineum* Allesch., 1895.

In vivo: *Colonies* amphigenous, brown. *Mycelium* internal and external, superficial, composed of sparingly branched hyphae, septate, 1–3 µm wide, pale olivaceous-brown or brown, thin-walled (≤ 0.5 µm), smooth or almost so to distinctly verruculose, fertile cells giving rise to conidiophores somewhat swollen at the branching point, up to 5 µm diam, and somewhat darker. *Stromata* lacking. *Conidiophores* erect, straight, filiform, up to 350 µm long, 2.5–4 µm wide, pluriseptate throughout, brown, darker below and paler above, thin-walled, smooth, apex penicillate, terminal cell of the conidiophore with 2–4 short denticle-like loci giving rise to sets of conidiogenous cells or ramoconidia that then form a sequence of new sets of ramoconidia on different levels, i.e., the loose to dense, metula-like branching system is composed of conidiogenous cells and ramoconidia which are often barely distinguishable and turn into each other; *conidiogenous loci* terminal or subterminal, usually 1–3(–4), subdenticulate, 1–2 µm diam, conical, apically truncate, unthickened or almost so, not to somewhat darkened-refractive. *Ramoconidia* with truncate base, barely attenuated, or ramoconidia distinctly attenuated at the truncate base, up to 20 × 2 µm, aseptate, at the apex with 2–3(–4) subdenticulate hila, subcylindrical,

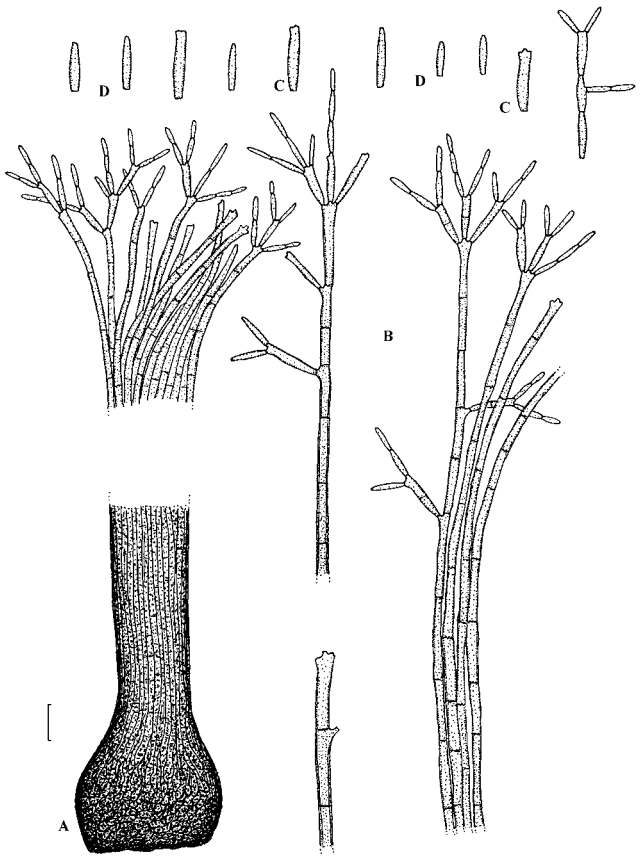

Fig. 10. *Penidiella cubensis* (type material). A. Swollen stromatic base of synnema. B. Conidiophores. C. Ramoconidia. D. Secondary conidia. Scale bar = 10 µm. U. Braun *del*.

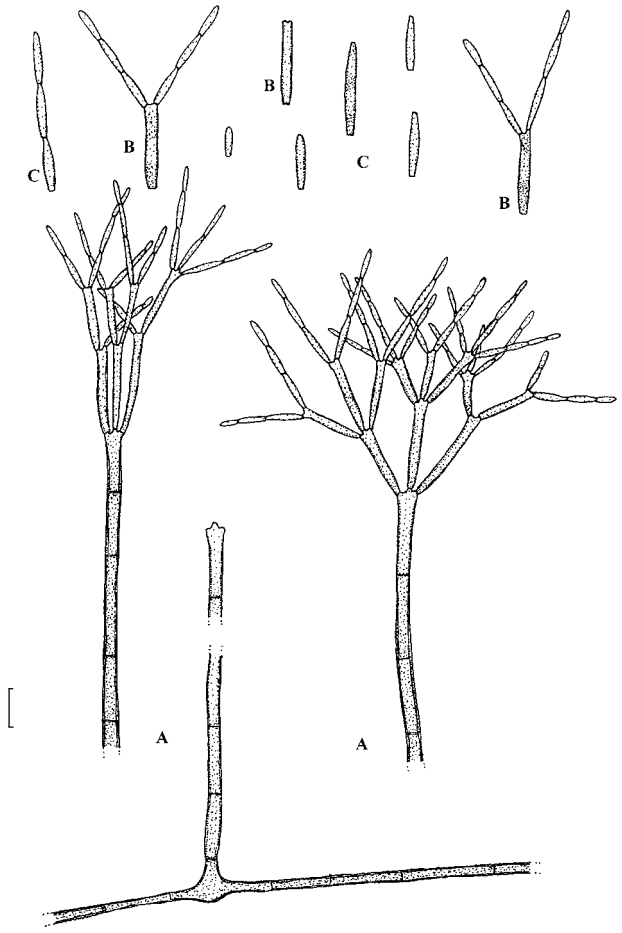

Fig. 11. *Penidiella nectandrae* (type material). A. Conidiophores. B. Ramoconidia. C. Secondary conidia. Scale bar = 10 µm. U. Braun *del*.

very pale olivaceous, olivaceous-brown to brown, sometimes with different shades of brown (heterochromatous), thin-walled (≤ 0.5 µm), smooth to faintly verruculose. *Conidia* in long acropetal chains, narrowly ellipsoid-ovoid, fusiform to cylindrical, 5–16 × (1.5–)2(–2.5) µm, aseptate, very pale olivaceous, olivaceous-brown to brown, thin-walled, smooth to very faintly rough-walled, primary conidia with rounded apex and truncate base, somewhat attenuated, secondary conidia truncate at both ends, hila 1–1.5 µm diam, unthickened or almost so, at most slightly darkened-refractive.

Cultural characteristics: Colonies on PDA slimy, smooth, spreading; aerial mycelium absent, margins smooth, irregular; surface black with patches of cream. Colonies reaching 20 mm diam after 1 mo at 25 °C in the dark; colonies sterile on PDA, SNA and OA.

Specimen examined: **Cuba**, Matanzas, San Miguel de los Baños, isolated from living leaves of *Nectandra coriacea* (*Lauraceae*), 24 Jan. 1987, R.F. Castañeda and G. Arnold, **holotype** INIFAT C87/45, culture ex-type CBS 734.87, and HAL 2018 F (ex-holotype).

Notes: Although the ex-type strain of *Cladosporium ferrugineum* is sterile, its LSU DNA phylogeny reveals it to be unrelated to *Cladosporium s. str.* (see Fig. 1 in Crous *et al*. 2007a – this volume). Based on a re-examination of the type material it could clearly be shown that the morphology of this species fully agrees with the concept of the new genus *Penidiella*, which is supported by its phylogenetic position within *Capnodiales*.

Penidiella rigidophora Crous, R.F. Castañeda & U. Braun, **sp. nov.** MycoBank MB504513. Figs 12–13.

≡ *Cladosporium rigidophorum* R.F. Castañeda, nom. nud. (herbarium name).

Differt a specibus Penidiellae conidiophoris dimorphosis, hyphis et conidiis ad septa saepe distincte constrictis.

Mycelium consisting of strongly branched, septate, smooth or almost so, pale olivaceous to medium brown, guttulate, commonly constricted at septa, 2–6 µm wide hyphae, swollen cells up to 8 µm wide, wall up to 1(–1.5) µm wide. *Conidiophores* dimorphic. *Macronematous conidiophores* separate, erect, subcylindrical, predominantly straight to slightly curved, terminally loosely penicillate, up to 120 µm long, and 4–5 µm wide at the base, which is neither lobed nor swollen, and lacks rhizoids, up to 10-septate, medium to dark brown, wall up to 1(–1.5) µm wide. *Micronematous conidiophores* erect, subcylindrical, up to 40 µm tall, 3–4 µm wide, 1–3-septate, pale to medium brown (concolorous with hyphae). *Conidiogenous cells* predominantly terminal, rarely intercalary, medium brown, smooth, subcylindrical, but frequently swollen at apex, 10–20 × 5–6 µm, loci (predominantly single in micronematous conidiophores, but up to 4 in macronematous conidiophores) flat-tipped, sub-denticulate or not, 1–1.5 µm wide, barely to slightly darkened and thickened-refractive. *Conidia* in branched chains, medium brown, verruculose, (appearing like small spines under light microscope), ellipsoid to cylindrical-oblong, up to 1(–1.5) µm wide, frequently constricted at septa, which turn dark with age; ramoconidia (10–)13–17(–25) × 3–4(–5) µm, 1(–3)-septate; secondary conidia (7–)8–10(–12) × 3–4(–5); hila unthickened to very slightly thickened and darkened, not refractive, (0.5–)1(–1.5) µm.

Cultural characteristics: Colonies on PDA erumpent, spreading, with lobate margins and moderate aerial mycelium; iron-grey (surface), with a greenish black margin; reverse greenish black. Colonies reaching 20 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

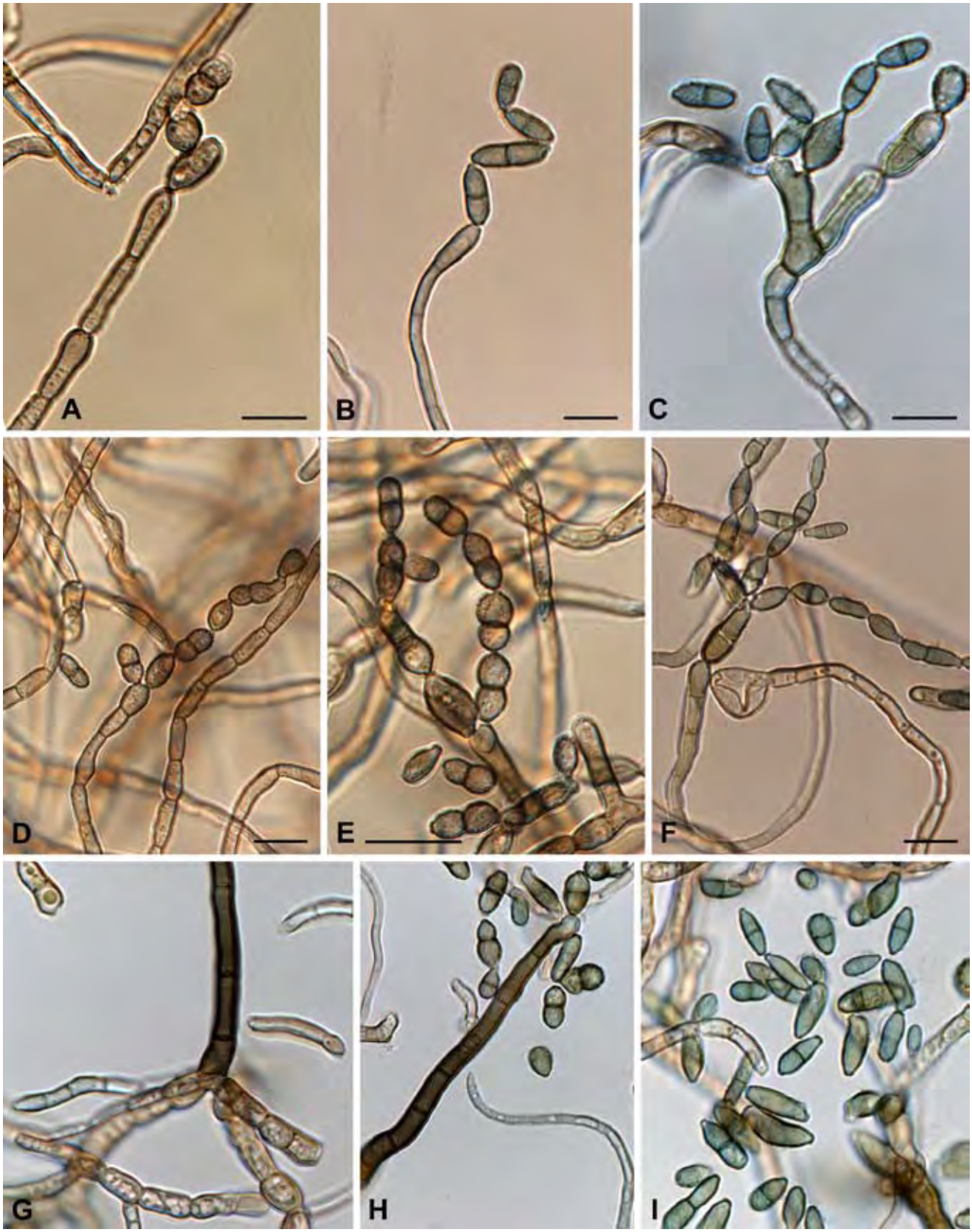

Fig. 12. *Penidiella rigidophora* (type material). A–F. Micronematous conidiophores giving rise to chains of conidia. G–H. Macronematous conidiophores (note base in G, and apex in H). I. Conidia. Scale bars = 10 µm.

Specimen examined: **Cuba**, isolated from leaf litter of *Smilax* sp. (*Smilacaceae*), 6. Nov. 1994, R.F. Castañeda, **holotype** CBS H-19938, culture ex-type CBS 314.95.

Notes: *Cladosporium rigidophorum* is a herbarium name, which was never validly published. The ex-type strain, however, represents a new species of *Penidiella*, for which a valid name with Latin diagnosis is herewith provided. This species is easily distinguishable from all other taxa of *Penidiella* by forming distinct constrictions at hyphal and conidial septa as well as micronematous conidiophores (except for *P. venezuelensis* in which a few micronematous conidiophores have been observed). It is also phylogenetically distinct from the other taxa of *Penidiella* (see Fig. 1 in Crous *et al*. 2007a – this volume).

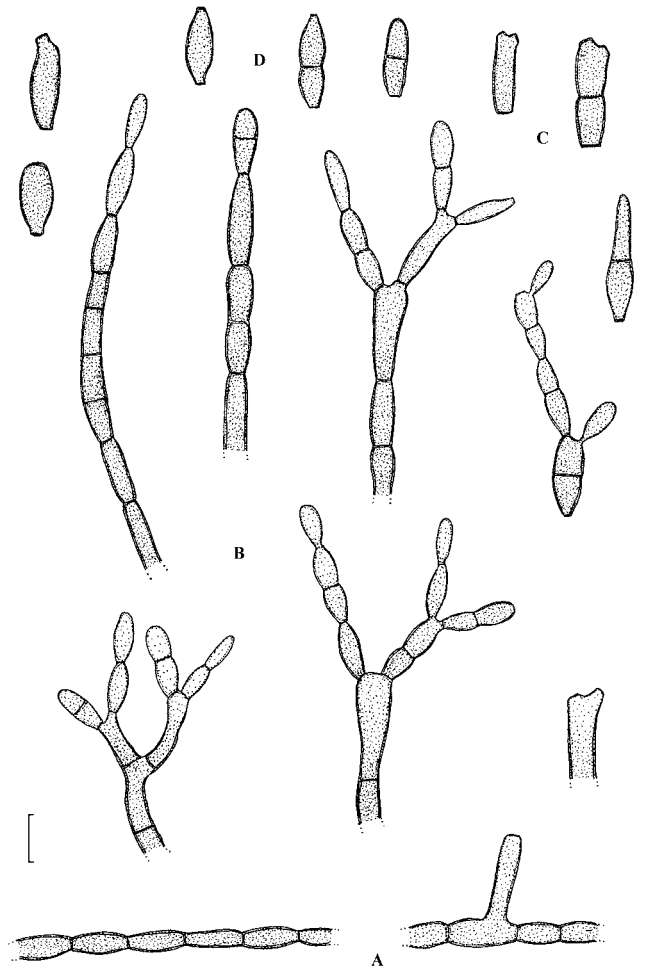

Fig. 13. *Penidiella rigidophora* (type material). A. Hyphae. B. Conidiophores. C. Ramoconidia. D. Secondary conidia. Scale bar = 10 µm. U. Braun *del*.

Penidiella strumelloidea (Milko & Dunaev) Crous & U. Braun, **comb. nov.** MycoBank MB504514. Figs 14–15.
Basionym: *Cladosporium strumelloideum* Milko & Dunaev, Novosti Sist. Nizsh. Rast. 23: 134. 1986.

Mycelium consisting of branched, septate, smooth, hyaline to pale olivaceous, 1–4 µm wide hyphae, sometimes constricted at somewhat darker septa. *Conidiophores* solitary, erect, arising from superficial mycelium, micronematous, i.e., reduced to conidiogenous cells, or macronematous, subcylindrical, straight to slightly curved, subcylindrical throughout or often somewhat attenuated towards the apex, 12–80 × (2–)2.5–4 µm, 0–6-septate, medium brown, smooth, wall ≤ 0.75 µm, penicillate apex formed by a terminal conidiogenous cell giving rise to a single set of ramoconidia. *Conidiogenous cells* terminal, integrated, subcylindrical, straight, 8–12 × 1.5–2(–2.5) µm, pale brown, thin-walled, smooth, apex obtusely rounded to somewhat clavate; loci terminal, occasionally subterminal or lateral, unthickened or almost so to slightly thickened and darkened, not refractive, 1–1.5 µm wide. *Conidia* in branched chains; ramoconidia subcylindrical, with 1–3 terminal loci, olivaceous-brown, smooth; secondary conidia ellipsoidal, with one side frequently straight and the other convex, straight to slightly curved, (8–)10–12(–20) × 2(–3) µm, subhyaline to olivaceous-brown, smooth, thin-walled; hila unthickened or almost so to somewhat thickened and darkened, not refractive, 1 µm wide.

Cultural characteristics: Colonies on PDA erumpent, spreading, with abundant, dense to woolly aerial mycelium, and uneven, feathery margins; surface pale olivaceous grey, reverse iron-grey. Colonies reaching 25 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Russia**, Yaroslavl Region, Rybinsk Reservoir, mouth of Sutka River, isolated from leaf of *Carex* sp. (*Cyperaceae*), from stagnant water, S. Ozerskaya, **holotype** BKMF-2534, culture ex-type CBS 114484.

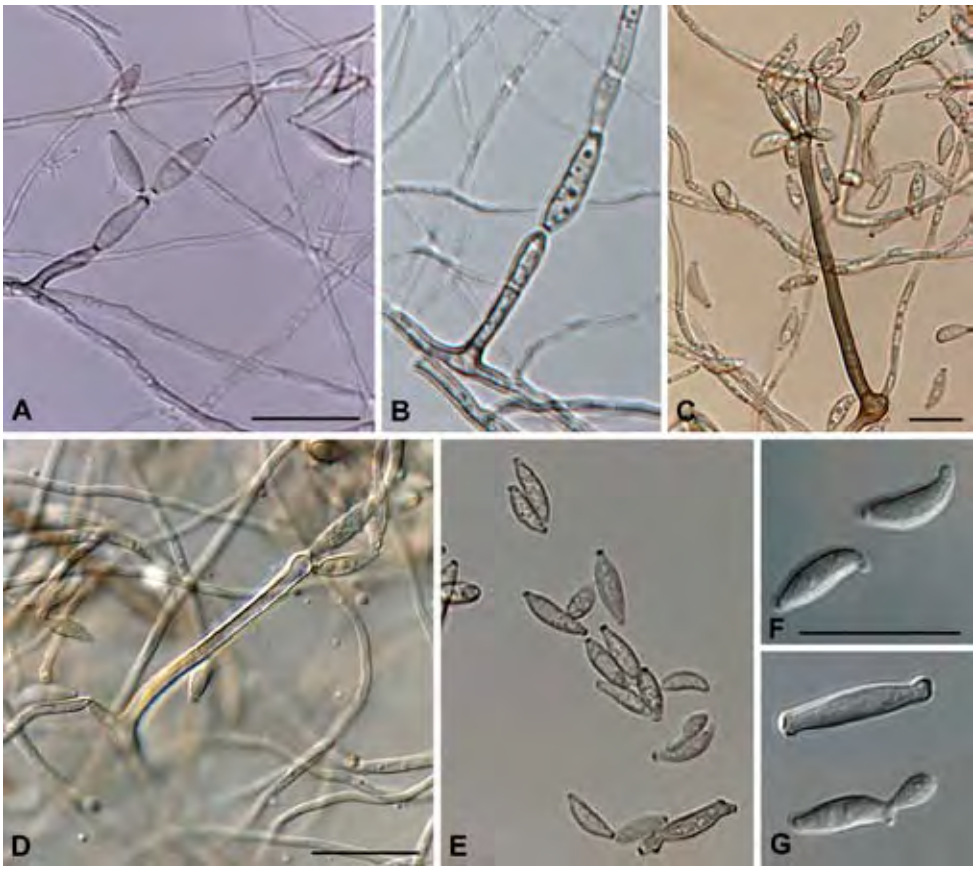

Fig. 14. *Penidiella strumelloidea* (type material). A–B. Micronematous conidiophores. C–D. Macronematous conidiophores. E–G. Conidia. Scale bars = 10 µm.

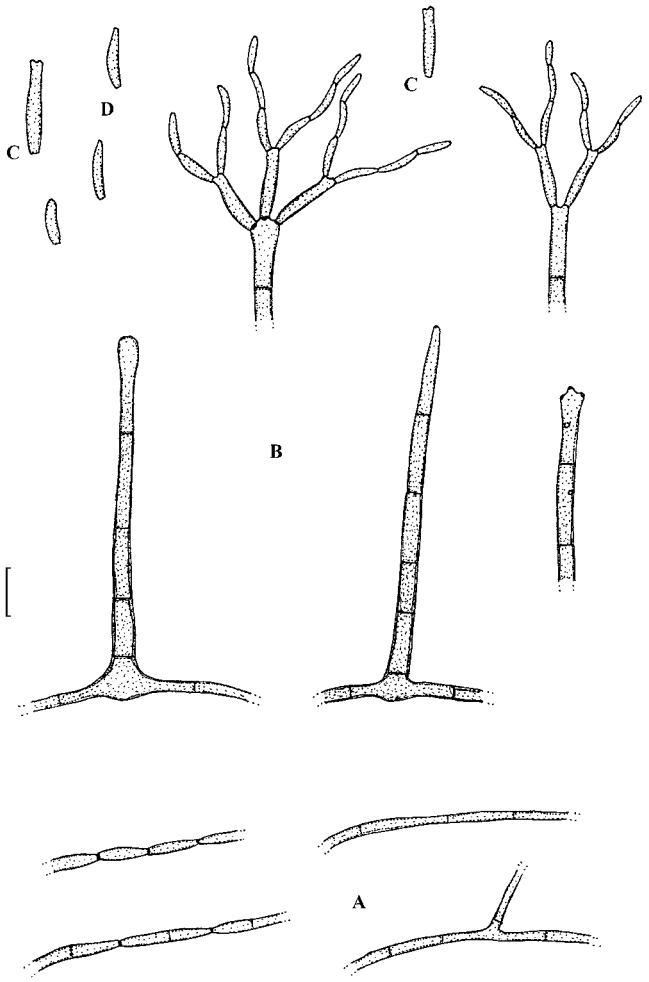

Fig. 15. *Penidiella strumelloidea* (type material). A. Hyphae. B. Conidiophores. C. Ramoconidia. D. Secondary conidia. Scale bars = 10 µm. U. Braun *del*.

Notes: *Penidiella strumelloidea* resembles other species of *Penidiella* by having penicillate conidiophores with a conidiogenous apparatus giving rise to branched conidial chains. It differs, however, from all other species of this genus in having a rather simple penicillate apex composed of a single terminal conidiogenous cell giving rise to one set of ramoconidia which form frequently somewhat curved conidia. It is also phylogenetically distinct from the other taxa of *Penidiella* (see Fig. 1 in Crous *et al*. 2007a – this volume).

Penidiella venezuelensis Crous & U. Braun, **sp. nov.** MycoBank MB504515. Figs 16–17.

Etymology: Named after the geographic location of its type strain, Venezuela.

Differt a P. columbiana conidiophoris bevioribus et angustioribus, ad 120 × 3–4 µm, subnodulosis, apice plus minusve laxe penicillatis et conidiis brevioribus, (4–)5–7(–8) µm longis.

Mycelium consisting of branched, septate, smooth to faintly rough-walled, thin-walled, subhyaline, pale olivaceous to medium brown, (1.5–)2–3 µm wide hyphae. *Conidiophores* solitary, erect, macronematous, subcylindrical, straight to flexuous to once geniculate, up to 120 µm long, 3–4 µm wide, 1–12-septate, pale to medium olivaceous-brown or brown, thin-walled (up to about 1 µm), terminally penicillate, branched portion composed of true branchlets and/or a single set or several sets of ramoconidia, branchlets up to 50 µm long; occasionally with a few additional micronematous conidiophores, about 10–15 × 2–3 µm. *Conidiogenous cells* terminal and intercalary, unbranched, subcylindrical, 5–12 × 3–4 µm, medium brown, smooth or almost so to finely verruculose, apex of conidiogenous cells frequently swollen, up to 6 µm diam, with 1–3(–4) flat-tipped, non to slightly thickened, non to slightly darkened-refractive loci, 1–1.5 µm wide, frequently appearing subdenticulate, up to 1.5 µm long, intercalary conidiogenous cells also slightly swollen at the conidiogenous portion just below the upper septum, which render the conidiophores subnodulose to nodulose, swellings round about the conidiophore axis or unilateral. *Conidia* ellipsoid-ovoid, subcylindrical, pale to medium olivaceous-brown or brown, finely verruculose, wall ≤ 0.5 µm wide, guttulate or not, occurring in branched chains. *Ramoconidia* 0–1(–3)-septate, 5–15(–22) × 3–4(–5) µm, with 1–3 subdenticulate apical hila; secondary conidia 0(–1)-septate, ellipsoid, obovoid to irregular, (4–)5–7(–8) × (2–)2.5–3(–4) µm; hila non to slightly thickened, non to slightly darkened-refractive, (0.5–)1(–1.5) µm wide.

Cultural characteristics: Colonies on OA erumpent, spreading, with dense, compact aerial mycelium, and even, smooth margins; olivaceous-grey (surface), margins iron-grey. Colonies reaching 22 mm diam after 1 mo at 25 °C in the dark.

Specimen examined: **Venezuela**, isolated from man with *tinea nigra*, Jan. 1975, D. Borelli, **holotype** CBS H-19934, culture ex-type CBS 106.75.

Notes: The type culture of *Penidiella venezuelensis* was originally determined as *Stenella araguata* from which it is, however, quite distinct by having smooth mycelium, long penicillate conidiophores with subdenticulate conidiogenous loci, smaller conidia, and agreeing with the concept of the genus *Penidiella*. It is phylogenetically distinct from the other taxa of *Penidiella* (see Fig. 1 in Crous *et al*. 2007a – this volume).

Pseudotaeniolina J.L. Crane & Schokn., Mycologia 78: 88. 1986.
? = *Friedmanniomyces* Onofri, Nova Hedwigia 68: 176. 1999.

Type species: *Pseudotaeniolina convolvuli* (Esfand.) J.L. Crane & Schokn., Mycologia 78: 88. 1986.

Description: Crane & Schoknecht (1986, figs 3–19).

Notes: No cultures or sequence data are available of the type species, and *Pseudotaeniolina globosa* De Leo, Urzì & de Hoog was placed in *Pseudotaeniolina* based on its morphology and ecology. The genus *Friedmanniomyces* is presently known from two species (Selbmann *et al*. 2005). Morphologically *Friedmanniomyces* is similar to *Pseudotaeniolina*, but fresh material of *Pseudotaeniolina convolvuli* needs to be recollected before this can be clarified.

Readeriella Syd. & P. Syd., Ann. Mycol. 6: 484. 1908.
= *Kirramyces* J. Walker, B. Sutton & Pascoe, Mycol. Res. 96: 919. 1992.
= *Colletogloeopsis* Crous & M.J. Wingf., Canad. J. Bot. 75: 668. 1997.
Synanamorphs: ***Cibiessia*** Crous, Fungal Diversity 26: 151. 2007; also pseudocercospora-like, see Crous (1998).

Type species: *Readeriella mirabilis* Syd. & P. Syd., Ann. Mycol. 6: 484. 1908.

Description: Crous *et al*. (2004b; figs 36–38).

Notes: Several coelomycete genera are presently available to accommodate anamorphs of *Capnodiales* that reside in *Teratosphaeriaceae*, for which *Readeriella* is the oldest name. Other genera such as *Phaeophleospora* Rangel, *Sonderhenia* H.J. Swart & J. Walker and *Lecanosticta* Syd. belong to *Mycosphaerellaceae*.

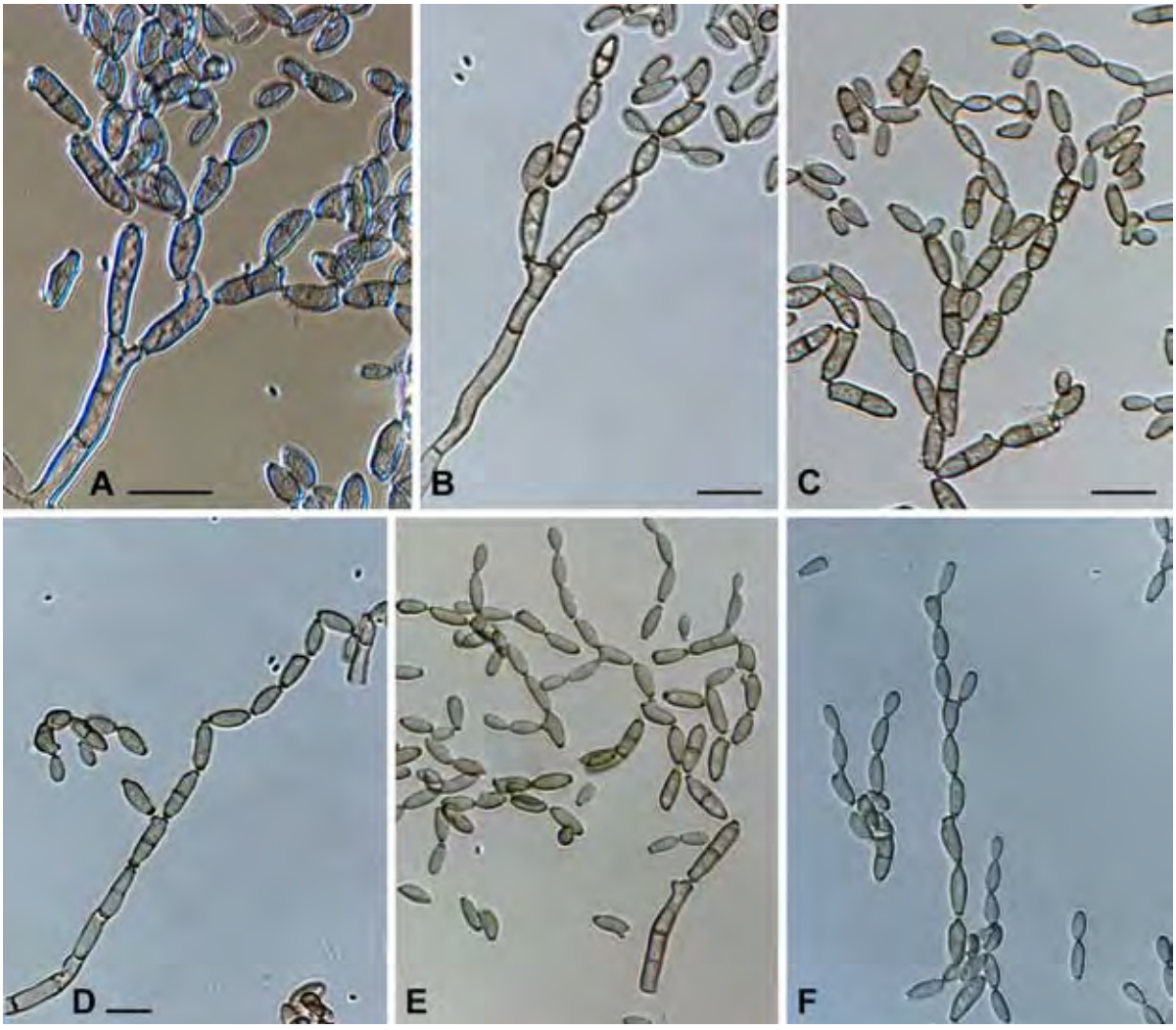

Fig. 16. *Penidiella venezuelensis* (type material). A. Microconidiophore. B. Apical part of macroconidiophore. C–F. Chains of conidia. Scale bars = 10 µm.

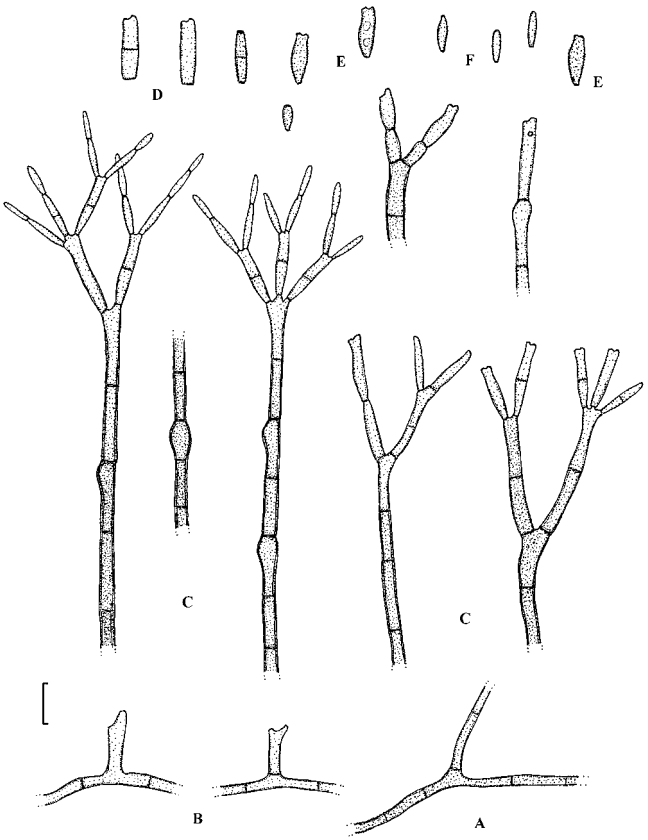

Fig. 17. *Penidiella venezuelensis* (type material). A. Hypha. B. Micronematous conidiophores. C. Macronematous conidiophores. D–E. Ramoconidia. F. Secondary conidia. Scale bar = 10 µm. U. Braun *del*.

Readeriella is polyphyletic within *Teratosphaeriaceae*. The recognition and circumscription (synonymy) of this genus follows the principles for anamorph genera within *Capnodiales* as outlined in the introduction to this volume. The only unifying character is conidial pigmentation, and the mode of conidiogenesis. Conidiogenous cells range from mono- to polyphialides with periclinal thickening, to phialides with percurrent proliferation, as observed in the type species, *R. mirabilis* (Fig. 18). Within the form genus conidia vary from aseptate to multiseptate, smooth to rough, and have a range of synanamorphs. *Readeriella mirabilis* has a synanamorph with cylindrical, aseptate conidia, while other species of *Readeriella* again have *Cibiessia* synanamorphs (scytalidium-like, with chains of dry, disarticulating conidia), suggesting the conidial morphology to be quite plastic. A re-examination of *R. readeriellophora* Crous & Mansilla revealed pycnidia to form a central cushion on which the conidiogenous cells are arranged (Fig. 18). This unique feature is commonly known in genera such as *Coniella* Höhn. and *Pilidiella* Petr. & Syd. (*Diaporthales*) (Van Niekerk *et al*. 2004), and has never been observed among anamorphs of the *Capnodiales*. Another species of *Readeriella*, namely "*Phaeophleospora*" *toledana* Crous & Bills, again forms paraphyses interspersed among conidiogenous cells, a rare feature in this group of fungi, while several species

have conidiomata ranging from acervuli to pycnidia (Cortinas *et al.* 2006). Phylogenetically this coelomycete morphology, with its characteristic conidiogenesis, has evolved several times in *Teratosphaeriaceae*.

Readeriella blakelyi (Crous & Summerell) Crous & U. Braun, **comb. nov.** MycoBank MB504516.
Basionym: *Colletogloeopsis blakelyi* Crous & Summerell, Fungal Diversity 23: 342. 2006.

Readeriella brunneotingens Crous & Summerell, **sp. nov.** MycoBank MB504517. Fig. 19.

Etymology: Named after the diffuse brown pigment visible in agar when cultivated on MEA.

Readeriellae gauchensi similis, sed coloniis viridi-atris et pigmento brunneo in agaro diffundente distinguenda.

Leaf spots amphigenous, irregular specks up to 3 mm diam, medium brown with a thin, raised, concolorous border. *Conidiomata* amphigenous, substomatal, exuding conidia in black masses; conidiomata pycnidial *in vivo* and *in vitro*, globose, brown to black, up to 120 µm diam; wall consisting of 3–4 cell layers of brown cells of *textura angularis*. *Conidiogenous cells* brown, verruculose, aseptate, doliiform to ampulliform, or reduced to inconspicuous loci on hyphae (*in vitro*), proliferating percurrently near the apex, 5–7 × 3–5 µm; sympodial proliferation also observed in culture. *Conidia* brown, smooth to finely verruculose, ellipsoidal to subcylindrical, apex obtuse to subobtuse, tapering to a subtruncate or truncate base (1–1.5 µm wide) with inconspicuous, minute marginal frill, (5–)6–7(–8) × 2–3(–3.5) µm *in vitro*, becoming 1-septate; in older cultures becoming swollen, and up to 2-septate, 15 µm long and 5 µm wide.

Cultural characteristics: *Colonies* on MEA reaching 20 mm diam after 2 mo at 25 °C; colonies erumpent, aerial mycelium sparse to absent, margins smooth but irregularly lobate; surface irregularly folded, greenish black, with profuse sporulation, visible as oozing black conidial masses; a diffuse dark-brown pigment is also produced, resulting in inoculated MEA plates appearing dark-brown.

Specimen examined: **Australia**, Queensland, Cairns, Eureka Creek, 48 km from Mareeba, S 17° 11' 13.2", E 145° 02' 27.4", 468 m, on leaves of *Eucalyptus tereticornis*, 26 Aug. 2006, P.W. Crous, CBS-H 19838 **holotype**, culture ex-type CPC 13303 = CBS 120747.

Notes: Conidial dimensions of *R. brunneotingens* closely match those of *Readeriella gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) Crous (Cortinas *et al*. 2006). The two species can be distinguished in culture, however, in that colonies of *R. brunneotingens* are greenish black in colour, sporulate profusely, and exude a diffuse, brown pigment into the agar, whereas colonies of *R. gauchensis* are more greenish olivaceous, and exude a yellow pigment into the agar (Cortinas *et al*. 2006).

Readeriella considenianae (Crous & Summerell) Crous & U. Braun, **comb. nov.** MycoBank MB504518.
Basionym: *Colletogloeopsis considenianae* Crous & Summerell, Fungal Diversity 23: 343. 2006.

Readeriella destructans (M.J. Wingf. & Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504519.
Basionym: *Kirramyces destructans* M.J. Wingf. & Crous, S. African J. Bot. 62: 325. 1996.
≡ *Phaeophleospora destructans* (M.J. Wingf. & Crous) Crous, F.A. Ferreira & B. Sutton, S. African J. Bot. 63: 113. 1997.

Readeriella dimorpha (Crous & Carnegie) Crous & U. Braun, **comb. nov.** MycoBank MB504520.
Basionym: *Colletogloeopsis dimorpha* Crous & Carnegie, Fungal Diversity 23: 345. 2006.

Readeriella gauchensis (M.-N. Cortinas, Crous & M.J. Wingf.) Crous & U. Braun, **comb. nov.** MycoBank MB504521.
Basionym: *Colletogloeopsis gauchensis* M.-N. Cortinas, Crous & M.J. Wingf., Stud. Mycol. 55: 143. 2006.

Readeriella pulcherrima (Gadgil & M. Dick) Crous & U. Braun, **comb. nov.** MycoBank MB504522.
Basionym: *Septoria pulcherrima* Gadgil & M. Dick, New Zealand J. Bot. 21: 49. 1983.
≡ *Stagonospora pulcherrima* (Gadgil & M. Dick) H.J. Swart, Trans. Brit. Mycol. Soc. 90: 285. 1988.
= *Cercospora eucalypti* Cooke & Massee, *Grevillea* 18: 7. 1889.
≡ *Kirramyces eucalypti* (Cooke & Massee) J. Walker, B. Sutton & Pascoe, Mycol. Res. 96: 920. 1992.
≡ *Phaeophleospora eucalypti* (Cooke & Massee) Crous, F.A. Ferreira & B. Sutton, S. African J. Bot. 63: 113. 1997.

Notes: The epithet "*eucalypti*" is preoccupied by *Readeriella eucalypti* (Gonz. Frag.) Crous (Summerell *et al*., 2006), and thus the synonym "*pulcherrima*" becomes the next available name for this species.

Readeriella readeriellophora, see ***Teratosphaeria readeriellophora***. Fig. 18.

Readeriella stellenboschiana (Crous) Crous & U. Braun, **comb. nov.** MycoBank MB504523.
Basionym: *Colletogloeopsis stellenboschiana* Crous, Stud. Mycol. 55: 110. 2006.

Readeriella zuluensis (M.J. Wingf., Crous & T.A. Cout.) Crous & U. Braun, **comb. nov.** MycoBank MB504524.
Basionym: *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout., Mycopathologia 136: 142. 1997.
≡ *Colletogloeopsis zuluensis* (M.J. Wingf., Crous & T.A. Cout.) M.-N. Cortinas, M.J. Wingf. & Crous (*zuluense*), Mycol. Res. 110: 235. 2006.

Staninwardia B. Sutton, Trans. Br. Mycol. Soc. 57: 540. 1971.

Type species: *Staninwardia breviuscula* B. Sutton, Trans. Br. Mycol. Soc. 57: 540. 1971.

Description: Sutton (1971; fig. 1).

Notes: The genus *Staninwardia* presently contains two species, namely *S. breviuscula* and *Staninwardia suttonii* Crous & Summerell (Summerell *et al*. 2006), though its placement in *Capnodiales* was less well resolved. The genus forms acervuli on brown leaf spots, with brown, catenulate conidia covered in a mucilaginous sheath.

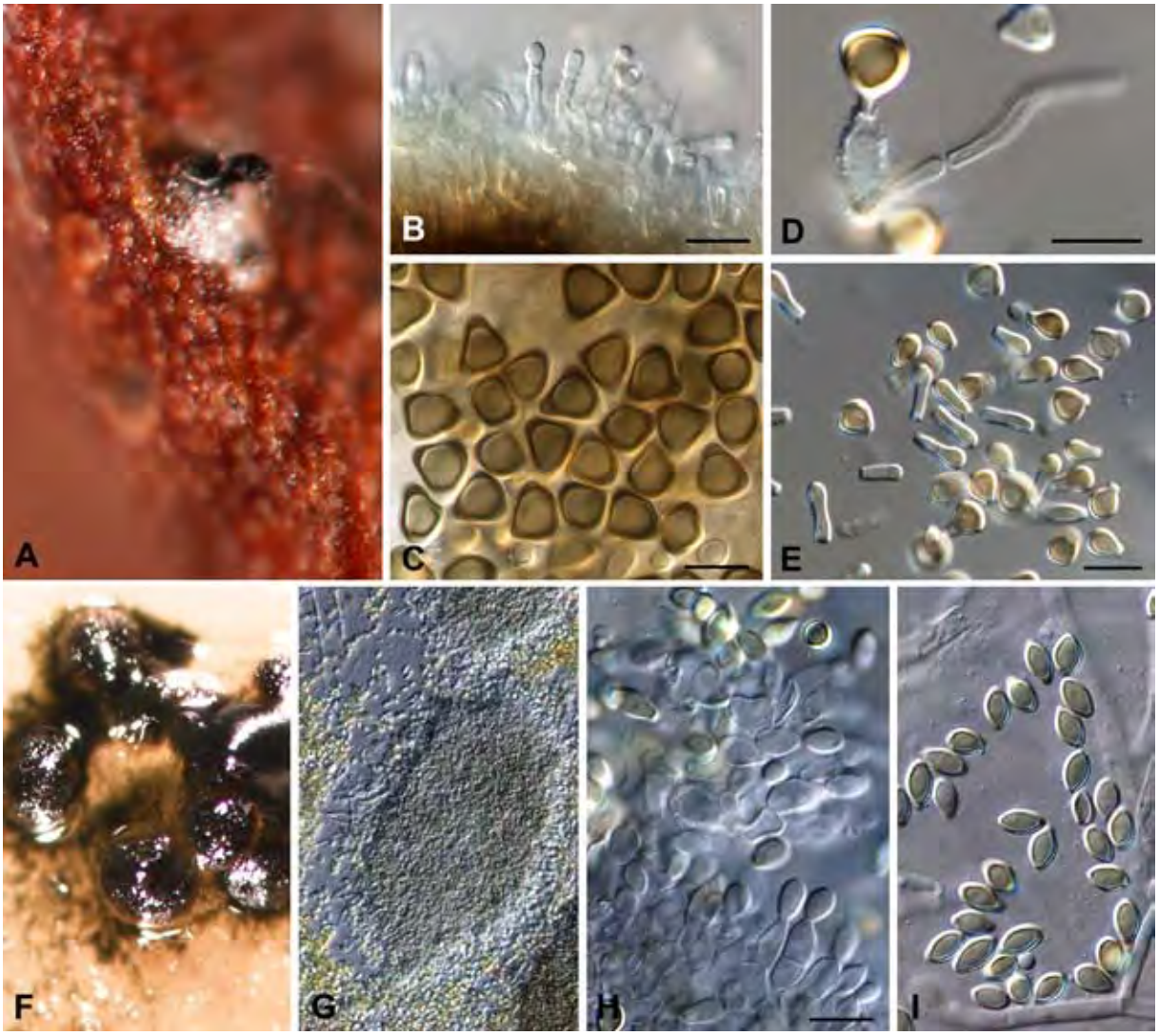

Fig. 18. *A–E. Readeriella mirabilis.* A. Conidium with conidial cirrus. B. Conidiogenous cells with percurrent proliferation. C. Macroconidia. D. Slightly pigmented, verruculose conidiogenous cell. E. Macro- and microconidia. F–I. *Readeriella readeriellophora* (type material). F. Colony on OA. G. Central stromatal tissue giving rise to conidiophores. H. Conidiogenous cells. I. Conidia. Scale bars = 10 µm.

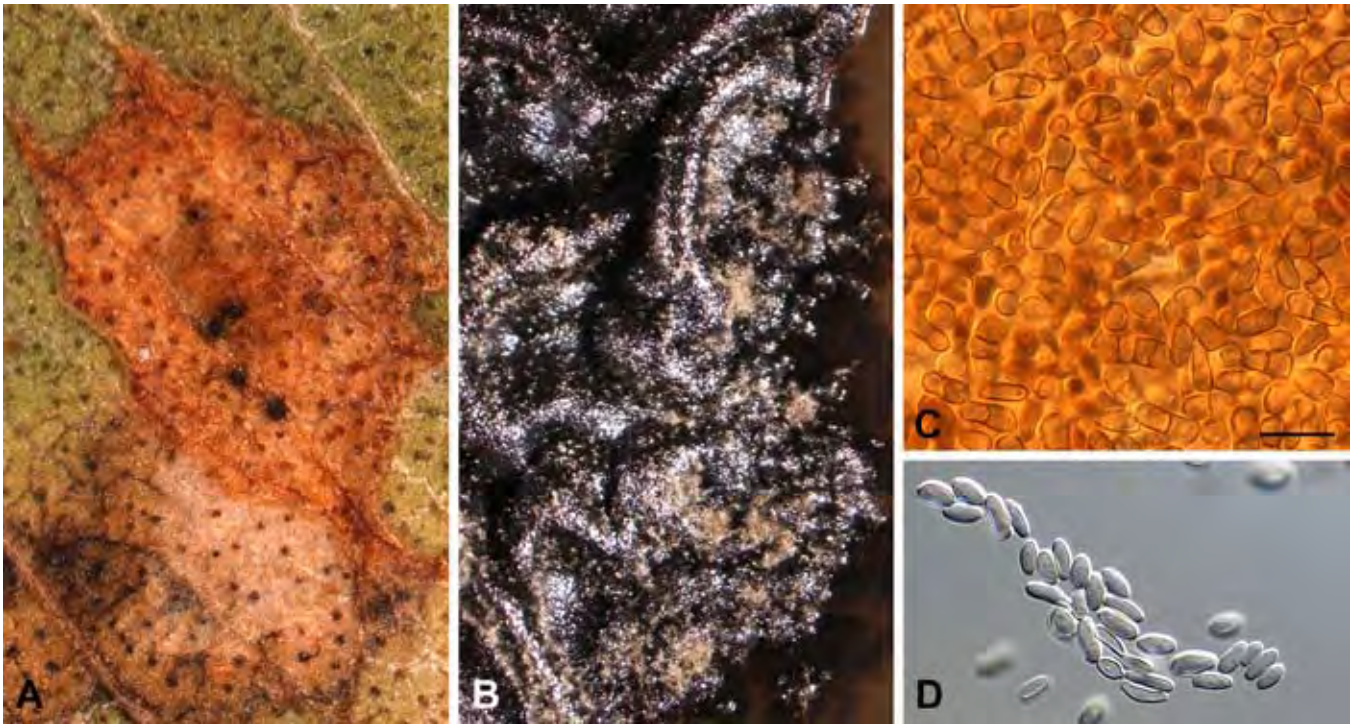

Fig. 19. *Readeriella brunneotingens* (type material). A. Leaf spot. B. Colony on MEA. C–D. Conidia. Scale bar = 10 µm.

Schizothyriaceae clade

Schizothyrium Desm., Ann. Sci. Nat., Bot., sér. 3: 11. 1849.

Type species: *Schizothyrium acerinum* Desm., Ann. Sci. Nat., Bot., sér. 3: 11. 1849.

Description: Batzer *et al.* (2007; figs 3–7).

Notes: Species of *Schizothyrium* (*Schizothyriaceae*) have *Zygophiala* E.W. Mason anamorphs, and were recently shown to be allied to *Mycosphaerellaceae* (Batzer *et al.* 2007). Although species of *Schizothyrium* have thyrothecia, they cluster among genera with pseudothecial ascomata, questioning the value of this character at the family level. Based on its bitunicate asci and 1-septate ascospores, the teleomorph is comparable to others in the *Capnodiales*.

Mycosphaerellaceae clade

Mycosphaerella subclade

Mycosphaerella Johanson, Öfvers. Förh. Kongl. Svenska Vetensk.-Akad. 41(9): 163. 1884.

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

Anamorph: *Ramularia endophylla* Verkley & U. Braun, Mycol. Res. 108: 1276. 2004.

Description: Verkley *et al*. (2004; figs 3–16).

Notes: The genus *Mycosphaerella* has in the past been linked to 23 anamorph genera (Crous *et al*. 2000), while additional genera have been linked via DNA-based studies, bringing the total to at least 30 genera (Crous & Braun 2003, Crous *et al*. 2007b). However, based on ITS and SSU DNA phylogenetic studies and a reassessment of morphological characters and conidiogenesis, several anamorph genera have recently been reduced to synonymy (Crous & Braun 2003, Crous *et al*. 2006a). Furthermore, the DNA sequence data generated to date clearly illustrate that the anamorph genera in *Mycosphaerella* are polyphyletic, residing in several clades within *Mycosphaerella*. If future collections not known from culture or DNA sequences are to be described in form genera, we recommend that the concepts as explained in Crous & Braun (2003) be used until such stage as they can be placed in *Mycosphaerella*, pending a modification of Art. 59 of the International Code of Botanical Nomenclature. The genus *Mycosphaerella* and its anamorphs represent a future topical issue of the *Studies in Mycology*, and will thus be treated separately.

Dissoconium subclade

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

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

? = *Uwebraunia* Crous & M.J. Wingf., Mycologia 88: 446. 1996.

Teleomorph: *Mycosphaerella*-like.

Description: de Hoog *et al.* (1983), Crous (1998), Crous *et al*. (2004b; figs 3–10).

Notes: The genus *Dissoconium* presently encompasses six species (Crous *et al.* 2007b), of which two, *M. lateralis* Crous & M.J. Wingf. (*D. dekkeri* de Hoog & Hijwegen), and *M. communis* Crous & Mansilla (*D. commune* Crous & Mansilla) are also known from their *Mycosphaerella*-like teleomorphs. No teleomorph genus will be introduced for this clade, however, until more sexual species have been collected to help clarify the morphological features of this genus. A further complication lies in the fact that yet other species, morphologically distinct from *Dissoconium*, also cluster in this clade (Crous, unpubl. data).

"Passalora" zambiae subclade

"Passalora" zambiae Crous & T.A. Cout., Stud. Mycol. 50: 209. 2004.

Description: Crous *et al.* (2004b; figs 32–33).

Notes: This fungus was placed in the form genus "*Passalora*" based on its smooth mycelium, giving rise to conidiophores forming branched chains of brown conidia with thickened, darkened, refractive hila. Although derived from single ascospores, the teleomorph material was lost, and thus it needs to be recollected before the relavance of its phylogenetic position can be fully understood.

Additional teleomorph genera considered

Coccodinium A. Massal., Atti Inst. Veneto Sci. Lett. Arti, Série 2, 5: 336. 1860. (Fig. 20).

Type species: *Coccodinium bartschii* A. Massal., Atti Inst. Veneto Sci. Lett. Arti, Série 2, 5: 337. 1860.

Description: Eriksson (1981, figs 34–35).

Notes: The genus *Coccodinium* (*Coccodiniaceae*) is characterised by having ascomata that are sessile on a subiculum, or somewhat immersed, semiglobose, collapsed when dry, brownish, uniloculate, with a centrum that stains blue in IKI (iodine potassium iodide). *Asci* are bitunicate, stalked, 8-spored, saccate, and have a thick, undifferentiated endotunica. *Periphyses* and *periphysoids* are well-developed and numerous. *Ascospores* are elongate, fusiform, ellipsoidal or clavate, transversely septate or muriform, hyaline or brownish (Eriksson 1981), and lack a mucous sheath. Based on a SSU sequence (GenBank accession U77668) derived from a strain identified as *C. bartschii* (Winka *et al*. 1998), *Coccodinium* appears to be allied to the taxa treated here in *Teratosphaeria*. Freshly collected cultures are relatively slow growing, and on MEA they form erumpent round, black colonies with sparse hyphal growth. On the surface of these colonies hyphal strands, consisting of brown, globose cells, give rise to conidia. Older cells (up to 15 µm diam) become fertile, giving rise to 1–3 conidia via inconspicuous phialidic loci. Conidia are fusoid-ellipsoidal to clavate, 3–5-septate, becoming constricted at the transverse septa, apex obtuse, base subtruncate, guttulate, smooth, widest in the upper third of the conidium, 15–40 × 4–7 µm. Phylogenetically *Coccodinium* is thus allied to the *Chaetothyriales* (Fig. 1), and not the *Teratosphaeriaceae*.

Fig. 20. *Coccodinium bartschii*. A. Ascomata on host. B. Ostiolar area. C. Periphysoids. D–E. Ascospores shot onto agar. F–I. Asci with thick ectotunica. J–K. Young ascospores. L–M. Mature ascospores. N. Colony on MEA. O–Q. Conidiogenous cells giving rise to conidia. R–S. Conidia. Scale bars: A, N = 250, B, D, F–G, I, L–M, O = 10 µm.

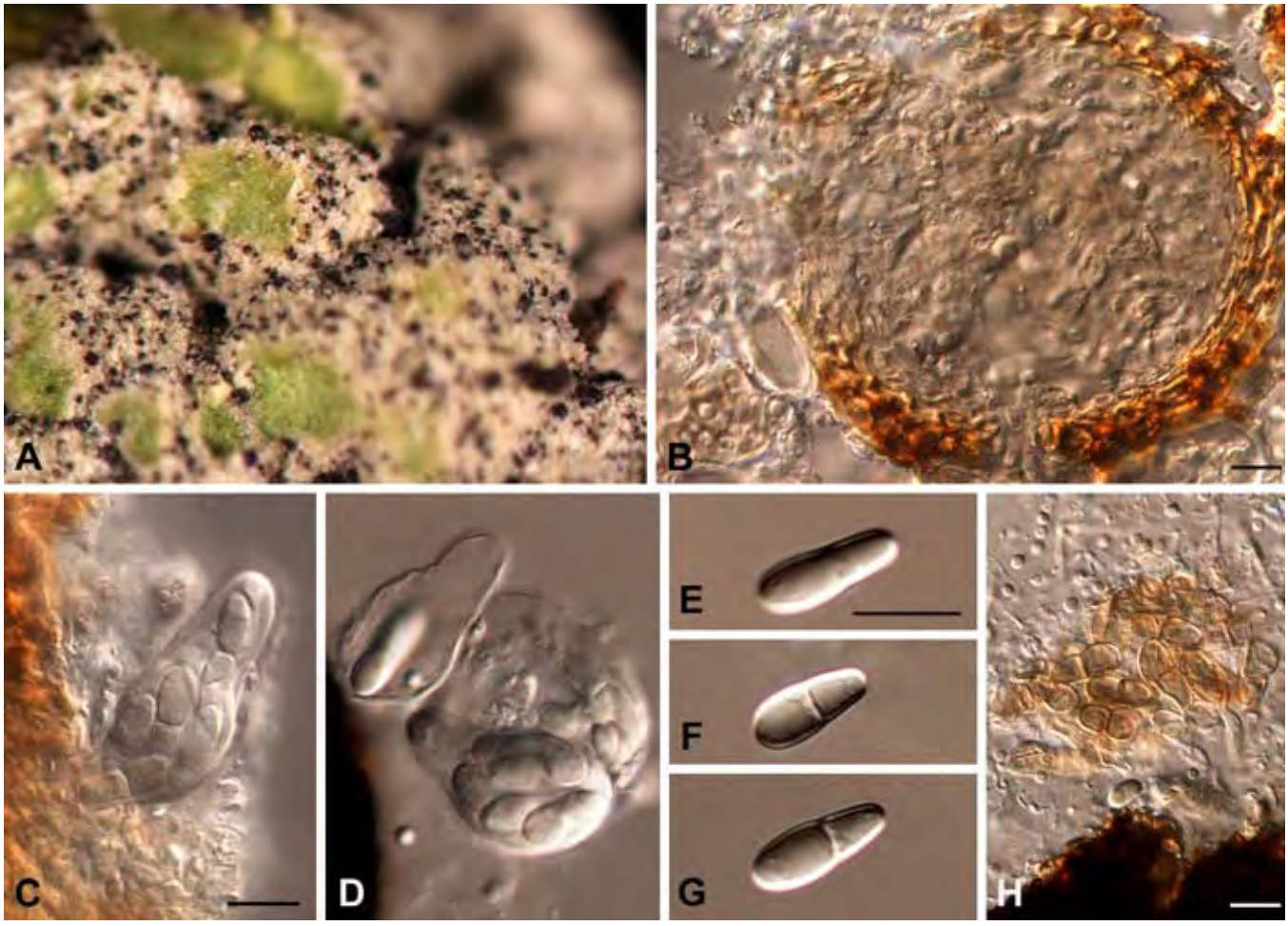

Fig. 21. *Stigmidium schaereri*. A. Lichenicolous habit on *Dacampia hookeri*. B. Vertical section through an ascoma. C–D. Asci. E–G. Ascospores. H. Older, brown ascospores. Scale bars = 10 µm.

Stigmidium Trevis., Consp. Verruc.: 17. 1860. (Fig. 21).

Type species: Stigmidium schaereri (A. Massal.) Trevis., Consp. Verruc.: 17. 1860.

Description: Roux & Triebel (1994, figs 47–50).

Notes: The type species of the genus is lichenicolous, characterised by semi-immersed, black, globose ascomata with ostiolar periphyses and periphysoids. *Asci* are 8-spored, fasciculate, bitunicate, (endotunica not giving a special reaction in Congo red or toluidine blue). *Ascospores* are fusoid-ellipsoidal, medianly 1-septate, guttulate, thin-walled, lacking a sheath. Presently no culture is available, and thus the placement of *Stigmidium* remains unresolved.

DISCUSSION

From the LSU sequence data presented here, it is clear that *Mycosphaerella* is not monophyletic as previously suggested (Crous *et al*. 2001, Goodwin *et al*. 2001). The first step to circumscribe natural genera within this complex was taken by Braun *et al*. (2003), who separated *Cladosporium* anamorphs from this complex, and erected *Davidiella* (*Davidiellaceae*; Schoch *et al*. 2006) to accommodate their teleomorphs. The present study reinstates the genus *Teratosphaeria* for a clade of largely extremotolerant fungi (Selbmann *et al*. 2005) and foliar pathogens of *Myrtaceae* and *Proteaceae* (Crous *et al*. 2004a, b, 2006b, 2007b), and further separates generic subclades within the *Mycosphaerellaceae*, while Batzer *et al*. (2007) again revealed *Schizothyrium* Desm. (*Schizothyriaceae*) to cluster within the *Mycosphaerellaceae*. Our results, however, provide support for recognition of *Schizothyrium* as a distinct genus, although *Schizothyriaceae* was less well supported as being separate from *Mycosphaerellaceae* (*Capnodiales*).

Although pleomorphism represents a rather unstudied phenomenon in this group of fungi, it has been observed in several species. Within the *Teratosphaeria* clade, Crous *et al*. (2007b) recently demonstrated teleomorphs to have *Readeriella* and *Cibiessia* synanamorphs, while the black yeast genera that belong to this clade, commonly have more than one anamorph state in culture. The present study also revealed *Readeriella mirabilis* to have two conidial types in culture, and to be highly plastic regarding its mode of conidiogenesis, and *Readeriella* to be the oldest generic name available for a large group of leaf-spotting coelomycetes in the *Teratosphaeriaceae* (*Capnodiales*).

Although not commonly documented, there are ample examples of synanamorphs in *Capnodiales*. Within *Mycosphaerella*, Beilharz *et al*. (2004) described *Passalora perplexa* Beilharz, Pascoe, M.J. Wingf. & Crous as a species with a coelomycete and yeast synanamorph, while Crous & Corlett (1998) described *Mycosphaerella stigmina-platani* F.A. Wolf to have a *Cercostigmina* U. Braun and *Xenostigmina* Crous synanamorph, and recent collections also revealed the presence of a similar species that has typical "*Stigmina*" (distoseptate conidia) and *Pseudocercospora* (euseptate conidia) synanamorphs (Crous, unpubl. data), and Crous (1998) reported *Readeriella epicoccoides* (coelomycete) to

have a *Cercostigmina* (hyphomycete) synanamorph in culture.

Although the *Mycosphaerella* complex encompasses thousands of names, it may appear strange that it is only now that more clarity is obtained regarding the phylogenetic relationships among taxa in this group. This is partly due to the fact that these organisms are cultivated with difficulty, and also that the first paper to address the taxonomy of this complex based on DNA sequence data was only relatively recently published (Stewart *et al.* 1999). In the latter study, the genus *Paracercospora* Deighton (scars minutely thickened along the rim), was shown to be synonymous with the older genus *Pseudocercospora*. Similarily, Crous *et al*. (2001) showed that *Cercostigmina* (rough, irregular percurrent proliferations) was also synonymous with *Pseudocercospora*. This led Crous & Braun (2003) to conclude that conidiomatal type, conidial catenulation, septation and proliferation of conidiogenous cells were of less importance in separating species at the generic level. *Mycovellosiella* Rangel and *Phaeoramularia* Munt.-Cvetk. were subsequently reduced to synonymy with the older name, *Passalora* Fr., and characters identified as significant at the generic level were pigmentation (*Cercospora vs*. *Passalora*), scar structure (*Passalora vs*. *Pseudocercospora*), and verruculose superficial hyphae (*Stenella vs*. *Passalora*). Due to the unavailability of cultures, no decision was made regarding *Stenella* (verrucose conidia and mycelium), *Stigmina* (distoseptate conidia), and several other, less well-known genera such as *Asperisporium* Maubl.*, Denticularia* Deighton*, Distocercospora* N. Pons & B. Sutton*, Prathigada* Subram.*, Ramulispora, Pseudocercosporidium* Deighton*, Stenellopsis* B. Huguenin and *Verrucisporota* D.E. Shaw & Alcorn. In a recent study, however, Crous *et al.* (2006a) were able to show that *Phaeoisariopsis* (synnemata, conidia with slightly thickened hila) and *Stigmina* (distoseptate conidia) were also synonyms of *Pseudocercospora*.

The present study shows that most anamorph genera are polyphyletic within *Teratosphaeria*, and paraphyletic within *Capnodiales*. In some cases, generic concepts of anamorphs based on morphology and conidium ontogeny conform well with phylogenetic relationships, though this is not true in all cases due to convergence. Nevertheless, anamorphs still convey valuable morphological information that is contained in the anamorph name, and naming anamorphs continue to provide a practical system to identify the various asexual taxa encountered.

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Delimiting *Cladosporium* from morphologically similar genera

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Abstract: The genus *Cladosporium* is restricted to dematiaceous hyphomycetes with a coronate scar type, and *Davidiella* teleomorphs. In the present study numerous cladosporium-like taxa are treated, and allocated to different genera based on their morphology and DNA phylogeny derived from the LSU nrRNA gene. Several species are introduced in new genera such as *Hyalodendriella, Ochrocladosporium, Rachicladosporium, Rhizocladosporium*, *Toxicocladosporium* and *Verrucocladosporium*. A further new taxon is described in *Devriesia* (*Teratosphaeriaceae*). Furthermore, *Cladosporium castellanii*, the etiological agent of *tinea nigra* in humans, is confirmed as synonym of *Stenella araguata*, while the type species of *Stenella* is shown to be linked to the *Teratosphaeriaceae* (*Capnodiales*), and not the *Mycosphaerellaceae* as formerly presumed.

Taxonomic novelties: *Devriesia americana* Crous & Dugan, sp. nov., *Hyalodendriella* Crous, gen. nov., *Hyalodendriella betulae* Crous sp. nov., *Ochrocladosporium* Crous & U. Braun, gen. nov., *Ochrocladosporium elatum* (Harz) Crous & U. Braun, comb. nov., *Ochrocladosporium frigidarii* Crous & U. Braun, sp. nov., *Rachicladosporium* Crous, U. Braun & Hill, gen. nov., *Rachicladosporium luculiae* Crous, U. Braun & Hill, sp. nov., *Rhizocladosporium* Crous & U. Braun, gen. nov., *Rhizocladosporium argillaceum* (Minoura) Crous & U. Braun, comb. nov., *Toxicocladosporium* Crous & U. Braun, gen. nov., *Toxicocladosporium irritans* Crous & U. Braun, sp. nov., *Verrucocladosporium* K. Schub., Aptroot & Crous, gen. nov., *Verrucocladosporium dirinae* K. Schub., Aptroot & Crous, sp. nov.
Key words: *Cladosporium*, *Davidiella*, food spoilage, hyphomycetes, indoor air, LSU phylogeny, taxonomy.

INTRODUCTION

Cladosporioid hyphomycetes are common, widespread fungi. The genus *Cladosporium* Link is based on the type species, *Cladosporium herbarum* (Pers. : Fr.) Link, which in turn has been linked to *Davidiella* Crous & U. Braun teleomorphs (Braun *et al*. 2003, Schubert *et al*. 2007b – this volume). *Cladosporium* is one of the largest, most heterogeneous genera of hyphomycetes, comprising more than 772 names (Dugan *et al*. 2004), and including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species. Species of this genus affect daily human life in various ways. The common saprobic members of *Cladosporium* occur on all kinds of senescing and dead leaves and stems of herbaceous and woody plants, as secondary invaders on necrotic leaf lesions caused by other fungi, are frequently isolated from air, soil, food stuffs, paint, textiles and other organic matters, are also known to be common endophytes (Riesen & Sieber 1985, Brown *et al*. 1998, El-Morsy 2000) as well as phylloplane fungi (Islam & Hasin 2000, De Jager *et al*. 2001, Inacio *et al*. 2002, Stohr & Dighton 2004, Levetin & Dorsey 2006). Furthermore, some *Cladosporium* species are known to be potential agents of medical relevance. *Cladosporium herbarum* is, for instance, a common contaminant in clinical laboratories and causes allergic lung mycoses (de Hoog *et al*. 2000, Schubert *et al*. 2007b – this volume).

In spite of the enormous relevance of this genus, there is no comprehensive modern revision of *Cladosporium*, but some attempts to revise and monograph parts of it have been initiated during the last decade (David 1997, Partridge & Morgan-Jones 2002, Wirsel *et al*. 2002, Braun *et al*. 2003, Dugan *et al*. 2004, Park *et al*. 2004, Seifert *et al*. 2004, Schubert & Braun 2004, 2005a, b, 2007, Heuchert *et al*. 2005, Schubert 2005a, b, Schubert *et al*. 2006).

Previous molecular studies employing rDNA ITS sequence data (Crous *et al*. 2001) have shown *Cladosporium* spp. to cluster adjacent to the main monophyletic *Mycosphaerella* Johanson cluster, suggesting a position apart from the latter genus. Braun *et al.* (2003) carried out more comprehensive sequence analyses, based on ITS (ITS-1, 5.8S and ITS-2) and 18S rDNA data, providing further evidence that *Cladosporium s. str.* represents a sister clade of *Mycosphaerella*.

Various authors discussed the taxonomy and circumscription of *Cladosporium* (von Arx 1981, 1983, McKemy & Morgan-Jones 1990, Braun 1995), reaching different conclusions. However, a first decisive revision of *Cladosporium*, leading to a more natural concept of this genus, was published by David (1997), who carried out comprehensive scanning electron microscopic examinations of the scar and hilum structure in *Cladosporium* and *Heterosporium* Klotzsch ex Cook. The first Scanning Electron Micrograph (SEM) studies of these structures, published by Roquebert (1981), indicated that the conidiogenous loci and conidial hila in *Cladosporium* are characterised by having a unique structure. David (1997) confirmed these observations, based on a wide range of *Cladosporium* and *Heterosporium* species, and demonstrated that the structures of the conidiogenous loci and hila in the latter genus fully agree with those of *Cladosporium*, proving that *Heterosporium* was indeed a synonym of *Cladosporium s. str*. He introduced the term "coronate" for the *Cladosporium* scar type, which is characterised by having a central convex part (dome), surrounded by a raised periclinal rim (David 1997), and showed that this type is confined to anamorphs, as far as experimentally proven, connected with teleomorphs belonging in "*Mycosphaerella*" *s. lat*. These results were confirmed in a later phylogenetic study by Braun *et al*. (2003). *Cladosporium s. str*. was shown to be a sister clade to *Mycosphaerella s. str.*, for which the new teleomorph genus *Davidiella* was proposed. Although no clear morphological differences were reported between *Davidiella* and *Mycosphaerella*, a further study by Aptroot (2006) found ascospores of *Davidiella* to have characteristic irregular cellular inclusions (lumina), which are absent in species of *Mycosphaerella*, along with periphysoids and pseudoparaphyses (Schubert *et al*. 2007b – this volume). Furthermore, a higher order phylogeny study by Schoch *et al*. (2006), which employed DNA sequence data of four loci (SSU nrDNA, LSU nrDNA, EF-1α, RPB2), revealed species of *Davidiella* to cluster in a separate family (*Davidiellaceae*) from species of *Mycosphaerella* (*Mycosphaerellaceae*), with both families residing in the *Capnodiales* (*Dothideomycetes*), and not *Dothideales* as always presumed.

Table 1. Isolates for which new sequences were generated.

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank numbers[2] (ITS, LSU)
Cladoriella eucalypti		CBS 115899*; CPC 10954	*Eucalyptus* sp.	South Africa	P.W. Crous	EU040224, EU040224
Coniothyrium palmarum		CBS 758.73; CMW 5283	*Phoenix dactylifera*	Israel	Y. Pinkas	DQ240000, EU040225
Devriesia acadiensis		CBS 115874; DAOM 232211	Soil	Canada	N. Nickerson	AY692095, EU040226
Devriesia americana		CBS 117726; ATCC 96545; CPC 5121	Air	U.S.A.	F.M. Dugan	AY251068, EU040227
Devriesia shelburniensis		CBS 115876; DAOM 232217	Soil	Canada	N. Nickerson	AY692093, EU040228
Devriesia thermodurans		CBS 115878*; DAOM 225330	Soil	Canada	N. Nickerson	AY692087, EU040229
Hormoconis resinae		CBS 365.86	–	–	–	EU040230, EU040230
		CBS 184.54; ATCC 11841; CPC 3692; IMI 089837; IFO 31706	Creosote-treated wooden pole	U.S.A.	–	AY251067, EU040231
Hyalodendriella betulae		CBS 261.82*	*Alnus glutinosa*	Netherlands	W. Gams	EU040232, EU040232
Ochrocladosporium elatum		CBS 146.33*; ATCC 11280; ATHUM 2862; IFO 6372; IMI 049629; MUCL 10094	Wood pulp	Sweden	E. Melin	EU040233, EU040233
Ochrocladosporium frigidarii		CBS 103.81*	Cooled room	Germany	B. Ahlert	EU040234, EU040234
Parapleurotheciopsis inaequiseptata		MUCL 41089; INIFAT C98/30-1	Rotten leaf	Brazil	R.F. Castañeda	EU040235, EU040235
Passalora daleae		CBS 113031*	*Dalea spinosa*	Mexico	L.B. Sparrius	EU040236, EU040236
Rachicladosporium luculiae		CPC 11407*	*Luculia* sp.	New Zealand	F. Hill	EU040237, EU040237
Ramularia aplospora	*Mycosphaerella alchemillicola*	CBS 545.82*	Powdery mildew on *Alchemilla vulgaris*	Germany	T. Hijwegen	EU040238, EU040238
Retroconis fusiformis		CBS 330.81; IMI 170799	*Gossypium* sp.	Pakistan	–	EU040239, EU040239
Rhizocladosporium argillaceum		CBS 241.67*; ATCC 38103; IFO 7055; OUT 4262	Decayed myxomycete	Japan	K. Tubaki	EU040240, EU040240
Subramaniomyces fusisaprophyticus		CBS 418.95; INIFAT C94/134	Leaf litter	Cuba	R.F. Castañeda	EU040241, EU040241
Thedgonia ligustrina		W1877	*Ligustrum* sp.	–	H. Evans	EU040242, EU040242
Toxicocladosporium irritans		CBS 185.58*	Mouldy paint	Suriname	M.B. Schol-Schwarz	EU040243, EU040243
Verrucocladosporium dirinae		CBS 112794*	*Dirina massiliensis*	U.K.	A. Aptroot	EU040244, EU040244

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; ATHUM: Culture Collection of Fungi, University of Athens, Department of Biology, Section of Ecology and Systematics, Athens, Greece; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Culture collection of Mike Wingfield, housed at FABI, Pretoria, South Africa; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; IFO: Institute For Fermentation, Osaka, Japan; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; INIFAT: Alexander Humboldt Institute for Basic Research in Tropical Agriculture, Ciudad de La Habana, Cuba; MUCL: Mycotheque de l' Université Catholique de Louvain, Louvain-la-Neuve, Belgium; OUT: Department of Fermentation Technology, Faculty of Engineering, Osaka University, Yamadaue, Suita-shi, Osaka, Japan.

[2]ITS: internal transcribed spacer regions and 5.8S rRNA gene; LSU: partial 28S rRNA gene.

*Ex-type cultures.

The current circumscription of *Cladosporium* emend. can be summarised as follows: *Dematiaceous hyphomycetes;* Davidiella *anamorphs; mycelium internal and external; hyphae branched, septate, pigmented; stromata lacking or occasionally present; conidiophores mononematous, solitary to fasciculate, cylindrical, geniculate-sinuous to nodulose, simple to branched, subhyaline to usually distinctly pigmented, continuous to septate, smooth to verruculose; conidiogenous cells integrated, terminal and intercalary, usually sympodial, with a single to several scars; conidiogenesis holoblastic; conidiogenous loci coronate, i.e., more or less protuberant, composed of a central convex dome, surrounded by a raised periclinal rim, barely to distinctly darkened; conidia solitary or in short to long, simple to branched acropetal chains, amero- to phragmosporous, subhyaline to usually distinctly pigmented, smooth, verruculose, verrucose, echinulate, cristate, hila coronate, more or less protuberant.*

The new concept of *Cladosporium s. str.*, supported by molecular data and typical coronate conidiogenous loci and conidial hila, rendered it possible to initiate a comprehensive revision of *Cladosporium s. lat*. The preparation of a general, annotated check-list of *Cladosporium s. lat.* was the first step in this direction (Dugan *et al*. 2004). The aim of the present study, therefore, was to delineate *Cladosporium s. str*. from other taxa that have in recent years been described in *Cladosporium s. lat*. To attain this goal isolates were studied under standardised conditions on a set of predescribed media (Schubert *et al*. 2007b – this volume), and subjected to DNA sequence analysis of the LSU nrRNA gene.

MATERIALS AND METHODS

Isolates

Isolates used were obtained from the Centraalbureau voor Schimmelcultures (CBS), or freshly isolated from various substrates (Table 1). Strains were cultured on 2 % malt extract plates (MEA; Gams *et al*. 2007), by obtaining single conidial colonies as explained in Crous (1998). Colonies were subcultured onto fresh MEA, oatmeal agar (OA), potato-dextrose agar (PDA) and synthetic nutrient-poor agar (SNA) (Gams *et al*. 2007), and incubated under near-ultraviolet light to study their morphology. Cultural characteristics were assessed after 2–4 wk on OA and PDA at 25 °C in the dark, using the colour charts of Rayner (1970). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org).

DNA isolation, sequencing and phylogeny

Fungal colonies were established on agar plates, and genomic DNA was isolated following the CTAB-based protocol described in Gams *et al*. (2007). The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene (LSU). Four internal primers, namely ITS4 (White *et al.* 1990), LR0R (Rehner & Samuels 1994), LR3R (www.biology.duke.edu/fungi/mycolab/primers.htm), and LR16 (Moncalvo *et al*. 1993), were used for sequencing to ensure that good quality overlapping sequences are obtained. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous *et al*. (2006d). The ITS1, ITS2 and 5.8S rRNA gene (ITS) were only sequenced for isolates of which these data were not available. The ITS data were not included in the analyses but deposited in GenBank where applicable. Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as missing data. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Morphology

Wherever possible, 30 measurements (× 1 000 magnification) were made of structures mounted in lactic acid or Shear's solution (Gams *et al*. 2007), with the extremes of spore measurements given in parentheses. Microscopic observations were made from colonies cultivated for 7 d under continuous near-ultraviolet light at 25 °C on SNA as explained in Schubert *et al*. (2007b – this volume). Three classes of conidia are distinguished. *Ramoconidia* are defined as short apical branches (often conidiogenous cells) of a conidiophore which secede and function as conidia. They are characterised by having a truncate, undifferentiated base, i.e., they differ from true conidia by lacking characteristic basal hila caused by conidiogenesis. Ramoconidia give rise to branched or unbranched conidia. *Secondary ramoconidia* are branched conidia with a narrowed base, bearing a true hilum, that can occur in chains, giving rise to *conidia*, which differ from secondary ramoconidia with regards to shape, size and septation. In previous literature on *Cladosporium* and allied genera, the true ramoconidia have often been classified as "ramoconidia *s. str*." whereas the secondary ramoconidia have been named "ramoconidia *s. lat*."

RESULTS

DNA extraction, amplification and phylogeny

Amplicons of approximately 1 700 bases were obtained for the isolates listed in Table 1. The newly generated sequences were used to obtain additional sequences from GenBank, which were added to the alignment. The manually adjusted LSU alignment contained 73 sequences (including the two outgroup sequences) and 996 characters including alignment gaps. Of the 849 characters used in the phylogenetic analysis, 336 were parsimony-informative, 77 were variable and parsimony-uninformative, and 436 were constant. Neighbour-joining analyses using three substitution models on the sequence data yielded trees with identical topologies to one another. The neighbour-joining trees support the same clades as obtained from the parsimony analysis, but with a different arrangement at the deeper nodes, which were poorly supported in the bootstrap analyses or not at all (for example, the *Helotiales* and *Pleosporales* are swapped around). Performing a parsimony analysis with gaps treated as new characters increases the number of equally parsimonious trees to 94; the same topology is observed but with less resolution for the taxa in the *Helotiales* (data not shown). Forty-four equally most parsimonious trees (TL = 1 572 steps; CI = 0.436; RI = 0.789; RC = 0.344), one of which is shown in Fig. 1, were obtained from the parsimony analysis of the LSU sequence data. The cladosporium-like taxa were found to belong to the *Helotiales*, *Pleosporales*, *Sordariales* and as sister taxa to the *Davidiellaceae* in the *Capnodiales*.

The LSU alignment used for parsimony and distance analysis was supplemented with sequences for *Parapleurotheciopsis inaequiseptata* (Matsush.) P.M. Kirk and *Subramaniomyces fusisaprophyticus* (Matsush.) P.M. Kirk, as well as related sequences

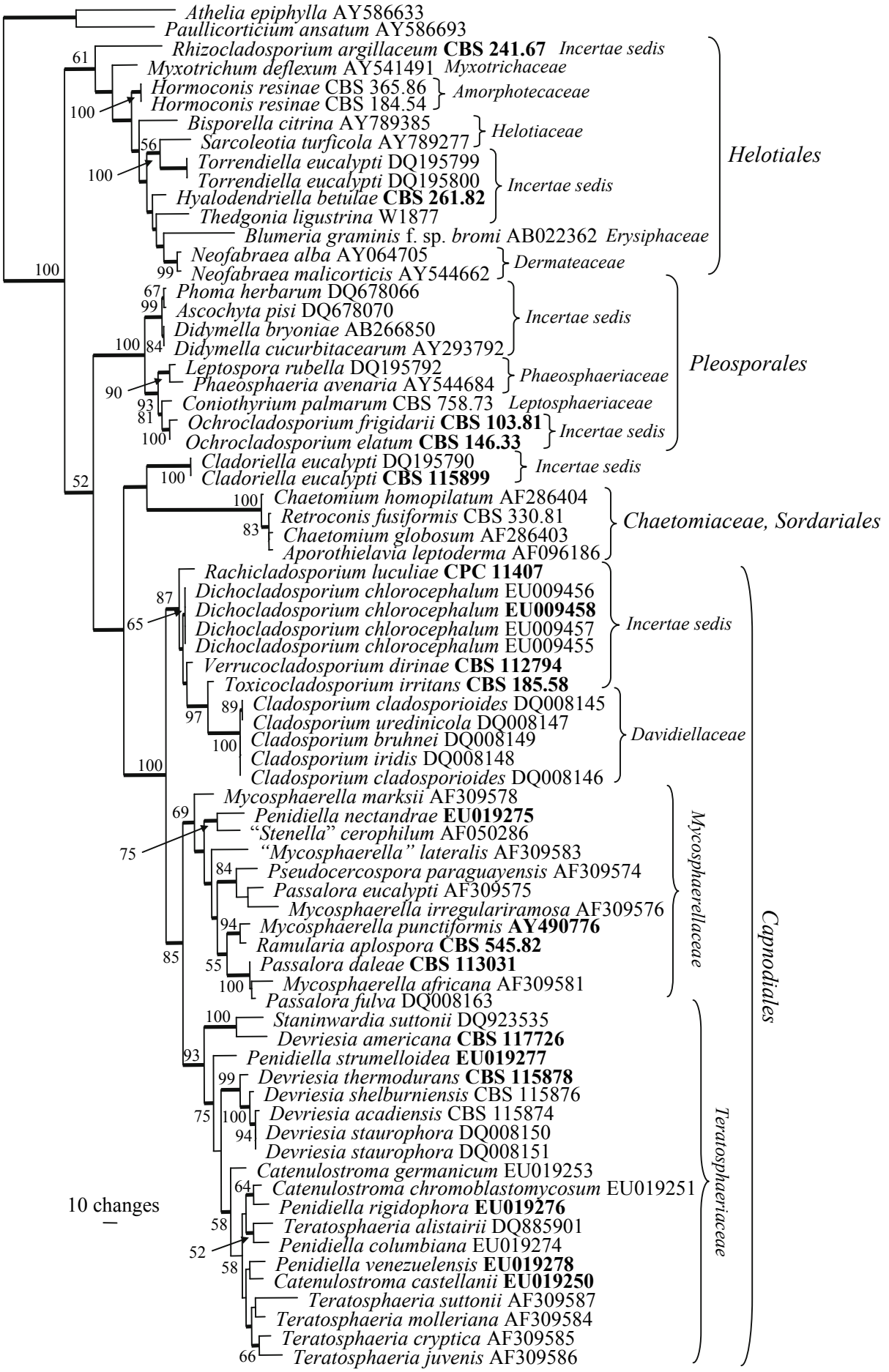

Fig. 1. One of 44 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

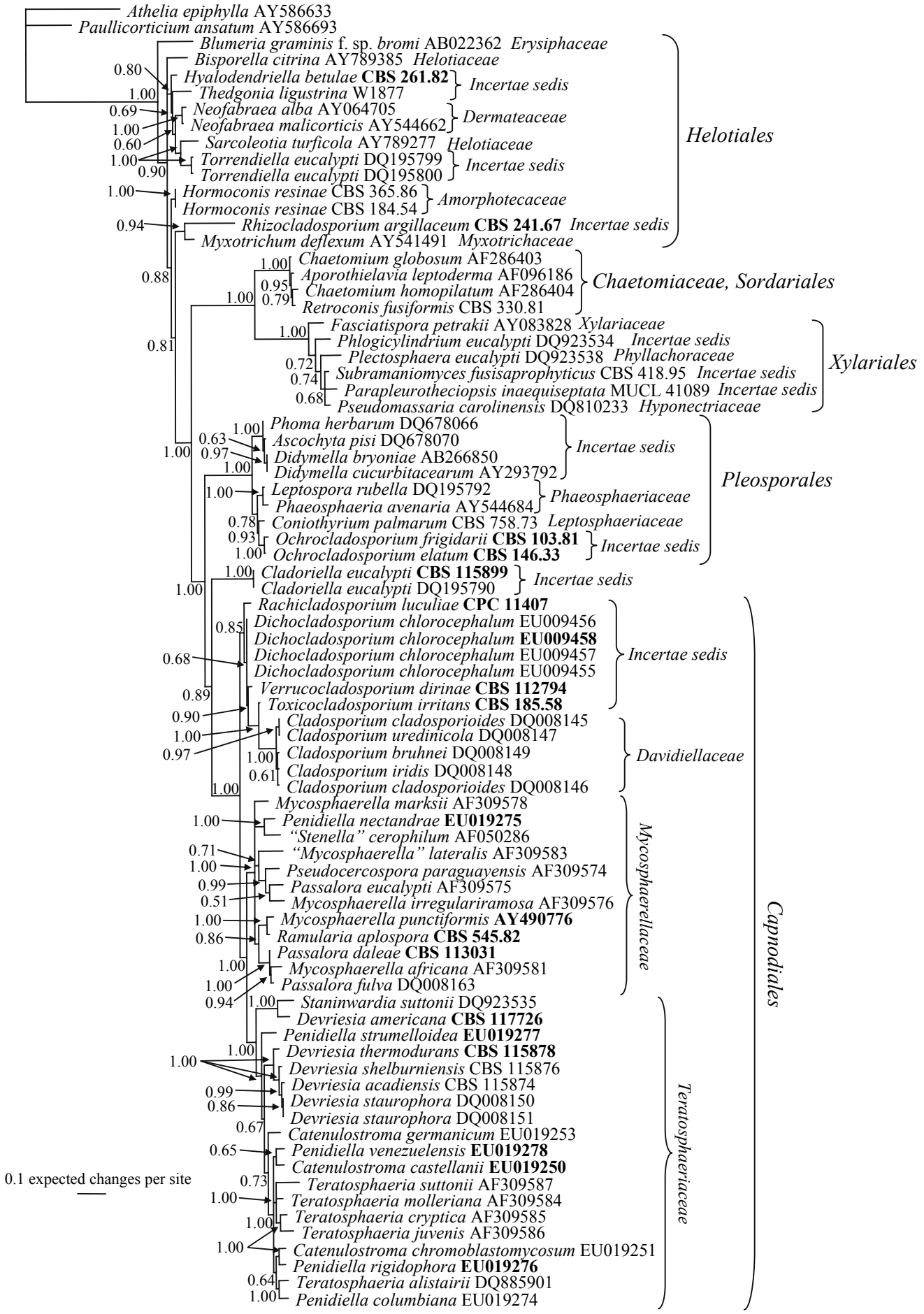

Fig. 2. Consensus phylogram (50 % majority rule) of 800 trees resulting from a Bayesian analysis of the LSU sequence alignment using MrBayes v. 3.1.2. Bayesian posterior probabilities are indicated at the nodes. Ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

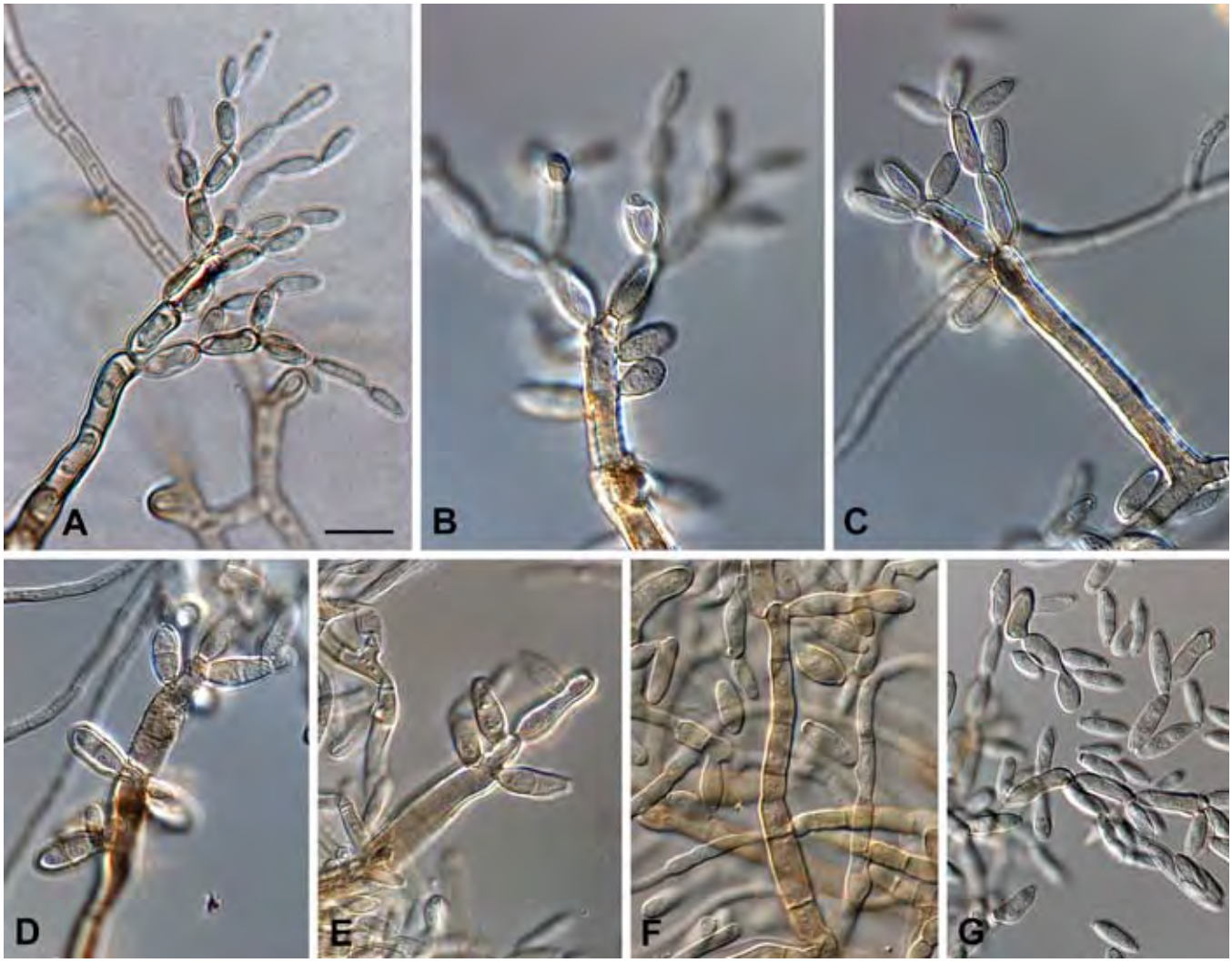

Fig. 3. *Rachicladosporium luculiae* (type material). A–F. Conidiophores with conidial chains, and conidiogenous loci aggregated in the upper region. G. Conidia. Scale bar = 10 µm.

from GenBank. This alignment was subjected to a Bayesian analysis using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies and the temp value set to 0.5. The Markov Chain Monte Carlo (MCMC) analysis of 4 chains started from a random tree topology and lasted 1 000 000 generations. Trees were saved each 1 000 generations, resulting in 1 000 trees. Burn-in was set at 200 000 generations after which the likelihood values were stationary, leaving 800 trees from which the consensus tree (Fig. 2) and posterior probabilities (PP's) were calculated. The average standard deviation of split frequencies was 0.018459 at the end of the run. The same overall topology as that observed using parsimony was obtained, with the main exception that the *Helotiales* and *Pleosporales* swapped around, as observed with the distance analysis.

Taxonomy

The present study has delineated several cladosporium-like genera which are phylogenetically unrelated to, and morphologically distinct from *Cladosporium s. str.* (*Davidiellaceae, Capnodiales*). These are treated below:

Capnodiales, incertae sedis

Rachicladosporium Crous, U. Braun & C.F. Hill, **gen. nov.** MycoBank MB504430.

Etymology: Named after the apical rachis on conidiophores, and its cladosporium-like appearance.

Differt a Cladosporio conidiophoris cum rachibus terminalibus, locis conidiogenis inconspicuis vel subconspicuis, margine leviter incrassatis, non fuscatis et non refractivis, hilis inconspicuis.

Mycelium consisting of branched, septate, smooth, hyaline to pale brown, thin-walled hyphae. *Conidiophores* erect, solitary, macronematous, arising from superficial hyphae, subcylindrical, straight to somewhat geniculate-sinuous, medium brown, finely verruculose; basal foot cell without swelling or rhizoids. *Conidiogenous cells* integrated, terminal, subcylindrical or tips slightly swollen, forming an apical rachis, multilocal, loci terminal and lateral, without evident sympodial proliferation (non-geniculate); conidiogenous loci inconspicuous or subconspicuous by being very slightly thickened along the rim, but neither darkened nor refractive, giving rise to simple or branched chains or solitary conidia. *Ramoconidia* medium brown, finely verruculose, 0–1-septate, subcylindrical to narrowly ellipsoid; *conidia* ellipsoid, pale brown, 0(–1)-septate, smooth to finely verruculose; hila inconspicuous; secession schizolytic.

Type species: *Rachicladosporium luculiae* Crous, U. Braun & C.F. Hill, sp. nov.

Rachicladosporium luculiae Crous, U. Braun & C.F. Hill, **sp. nov.** MycoBank MB504431. Fig. 3.

Etymology: Named after its host genus, *Luculia.*

Mycelium ex hyphis ramosis, septatis, levibus, hyalinis vel pallide brunneis, 2–3 µm latis compositum. Conidiophora erecta, solitaria, macronemata, ex hyphis superficialibis oriunda, subcylindrica, recta to geniculata-sinuosa, ad 60 µm longa et 6 µm lata, 3–6-septata, modice brunnea, subtiliter verruculosa, non crassitunicata, ad basim non inflatae et non rhizoideae. Cellulae conidiogenae integratae, terminales, 8–15 × 4–5 µm, subcylindricae, apicem versus attenuatae, apice obtuso, rachidi terminali, locis conidialibus numerosis, 1–2 µm latis, margine leviter incrassatis, non fuscatis et non refractivis. Conidia catenata vel solitaria. Ramoconidia modice brunnea, subtile verruculosa, 0–1-septata, subcylindrica vel anguste ellipsoidea, 10–17 × 4–5 µm; conidia secundaria ellipsoidea, pallide brunnea, 0(–1)-septata, levia vel subtile verruculosa, interdum guttulata, (7–)9–12(–15) × 3(–4) µm; hila inconspicua.

Mycelium consisting of branched, septate, smooth, thin-walled, hyaline to pale brown, 2–3 µm wide hyphae. *Conidiophores* erect, solitary, macronematous, arising from superficial hyphae, subcylindrical, straight to somewhat geniculate-sinuous, up to 60 µm long, and 6 µm wide, 3–6-septate, medium brown, finely verruculose, thin-walled (≤ 1 µm), rarely with a single percurrent proliferation; basal foot cell without swelling or rhizoids. *Conidiogenous cells* integrated, terminal, 8–15 × 4–5 µm, subcylindrical, tapering to an obtuse apex, occasionally slightly swollen at the tip, without distinct sympodial proliferation (non-geniculate), forming a rachis, with several conidiogenous loci, terminal and lateral, 1–2 µm wide, non-protuberant, quite inconspicuous to subconspicuous, very slightly thickened along the rim, but not darkened and refractive; giving rise to simple or branched chains or solitary conidia, thin-walled (≤ 0.75 µm). *Ramoconidia* medium brown, finely verruculose, 0–1-septate, subcylindrical to narrowly ellipsoid, 10–17 × 4–5 µm; *conidia* ellipsoid, pale brown, 0(–1)-septate, smooth to finely verruculose, at times guttulate, (7–)9–12(–15) × 3(–4) µm; hila inconspicuous, neither thickened nor darkened-refractive.

Cultural characteristics: Colonies on PDA erumpent, spreading, with moderate aerial mycelium and smooth, even margins; iron-grey in the centre, olivaceous-grey in the outer region (surface); iron-grey underneath. Colonies reaching 4 cm diam after 1 mo at 25 °C in the dark.

Specimen examined: **New Zealand**, Auckland, isolated from leaf spots on *Luculia* sp. (*Rubiaceae*), 25 Jul. 2004, F. Hill 1059, **holotype** CBS H-19891, culture ex-type CBS 121620 = CPC 11407.

Notes: *Rachicladosporium* is morphologically quite distinct from *Cladosporium s. str.* and allied cladosporioid genera by having an apical conidiophore rachis with inconspicuous to subconspicuous scars and unthickened, not darkened-refractive conidial hila. Due to the structure of the conidiogenous cells, *R. luculiae* superficially resembles species of the tretic genus *Diplococcium* Grove (Ellis 1971, 1976; Goh & Hyde 1998). However, there is no evidence for a tretic conidiogenesis in *R. luculiae*. The conidia are formed holoblastically and separated by a thin septum. Furthermore, in *Diplococcium* the conidiogenous cells are terminal as well as intercalary, the conidiophores are often branched, and branched conidial chains are lacking or at least less common. Molecular sequence data about *Diplococcium* species are not yet available, though taxa that have been analysed show affinities to the *Pleosporaceae* and *Helotiales* (Wang *et al*., unpubl. data), whereas *Rachicladosporium* is allied with the *Capnodiales*. The ecology of *R. luculiae* is still unclear, although it has been isolated from lesions on *Luculia* sp. Fruiting of this species *in vivo* has not yet been observed, and its pathogenicity remains unproven.

Toxicocladosporium Crous & U. Braun, **gen. nov.** MycoBank MB504426.

Etymology: Named after ample volatile metabolites produced in culture, and cladosporium-like morphology.

Differt a Cladosporio locis conidiogenis denticulatis, incrassatis et fuscatis-refractivis, sed non coronatis, conidiophoris et conidiis cum septis incrassatis et atrofuscis, et culturis cum metabolitis volaticis toxicis.

Mycelium consisting of branched, septate, dark brown, finely verruculose hyphae. *Conidiophores* solitary, dimorphic, solitary, macronematous or micronematous, reduced to conidiogenous cells. *Macronematous conidiophores* subcylindrical, straight to geniculate-sinuous, or irregularly curved, unbranched or branched above, septate, dark brown, finely verruculose, walls thick, septa dark brown; *micronematous conidiophores* reduced to conidiogenous cells, erect, doliiform to subcylindrical, with slight taper towards the apex. *Conidiogenous cells* integrated, terminal or lateral, subcylindrical with slight taper towards apex; proliferating sympodially with apical loci protruding and denticle-like, thickened, darkened and refractive, but not coronate. *Conidia* catenulate in branched or unbranched chains, medium to dark brown, thick-walled, with dark, thick septa, smooth to finely verruculose; *ramoconidia* septate, prominently constricted at septa, broadly ellipsoid to subcylindrical; conidia ellipsoid to ovoid, pale to medium brown, 0(–1)-septate; hila not coronate, but protruding, thickened, darkened and refractive in ramoconidia, but less obvious in young conidia.

Type species: *Toxicocladosporium irritans* Crous & U. Braun, sp. nov.

Toxicocladosporium irritans Crous & U. Braun, **sp. nov.** MycoBank MB504427. Fig. 4.

Etymology: Named after the skin irritation resulting from exposure to the fungus.

Mycelium (in PDA) ex hyphis ramosis, septatis, atro-brunneis, minute verruculosis, (2–)3–4 µm latis, ultimo crassitunicatis et crassiseptatis. Conidiophora solitaria, dimorphosa, macronemata et solitaria vel micronemata. Conidiophora macronemata ex hyphis modice brunneis lateraliter oriunda, erecta, subcylindrica, recta, geniculata-sinuosa vel irregulariter curvata, non ramosa vel ad apicem ramosa, 2–7-septata, atro-brunnea, leviter verruculosa, crassitunicata, septa atro-brunnea, 30–60 × 4–6 µm; conidiophora micronemata saepe non septata, raro 1–2-septata, erecta, doliiformes vel subcylindrica, apicem versus leviter attenuata, 10–30 × 2.5–4 µm. Cellulae conidiogenae integratae, terminales vel laterales, subcylindricae, apicem versus leviter attenuatae, 7–12 × 3–4 µm, sympodiales, cum 1–3 locis conidiogenibus, denticulatis, 1–1.5 µm latis, incrassatis, fuscatis-refractivis. Conidia catenulata vel rami-catenulata, modice vel atro-brunnea, crassitunicata, septis incrassatis, fuscatis, levia vel subtile verruculosa; ramoconidia (0–)1(–3)-septata, constricta, late ellipsoidea vel subcylindrica, 7–15 × 3–5 µm; conidia secundaria ellipsoidea vel ovoidea, pallide vel modice brunnea, 0(–1)-septata, (5–)6–8(–10) × (3–)4(–5) µm; hila protuberantes, 1–1.5 µm lata, hila ramoconidiorum incrassata et fuscata-refractiva, vel hila conidiorum secundariorum 0.5–1 µm lata et subconspicua.

Mycelium on PDA consisting of branched, septate, dark brown, finely verruculose, (2–)3–4 µm wide hyphae; walls and septa becoming thickened and darkened with age. *Conidiophores* solitary, dimorphic, macronematous and solitary, or micronematous, reduced to conidiogenous cells. *Macronematous conidiophores* subcylindrical, straight to geniculate-sinuous, or irregularly curved, unbranched or branched above, 2–7-septate, dark brown, finely verruculose, walls thick, septa dark brown, 30–60 × 4–6 µm; medium brown hyphae giving rise to lateral, erect branches that become swollen, dark brown, and develop into macronematous conidiophores with thick-walled and dark septa; *micronematous conidiophores*

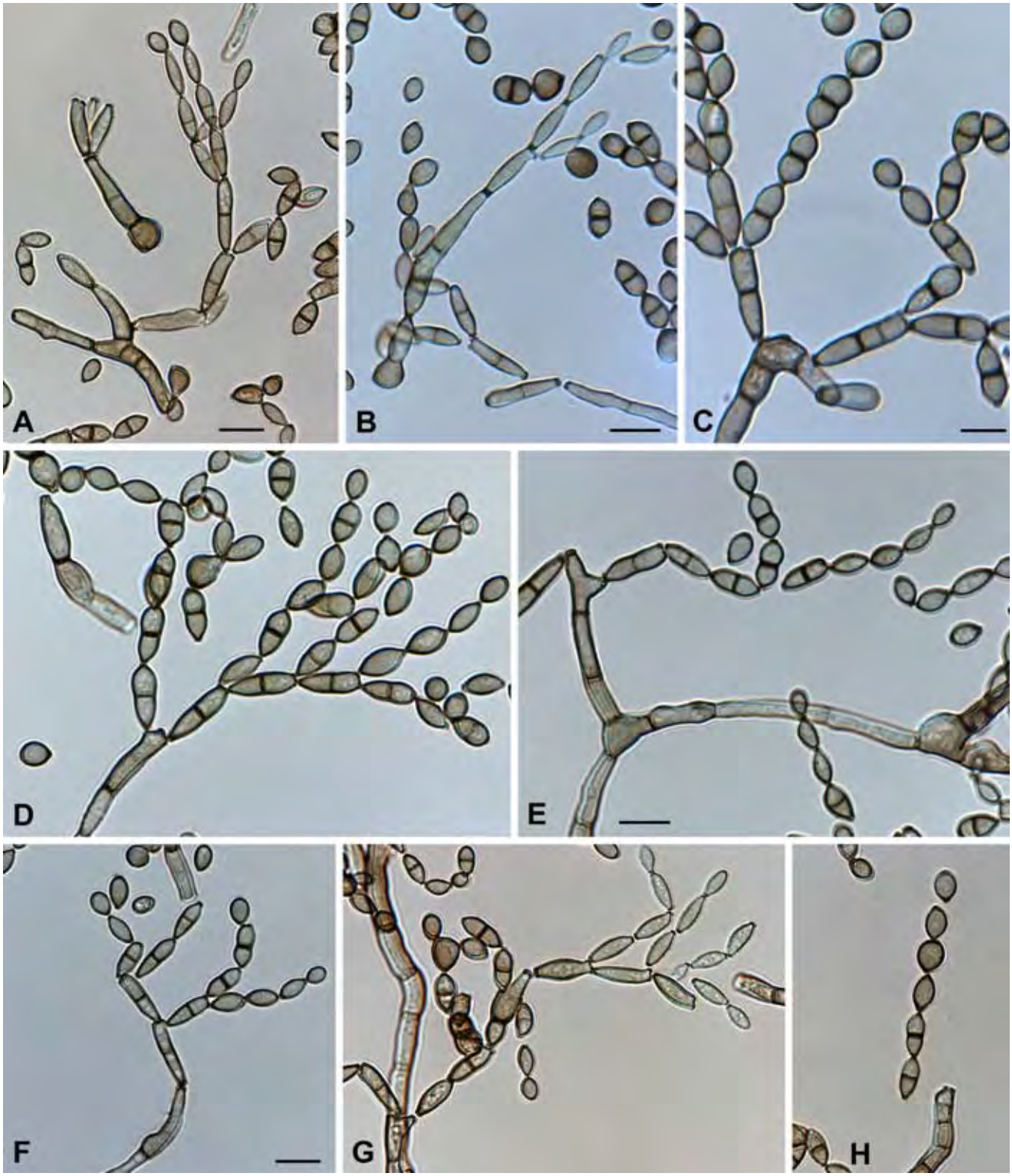

Fig. 4. *Toxicocladosporium irritans* (type material). A–B, F. Microconidiophores. C–E. Macroconidiophores. G–H. Ramoconidia and conidia. Scale bars = 10 µm.

aseptate, reduced to conidiogenous cells (rarely 1–2-septate, i.e., with 1–2 supporting cells), erect, doliiform to subcylindrical, with slight taper towards the apex, 10–30 × 2.5–4 µm. *Conidiogenous cells* integrated, terminal or lateral, subcylindrical with slight taper towards apex, 7–12 × 3–4 µm; proliferating sympodially with 1–3 apical loci that can be slightly protruding and denticle-like, 1–1.5 µm wide, thickened, darkened and refractive. *Conidia* catenulate in branched or unbranched chains, medium to dark brown, thick-walled, with dark, thick septa, smooth to finely verruculose; ramoconidia (0–)1(–3)-septate, prominently constricted at septa, broadly ellipsoid to subcylindrical, 7–15 × 3–5 µm; conidia ellipsoid to ovoid, younger apical conidia pale to medium brown, 0(–1)-septate, (5–)6–8(–10) × (3–)4(–5) µm; hila protruding, 1–1.5 µm wide, thickened, darkened and refractive in ramoconidia, but less obvious in young conidia, where hila are 0.5–1 µm wide.

Cultural characteristics: Colonies on PDA erumpent, spreading, with dense aerial mycelium and smooth, even margins; surface olivaceous-black (centre), olivaceous-grey in outer region; reverse olivaceous-black. Colonies reaching 35 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Suriname**, Paramaribo, isolated from mouldy paint, Feb. 1958, M.B. Schol-Schwarz, **holotype** CBS-H 19892, culture ex-type CBS 185.58.

Notes: *Toxicocladosporium irritans* produces ample amounts of volatile metabolites, which cause a skin rash within minutes of opening an inoculated dish for microscopic examination. Morphologically and phylogenetically it is very similar to *Cladosporium s. str*., and produces dimorphic conidiophores, which is also commonly observed in *Cladosporium*. It is distinct by having dark, thick-walled conidial and conidiophore septa, and lacking the typical coronate *Cladosporium* scar type (David 1997).

Verrucocladosporium K. Schub., Aptroot & Crous, **gen. nov.** MycoBank MB504432.

Etymology: Named after its frequently coarsely verrucose to warted hyphae, conidiophores and conidia, and cladosporium-like morphology.

Differt a Cladosporio hyphis saepe verrucosis, hyalinis, conidiophoris cylindraceis-filiformibus, rectis, non vel vix geniculatis, non nodulosis, locis conidiogenis leviter incrassatis, distincte fuscatis-refractivis, sed non coronatis, conidiis saepe valde variantibus, saepe irregulariter formatis, grosse verrucosis-rugosis.

Mycelium sparingly branched, hyphae septate, not constricted at septa, hyaline, almost smooth to irregularly rough-walled, coarsely verrucose to warted. *Conidiophores* arising laterally from creeping hyphae, erect, straight, or somewhat flexuous, narrowly cylindrical to filiform, neither geniculate nor nodulose, unbranched, septate, pale brown, thin-walled, smooth to often irregularly rough-walled or verrucose. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, polyblastic, with sympodial proliferation, with loci often crowded at the apex, truncate, barely to slightly thickened, but distinctly darkened-refractive. *Ramoconidia* cylindrical, aseptate, concolorous with conidiophores, thin-walled, irregularly rough-walled, coarsely verruculose to verrucose-rugose; hila unthickened but somewhat refractive. *Conidia* in long unbranched or loosely branched chains, obovoid, ellipsoid, fusiform to subcylindrical, with swollen and constricted parts, often appearing irregular in shape and outline, 0–1-septate, pale brown, thin-walled and irregularly rough-walled, verruculose-rugose; hila truncate, barely to slightly thickened, but distinctly darkened-refractive.

Type species: *Verrucocladosporium dirinae* K. Schub., Aptroot & Crous, sp. nov.

Verrucocladosporium dirinae K. Schub., Aptroot & Crous, **sp. nov.** MycoBank MB504433. Fig. 5.

Etymology: Named after its host, *Dirina massiliensis.*

Mycelium sparse ramosum. Hyphae 1–3 µm latae, septatae, non constrictae, hyalinae, leviae, vel irregulariter verruculosae, interdum verrucosae, tuberculatae, tenuitunicatae. Conidiophora ex hyphis repentibus lateraliter oriunda, erecta, recta, interdum leviter flexuosa, anguste cylindrica vel filiformes, non geniculta, non nodulosa, non ramosa, ad 85 µm longa, 2–3 µm lata, septata, tenuitunicata (≤ 0.75 µm), pallide brunnea, levia vel saepe irregulariter verrucosa, leviter crassitunicata. Cellulae conidiogenae integratae, saepe terminales, interdum intercalares, cylindricae, angustae, 9–20 µm longae, holoblasticae, sympodiales, locis conidiogenibus 1–3, saepe ad apicem aggregatis, interdum protuberantibus, truncatis, 1–1.8(–2) µm latis, incrassatis et fuscatis-refractivis. Ramoconidia cylindrica, 16–21 × (2–)2.5–3 µm, non septata, pallide brunnea, tenuitunicata, irregulariter verruculosa vel crosse verrucosa-rugosa, ad 4 hilis terminalibus, ad basim late truncata, non attenuata, 2–2.5 µm lata, non incrassata, sed leviter refractiva. Conidia catenata, in catenis longis, non ramosis vel laxe ramosis, plus minusve recta, obovoidea, ellipsoidea, fusiformes vel subcylindricae, sed saepe irregulares, 4–18(–23) × (2–)2.5–3.5 µm, 0–1-septata, ad septa interdum constricta, pallide brunnea, tenuitunicata (≤ 0.5 µm), irregulariter verruculosa-rugosa, utrinque leviter attenuata, hila truncata, (0.5–)0.8–1.5(–2) µm lata, vix vel leniter incrassata, sed distincte fuscata-refractiva.

Mycelium sparingly branched; hyphae 1–3 µm wide, septate, not constricted at septa, hyaline, smooth to irregularly rough-walled, sometimes coarsely verrucose, with small to large drop-like, tuberculate warts, walls unthickened. *Conidiophores* arising laterally from creeping hyphae, erect, straight, sometimes slightly flexuous, narrowly cylindrical to filiform, not geniculate, non nodulose, unbranched, up to 85 µm long, 2–3 µm wide, septate, thin-walled (≤ 0.75 µm), pale brown, smooth to often irregularly rough-walled, verrucose, walls slightly thickened. *Conidiogenous cells* integrated, mostly terminal, sometimes also intercalary, cylindrical, narrow, 9–20 µm long, conidiogenesis holoblastic, proliferation sympodial, with a single or up to three conidiogenous loci, often crowded at the apex, sometimes situated on small lateral prolongations, loci truncate, 1–1.8(–2) µm wide, thickened and darkened-refractive. *Ramoconidia* cylindrical, 16–21 × (2–)2.5–3 µm, aseptate, concolorous with conidiophores, thin-walled, irregularly rough-walled, verruculose to coarsely verrucose-rugose, apically with up to 4 hila, with a broadly truncate, non-attenuated base, 2–2.5 µm wide, unthickened but somewhat refractive. *Conidia* catenate, in long unbranched or loosely branched chains, more or less straight, obovoid, ellipsoid, fusiform to subcylindrical, but often appearing to form band-like structures, with swollen and constricted parts, accordion or fir tree-like and also due to ornamentation often appearing irregular in shape and outline, 4–18(–23) × (2–)2.5–3.5 µm, 0–1-septate, sometimes constricted at the more or less median septum, pale brown, thin-walled (≤ 0.5 µm), irregularly rough-walled, verruculose-rugose, somewhat attenuated towards apex and base, hila truncate, (0.5–)0.8–1.5(–2) µm wide, barely or slightly thickened, but distinctly darkened-refractive; microcyclic conidiogenesis not observed.

Cultural characteristics: Colonies erumpent, spreading, with catenate, feathery margins and moderate aerial mycelium on PDA. Surface grey-olivaceous, reverse iron-grey. Colonies reaching 25 mm after 1 mo at 25 °C.

Specimen examined: **U.K.**, Somerset, Kingsbury Episcopi, isolated from the lichen *Dirina massiliensis* (*Roccelaceae*, *Arthoniales*), Mar. 2003, A. Aptroot, **holotype** CBS-H 19883, culture ex-type CBS 112794.

Notes: *Verrucocladosporium dirinae* was deposited as *Cladosporium arthoniae* M. Christ. & D. Hawksw., but the name was misapplied. The latter species, described from apothecia of *Arthonia impolita* on *Quercus* from Sweden, does not possess clearly visible, distinct conidiogenous loci and hila, and therefore has to be excluded from the genus *Cladosporium s. str*. and is also easily distinguishable from the newly introduced species above. Furthermore the conidiophores are apically frequently branched and the catenate, ellipsoid conidia are smaller and wider, 6–10 × 4–5 µm (Hawksworth 1979). Due to the conidiogenesis and the structure of the conidiogenous loci and conidia, *C. arthoniae* is rather close to lichenicolous *Taeniolella* S. Hughes species. The unique feature of the new genus *Verrucocladosporium* is its unusual conidial and hyphal ornamentation. Furthermore, it differs from *Cladosporium s. str.* in having cylindrical-filiform conidiophores, which are neither geniculate nor nodulose, quite distinct, thickened and darkened, but non-coronate conidiogenous loci and often irregularly shaped conidia. Phylogenetially, it is also distinct as a sister taxon to *Cladosporium s. str*. Concerning differences to other cladosporioid genera, see “key to the genera”. *Verrucocladosporium dirinae* has been isolated from the lichen species *Dirina massiliensis*, i.e., this species is probably lichenicolous, although its ecology is not quite clear. Fruiting of this species *in vivo* has not yet been observed. A second unnamed, taeniolella-like, lichenicolous hyphomycete was also present on the thallus of this lichen.

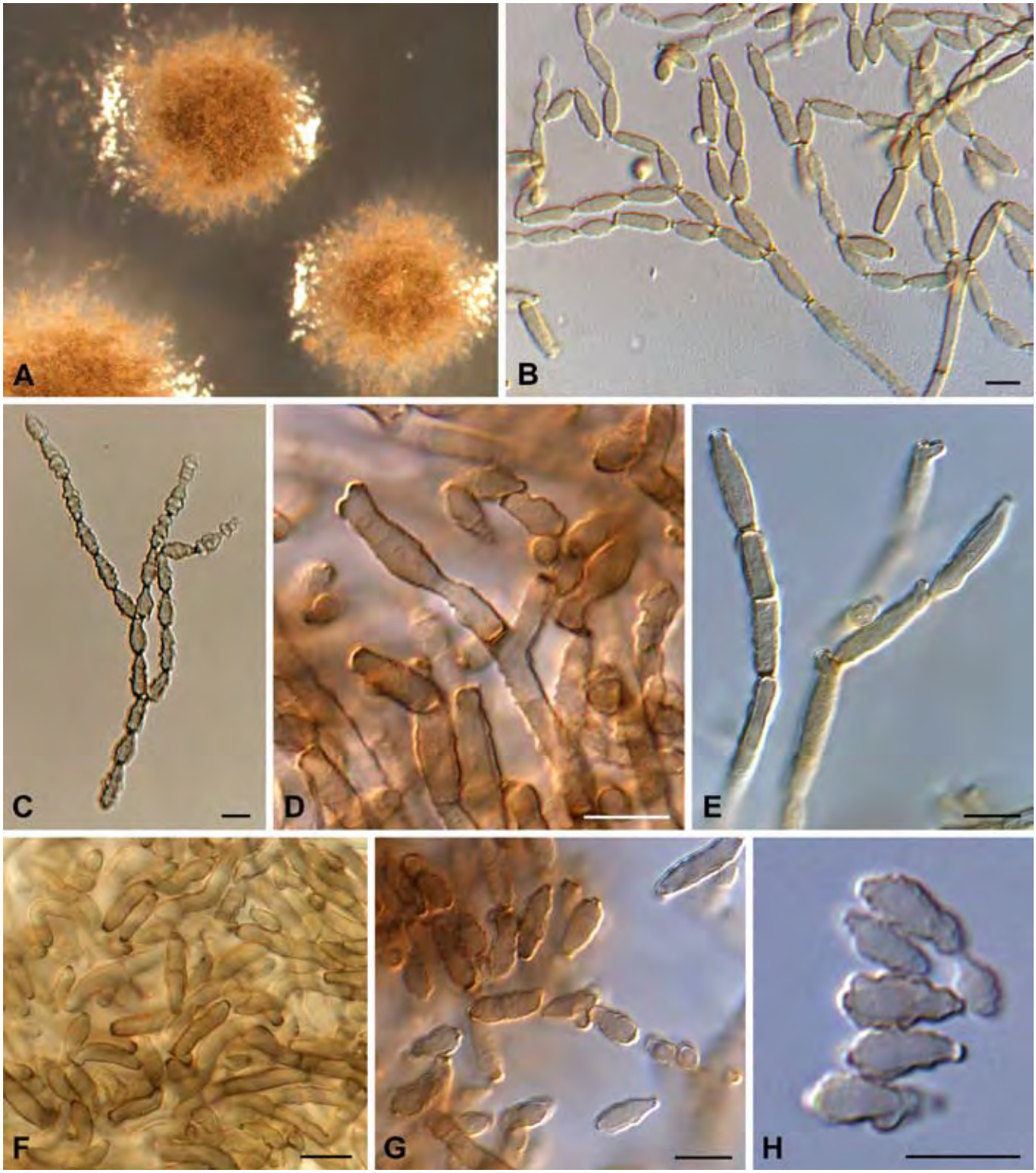

Fig. 5. *Verrucocladosporium dirinae* (type material). A. Colonies on MEA. B–C. Conidial chains. D–H. Ramoconidia and conidia. Scale bars = 10 µm.

Capnodiales, Teratosphaeriaceae

Devriesia americana Crous & Dugan, **sp. nov.** MycoBank MB504434. Fig. 6.

Etymology: Named after the geographic location of its type strain, New York, U.S.A.

Differt a D. shelburniensi conidiophoris brevioribus (ad 30 µm longis), leviter latioribus (2–3 µm), ramoconidiis saepe nullis et conidiis 0–1-septatis.

Mycelium consisting of branched, septate, 1.5–3 µm wide hyphae, irregular in width, predominantly guttulate, smooth, forming hyphal strands and hyphal coils; hyphae frequently forming dark brown, thick-walled, intercalary, muriformly septate chlamydospores on PDA in culture. *Conidiophores* subcylindrical, medium brown, straight to irregularly curved, up to 7-septate and 30 µm tall, 2–3 µm wide, or reduced to conidiogenous cells. *Conidiogenous cells* terminal or lateral on hyphae, 5–12 × 2–3 µm, medium brown, smooth, guttulate, subcylindrical, mono- to polyblastic; scars somewhat darkened and thickened, but not refractive. *Conidia* medium brown, guttulate, smooth, in mostly unbranched chains, subcylindrical to narrowly ellipsoidal, tapering towards subtruncate ends, 0–1-septate, (7–)8–12(–16) × 2(–2.5) µm; hila darkened, somewhat thickened, not refractive, 1–1.5 µm wide.

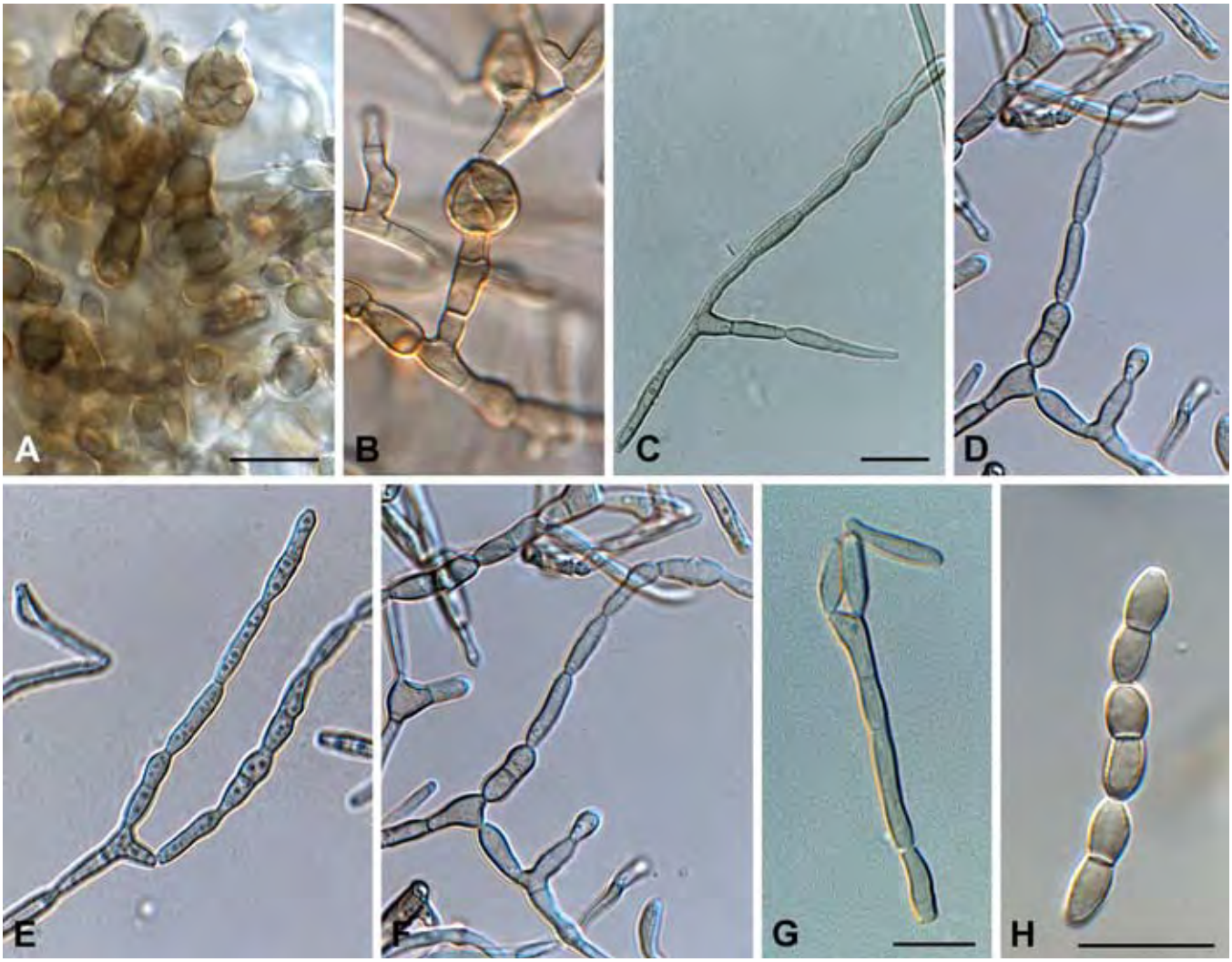

Fig. 6. *Devriesia americana* (type material). A–B. Chlamydospore-like structures formed in culture. C–F. Conidiophores giving rise to conidial chains. G–H. Conidia. Scale bars = 10 µm

Cultural characteristics: Colonies erumpent, with sparse aerial mycelium on PDA, and smooth, uneven, wide margins, submerged under the agar surface; greenish-black (surface); reverse olivaceous-black; on OA iron-grey (surface). Colonies reaching 8–15 mm diam on PDA after 14 d at 25 °C in the dark; colonies fertile, but sporulation sparse.

Specimen examined: **U.S.A.**, New York, Long Island, isolated from air, F.M. Dugan, **holotype** CBS-H 19894, culture ex-type CBS 117726 = ATCC 96545 = CPC 5121.

Notes: Until recently, this species was treated as part of the "*Phaeoramularia" hachijoensis* species complex (Braun *et al*. 2003). The present strain has conidia that are smaller than those of "*Phaeoramularia" hachijoensis*, which has ramoconidia that are 1–3-septate, up to 30 µm long, and conidia that are predominantly 1-septate, 10–21 × 2–4 µm (Matsushima 1975). From the illustration provided by Matsushima, it appears that "*Phaeoramularia" hachijoensis* is indeed a species of *Pseudocladosporium* U. Braun, a finding which is in agreement with the name *Pseudocladosporium hachijoense* (Matsush.) U. Braun proposed by Braun (1998).

Devriesia americana is both morphologically and phylogenetically more allied to *Teratosphaeria* Syd. & P. Syd. than *Venturia* Sacc. Based on its pigmented conidiophores and catenulate conidia, and scars that are somewhat darkened and thickened, and the formation of chlamydospores in culture, it is allocated to *Devriesia* Seifert & N.L. Nick. Species of the genus *Devriesia* are ecologically different, however (Seifert *et al*. 2004), being soil-borne and thermotolerant. It is possible, therefore, that further collections of this fungus may eventually indicate that it needs to be placed in a distinct genus within the *Teratosphaeriaceae*. *Devriesia americana* is the second species of *Devriesia* with muriform chlamydospores, beside *D. shelburniensis* N.L. Nick. & Seifert, but the latter species is easily distinguishable by its long and narrow conidiophores (*ca* 100–200 × 1.5–2.5 µm) and abundant ramoconidia, up to 25.5 µm long, with 0–3 septa. Furthermore, *D. shelburniensis* is a thermotolerant soil-borne hyphomycete.

Stenella araguata Syd., Ann. Mycol. 28(1/2): 205. 1930. Figs 7–8.
≡ *Cladosporium araguatum* (Syd.) Arx, *Genera of Fungi Sporulating in pure Culture*, Edn 2 (Vaduz): 224. 1974.
= *Cladosporium castellanii* Borelli & Marcano, Castellania 1: 154. 1973.

Leaf spots hypophyllous, irregular to subcircular, up to 8 mm diam, indistinct, yellow to pale brown with indistinct margins on IMI 15728(a); on IMI 34905 (Fig. 7) lesions are amphigenous, and fascicles and sporodochia are rare, with superficial mycelium being predominant. *Mycelium* consisting of internal and external, medium brown, septate, branched, verruculose, 3–4 µm wide hyphae. *Caespituli* fasciculate to sporodochial, hypophyllous, medium brown, up to 120 µm wide and 60 µm high. *Conidiophores* arising singly from superficial mycelium, or aggregated in loose to dense fascicles arising from the upper cells of a brown stroma up to 70 µm wide and 30 µm high; conidiophores medium brown, finely verruculose, 1–5-septate, subcylindrical, straight to geniculate-sinuous, unbranched

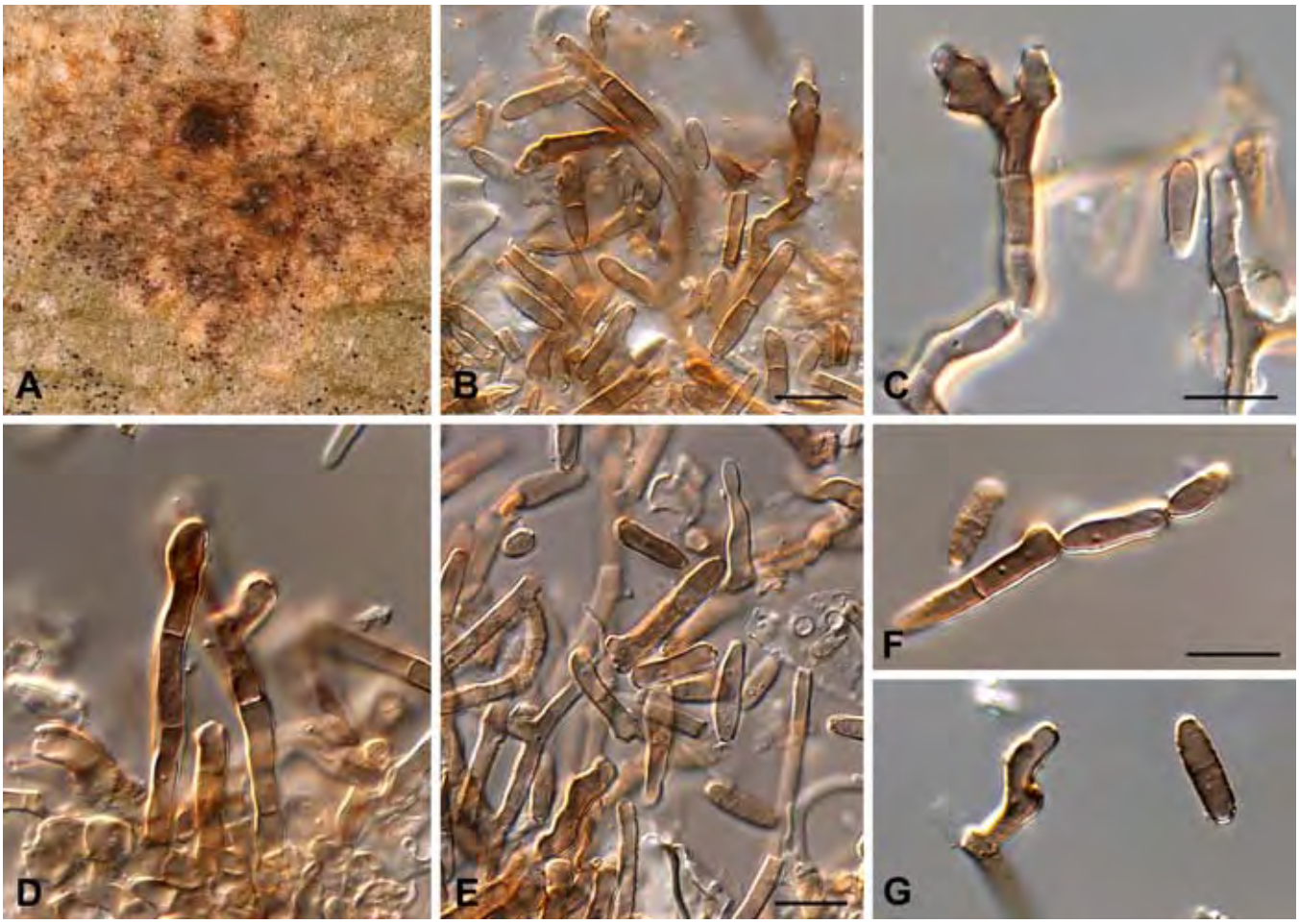

Fig. 7. *Stenella araguata* (syntype material, IMI 34905). A. Leaf spot. B. Conidiophore, conidia and verruculose hypha on leaf surface. C–D. Conidiophore with terminal conidiogenous cells. E–G. Ramoconidia and conidia. Scale bars = 10 µm.

or branched, 20–40 × 3–4 µm. *Conidiogenous cells* terminal or lateral, unbranched, medium brown, finely verruculose, tapering to slightly or flat-tipped loci, proliferating sympodially, 5–20 × 3–4 µm; scars thickened, darkened and refractive. *Conidia* solitary or catenulate, in simple chains, medium brown, verruculose, subcylindrical to narrowly obclavate, apex obtuse, base bluntly rounded with truncate hilum, straight, 0–3-septate, (7–)13–20(–25) × 3(–3.5) µm; hila thickened, darkened, refractive, 1–1.5 µm wide.

Description based on CBS 105.75 (Fig. 8): *Mycelium* consisting of branched, septate, verruculose, medium brown, 2–4 µm wide hyphae. *Conidiomata* brown, superficial, sporodochial, up to 200 µm diam *Conidiophores* solitary, erect, micro- to macronematous, 1–12-septate, subcylindrical, straight to geniculate-sinuous or irregularly curved, 10–70 × 3–4 µm; frequently swollen and constricted at septa, thick-walled, medium brown, verruculose. *Conidiogenous cells* terminal and intercalary, subcylindrical, straight, but frequently branched laterally, 6–20 × 3–4 µm, with 1–3 flat-tipped loci that can be subdenticulate, 1.5–2 µm wide, somewhat darkened and thickened, not prominently refractive. *Conidia* medium brown, thick-walled, verruculose, septa becoming darkly pigmented, occurring in branched chains. *Ramoconidia* subcylindrical to narrowly ellipsoid, 12–25 × 3.5–4(–5) µm, 1(–4)-septate. *Conidia* occurring in short chains (–8), subcylindrical to narrowly ellipsoid, 0–1(–3)-septate, (7–)10–15(–20) × (2–)3–3.5(–4) µm; hila somewhat thickened, darkened but not refractive, 1.5–2(–2.5) µm wide.

Cultural characteristics: Colonies on OA erumpent, spreading, with moderate aerial mycelium and smooth, even margins; olivaceous-grey (surface); on PDA olivaceous-black (surface), margins feathery, uneven, with moderate aerial mycelium; reverse iron-grey. Colonies reaching 20 mm diam after 1 mo at 25°C in the dark on OA.

Specimens examined: **Venezuela**, Aragua, La Victoria, on leaf spots of *Pithecellobium lanceolatum* (*Mimosaceae*), Jan. 1928, H. Sydow, **lectotype of *S. araguata*** (selected here!) IMI 15728(a); 3 Feb. 1928, syntype of *S. araguata*, IMI 34905. **Venezuela**, isolated from man with *tinea nigra*, 1973, D. Borelli, **holotype of *C. castellanii***, IMI 183818, culture ex-type CBS 105.75.

Notes: *Stenella araguata* is a leaf spot pathogen of *Pithecellobium* in Venezuela, and represents the type species of the genus *Stenella* [Two collections were cited, viz. no. 407, 'La Victoria', and no. 370, 'inter La Victoria et Suata', both without any date, and without any specific type indication. Thus, the two collections have to be considered syntypes. The two IMI collections with different dates are parts of the syntypes, of which IMI 15728(a) is proposed here to serve as lectotype]. *Stenella araguata* was incorrectly seen as a species of *Cladosporium* by von Arx (1974), which has recently been morphologically circumscribed (Braun *et al*. 2003, Schubert *et al.* 2007b – this volume), and is linked to *Davidiella* teleomorphs.

In a study by McGinnis & Padhye (1978), *Cladosporium castellanii* (*tinea nigra* of human in Venezuela) was shown to be synonymous to *Stenella araguata* (leaf spots of *Pithecellobium lanceolatum* in Venezuela). In the present study we re-examined the ex-type strain of *C. castellanii* (CBS 105.75), and found conidia to be 0–1(–3)-septate, (7–)10–15(–20) × (2–)3–3.5(–4) µm, while those of the type specimen of *S. araguata* were similar, namely 0–3-septate, (7–)13–20(–25) × 3(–3.5) µm. Furthermore, both collections have verruculose hyphae, which is the primary feature distinguishing *Stenella* from *Passalora* Fr. (Crous & Braun 2003).

Fig. 8. *Stenella araguata* (CBS 105.75). A–B. Conidiophore fascicles on a pine needle and tap-water agar, respectively. C–D, G. Conidiophores giving rise to conidial chains. E–F, H–J. Conidial chains with ramoconidia and conidia. Scale bars = 10 µm.

Stenella has always been used for anamorphs of *Mycosphaerella* (Crous *et al*. 2004, 2006c), and the fact that it belongs to *Teratosphaeria* (*Teratosphaeriaceae*), and not *Mycosphaerella* (*Mycosphaerellaceae*), raised the question of how to treat stenella-like anamorphs in *Mycosphaerella*. Due to insufficient availability of cultures (Crous *et al*. 2000, 2001), the status of *Stenella* was left unresolved (Crous & Braun 2003). Presently (Crous & Groenewald, unpubl. data), it is clear that the stenella-like morphology type is polyphyletic within the *Mycosphaerellaceae*, and paraphyletic within the *Capnodiales*. Several species are known that represent morphological transitions between *Stenella* and *Passalora*. It seems logical, therefore, that future studies should favour using *Passalora* to also accommodate *Mycosphaerella* anamorphs with superficial, verruculose hyphae, which have traditionally been placed in *Stenella*. This is in spite of the fact that there are other generic names available within the *Mycosphaerellaceae* for taxa with a stenella-like morphology (pigmented structures, darkened, thickened, refractive scars, and superficial, verruculose mycelium), namely *Zasmidium* Fr. (1849) (see Arzanlou *et al*. 2007 – this volume), and *Verrucisporota* D.E. Shaw & Alcorn (1993). Based on the phylogenetic position of the type species, *Stenella s. str.* is an anamorph of *Teratosphaeria* (*Teratosphaeriaceae*). Using the generic concept as employed in this volume of the *Studies in Mycology*, however, the anamorph genus is accepted as being poly- and paraphyletic within the order *Capnodiales*.

Helotiales, incertae sedis

Hyalodendriella Crous, **gen. nov.** MycoBank MB504435.

Etymology: Morphologically similar to *Hyalodendron* Diddens.

Differt a Hyalodendro et Retroconi conidiophoris dimorphis, cicatricibus incrassatis et conidiis ultimo brunneis.

Morphologically similar to *Hyalodendron* and *Retroconis*, but distinct in that it has dimorphic conidiophores, conidia that turn brown with age, and have thickened scars. *Microconidiophores* forming as lateral branches on hyphae, subcylindrical, subhyaline to pale brown, smooth, septate, with terminal conidiogenous cells. *Macroconidiophores* septate, subcylindrical, straight to curved, subhyaline to pale brown, smooth, with an apical rachis that is pale brown, smooth, subcylindrical, with numerous, aggregated loci. *Conidia* limoniform to ellipsoid, aseptate, smooth, pale brown, in short chains, tapering towards ends that are prominently apiculate, prominently thickened and darkened, but not refractive.

Type species: *Hyalodendriella betulae* Crous, sp. nov.

Hyalodendriella betulae Crous **sp. nov.** MycoBank MB504436. Fig. 9.

Mycelium ex hyphis ramosis, septatis, 1.5–2 µm latis, levibus, hyalinis vel pallide brunnei compositum. Conidiophora dimorphosa: (A) Conidiophora ex hyphis lateraliter oriunda, subcylindrical, subhyalina vel pallide brunnea, levia, 1–6-septata, ad 40 µm longa et 2–3 µm lata. Cellulae conidiogenae terminales, 5–15 × 2–3 µm, loco conidiogeno singulare et terminale, cellula ellipsoidea (conidio ?), persistente, interdum cellulis catenulatis (ad 6), pallide brunneo, apice subacute rotundato, basi truncata, 5–7 × 3–4 µm. (B) Conidiophoris 10–20 × 2–3 µm, 1–2-septatis, subcylindraceis, rectis vel curvatis, subhyalinis vel pallide brunneis, levibus. Cellulae conidiogenae pallide brunneae, leviae, subcylindraceae, locis numerosis, aggregatis, inconspicuis vel subdenticulatis, leviter protuberantes, 0.5 µm diam, incrassatis et fuscatis. Conidia catenulata (2–3), (4–)5–6(–7) × 2.5–3 µm, limoniformes vel ellipsoidea, non septata, levia, pallide brunnea, utrinque attenuata, apiculata, 0.5–1 × 0.5 µm, incrassata et fuscata, non refractiva.

Mycelium consisting of branched, septate, 1.5–2 µm wide hyphae, smooth, hyaline to pale brown. *Conidiophores* dimorphic. Type A: *Conidiophores* forming as lateral branches on hyphae, subcylindrical, subhyaline to pale brown, smooth, 1–6-septate, up to 40 µm long, and 2–3 µm wide. *Conidiogenous cells* terminal, 5–15 × 2–3 µm, with a single, apical locus, giving rise to an ellipsoidal cell (conidium?) which mostly remains attached, pale brown, with a subacutely rounded apex and truncate base, 5–7 × 3–4 µm, at times forming chains of up to 6 such cells. Type B: *Conidiophores* 10–20 × 2–3 µm, 1–2-septate, subcylindrical, straight to curved, subhyaline to pale brown, smooth. *Conidiogenous cells* pale brown, smooth, subcylindrical with numerous, aggregated loci, inconspicuous to subdenticulate and somewhat protruding, 0.5 µm wide, somewhat thickened and darkened. *Conidia* in chains of 2–3, limoniform to ellipsoid, widest in the middle, aseptate, smooth, pale brown, tapering towards ends that are prominently apiculate, 0.5–1 µm long, 0.5 µm wide, prominently thickened and darkened, but not refractive.

Cultural characteristics: Colonies on PDA slimy, spreading, somewhat erumpent in the centre, with even, catenulate margins, lacking aerial mycelium; surface fuscous-black to olivaceous-black, with patches of cream; reverse fuscous-black with patches of cream. Colonies reaching 25 mm diam on PDA after 1 mo at 25 °C in the dark; colonies fertile with profuse sporulation on SNA.

Specimen examined: **Netherlands**, Oostelijk Flevoland, Jagersveld, isolated from *Alnus glutinosa* (*Betulaceae*), May 1982, W. Gams, **holotype** CBS-H 19895, culture ex-type CBS 261.82.

Notes: Morphologically *Hyalodendriella* resembles the genera *Hyalodendron* and *Retroconis* de Hoog & Bat. Vegte (de Hoog & Batenburg van der Vegte 1989). It is distinct, however, in its pigmentation, dimorphic conidiophores and conidia. Furthermore, a strain of *Retroconis fusiformis* (S.M. Reddy & Bilgrami) de Hoog & Bat. Vegte (CBS 330.81) clusters apart from *Hyalodendriella*, namely in the *Chaetomiaceae, Sordariales*.

Pleosporales, incertae sedis

Ochrocladosporium Crous & U. Braun, **gen. nov.** MycoBank MB504437.

Etymology: Named after its pale brown, cladosporium-like conidia.

Differt a Cladosporio et generis cladosporioidibus diversis conidiophoris cum cellulis basalibus T-formibus et/vel cicatricibus non incrassatis, non vel leviter fuscatis-refractivis.

Mycelium consisting of branched, septate hyphae, subhyaline to pale brown, smooth, giving rise to two types of conidiophores. *Macronematous conidiophores* solitary, erect, arising from superficial hyphae, composed of a subcylindrical stipe, without a swollen or lobed base or rhizoids, with or without a T-shaped foot cell, pale to dark brown; apical conidiogenous apparatus with or without additional branches, branched part, if present, with short branchlets composed of conidiogenous cells and ramoconidia, continuous to septate, wall thin or slightly thicked, pale brown. *Conidiogenous cells* integrated, terminal or intercalary, subcylindrical to doliiform, pale brown, thin-walled, smooth; unilocal or multilocal, determinate to sympodial, loci conically truncate, subdenticulate, neither thickened, nor darkened-refractive or only slightly darkened-refractive. *Micronematous conidiophores* integrated in hyphae, reduced to a lateral peg-like locus or erect, frequently reduced to conidiogenous cells, pale brown, smooth, subcylindrical. *Conidia* occurring in branched chains, fusiform, ellipsoid-ovoid to subcylindrical, 0(–1)-septate, ramoconidia present, pale brown, thin-walled, smooth to finely verruculose, ends attenuated, hila obconically truncate to almost pointed, neither thickened nor darkened-refractive.

Type species: *Ochrocladosporium elatum* (Harz) Crous & U. Braun, comb. nov.

Ochrocladosporium elatum (Harz) Crous & U. Braun, **comb. nov.** MycoBank MB504438. Fig. 10.

Basionym: *Hormodendrum elatum* Harz, Bull. Soc. Imp. Naturalistes Moscou 44: 140. 1871.

≡ *Cladosporium elatum* (Harz) Nannf., in Melin & Nannfeldt, Svenska Skogsvardsfoereren Tidskr. 32: 397. 1934.

≡ *Cadophora elatum* (Harz) Nannf., in Melin & Nannfeldt, Svenska Skogsvardsfoereren Tidskr. 32: 422. 1934.

Mycelium consisting of branched, septate, smooth, hyaline, 2–4 µm wide, thin-walled, hyphae, becoming darker brown in places, giving rise to erect conidiophores. *Conidiophores* either reduced to conidiogenous cells, or well-differentiated, terminal and lateral on hyphae, erect, highly variable, arising from superficial and submerged hyphae, reduced to subdenticulate loci, 1–1.5 µm wide, or well-differentiated, up to 60 µm long, 1–3-septate, 3–4 µm wide, hyaline to medium brown, smooth, thin-walled (≤ 1 µm). *Conidiogenous cells* integrated as lateral peg-like loci on hyphal cells, or erect, subcylindrical, up to 25 µm long, 2.5–4 µm wide, with 1–3 terminal loci, occasionally also lateral, 1–1.5 µm wide, not thickened and darkened, but frequently somewhat refractive (mounted in Shear's solution, not lactic acid). *Ramoconidia*

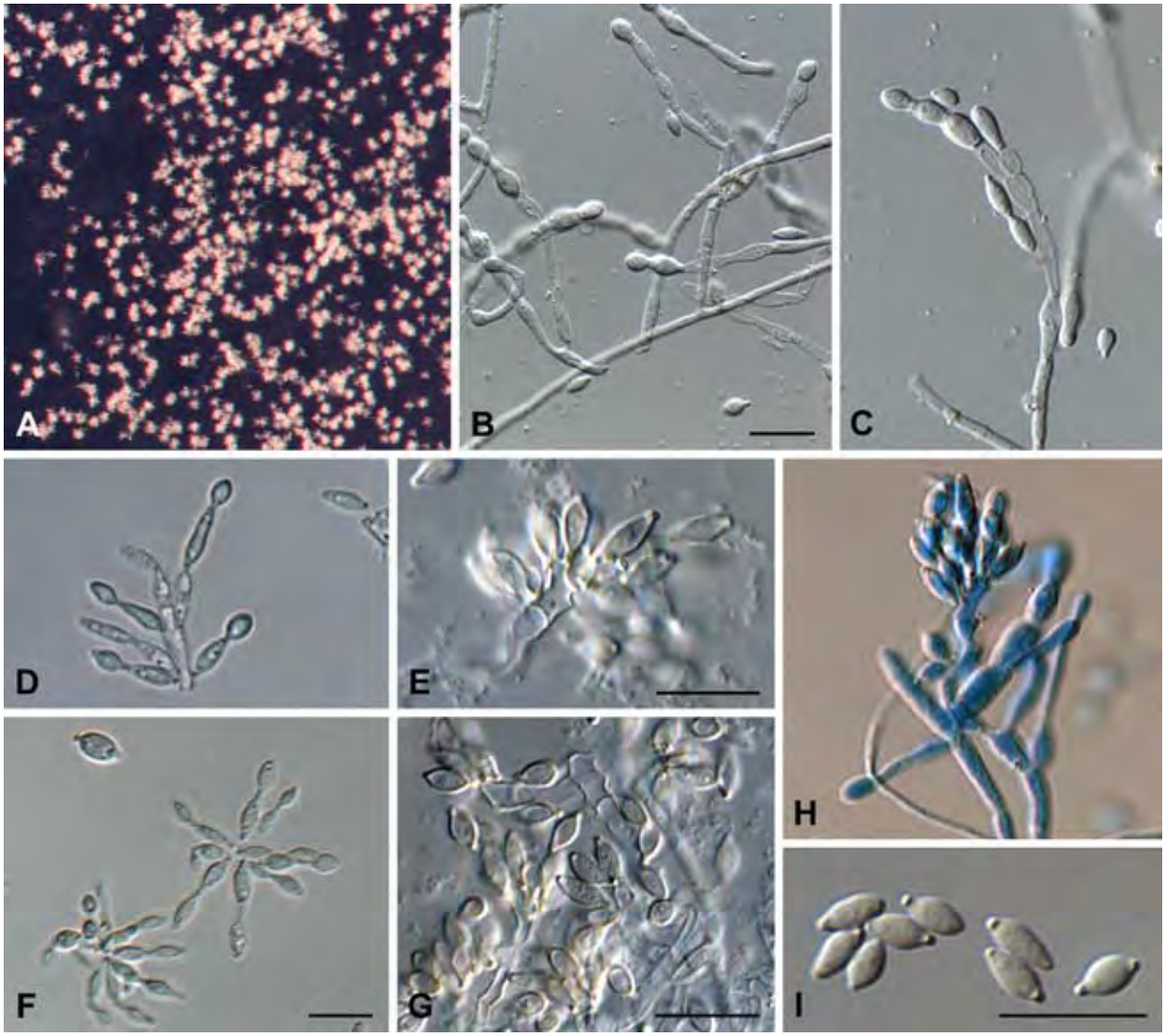

Fig. 9. *Hyalodendriella betulae* (type material). A. Conidiophores on PDA. B–C. Microconidiophores. D–H. Macroconidiophores with fascicles of conidiogenous cells. I. Conidia with darkened, thickened hila. Scale bars = 10 µm.

subcylindrical to ellipsoid, hyaline to pale brown, smooth to finely verruculose, 10–40 × 3–5 µm, 0(–1)-septate, giving rise to branched chains of conidia (up to 20 per chain) that are subcylindrical to ellipsoid, aseptate, (7–)8–10(–14) × (3–)4(–4.5) µm, smooth to finely verruculose, olivaceous-brown, thin-walled (up to 0.5 µm), hila 0.5–1 µm wide, neither thickened nor, or barely, darkened refractive.

Cultural characteristics: Colonies erumpent, spreading, fast growing, covering the plate within 1 mo at 25 °C; aerial mycelium abundant, margins smooth on PDA; surface isabelline in centre, umber in outer region; olivaceous-black in reverse.

Specimen examined: **Sweden**, Iggesund, isolated from wood pulp, Jan. 1976, E. Melin, specimen CBS-H 19896, culture CBS 146.33.

Notes: "*Hormodendrum" elatum* was originally described from a wooden stump in Germany. The culture examined here was deposited by Melin in 1933 as culture 389:14, isolated from wood chips in Sweden, and described by Nannfeldt, and has since been accepted as authentic for the species. Earlier publications (de Vries 1952, Ho *et al*. 1999, de Hoog *et al*. 2000), clearly state that this species does not belong in *Cladosporium s. str.,* and this statement is supported by the phylogenetic analysis placing it in the *Pleosporales*.

Ochrocladosporium frigidarii Crous & U. Braun, **sp. nov.** MycoBank MB504439. Fig. 11.

Etymology: Named after it collection site, within a cooled incubation room.

Differt a O. elato conidiophoris distincte dimorphis, macroconidiophoris majoribus, ad 600 × 5–7 µm, septis incrassates, cellulis basalibus T-formibus et conidiis leniter brevioribus et latioribus, (6–)7–8(–10) × (4–)4.5–5(–6) µm.

Mycelium consisting of branched, septate, 2–7 µm wide hyphae, occasionally constricted at septa with hyphal swellings, subhyaline to pale brown, smooth, thin-walled, giving rise to two types of conidiophores. *Macronematous conidiophores* solitary, erect, arising from superficial hyphae, up to 600 µm long, composed of a subcylindrical stipe, 5–7 µm wide, 10–15(–20)-septate, without a swollen or lobed base or rhizoids, but with a T-shaped foot cell, wall ≤ 1 µm wide, guttulate, with thick septa, dark brown, finely verruculose, apical 1–2 cells at times medium brown, giving rise to 1–2 primary branches, 0–1-septate, subcylindrical, thin-walled, pale brown, smooth to finely verruculose, 10–20 × 4–6 µm, giving rise to (1–)2–4 secondary branches, 0–1-septate, subcylindrical, 8–13(–20) × 4–5 µm, or giving rise directly to conidiogenous cells. *Conidiogenous cells* subcylindrical to doliiform, pale brown, smooth, 8–15 × 3–4 µm, loci somewhat protruding 1–2 µm wide, neither

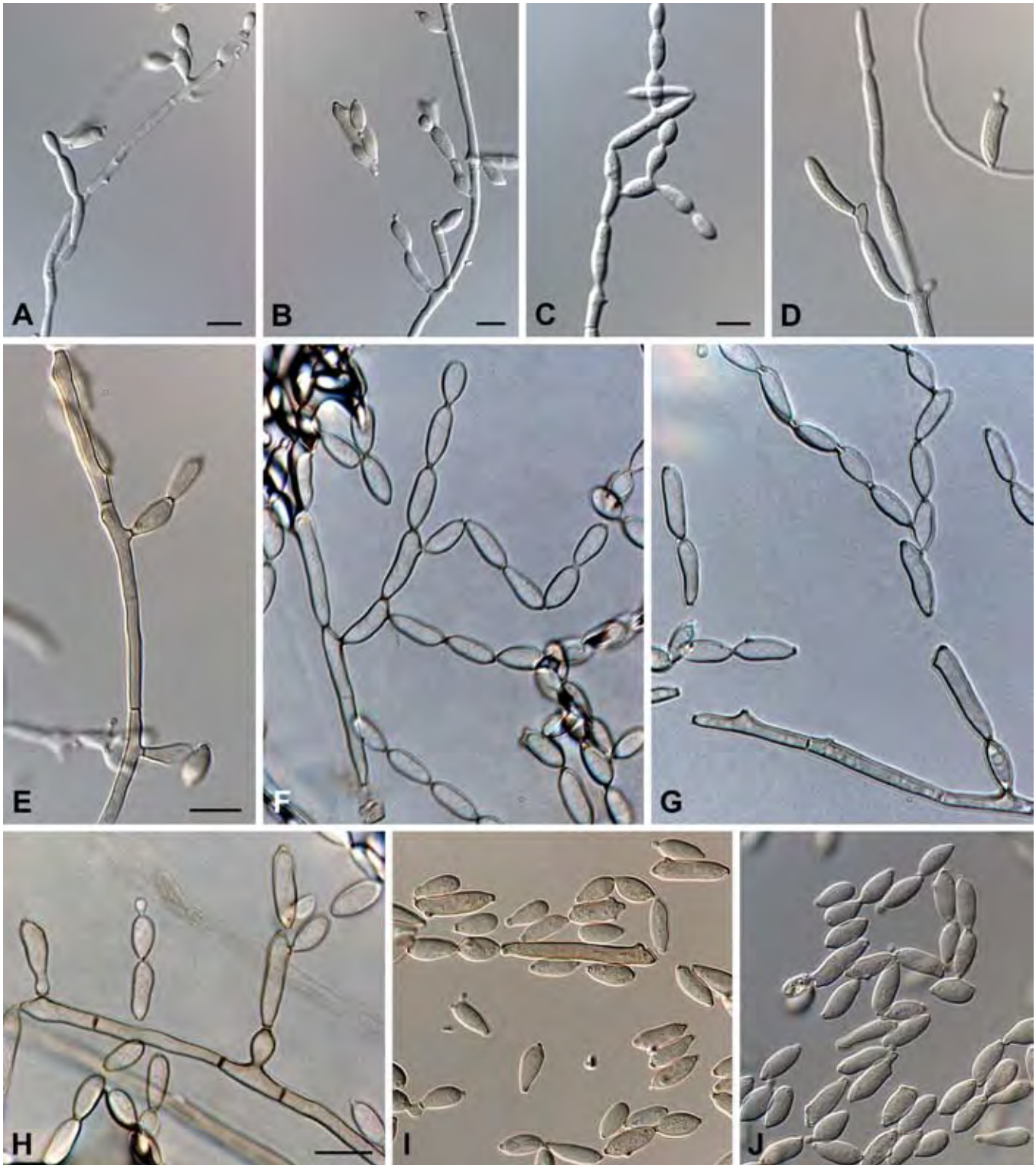

Fig. 10. *Ochrocladosporium elatum* (CBS 146.33). A–C, E. Microconidiophores. D. Macro- and microconidiophore. F–H. Macroconidiophores. I–J. Ramoconidia and conidia. Scale bars = 10 µm.

thickened, darkened, nor refractive. *Micronematous conidiophores* erect, pale brown, smooth, subcylindrical, reduced to conidiogenous cells, or up to 4-septate, 15–90 × 2–3.5 µm, mostly unbranched, rarely branched below; conidiogenous cells subcylindrical, pale brown, smooth to finely verruculose, tapering at apex and sometimes at base, proliferating sympodially via 1(–3) loci, 1–1.5 µm wide, denticle-like, which can appear somewhat darkened; micronematous conidiophores frequently occurring at the base of macronematous conidiophores. *Ramoconidia*, if present, up to 30 µm long, 0–1-septate. *Conidia* and ramoconidia ellipsoid to ovoid, aseptate, pale brown, thin-walled (≤ 0.75 µm), finely verruculose, occurring in branched chains; conidia (6–)7–8(–10) × (4–)4.5–5(–6) µm; hila 0.5–1 µm wide, not darkened, thickened or refractive.

Cultural characteristics: Colonies on PDA erumpent, spreading, with profuse sporulation and moderate aerial mycelium, even margins, olivaceous-grey (surface); reverse olivaceous-black. Colonies covering the dish after 1 mo at 25 °C in the dark.

Specimen examined: **Germany**, Hannover, isolated from a cooled room, Jan. 1981, B. Ahlert, **holotype** CBS-H 19897, culture ex-type CBS 103.81.

Notes: *Ochrocladosporium frigidarii* is characterised by its dimorphic fruiting, and inconspicuous scars and conidial hila, which

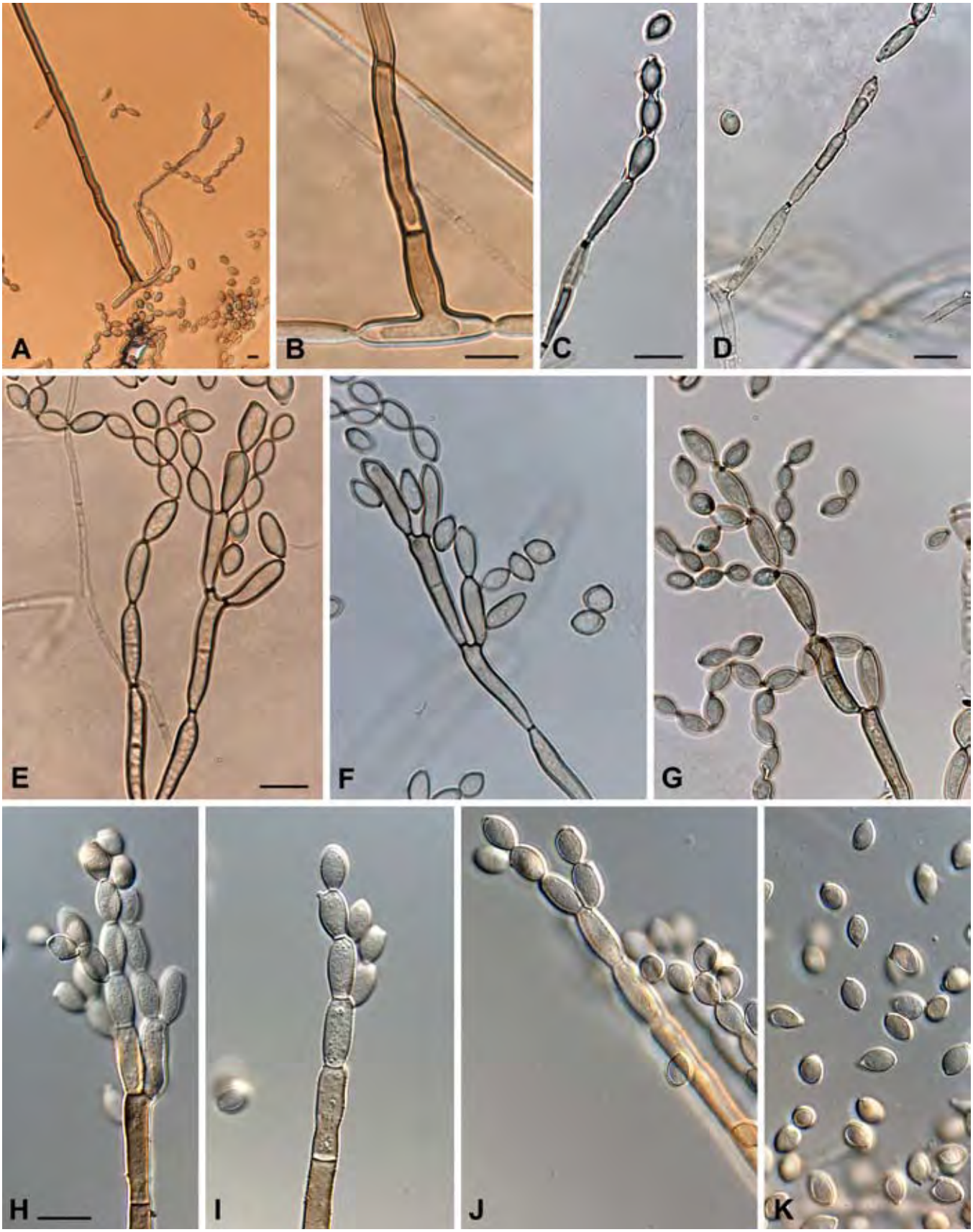

Fig. 11. *Ochrocladosporium frigidarii* (type material). A. Macro- and microconidiophores. B. Foot cell of macroconidiophore. C–D. Microconidiophore. E–J. Macroconidiophores. K. Conidia. Scale bars = 10 µm.

are distinct from *Cladosporium s. str.* The phylogenetic analysis of its LSU sequence places it in the *Pleosporales*, together with *O. elatum*.

The dimorphic conidiophores seen in *O. frigidarii* (CBS 103.81) are less obvious in *O. elatum* (CBS 146.33), but the scars and hila are similar. The macronematous conidiophores of *O. frigidarii* are much longer and wider and the conidia are shorter and slightly wider, (6–)7–8(–10) × (4–)4.5–5(–6) µm, than those of *O. elatum* which are (7–)8–10(–14) × (3–)4(–4.5) µm.

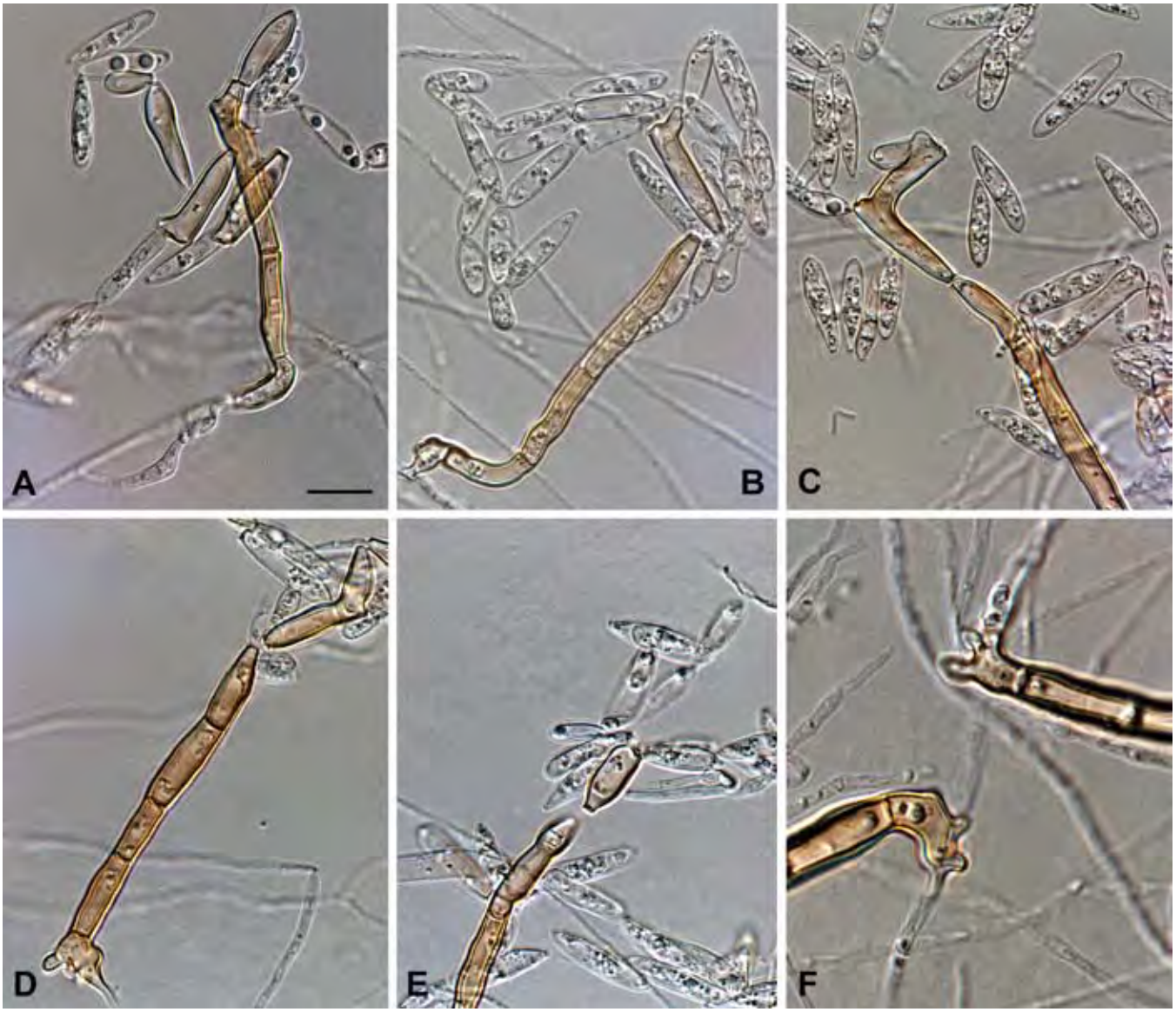

Fig. 12. *Rhizocladosporium argillaceum* (type material). A–E. Conidiophores with pigmented ramoconidia and hyaline conidia. F. Rhizoids forming at the foot cells of macroconidiophores. Scale bar = 10 µm.

Incertae sedis

Rhizocladosporium Crous & U. Braun, **gen. nov.** MycoBank MB504440.

Etymology: Named after the presence of rhizoids on its conidiophores, and cladosporium-like conidia.

Differt a Cladosporio et generis cladosporioidibus diversis hyphis hyalinis, conidiophoris cum cellulis basalibus lobatis vel rhizoidibus, cellulis conidiogenis monoblasticis, determinatis, locis margine leviter incrassatis et fuscatis, non refractivis, non coronatis, ramoconidiis brunneis sed conidiis hyalinis, hilis non incrassatis, non fuscatis-refractivis.

Mycelium consisting of branched, septate, smooth, hyaline hyphae. *Conidiophores* solitary, macronematous, subcylindrical, erect, arising from superficial mycelium, septate, pigmented, smooth; base somewhat inflated, lobed or with rhizoids. *Conidiogenous cells* integrated, terminal, monoblastic, determinate, subcylindrical, tapering towards a single flat-tipped locus, straight to once geniculate, occasionally with two loci, pigmented, smooth; locus flattened, undifferentiated to somewhat darkened and thickened along the rim, not refractive, giving rise to a single conidial chain or a single ramoconidium with several simple acropetal chains of secondary ramoconidia or conidia. *Conidia* occurring in branched chains; *ramoconidia* subcylindrical to narrowly ellipsoidal, straight to geniculate-sinuous, with apical and lateral conidial hila; ramoconidia with broadly truncate base medium brown; *secondary ramoconidia* with narrowed base subhyaline or hyaline, smooth; *conidia* aseptate, in chains, hyaline, guttulate, ellipsoidal with obtuse ends; hila inconspicuous, neither darkened nor refractive or thickened.

Type species: *Rhizocladosporium argillaceum* (Minoura) Crous & U. Braun, comb. nov.

Rhizocladosporium argillaceum (Minoura) Crous & U. Braun, **comb. nov.** MycoBank MB504441. Fig. 12.
Basionym: *Cladosporium argillaceum* Minoura, J. Ferment. Technol. 44: 140. 1966.

Mycelium consisting of branched, septate, smooth, hyaline, thin-walled, 1.5–2 µm wide hyphae. *Conidiophores* solitary, macronematous, erect, arising from superficial mycelium; base somewhat inflated, lobed or with rhizoids, up to 10 µm wide; conidiophore stipe subcylindrical, straight to curved, rarely geniculate-sinuous, wall up to 1 µm wide, medium brown, sometimes paler towards the tip, smooth, 1–6-septate, 35–160

µm tall, 4–6 µm wide. *Conidiogenous cells* terminal, straight, subcylindrical, tapering towards a flat-tipped locus, occasionally once geniculate, with two loci, medium brown, smooth, 15–35 × 4–6 µm; locus flattened, undifferentiated or very slightly darkened and thickened along the rim, not refractive, 1.5–2 µm wide. *Conidia* occurring in branched chains. *Ramoconidia* subcylindrical to narrowly ellipsoidal, straight to geniculate-sinuous, 17–35 × 4–5 µm, medium brown, smooth, thin-walled, frequently branching laterally, with apical and lateral subdenticulate conidial hila, 1.5–2.5 µm wide; *secondary ramoconidia* hyaline or subhyaline. *Conidia* aseptate, (10–)12–17(–20) × (3.5–)4(–4.5) µm, in branched chains (–6), hyaline or subhyaline, guttulate, ellipsoidal-fusiform, with obtuse ends, or tapering to obconically subtruncate ends with hila that are inconspicuous (neither darkened nor refractive or thickened), 0.5–1 µm wide.

Cultural characteristics: Colonies on PDA spreading, erumpent, with smooth, even margins and sparse to moderate aerial mycelium; hazel to fawn (surface); reverse hazel to fawn. Colonies reaching 35 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Japan**, Yoku Island, isolated from decayed myxomycete, 21 Oct. 1961, K. Tubaki No. 4262 **holotype**, culture ex-type CBS 241.67 = IFO 7055.

Notes: The lobed-rhizoid conidiophore base, and brown, disarticulating ramoconidia, with hyaline chains of conidia, are characteristic of *Rhizocladosporium*. Although Minoura (1966) illustrated some conidiophores that were micronematous (reduced to conidiogenous cells on superficial mycelium), these were not observed in the present study. *Metulocladosporiella* Crous, Schroers, J.Z. Groenew., U. Braun & K. Schub. (Crous *et al*. 2006a) (*Herpotrichiellaceae*), comprising two banana leaf-spotting pathogens, is another cladosporioid hyphomycete genus having distinct rhizoid hyphae at the swollen base of conidiophores. It differs, however, in having conidiophores terminally branched in a metula-like manner and distinct conidiogenous loci and conidial hila. *Pleurotheciopsis* B. Sutton (Ellis 1976) is also characterised by having pigmented conidiophores and hyaline or pale, septate conidia formed in acropetal chains, but the conidiophores proliferate percurrently, the conidiogenous cells are polyblastic and ramoconidia are lacking, i.e., the conidia are formed in unbranched chains. *Parapleurotheciopsis* P.M. Kirk (Kirk 1982) is very similar to *Rhizocladosporium*. The conidiophores possess a single terminal unilocal conidiogenous cell giving rise to a single ramoconidium which forms several chains of acropetal, aseptate, hyaline to pale olivaceous conidia. The base of the conidiophores is somewhat swollen and lobed [except for *Parapleurotheciopsis coccolobae* R.F. Castañeda & B. Kendr., Castañeda & Kendrick (1990), with at most slightly swollen, but unlobed base]. However, *R. argillaceum* occasionally has once-geniculate conidiogenous cells with two loci. Furthermore, it clusters in the *Helotiales* (Fig. 1), whereas a sequenced strain of *Parapleurotheciopsis inaequiseptata* (MUCL 41089), belongs to the *Xylariales* (Fig. 2). The occasionally occurring conidiogenous cells with two loci and the aseptate conidia connect *Rhizocladosporium* with *Subramaniomyces* Varghese & V.G. Rao (Varghese & Rao 1979, Kirk 1982) in which, however, terminal ramoconidia are lacking. Furthermore, the type species, *S. fusisaprophyticus* (Matsush.) P.M. Kirk, frequently has branched conidiophores. *Subramaniomyces simplex* U. Braun & C.F. Hill (Braun & Hill 2002), a species with unbranched conidiophores is, however, morphologically similar to *R. argillaceum*, but the genus *Subramaniomyces* is phylogenetically distinct and also belongs to the *Xylariales* (CBS 418.95, Fig. 2).

Key to *Cladosporium* and morphologically similar genera

(bearing simple or branched acropetal chains of amero- to phragmosporous blastoconidia)

1. Conidiophores and conidia hyaline 2
1. At least conidiophores pigmented 4

2. Conidia in simple chains ..... ***Hormiactis***
2. Conidia in branched chains 3

3. Conidiogenous cells sympodial, with distinct conidiogenous loci (scars), thickened and darkened; conidia amero- to phragmosporous; plant pathogenic, leaf-spotting fungi (*Mycosphaerella* anamorphs; *Mycosphaerellaceae*) ..... ***Ramularia***
3. Terminal conidiogenous cells with denticle-like loci, giving rise to ramoconidia which form simple or branched conidial chains; lignicolous ..... ***Hyalodendron***
   [Conidiophores dimorphic; mycelium, conidiophores and conidia at first hyaline, later turning pale brown; conidia in short chains, see *Hyalodendriella*]

4(1). Conidia distoseptate, in simple chains ..... ***Lylea***
4. Conidia aseptate or euseptate 5

5. Conidiophores little differentiated, micronematous to semimacronematous; conidiogenous loci undifferentiated, truncate, neither distinctly thickened nor darkened or only very slightly so 6
5. Conidiophores well-differentiated, semimacronematous (but multilocal and/or conidiogenous loci well-differentiated) to macronematous 12

6. Conidiophores and conidia delicate, thin-walled, in long, easily disarticulating chains 7
6. Conidiophores and conidia robust, wall thickened, dark, conidial chains often seceding with difficulty 9

7. Conidiophores semimacronematous, simple to often irregularly branched; conidia delicate, narrow, 1–3 µm wide, hyaline to pale olivaceous ..... ***Polyscytalum***

7. Conidiophores unbranched, micronematous or semimicronematous, integrated in ordinary hyphae, forming minute, lateral, monoblastic, determinate, peg-like protuberances to semimacronematous, forming short lateral branches (conidiophores) with several inconspicuous to denticle-like loci 8

8. Phialidic synanamorphs often present, but sometimes also lacking; saprobic, rarely plant pathogenic, often human pathogenic (*Herpotrichiellaceae*, *Chaetothyriales*) ..... ***Cladophialophora***
8. Without phialidic synanamorphs; saprobic or plant pathogenic (*Venturia*, *Venturiaceae*) ..... ***Fusicladium s. lat.*** (incl. *Pseudocladosporium*)

9(6). Conidia aseptate, rarely 1-septate; lignicolous, on dead wood ..... ***Xylohypha***
9. Conidia septate 10

10. Conidia 1-septate, with a dark brown to blackish band at the septum; on dead wood ..... ***Bispora***
10. Conidia at least partly 2- to pluriseptate and/or without dark brown to blackish band at the septum 11

11. Conidia branched ..... ***Taeniolina***
11. Conidia unbranched ..... ***Taeniolella***

12(5). Conidiogenous loci and conidial hila distinctly coronate, i.e., composed of a central convex dome surrounded by a periclinal raised rim, mostly at least somewhat protuberant (anamorphs of *Davidiella*, *Davidiellaceae*, *Capnodiales*) ..... ***Cladosporium s. str.***
12. Conidiogenous loci non-coronate (either inconspicuous, thickened and darkened or denticle-like) 13

13. Mycelium, conidiophores and conidia at first hyaline or subhyaline, later turning pale brown; conidiophores dimorphic, either conidiogenous cells with a single conidiogenous locus, giving rise to an ellipsoid cell (conidium?) which mostly remains attached, base truncate, apex subacutely rounded, at times forming chains of such cells; or conidiophores with numerous aggregated loci, inconspicuous to subdenticulate; conidia in short chains, of mostly 2–3 (isolated from *Alnus* in Europe) ..... ***Hyalodendriella***
13. Fruiting different; at least conidiophores consistently pigmented or conidiophores uniform or loci distinct; conidia mostly in long, often branched chains 14

14. Conidiophores with verruculose conidiogenous apices, otherwise smooth; conidia distinctly verruculose-verrucose; conidiogenous loci and conidial hila inconspicuous 15
14. Conidiophores either smooth throughout or verruculose below and smooth above or verruculose throughout; and/or conidiogenous loci conspicuous, i.e., thickened and darkened or denticle-like 16

15. Conidiophores macronematous, unbranched, base swollen, with percurrent regenerative proliferations, unrelated to conidiation; conidiogenous cells terminal, occasionally also subterminal; conidia terminally and laterally formed, aseptate (saprobic on leaves) ..... ***Castanedaea***
15. Conidiophores little differentiated, semimacronematous, unbranched or with short lateral branchlets, base undifferentiated, without percurrent proliferations; conidiogenous cells terminal and occasionally intercalary-pleurogenous; conidia terminally and subterminally formed, 0–2-septate (lignicolous, on decorticated wood) ..... ***Websteromyces***

16(14). Conidiophores unbranched, with a simple terminal conidiogenous cell, non-geniculate-sinuous, subcylindrical to somewhat inflated at the tip; conidiogenous loci terminal and lateral, inconspicuous or subconspicuous, neither thickened nor darkened, non-protuberant; conidia attached with a very narrow, pointed hilum 17
16. Conidiophores with a branched terminal conidiogenous apparatus, composed of conidiogenous cells and/or ramoconidia or conidiophores unbranched, with a single terminal conidiogenous cell or additional intercalary ones, but conidiogenous loci different, conspicuous, thickened and darkened or denticle-like 18

17. Conidiophores with distinct rhizoid-digitate base; tips of the conidiogenous cells somewhat swollen, usually unilaterally swollen or somewhat curved; conidia solitary or only in very short unbranched chains; hyperparasitic on rusts ..... ***Digitopodium***
17. Conidiophores without rhizoid-digitate base; tips of the conidiogenous cells subcylindrical to somewhat swollen, but swellings not unilateral and not curved; conidia solitary and in simple or branched chains; associated with leaf spots ..... ***Rachicladosporium***

18(16). Conidiophores in synnematous conidiomata 19
18. Synnemata lacking 20

19. Conidiogenous cells with a single or several truncate to subdenticulate, relatively broad conidiogenous loci; conidia with truncate, flat hila; on wood, resin ..... ***Sorocybe***
19. Conidiogenous loci with few, mostly 1–2 conidiogenous loci formed as minute spicules; conidia with narrow hila (shallowly apiculate); plant pathogenic, causing bud blast and twig blight ..... ***Seifertia***

20(18). Conidiophores unbranched or occasionally branched; conidiogenous cells distinctly inflated, ampulliform, doliiform or clavate, non-denticulate; conidia at least partly globose, dark brown when mature; colonies effuse, dark; wood-inhabiting ..... ***Phaeoblastophora***

20. Conidiogenous cells not inflated, if somewhat inflated, loci denticle-like or conidia non-globose 21

21. Conidiophores penicillate, i.e., with an unbranched stipe and distinct terminal branched "head" composed of branchlets, conidiogenous cells and/or ramoconidia 22
21. Conidiophores non-penicillate, i.e., irregularly and loosely branched, branchings not confined to the apical portion, sometimes only with short lateral branchlets, or unbranched 27

22. Penicillate apex simple, only composed of a single terminal conidiogenous cells giving rise to several ramoconidia which form secondary ramoconidia and conidia ..... ***Penidiella*** **p.p.** [*P. strumelloidea*]
22. Penicillate apex more complex, composed of true branchlets and/or conidiogenous cells and ramoconidia 23

23. Conidiophores with a compact, dense, subglobose to broadly ovoid head; conidiogenous loci and conidial hila unthickened or almost so, but distinct by being darkened-refractive [fruiting dimorphic, periconioid branched conidiophores formed on overwintered stem of *Paeonia* spp., unbranched cladosporioid conidiophores on leaf spots, biotrophic] (belonging to the *Capnodiales*) ..... ***Dichocladosporium***
23. Penicillate apex looser, neither compact nor subglobose 24

24. Branched head composed of short branchlets and conidiogenous cells; ramoconidia lacking; conidiogenous cells subcylindrical to subclavate, non-geniculate; conidiogenous loci usually numerous and aggregated, terminal and lateral, non-protuberant, flat, conspicuous, thickened and darkened, at least around the rim; conidia solitary or in short chains ..... ***Periconiella***
24. Ramoconidia often present; conidiogenous cells distinct, sympodial, somewhat geniculate or subdenticulate; conidiogenous loci inconspicuous or somewhat protruding, denticle-like, unthickened or almost so, not or somewhat darkened-refractive; conidia in long, often branched chains 25

25. Branched apex composed of short branchlets consisting of conidiogenous cells or ramoconidia, in pairs or whorls of 3–4, mostly distinctly constricted at the base; hyperparasitic on *Asterina* spp. ..... ***Parapericoniella***
25. Branched apex distinct, composed of branchlets, conidiogenous cells and/or ramoconidia, if true branchlets lacking conidiogenous cells and ramoconidia not in whorls and not distinctly constricted at the base; saprobic or biotrophic 26

26. Penicillate apex of the conidiophores loosely to densely branched, occasionally metula-like, base of the conidiophores simple, undifferentiated; saprobic or biotrophic (*Teratosphaeriaceae*, *Capnodiales*) ..... ***Penidiella***
26. Penicillate apex always dense, metula-like, base of the conidiophores swollen or lobed, often with rhizoid hyphae; plant pathogenic [on banana] (*Chaetothyriales*) ..... ***Metulocladosporiella***

27(21). Conidiophores simple or branched; septa of the conidiophores and conidia becoming thick-walled and dark; conidiogenous loci subdenticulate, somewhat thickened and conspicuously darkened-refractive; cultures producing ample amounts of volatile metabolites causing skin irritation after exposure to the fungus; saprobic (isolated from mouldy paint) ..... ***Toxicocladosporium***
27. Without conspicuously thickened-darkened septa; cultures without irritant, volatile metabolites 28

28. Conidiogenous loci conspicuous, distinctly thickened and darkened (visible as small dark circles when viewed upon the scar), sometimes on small shoulders formed by sympodial proliferation, but not distinctly denticulate (*Capnodiales*) 29
28. Conidiogenous loci inconspicuous or conspicuous by being denticle-like, not or barely thickened, not darkened or at most upper truncate end very slightly thickened and somewhat darkened-refractive 31

29. Mycelium smooth; conidiophores and conidia smooth or almost so, at most faintly rough-walled; conidiophores solitary, fasciculate, sporodochial to synnematous; biotrophic, usually leaf-spotting (*Mycosphaerella* anamorphs, *Mycosphaerellaceae*) ..... ***Passalora*** **emend.** (incl. *Mycovellosiella*, *Phaeoramularia*, etc.)
29. At least mycelium distinctly verruculose 30

30. Mycelium, conidiophores and conidia coarsely verruculose-verrucose; conidial shape variable, often irregular; isolated from a lichen (*Dirina*) ..... ***Verrucocladosporium***
30. Mycelium verruculose; conidiophores mostly smooth, sometimes somewhat rough-walled, conidia smooth to distinctly verruculose; biotrophic, often leaf-spotting ..... ***Stenella***

31(28). Conidiophores with swollen, often lobed base 32
31. Conidiophores without swollen base, at most slightly swollen, but not lobed 35

32. Conidia septate 33
32. Conidia aseptate 34

33. Conidiophores with a single, terminal, monoblastic, determinate conidiogenous cell giving rise to a single ramoconidium that forms simple or branched chains of conidia ..... ***Parapleurotheciopsis***

33. Terminal conidiogenous cells polyblastic, with several denticle-like conidiogenous loci ..... ***Anungitea* p.p.** (e.g. *A. longicatenata*)

34(32). Conidiogenous cells terminal, monoblastic, with a single ramoconidium giving rise to conidial chains or occasionally with 2(–3) denticle-like loci; base of the conidiophores often with rhizoid hyphae ..... ***Rhizocladosporium***
34. Conidiogenous cells polyblastic, with two or several denticle-like loci; base of the conidiophores without rhizoid hyphae ..... ***Subramaniomyces***

35(31). Conidiophores unbranched, with a terminal monoblastic conidiogenous cell, determinate or percurrent 36
35. Conidiophores branched or unbranched, but conidiogenous cells at least partly polyblastic 38

36. Conidiogenous cell giving rise to a single ramoconidium which forms simple or branched chains of 0(–1)-septate conidia ..... ***Parapleurotheciopsis* p.p.** (*P. coccolobae*)
36. Conidiogenous cells giving rise to simple conidial chains without ramoconidia; conidia septate 37

37. Conidiophores sometimes with percurrent proliferations; conidiophores and conidia with somewhat thickened, dark walls; conidia 1–10-septate, width usually exceeding 4 µm ..... ***Heteroconium***
37. Percurrent proliferations lacking; conidiophores and conidia delicate, thin-walled and paler; conidia usually 0–1(–3)-septate and narrow, usually below 4 µm wide (*Chaetothyriales*) ..... ***Cladophialophora* p.p.** (e.g. *C. chaetospira*) [similar anamorphs of the *Venturiaceae*, see *Fusicladium* (incl. *Pseudocladosporium*)]

38(35). Conidiophores often branched; conidiogenous loci distinctly denticle-like or subdenticulate; conidia aseptate; lignicolous, on dead wood, resin or isolated from hydrocarbone-rich substrates (jet-fuel, cosmetics, etc.) 39
38. Either with unbranched conidiophores or conidiogenous loci not distinctly denticle-like, or conidia septate, or on other substrates 42

39. Conidiogenous cells distinctly denticulate; conidia rather broad, approx. 7–13 µm ..... ***Haplotrichum***
39. Conidiogenous cells non-denticulate or at most subdenticulate; conidia narrower, approx. 3–6 µm 40

40. Colonies effuse, dense, but felted, black, brittle and appearing carbonaceous when dry; conidiophores solitary, brown; conidiogenous cells terminal and pleurogenous; conidia pale to dark brown, lateral walls conspicuously thicker than the hila; on conifer resin ..... ***Sorocybe*** (mononematous form, *Hormodendrum resinae*)
40. Colonies effuse, dense, resupinate, hypochnoid, powdery, chocolate-brown and/or conidiophores lightly pigmented; conidia subhyaline to lightly pigmented and/or lateral walls not thickener than poles; on dead wood or isolated from hydrocarbone-rich substrates (jet-fuel, cosmetics, etc.) 41

41. Colonies effuse, dense, resupinate, hypochnoid, powdery, chocolate-brown; conidiophores smooth; conidia subhyaline to very pale yellowish, hila very thin; on dead wood ..... ***Parahaplotrichum***
41. Colonies neither resupinate nor hypochnoid; conidiophores warty; lateral walls of the conidia not thicker than the hila; isolated from hydrocarbone-rich substrates (jet-fuel, cosmetics, etc.) ..... ***Hormoconis***

42(38). Conidiophores simple or branched; conidiogenous cells monoblastic or occasionally polyblastic; conidiogenous loci subdenticulate, neither thickened nor darkened, forming simple or branched chains of regular conidia, uniform in shape, size and septation ..... ***Septonema***
42. Conidia not uniform in shape, size and septation; conidiogenous loci flat-tipped, subdenticulate, unthickened or slightly so, not to somewhat darkened-refractive 43

43. Conidiophores simple or branched; in culture forming abundant chlamydospores; mostly soil-borne and heat-resistant (*Teratosphaeriaceae*, *Capnodiales*) ..... ***Devriesia***
43. Without chlamydospores in culture; phylogenetically distinct 44

44. Conidiophores dimorphic; conidia mostly aseptate, hila inconspicuous, neither thickened nor darkened (*Pleosporales*) ..... ***Ochrocladosporium***
44. Conidiophores either uniform or conidia at least partly septate or hila more conspicuous by being slightly thickened or at least somewhat darkened or refractive; phylogenetically distinct 45

45. Phialidic synanamorphs often present, but sometimes also lacking; saprobic, rarely plant pathogenic, often human pathogenic (*Herpotrichiellaceae*, *Chaetothyriales*) ..... ***Cladophialophora***
45. Without phialidic synanamorphs; saprobic or plant pathogenic; phylogenetically distinct 46

46. Conidiophores usually unbranched (*Venturia*, *Venturiaceae*) ..... ***Fusicladium s. lat.*** (incl. *Pseudocladosporium*) [similar, barely distinguishable taxa, also clustering in the *Venturiaceae*, but apart from the *Venturia* clade are tentatively referred to as *Anungitea* until this genus will be resolved by sequences of its type species]

46. Conidiophores simple to often irregularly branched; conidia delicate, narrow, 1–3 µm wide, hyaline to pale olivaceous (not belonging to the *Venturiaceae*) ..... ***Polyscytalum***

DISCUSSION

Phylogenetic studies conducted on species of *Cladosporium s. lat*. proved the genus to be highly heterogeneous (Braun *et al*. 2003). It could be demonstrated that various anamorphs, previously referred to as *Cladosporium*, e.g. *Cladosporium fulvum* Cooke [≡ *Passalora fulva* (Cooke) U. Braun & Crous], have to be excluded since they clustered in the *Mycosphaerella* clade (*Mycosphaerellaceae*). Previous re-examinations and reassessments of human pathogenic *Cladosporium* species, including morphology, biology/ecology, physiology and molecular data (Masclaux *et al*. 1995, Untereiner 1997, Gerrits van den Ende & de Hoog 1999, Untereiner & Naveau 1999, Untereiner *et al*. 1999; de Hoog *et al*. 2000), could also be confirmed. In all phylogenetic analyses, it could be shown that the human pathogenic fungi concerned formed a clade belonging to the *Herpotrichiellaceae* (*Capronia* Sacc./*Cladophialophora* Borelli). *Venturia* anamorphs with catenate conidia, previously often assigned to *Cladosporium s. lat.*, clustered together with other anamorphs of the *Venturiaceae,* and formed a monophyletic clade (Braun *et al.* 2003, Schubert *et al.* 2003, Beck *et al.* 2005). *Venturia* has now also been shown to accommodate less well-known anamorph genera such as *Pseudocladosporium*, which represent an additional synonym of *Fusicladium* Bonord. (Crous *et al*. 2007 – this volume).

Seifert *et al.* (2004) examined morphological, ecological and molecular characters of *Cladosporium staurophorum* (W.B. Kendr.) M.B. Ellis and three allied heat-resistant species and placed them in the new genus *Devriesia*, which formed a monophyletic group apart from the *Cladosporium* clade. Crous *et al*. (2006b) erected the genus *Cladoriella* Crous for a saprobic species (*incertae sedis*) characterised by having narrowly ellipsoidal to cylindrical or fusoid, 0–1-septate, medium brown, thick-walled, finely verruculose conidia arranged in simple or branched chains, with thickened, darkened, refractive hila, with a minute central pore. *Cladosporium musae* E.W. Mason, the causal agent of banana speckle disease, has recently been shown to be allied to the *Chaetothyriales* (Crous *et al.* 2006a), and was placed in a new genus, *Metulocladosporiella* with *C. musae* as type species. *Digitopodium* U. Braun, Heuchert & K. Schub. (type species: *Cladosporium hemileiae* Steyaert) and *Parapericoniella* U. Braun, Heuchert & K. Schub. (type species: *Cladosporium asterinae* Deighton) represent two new genera of hyperparasitic hyphomycetes, introduced due to unique morphological features and striking differences to *Cladosporium s. str*. (Heuchert *et al*. 2005), but have as yet been excluded from DNA-based studies due to the absence of cultures. Schubert *et al.* (2007a – this volume) introduced a new genus, *Dichocladosporium* K. Schub., U. Braun & Crous (allied to the *Davidiellaceae*, *Capnodiales*) to accommodate a fungus with dimorphic fruiting that is pathogenic to *Paeonia* spp. The present study introduced yet several additional cladosporium-like genera, which could be distinguished based on their morphology and distinct DNA phylogeny, namely *Ochrocladosporium* (*Pleosporales*), *Rhizocladosporium* (*incertae sedis*), *Rachicladosporium, Toxicocladosporium* and *Verrucocladosporium* (*Capnodiales*).

Although all these genera are cladosporium-like, and many have in the past been confused with *Cladosporium s. str*., the unique coronate scar type of *Cladosporium s. str*. allows a critical revision of cladosporioid hyphomycetes, based on reliable, distinctive morphological characters. In all cases where cladosporium-like (*Cladosporium s. lat*.) hyphomycetes clearly clustered apart from *Cladosporium s. str*. in the phylogenetic analyses, it could be demonstrated that the fungal groups concerned were also morphologically unambiguously distinguished, above all with regard to the structure of the conidiogenous hila. Hence, the excluded groups of species, belonging in other genera, sometimes even in new genera, are genetically as well as morphologically clearly distinct from *Cladosporium s. str.*

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Phylogenetic and morphotaxonomic revision of *Ramichloridium* and allied genera

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Abstract: The phylogeny of the genera *Periconiella, Ramichloridium, Rhinocladiella* and *Veronaea* was explored by means of partial sequences of the 28S (LSU) rRNA gene and the ITS region (ITS1, 5.8S rDNA and ITS2). Based on the LSU sequence data, ramichloridium-like species segregate into eight distinct clusters. These include the *Capnodiales* (*Mycosphaerellaceae* and *Teratosphaeriaceae*), the *Chaetothyriales* (*Herpotrichiellaceae*), the *Pleosporales*, and five ascomycete clades with uncertain affinities. The type species of *Ramichloridium*, *R. apiculatum*, together with *R. musae, R. biverticillatum, R. cerophilum, R. verrucosum, R. pini*, and three new species isolated from *Strelitzia*, *Musa* and forest soil, respectively, reside in the *Capnodiales* clade. The human-pathogenic species *R. mackenziei* and *R. basitonum*, together with *R. fasciculatum* and *R. anceps*, cluster with *Rhinocladiella* (type species: *Rh. atrovirens*, *Herpotrichiellaceae*, *Chaetothyriales*), and are allocated to this genus. *Veronaea botryosa,* the type species of the genus *Veronaea*, also resides in the *Chaetothyriales* clade, whereas *Veronaea simplex* clusters as a sister taxon to the *Venturiaceae* (*Pleosporales*), and is placed in a new genus, *Veronaeopsis*. *Ramichloridium obovoideum* clusters with *Carpoligna pleurothecii* (anamorph: *Pleurothecium* sp., *Chaetosphaeriales*), and a new combination is proposed in *Pleurothecium*. Other ramichloridium-like clades include *R. subulatum* and *R. epichloës* (*incertae sedis*, *Sordariomycetes*), for which a new genus, *Radulidium* is erected. *Ramichloridium schulzeri* and its varieties are placed in a new genus, *Myrmecridium* (*incertae sedis*, *Sordariomycetes*). The genus *Pseudovirgaria* (*incertae sedis*) is introduced to accommodate ramichloridium-like isolates occurring on various species of rust fungi. A veronaea-like isolate from *Bertia moriformis* with phylogenetic affinity to the *Annulatascaceae* (*Sordariomycetidae*) is placed in a new genus, *Rhodoveronaea*. Besides *Ramichloridium*, *Periconiella* is also polyphyletic. *Thysanorea* is introduced to accommodate *Periconiella papuana* (*Herpotrichiellaceae*), which is unrelated to the type species*, P. velutina* (*Mycosphaerellaceae*).

Taxonomic novelties: *Myrmecridium* Arzanlou, W. Gams & Crous, gen. nov., *Myrmecridium flexuosum* (de Hoog) Arzanlou, W. Gams & Crous, comb. et stat. nov., *Myrmecridium schulzeri* (Sacc.) Arzanlou, W. Gams & Crous var. *schulzeri*, comb. nov., *Myrmecridium schulzeri* var. *tritici* (M.B. Ellis) Arzanlou, W. Gams & Crous, comb. nov., *Periconiella arcuata* Arzanlou, S. Lee & Crous, sp. nov., *Periconiella levispora* Arzanlou, W. Gams & Crous, sp. nov., *Pleurothecium obovoideum* (Matsush.) Arzanlou & Crous, comb. nov., *Pseudovirgaria* H.D. Shin, U. Braun, Arzanlou & Crous, gen. nov., *Pseudovirgaria hyperparasitica* H.D. Shin, U. Braun, Arzanlou & Crous, sp. nov., *Radulidium* Arzanlou, W. Gams & Crous, gen. nov., *Radulidium epichloës* (Ellis & Dearn.) Arzanlou, W. Gams & Crous, comb. nov., *Radulidium subulatum* (de Hoog) Arzanlou, W. Gams & Crous, comb. nov., *Ramichloridium australiense* Arzanlou & Crous, sp. nov., *Ramichloridium biverticillatum* Arzanlou & Crous, nom. nov., *Ramichloridium brasilianum* Arzanlou & Crous, sp. nov., *Ramichloridium strelitziae* Arzanlou, W. Gams & Crous, sp. nov., *Rhinocladiella basitona* (de Hoog) Arzanlou & Crous, comb. nov., *Rhinocladiella fasciculata* (V. Rao & de Hoog) Arzanlou & Crous, comb. nov., *Rhinocladiella mackenziei* (C.K. Campb. & Al-Hedaithy) Arzanlou & Crous, comb. nov., *Rhodoveronaea* Arzanlou, W. Gams & Crous, gen. nov., *Rhodoveronaea varioseptata* Arzanlou, W. Gams & Crous, sp. nov., *Thysanorea* Arzanlou, W. Gams & Crous, gen. nov., *Thysanorea papuana* (Aptroot) Arzanlou, W. Gams & Crous, comb. nov., *Veronaea japonica* Arzanlou, W. Gams & Crous, sp. nov., *Veronaeopsis* Arzanlou & Crous, gen. nov., *Veronaeopsis simplex* (Papendorf) Arzanlou & Crous, comb.nov.
Key words: *Capnodiales*, *Chaetothyriales*, *Mycosphaerella*, *Periconiella*, phylogeny, *Rhinocladiella*, *Veronaea*.

INTRODUCTION

The anamorph genus *Ramichloridium* Stahel ex de Hoog 1977 presently accommodates a wide range of species with erect, dark, more or less differentiated, branched or unbranched conidiophores and predominantly aseptate conidia produced on a sympodially proliferating rachis (de Hoog 1977). This heterogeneous group of anamorphic fungi includes species with diverse life styles, viz. saprobes, human and plant pathogens, most of which were classified by Schol-Schwarz (1968) in *Rhinocladiella* Nannf. according to a very broad generic concept. *Ramichloridium* was originally erected by Stahel (1937) with *R. musae* Stahel as type species. However, because his publication lacked a Latin diagnosis, the genus was invalid. Stahel also invalidly described *Chloridium musae* Stahel for a fungus causing leaf spots (tropical speckle disease) on banana. Ellis (1976) validated *Chloridium musae* as *Veronaea musae* M.B. Ellis, and *Ramichloridium musae* as *Periconiella musae* Stahel ex M.B. Ellis.

Periconiella Sacc. (1885) [type species *P. velutina* (G. Winter) Sacc.] differs from *Veronaea* Cif. & Montemart. chiefly based on its dark brown, apically branched conidiophores. However, de Hoog (1977) observed numerous specimens of *V. musae* to exhibit branched conidiophores in culture, as did Stahel (1937) for *Ramichloridium musae*. De Hoog (1977) subsequently re-introduced *Ramichloridium*, but typified it with *R. apiculatum* (J.H. Mill., Giddens & A.A. Foster) de Hoog. He regarded *V. musae* and *P. musae* to be conspecific, and applied the name *R. musae* (Stahel ex M.B. Ellis) de Hoog to both species, regarding *Periconiella musae* as basionym. The circumscription by de Hoog was based on their similar morphology and ecology. Central in his genus concept was the observed presence of more or less differentiated and pigmented conidiophores, with predominantly aseptate conidia produced on a sympodially proliferating rachis. De Hoog (1977) also used some ecological features as additional characters to discriminate *Ramichloridium* from other genera, noting, for instance, that species in *Ramichloridium* were non-pathogenic to humans (de Hoog 1977, Campbell & Al-Hedaithy 1993). This delimitation, however, was not commonly accepted (McGinnis & Schell 1980). De Hoog *et al.* (1983) further discussed the problematic separation of *Ramichloridium* from genera such as *Rhinocladiella*, *Veronaea* and *Cladosporium* Link. It was further noted that the main feature to distinguish *Ramichloridium* from *Rhinocladiella*, was the presence of exophiala-type budding cells in species of *Rhinocladiella* (de Hoog 1977, de Hoog *et al*. 1983, Veerkamp & Gams 1983). The separation of *Veronaea* from this complex is more problematic, as the circumscriptions provided by Ellis (1976) and Morgan-Jones (1979, 1982) overlap with that of *Ramichloridium sensu* de Hoog (1977). *Cladosporium* is more distinct, having very conspicuous, protuberant, darkened and thickened, coronate conidial scars, and catenate conidia (David 1997, Braun *et al.* 2003, Schubert *et al*. 2007 – this volume).

To date 26 species have been named in *Ramichloridium*; they not only differ in morphology, but also in life style. *Ramichloridium mackenziei* C.K. Campb. & Al-Hedaithy is a serious human pathogen, causing cerebral phaeohyphomycosis (Al-Hedaithy *et al.* 1988, Campbell & Al-Hedaithy 1993), whereas *R. musae* causes tropical speckle disease on members of the *Musaceae* (Stahel 1937, Jones 2000). Another plant-pathogenic species, *R. pini* de Hoog & Rahman, causes a needle disease on *Pinus contorta* (de Hoog *et al*. 1983). Other clinically relevant species of *Ramichloridium* are *R. basitonum* de Hoog and occasionally *R. schulzeri* (Sacc.) de Hoog, while the remaining species tend to be common soil saprobes.

No teleomorph has thus far been linked to species of *Ramichloridium*. The main question that remains is whether shared morphology among the species in this genus reflects common ancestry (Seifert 1993, Untereiner & Naveau 1999). To delineate anamorphic genera adequately, morphology and conidial ontogeny alone are no longer satisfactory (Crous *et al*. 2006a, b), and DNA data provide additional characters to help delineate species and genera (Taylor *et al.* 2000, Mostert *et al*. 2006, Zipfel *et al*. 2006). The aim of the present study was to integrate morphological and cultural features with DNA sequence data to resolve the species concepts and generic limits of the taxa currently placed in *Periconiella*, *Ramichloridium*, *Rhinocladiella* and *Veronaea*, and to resolve the status of several new cultures that were isolated during the course of this study.

MATERIALS AND METHODS

Isolates

Species names, substrates, geographical origins and GenBank accession numbers of the isolates included in this study are listed in Table 1. Fungal isolates are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands.

DNA extraction, amplification and sequence analysis

Genomic DNA was extracted from colonies grown on 2 % malt extract agar (MEA, Difco) (Gams *et al*. 2007) using the FastDNA kit (BIO101, Carlsbad, CA, U.S.A.). The primers ITS1 and ITS4 (White *et al.* 1990) were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including: the 3' end of the 18S rRNA gene, the first internal transcribed spacer region (ITS1), the 5.8S rRNA gene, the second internal transcribed spacer region (ITS2) and the 5' end of 28S rRNA gene. Part of the large subunit 28S rRNA (LSU) gene was amplified with primers LR0R (Rehner & Samuels 1994) and LR5 (Vilgalys & Hester 1990). The ITS region was sequenced only for those isolates for which these data were not available. The ITS analyses confirmed the proposed classification based on LSU analysis for each major clade and are not presented here in detail; but the sequences are deposited in GenBank where applicable. The PCR reaction was performed in a mixture with 0.5 units *Taq* polymerase (Bioline, London, U.K.), 1× PCR buffer, 0.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5 pmol of each primer, approximately 10–15 ng of fungal genomic DNA, with the total volume adjusted to 25 µL with sterile water. Reactions were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with cycling conditions consisting of 5 min at 96 °C for primary denaturation, followed by 36 cycles at 96 °C (30 s), 52 °C (30 s), and 72 °C (60 s), with a final 7 min extension step at 72 °C to complete the reaction. The amplicons were sequenced using BigDye Terminator v. 3.1 (Applied Biosystems, Foster City, CA) or DYEnamicET Terminator (Amersham Biosciences, Freiburg, Germany) Cycle Sequencing Kits and analysed on an ABI Prism 3700 (Applied Biosystems, Foster City, CA) under conditions recommended by the manufacturer. Newly generated sequences were subjected to a Blast search of the NCBI databases, sequences with high similarity were downloaded from GenBank and comparisons were made based on the alignment of the obtained sequences. Sequences from GenBank were also selected for similar taxa. The LSU tree was rooted using sequences of *Athelia epiphylla* Pers. and *Paullicorticium ansatum* Liberta as outgroups. Phylogenetic analysis was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003), using the neighbour-joining algorithm with the uncorrected ("p"), the Kimura 2-parameter and the HKY85 substitution models. Alignment gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as missing data. Any ties were broken randomly when encountered. Phylogenetic relationships were also inferred with the parsimony algorithm using the heuristic search option with simple taxon additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm; alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Only the first 5 000 equally most parsimonious trees were saved. Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC, respectively). The robustness of the obtained trees was evaluated by 1 000 bootstrap replications. Bayesian analysis was performed following the methods of Crous *et al*. (2006c). The best nucleotide substitution model was determined using MrModeltest v. 2.2 (Nylander 2004). MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) was used to perform phylogenetic analyses, using a general time-reversible (GTR) substitution model with inverse gamma rates, dirichlet base frequencies and the temp value set to 0.5. New sequences were lodged with NCBI's GenBank (Table 1) and the alignment and trees with TreeBASE (www.treebase.org).

Morphology

Cultural growth rates and morphology were recorded from colonies grown on MEA for 2 wk at 24 ºC in the dark, and colony colours were determined by reference to the colour charts of Rayner (1970). Microscopic observations were made from colonies cultivated on MEA and OA (oatmeal agar, Gams *et al.* 2007), using a slide culture technique. Slide cultures were set up in Petri dishes containing 2 mL of sterile water, into which a U-shaped glass rod was placed, extending above the water surface. A block of freshly growing fungal colony, approx. 1 cm square was placed onto a sterile microscope slide, covered with a somewhat larger, sterile glass cover slip, and incubated in the moist chamber. Fungal sporulation was monitored over time, and when optimal, images were captured by means of a Nikon camera system (Digital Sight DS-5M, Nikon Corporation, Japan). Structures were mounted in lactic acid, and 30 measurements (× 1 000 magnification) determined wherever possible, with the extremes of spore measurements given in parentheses.

Table 1. Isolates of *Ramichloridium* and similar genera used for DNA analysis and morphological studies.

Species	Accession number[1]	Source	Origin	GenBank numbers (LSU, ITS)
Myrmecridium flexuosum	CBS 398.76*; IMI 203547	Soil	Suriname	EU041825, EU041768
Myrmecridium schulzeri	CBS 100.54; JCM 6974	Soil	Zaire	EU041826, EU041769
	CBS 134.68; ATCC 16310	Soil	Germany	EU041827, EU041770
	CBS 156.63	*Homo sapiens*	Netherlands	EU041828, EU041771
	CBS 188.96	Soil	Papua New Guinea	EU041829, EU041772
	CBS 304.73	Wheat straw	South Africa	EU041830, EU041773
	CBS 305.73; JCM 6967	Wheat straw	South Africa	EU041831, EU041774
	CBS 325.74; JCM 7234	*Triticum aestivum*	Netherlands	EU041832, EU041775
	CBS 381.87	—	Australia	EU041833, EU041776
	CBS 642.76	*Malus sylvestris*	Switzerland	EU041834, EU041777
	CBS 114996	*Cannomois virgata*	South Africa	EU041835, EU041778
Periconiella arcuata	CBS 113477*	*Ischyrolepsis subverticellata*	South Africa	EU041836, EU041779
Periconiella levispora	CBS 873.73*	*Turpinia pomifera*	Sri Lanka	EU041837, EU041780
Periconiella velutina	CBS 101948*; CPC 2262	*Brabejum stellatifolium*	South Africa	EU041838, EU041781
	CBS 101949; CPC 2263	*Brabejum stellatifolium*	South Africa	EU041839, EU041782
	CBS 101950; CPC 2264	*Brabejum stellatifolium*	South Africa	EU041840, EU041783
Pleurothecium obovoideum	CBS 209.95*; MFC 12477	*Pasania edulis*	Japan	EU041841, EU041784
Pseudovirgaria hyperparasitica	CBS 121735; CPC 10702	On *Phragmidium* sp. on *Rubus coreanus*	Korea	EU041822, EU041765
	CBS 121738; CPC 10704	On *Phragmidium* sp. on *Rubus coreanus*	Korea	EU041823, EU041766
	CBS 121739*; CPC 10753	On *Pucciniastrum agrimoniae* on *Agrimonia pilosa*	Korea	EU041824, EU041767
Radulidium epichloës	CBS 361.63*; MUCL 3124	*Epichloë typhina*	U.S.A.	EU041842, EU041785
Radulidium sp.	CBS 115704	*Poaceae*	Guyana	EU041843, EU041786
Radulidium subulatum	CBS 287.84	*Puccinia allii*	U.K.	EU041844, EU041787
	CBS 405.76*	*Phragmites australis*	Czech Republic	EU041845, EU041788
	CBS 912.96	Incubator for cell cultures	Germany	EU041846, EU041789
	CBS 101010	*Lasioptera arundinis*	Czech Republic	EU041847, EU041790
Ramichloridium apiculatum	CBS 156.59*; ATCC 13211; IMI 100716; JCM 6972; MUCL 7991; MUCL 15753; QM 7716	Forest soil	U.S.A.	EU041848, EU041791
	CBS 390.67	*Cucumis sativus*	South Africa	EU041849, EU041792
	CBS 391.67; JCM 6966	*Aloe* sp.	South Africa	EU041850, EU041793
	CBS 400.76; IMI 088021	Soil	Pakistan	EU041851, EU041794
Ramichloridium australiense	CBS 121710	*Musa banksii*	Australia	EU041852, EU041795
Ramichloridium biverticillatum	CBS 335.36	*Musa sapientum*	—	EU041853, EU041796
Ramichloridium brasilianum	CBS 283.92*	Forest soil	Brazil	EU041854, EU041797
Ramichloridium cerophilum	CBS 103.59*	*Sasa* sp.	Japan	EU041855, EU041798
Ramichloridium indicum	CBS 171.96	—	—	EU041856, EU041799
Ramichloridium musae	CBS 190.63; MUCL 9557	*Musa sapientum*	—	EU041857, EU041800
	CBS 365.36*; JCM 6973; MUCL 9556	*Musa sapientum*	Surinam	EU041858, EU041801
Ramichloridium pini	CBS 461.82*; MUCL 28942	*Pinus contorta*	U.K.	EU041859, EU041802
Ramichloridium strelitziae	CBS 121711	*Strelitzia* sp.	South Africa	EU041860, EU041803
Rhinocladiella anceps	CBS 157.54; ATCC 15680; MUCL 1081; MUCL 7992; MUCL 15756	*Fagus sylvatica*	France	EU041861, EU041804
	CBS 181.65*; ATCC 18655; DAOM 84422; IMI 134453; MUCL 8233; OAC 10215	Soil	Canada	EU041862, EU041805
Rhinocladiella basitona	CBS 101460*; IFM 47593	*Homo sapiens*	Japan	EU041863, EU041806
Rhinocladiella fasciculata	CBS 132.86*	Decayed wood	India	EU041864, EU041807
Rhinocladiella mackenziei	CBS 367.92; NCPF 2738; UTMB 3169	*Homo sapiens*	Israel	EU041865, EU041808

Table 1. (Continued).

Species	Accession number[1]	Source	Origin	GenBank numbers (LSU, ITS)
	CBS 368.92; UTMB 3170	*Homo sapiens*	Israel	EU041866, EU041809
	CBS 102590; NCPF 2853	*Homo sapiens*	United Arab Emirates	EU041867, EU041810
Rhinocladiella phaeophora	CBS 496.78*; IMI 287527	Soil	Colombia	EU041868, EU041811
Rhinocladiella sp.	CBS 264.49; MUCL 9904	Honey	France	EU041869, EU041812
Rhodoveronaea varioseptata	CBS 431.88*	*Bertia moriformis*	Germany	EU041870, EU041813
Thysanorea papuana	CBS 212.96*	—	Papua New Guinea	EU041871, EU041814
Veronaea botryosa	CBS 121.92	*Xanthorrhoea preissii*	Australia	EU041872, EU041815
	CBS 254.57*; IMI 070233; MUCL 9821	—	Italy	EU041873, EU041816
	CBS 350.65; IMI 115127; MUCL 7972	Goat dung	India	EU041874, EU041817
Veronaea compacta	CBS 268.75*	—	South Africa	EU041876, EU041819
Veronaea japonica	CBS 776.83*	On dead bamboo culm	Japan	EU041875, EU041818
Veronaeopsis simplex	CBS 588.66*; IMI 203547	*Acacia karroo*	South Africa	EU041877, EU041820
Zasmidium cellare	CBS 146.36	Wine cellar	—	EU041878, EU041821

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; IFM: Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan; IMI: International Mycological Institute, CABI-Bioscience, U.K.; JCM: Japan Collection of Microorganism, RIKEN BioResource Center, Japan; MFC: Matsushima Fungus Collection, Kobe, Japan; MUCL: Mycotheque de l' Université Catholique de Louvain, Louvain-la-Neuve, Belgium; NCPF: The National Collection of Pathogenic Fungi, Holborn, London, U.K.; OAC: Department of Botany and Genetics, University of Guelph, Ont., Canada; QM: Quartermaster Research and Developement Center, U.S. Army, MA, U.S.A.; UTMB: University of Texas Medical Branch, Texas, U.S.A.

*Ex-type cultures.

RESULTS

Phylogeny

The manually adjusted alignment of the 28S rDNA data contained 137 sequences (including the two outgroups) and 995 characters including alignment gaps. Of the 748 characters used in the phylogenetic analysis, 373 were parsimony-informative, 61 were variable and parsimony-uninformative, and 314 were constant. Neighbour-joining analysis using the three substitution models on the LSU alignment yielded trees with similar topology and bootstrap values. Parsimony analysis of the alignment yielded 5 000 equally most parsimonious trees, one of which is shown in Fig. 1 (TL = 2 157, CI = 0.377, RI = 0.875, RC = 0.330). The Markov Chain Monte Carlo (MCMC) analysis of four chains started from a random tree topology and lasted 2 000 000 generations. Trees were saved each 1 000 generations, resulting in 2 000 trees. Burn-in was set at 500 000 generations after which the likelihood values were stationary, leaving 1 500 trees from which the consensus tree (Fig. 2) and posterior probabilities (PP's) were calculated. The average standard deviation of split frequencies was 0.043910 at the end of the run. Among the neighbour-joining, Bayesian and parsimony analyses, the trees differed in the hierarchical order of the main families and the support values (data not shown; e.g. the support within of the *Capnodiales* in Figs 1–2).

The phylogenetic trees (Figs 1–2) show that the *Ramichloridium* species segregate into eight distinct clades, residing in the *Capnodiales* (*Mycosphaerellaceae* and *Teratosphaeriaceae*), the *Chaetothyriales* (*Herpotrichiellaceae*), the *Pleosporales*, and five other clades of which the relationships remain to be elucidated. The type species of *Ramichloridium*, *R. apiculatum*, together with *R. musae*, *R. cerophilum* (Tubaki) de Hoog, *R. indicum* (Subram.) de Hoog, *R. pini* and three new species respectively isolated from *Musa banksii*, *Strelitzia nicolai*, and forest soil, reside in different parts of the *Capnodiales* clade (all in the *Mycosphaerellaceae*, except for the species from forest soil which clusters in the *Teratosphaeriaceae*). The second clade (in the *Chaetothyriomycetes* clade), including the human-pathogenic species *R. mackenziei* and *R. basitonum*, together with *R. fasciculatum* V. Rao & de Hoog and *R. anceps* (Sacc. & Ellis) de Hoog, groups together with *Rhinocladiella* in the *Herpotrichiellaceae*. The third clade (in the *Sordariomycetes* clade) includes *R. obovoideum* (Matsush.) de Hoog, which in a Blast search was found to have affinity with *Carpoligna pleurothecii* F.A. Fernández & Huhndorf (*Chaetosphaeriales*). The fourth clade (in the *Sordariomycetes* clade) includes a veronaea-like isolate from *Bertia moriformis,* with phylogenetic affinity to the *Annulatascaceae* (*Sordariomycetidae*). The fifth clade (in the *Sordariomycetes* clade) includes *R. schulzeri* var. *schulzeri* and *R. schulzeri* var. *flexuosum* de Hoog, the closest relatives being *Thyridium vestitum* (Fr.) Fuckel in the *Thyridiaceae* and *Magnaporthe grisea* (T.T. Hebert) M.E. Barr in the *Magnaporthaceae.* The sixth clade (in the *Incertae sedis* clade) includes *R. subulatum* de Hoog, *R. epichloës* (Ellis & Dearn.) de Hoog and a species isolated from the *Poaceae*. Three ramichloridium-like isolates from *Rubus coreanus* and *Agrimonia pilosa* form another unique clade (in the *Incertae sedis* clade) with uncertain affinity. *Veronaea simplex* Papendorf clusters as sister taxon to the *Venturiaceae* representing the eighth clade (*Dothideomycetes*). The type species of *Periconiella*, *P. velutina*, clusters within the *Mycosphaerellaceae* (*Capnodiales* clade), whereas *P. papuana* Aptroot resides in the *Herpotrichiellaceae* (*Chaetothyriales* clade). *Veronaea botryosa* Cif. & Montemart., the type species of *Veronaea*, also resides in the *Herpotrichiellaceae*.

Taxonomy

The species previously described in *Ramichloridium* share some morphological features, including erect, pigmented, more or less differentiated conidiophores, sympodially proliferating conidiogenous cells and predominantly aseptate conidia. Other than conidial morphology, features of the conidiogenous apparatus that

appear to be more phylogenetically informative include pigmentation of vegetative hyphae, conidiophores and conidia, denticle density on the rachis, and structure of the scars. By integrating these data with the molecular data set, more natural genera are delineated, which are discussed below.

Key to ramichloridium-like genera

1. Conidiogenous cells integrated, terminal and lateral on creeping or ascending hyphae (differentiation between branched vegetative hyphae and conidiophores barely possible); conidiogenous loci bulging, more or less umbonate, apex rounded; occurring on rust pustules ..... ***Pseudovirgaria***
1. Conidiogenous cells integrated in distinct conidiophores; conidiogenous loci non-umbonate (flat, not prominent; subcylindrical or conical denticles; or terminally flat-tipped; or thickened and darkened); rarely occurring on rust pustules, but if so, with a raduliform rachis and distinct denticles 2

2. Conidia 0–2(–3)-septate, conidial base truncate, retaining a marginal frill after liberation [anamorphs of *Sordariomycetes*] ..... ***Rhodoveronaea***
2. Conidial base without marginal frill 3

3. Conidiophores composed of a well-developed erect stalk and a terminal branched head 4
3. Conidiophores unbranched or, if branched, branches loose, irregular or dichotomous, but not distinctly separated into stalk and branched head 5

4. Conidiophores dimorphic, either macronematous, dark brown with a dense apical cluster of branches or micronematous, undifferentiated, resembling vegetative hyphae; both kinds with a denticulate rachis; conidia predominantly 1-septate [anamorph of *Chaetothyriales*] ..... ***Thysanorea***
4. Conidiophores monomorphic; branched head with fewer branches and looser; conidiogenous loci usually flat, non-prominent, less denticle-like; conidia aseptate to pluriseptate [anamorphs of *Capnodiales*] ..... ***Periconiella***

5. Rachis with denticles 1–1.5 µm long, denticles almost cylindrical; conidia at least partly in short chains ..... ***Pleurothecium***
5. Rachis with denticles less than 1 µm long, denticles not cylindrical or denticles lacking, rachis with flat, barely prominent scars 6

6. Conidia predominantly septate 7
6. Conidia predominantly aseptate 8

7. Conidiophores up to 200 µm long; rachis straight, not to slightly geniculate; conidiogenous loci more or less flat, barely prominent, unthickened, slightly darkened [anamorphs of *Chaetothyriales, Herpotrichiellaceae*] ..... ***Veronaea***
7. Conidiophores up to 60 µm long; rachis distinctly geniculate; conidiogenous loci denticle-like, prominent, up to 0.5 µm high, slightly thickened and darkened [anamorph of *Pleosporales*, *Venturiaceae*] ..... ***Veronaeopsis***

8. Vegetative mycelium entirely hyaline; rachis long, hyaline, with widely scattered pimple-shaped, terminally pointed, unpigmented denticles ..... ***Myrmecridium***
8. Vegetative mycelium at least partly pigmented; conidiogenous loci distinct, non-denticulate, somewhat darkened-refractive, or denticles, if present, neither pimple-shaped nor pointed 9

9. Rachis distinctly raduliform, with distinct, prominent blunt denticles, 0.5–1 µm long; scars and hila unthickened, but pigmented ..... ***Radulidium***
9. Rachis not distinctly raduliform, at most subdenticulate; scars flat or only slightly prominent (subdenticulate), shorter 10

10. Conidiophores usually poorly differentiated from the vegetative hyphae; conidial apparatus often loosely branched; exophiala-like budding cells usually present in culture [anamorphs of *Chaetothyriales*, *Herpotrichiellaceae*] ..... ***Rhinocladiella***
10. Conidiophores usually well differentiated from the vegetative mycelium (macronematous), usually unbranched; without exophiala-like states [anamorphs of *Capnodiales*] ..... ***Ramichloridium***

Capnodiales (*Mycosphaerellaceae, Teratosphaeriaceae*)

The type species of *Ramichloridium, R. apiculatum,* together with *R. indicum* cluster as a sister group to the *Dissoconium* de Hoog, Oorschot & Hijwegen clade in the *Mycosphaerellaceae*. Some other *Ramichloridium* species, including *R. musae*, *R. biverticillatum* Arzanlou & Crous, *R. pini* and *R. cerophilum*, are also allied with members of the *Mycosphaerellaceae*. Three additional new species are introduced for *Ramichloridium* isolates from *Musa banksii, Strelitzia nicolai*, and forest soil. *Periconiella velutina,* the type species of *Periconiella*, which also resides in the *Mycosphaerellaceae*, is morphologically sufficiently distinct to retain its generic status. Two new species of *Periconiella* are introduced for isolates obtained from *Turpinia pomifera* and *Ischyrolepis subverticellata* in South Africa. *Zasmidium cellare* (Pers.) Fr., the type species of *Zasmidium* (Pers.) Fr., is also shown to cluster within the *Mycosphaerellaceae.*

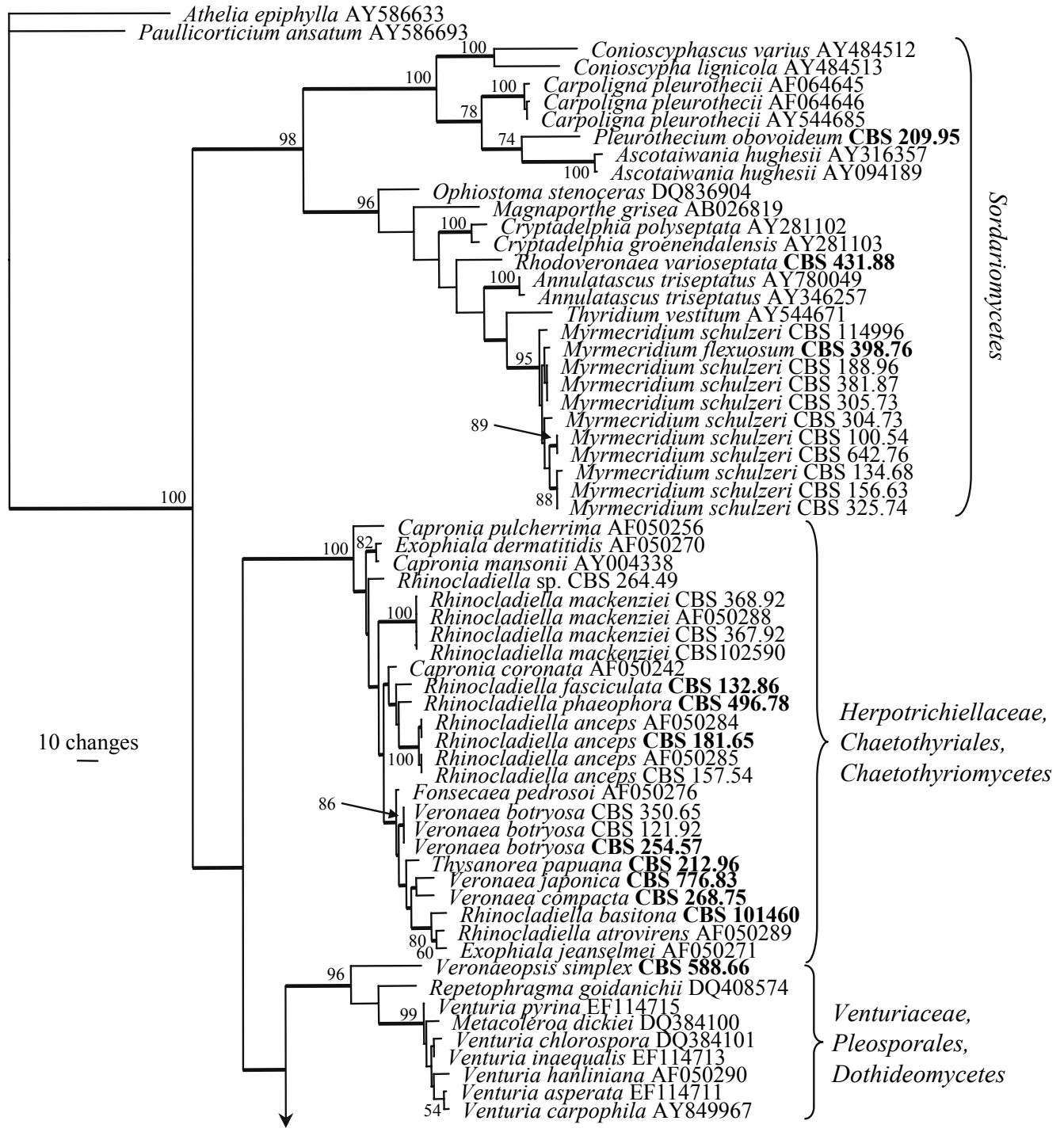

Fig. 1. (Page 62–63). One of 5 000 equally most parsimonious trees obtained from a heuristic search with simple taxon additions of the LSU sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes; bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

Periconiella Sacc., in Sacc. & Berlese, Atti Ist. Veneto Sci., Ser. 6, 3: 727. 1885.

In vitro: *Colonies* with entire margin; aerial mycelium rather compact, raised, velvety, olivaceous-grey; reverse olivaceous-black. *Submerged hyphae* verrucose, hyaline, thin-walled, 1–3 µm wide; *aerial hyphae* subhyaline, later becoming dark brown, thick-walled, smooth. *Conidiophores* arising vertically from creeping hyphae, straight or flexuose, up to 260 µm long, dark brown at the base, paler towards the apex, thick-walled; in the upper part bearing short branches. *Conidiogenous cells* terminally integrated, polyblastic, smooth or verrucose, subcylindrical, mostly not or barely geniculate-sinuous, variable in length, subhyaline, later becoming pale brown, fertile part as wide as the basal part, proliferating sympodially, sometimes becoming septate and forming a short, straight rachis with pigmented, slightly thickened and hardly prominent, more or less flat scars. *Conidia* solitary, occasionally in short chains, 0–multi-septate, subhyaline to rather pale olivaceous or olivaceous-brown, smooth to verrucose, globose, ellipsoidal to obovoid or obclavate, with a slightly darkened and thickened hilum; conidial secession schizolytic.

Type species: *P. velutina* (G. Winter) Sacc., Miscell. mycol. 2: 17. 1884.

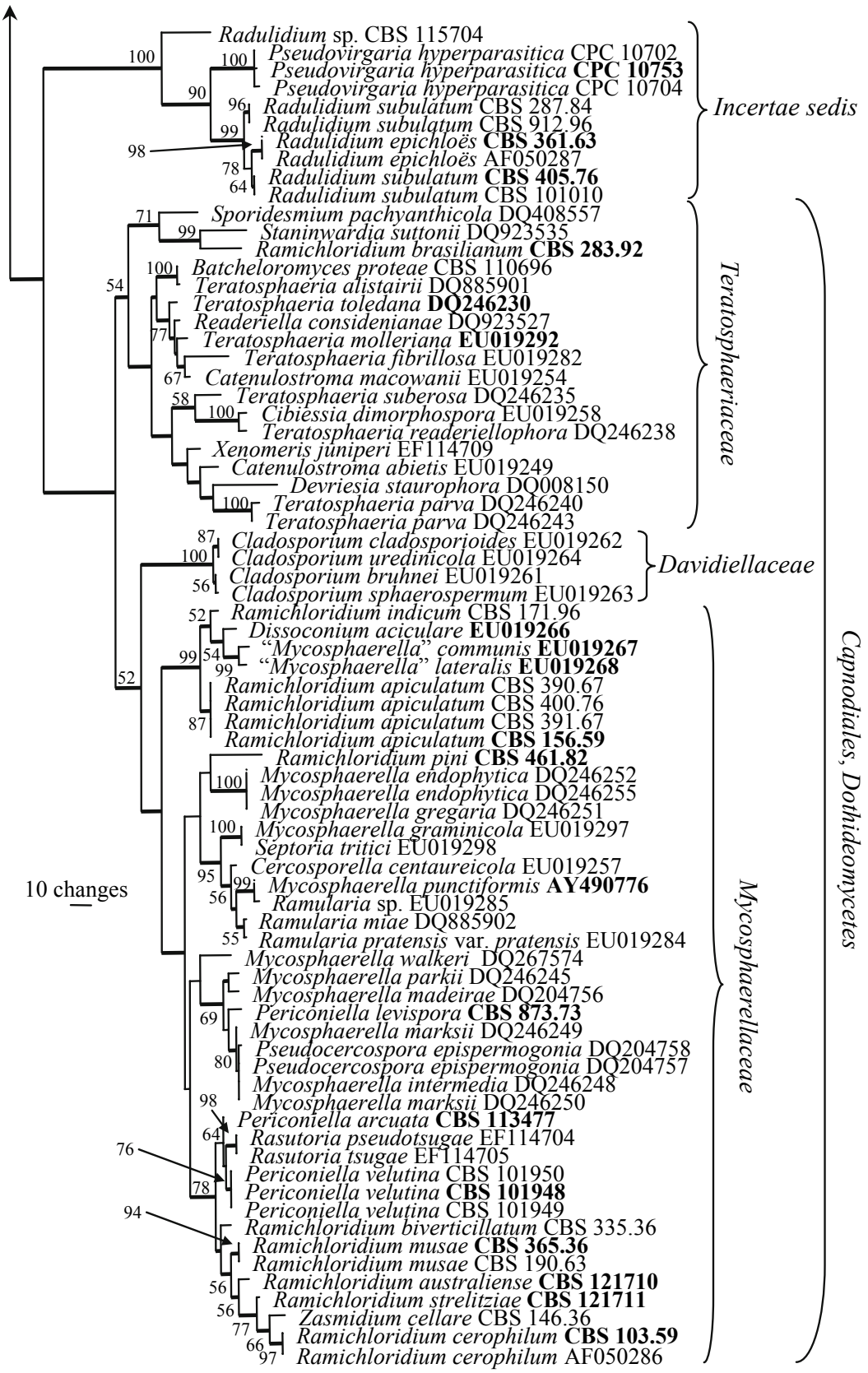

Fig. 1. (Continued).

Notes: *Periconiella* is distinct from other ramichloridium-like genera by its conidiophores that are prominently branched in the upper part, and by its darkened, thickened conidial scars, that are more or less flat and non-prominent. Although conidiophores are also branched in the upper part in *Thysanorea* Arzanlou, W. Gams & Crous, the branching pattern in the latter genus is different from that of *Periconiella*. *Thysanorea* has a complex head consisting of up to six levels of branches, while in *Periconiella* the branching is limited, with mainly primary and secondary branches. Furthermore, *Thysanorea* is characterised by having dimorphic conidiophores and more or less prominent denticle-like conidiogenous loci.

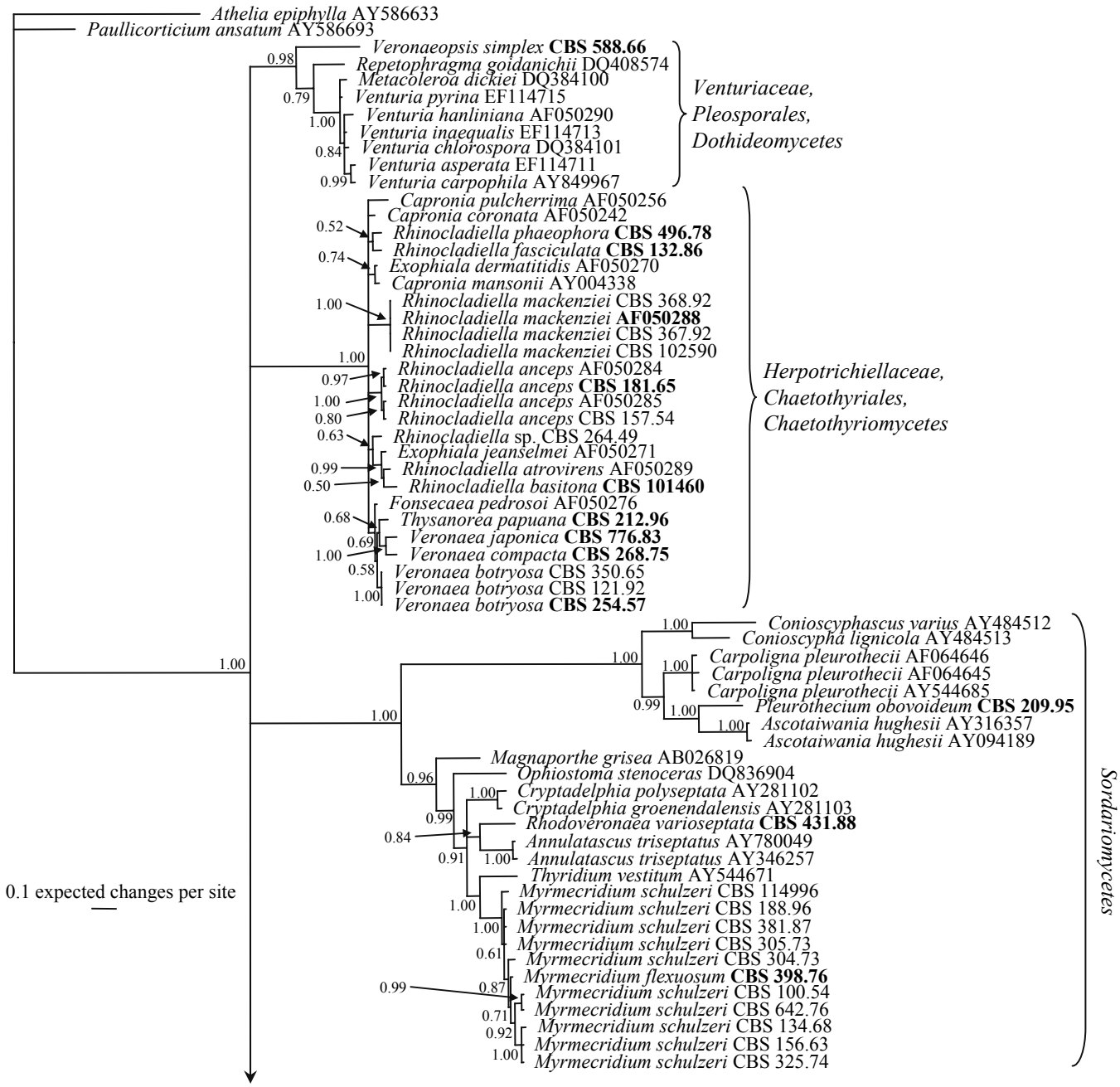

Fig. 2. (Page 64–65). Consensus phylogram (50 % majority rule) of 1 500 trees resulting from a Bayesian analysis of the LSU sequence alignment using MRBAYES v. 3.1.2. Bayesian posterior probabilities are indicated at the nodes. Ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

Periconiella velutina (G. Winter) Sacc., Miscell. mycol. 2: 17. 1884. Fig. 3.

Basionym: *Periconia velutina* G. Winter, Hedwigia 23: 174. 1884.

In vitro: *Submerged hyphae* verrucose, hyaline, thin-walled, 1–3 µm wide; *aerial hyphae* subhyaline, later becoming dark brown, thick-walled, smooth. *Conidiophores* arising vertically from creeping hyphae, straight or flexuose, up to 260 µm long, dark brown at the base, paler towards the apex, thick-walled; in the upper part bearing short branches, 10–35 µm long. *Conidiogenous cells* mostly terminally integrated, sometimes discrete, smooth or verrucose, cylindrical, variable in length, subhyaline, later becoming pale brown, fertile part as wide as the basal part, proliferating sympodially, sometimes becoming septate and forming a short, straight rachis with pigmented, slightly thickened and hardly prominent, more or less flat scars, less than 1 µm diam. *Conidia* 0(–1)-septate, subhyaline, thin-walled, verrucose or smooth, globose, ellipsoidal to obovoid, (7–)8–9(–11) × (2.5–)3(–4) µm, with a slightly darkened and thickened hilum, 1.5–2 µm diam.

Cultural characteristics: Colonies on MEA slow-growing, reaching 4 mm diam after 14 d at 24 °C, with entire margin; aerial mycelium rather compact, raised, velvety, olivaceous-grey; reverse olivaceous-black.

Specimens examined: **South Africa**, Cape Town, on *Brabejum stellatifolium*, P. MacOwan, herb. G. Winter (B), **lectotype selected here**; Cape Town, on leaves of *Brabejum stellatifolium* (= *B. stellatum*), P. Mac-Owan, PAD, F42165, F462166, **isolectotypes**; Stellenbosch, Jonkershoek Nature Reserve, on *Brabejum stellatifolium,* 21 Jan. 1999, J.E. Taylor, **epitype designated here** CBS H-15612, cultures ex-epitype CBS 101948–101950.

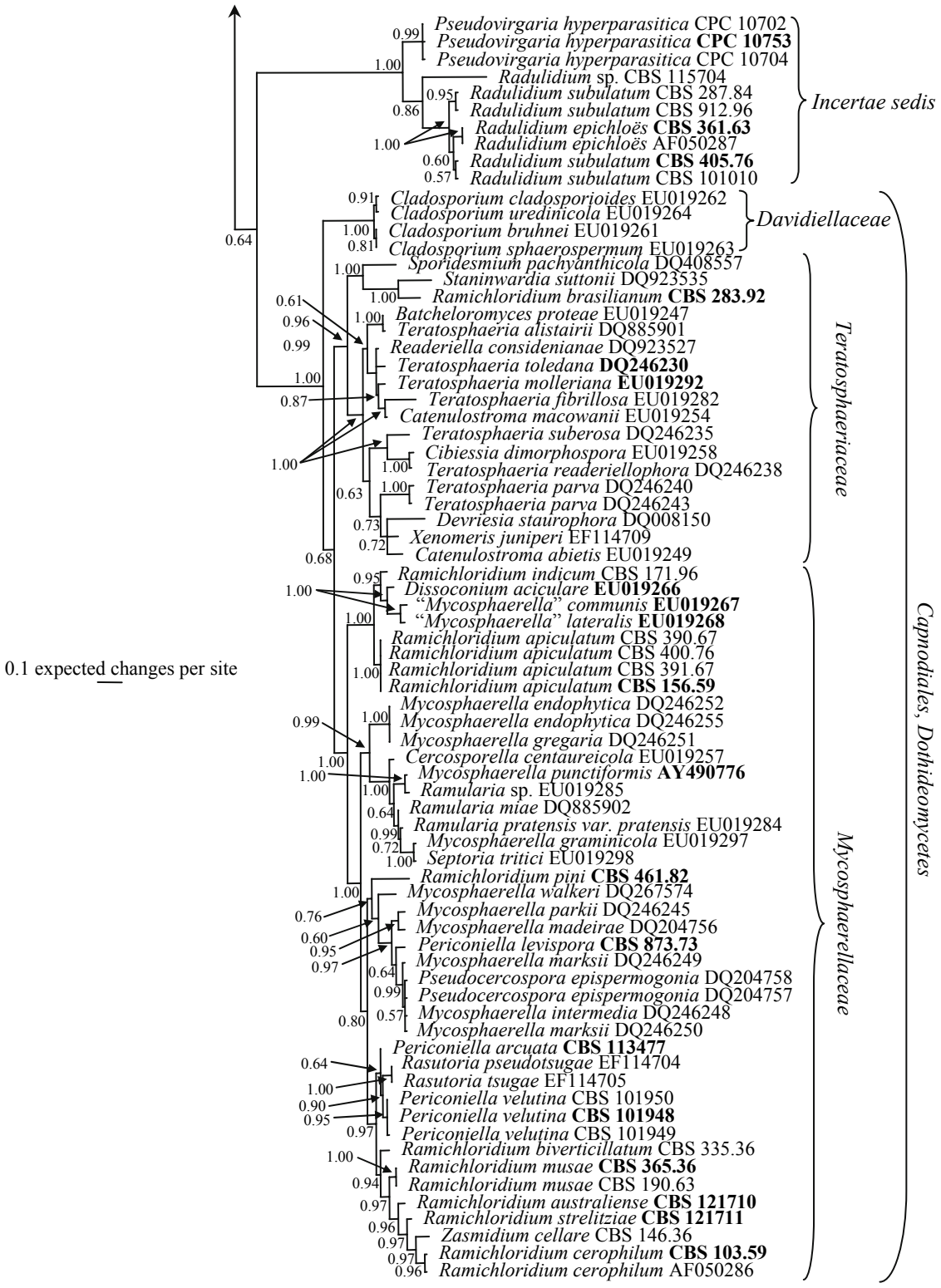

Fig. 2. (Continued).

Periconiella arcuata Arzanlou, S. Lee & Crous, **sp. nov.** MycoBank MB504547. Figs 4, 7A.

Etymology: Named after its curved conidia.

Ab aliis speciebus *Periconiellae* conidiis obclavatis, rectis vel curvatis, (30–)53–61(–79) × (3–)5(–7) µm, distinguenda.

Submerged hyphae smooth, hyaline, thin-walled, 2 µm wide; *aerial hyphae* pale brown, smooth or verrucose, slightly narrower. *Conidiophores* arising vertically from creeping hyphae, straight or flexuose, up to 300 µm long, dark brown at the base, paler towards the apex, thick-walled; loosely branched in the upper part, bearing short branches. *Conidiogenous cells* integrated, cylindrical, variable in length, 20–50 µm long, subhyaline, later becoming pale brown, fertile part as wide as the basal part, proliferating sympodially, forming a geniculate conidium-bearing rachis with pigmented and thickened, prominent, cone-shaped scars, 1 µm diam. *Conidia* formed singly, obclavate, straight or mostly curved, 0(–4)-septate, coarsely verrucose, pale olive, thin-walled, tapering towards the apex, (30–)53–61(–79) × (3–)5(–7) µm, with a narrowly truncate base and a darkened, hardly thickened hilum, 2 µm diam; microcyclic conidiation observed in culture.

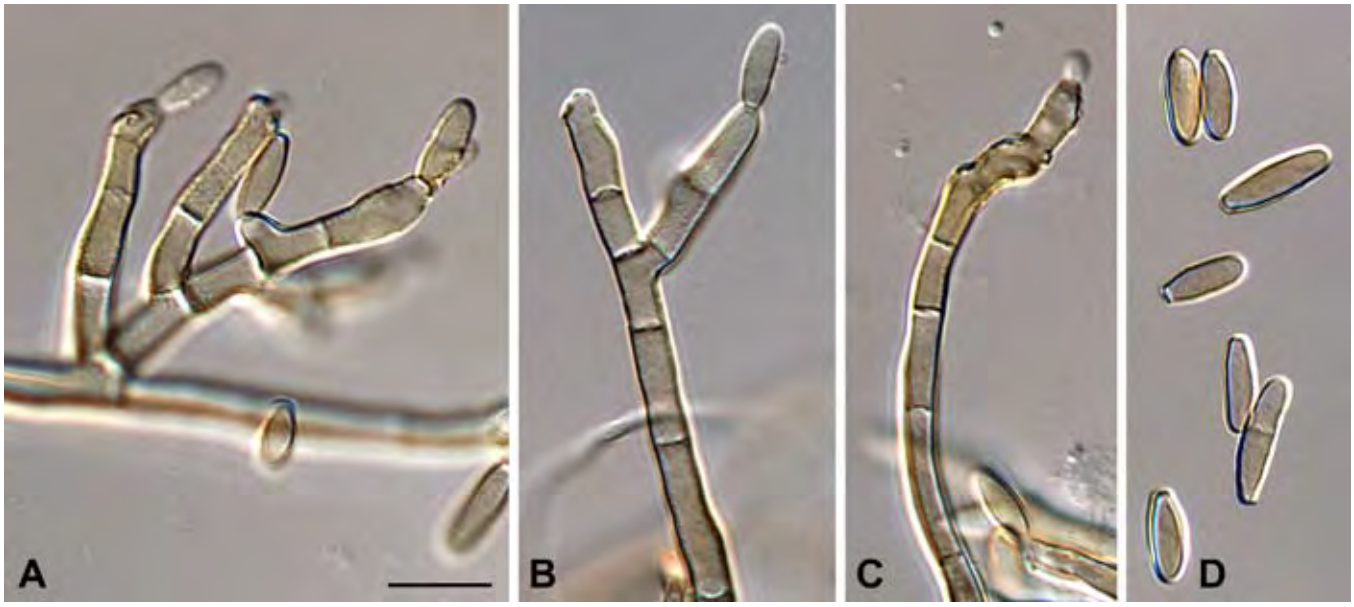

Fig. 3. *Periconiella velutina* (CBS 101948). A–B. Macronematous conidiophores with short branches in the upper part. C. Sympodially proliferating conidiogenous cell with darkened and slightly thickened scars. D. Conidia. Scale bar = 10 µm.

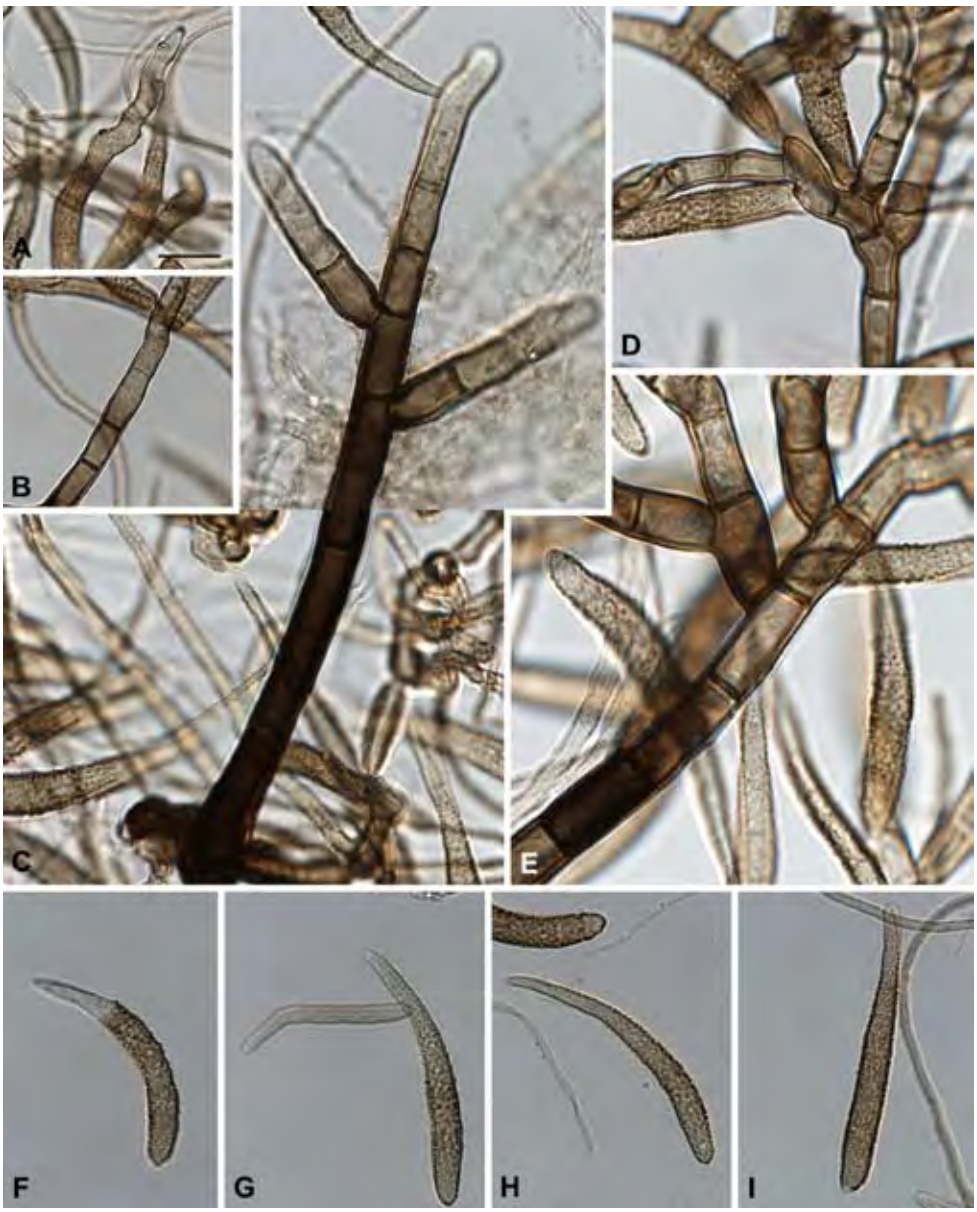

Fig. 4. *Periconiella arcuata* (CBS 113477). A–B. Sympodially proliferating conidiogenous cells with darkened, thickened and cone-shaped scars. C–E. Macronematous conidiophores with loose branches in the upper part. F–I. Conidia. Scale bar = 10 µm.

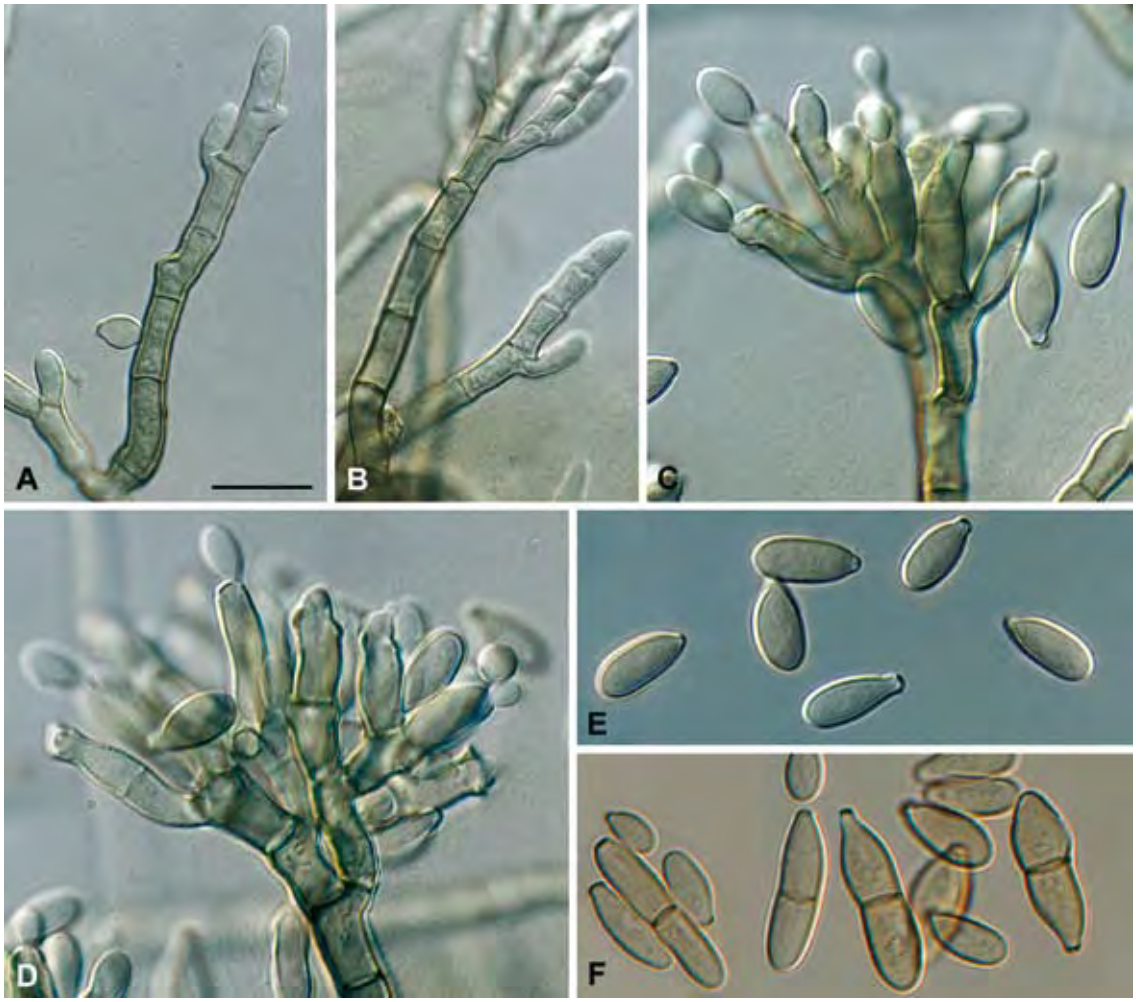

Fig. 5. *Periconiella levispora* (CBS 873.73). A–C. Conidial apparatus at different stages of development, which gives rise to macronematous conidiophores with dense branches in the upper part. D. Sympodially proliferating conidiogenous cells with darkened and somewhat protruding scars. E–F. Conidia with truncate base and darkened hilum. Scale bar = 10 µm.

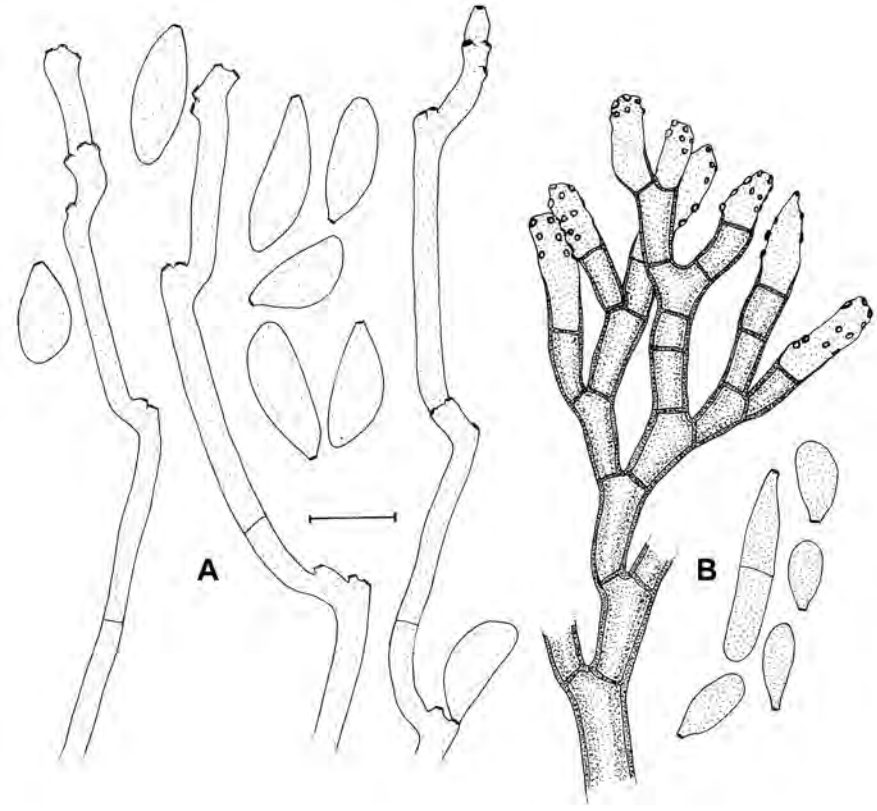

Fig. 6. A. *Pseudovirgaria hyperparasitica* (CBS 121739 = CPC 10753). B. *Periconiella levispora* (CBS 873.73). Scale bar = 10 µm.

Cultural characteristics: Colonies on MEA reaching 12 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium compacted, becoming hairy, colonies up to 1 mm high; surface olivaceous to olivaceous-grey, reverse dark grey-olivaceous to olivaceous-black.

Specimen examined: **South Africa**, Western Cape Province, Kogelberg, on dead culms of *Ischyrolepis subverticillata*, May 2001, S. Lee, **holotype** CBS H-19927, culture ex-type CBS 113477.

Periconiella levispora Arzanlou, W. Gams & Crous, **sp. nov.** MycoBank MB504546. Figs 5–6B.

Etymology: (Latin) *levis* = smooth.

A simili *Periconiella velutina* conidiis levibus et maioribus, ad 23 µm longis distinguenda.

In vitro: *Submerged hyphae* smooth, hyaline, thin-walled, 2–2.5 µm wide; *aerial hyphae* subhyaline, later becoming dark brown, thick-walled, smooth. *Conidiophores* arising vertically from creeping aerial hyphae, dark brown at the base, paler towards the apex, thick-walled; in the upper part bearing several short branches, up to 120 µm long. *Conidiogenous cells* integrated, occasionally discrete, cylindrical, variable in length, 10–20 µm long, subhyaline, later becoming pale brown, fertile part as wide as the basal part, proliferating sympodially, forming a short rachis with pigmented and slightly thickened, somewhat protruding scars, less than 1 µm diam. *Conidia* solitary, 0(–2)-septate, smooth, pale olivaceous, cylindrical, ellipsoidal, pyriform to clavate, (7–)11–14(–23) × (3–)4–5(–6) µm, with a truncate base and a darkened, slightly thickened hilum, 2 µm diam.

Cultural characteristics: Colonies on MEA slow-growing, reaching 5 mm diam after 14 d at 24 °C, with entire margin; aerial mycelium compact, raised, velvety, olivaceous-grey; reverse olivaceous-black.

Specimen examined: **Sri Lanka**, Hakgala Botanic Gardens, on dead leaves of *Turpinia pomifera*, Jan. 1973, W. Gams, **holotype** CBS H-15611, culture ex-type CBS 873.73.

Ramichloridium Stahel ex de Hoog, Stud. Mycol. 15: 59. 1977.

In vitro: *Colonies* flat to raised, with entire margin; surface olivaceous-green to olivaceous-black. *Mycelium* consisting of submerged and aerial hyphae; submerged hyphae hyaline to subhyaline, thin-walled, aerial hyphae smooth or verrucose. *Conidiophores* straight, unbranched, rarely branched, thick-walled, dark brown (darker than the subtending hyphae), continuous or with several additional thin septa. *Conidiogenous cells* integrated, terminal, polyblastic, smooth, thick-walled, golden-brown, apical part subhyaline, with sympodial proliferation, straight or flexuose, geniculate or nodose, with conspicuous conidiogenous loci; scars crowded or scattered, unthickened, unpigmented to faintly pigmented, or slightly prominent denticles. *Conidia* solitary, 0–1-septate, subhyaline to pale brown, smooth to coarsely verrucose, rather thin-walled, obovate, obconical or globose to ellipsoidal, fusiform, with a somewhat prominent, slightly pigmented hilum; conidial secession schizolytic.

Type species: *R. apiculatum* (J.H. Mill., Giddens & A.A. Foster) de Hoog, Stud. Mycol. 15: 69. 1977.

Ramichloridium apiculatum (J.H. Mill., Giddens & A.A. Foster) de Hoog, Stud. Mycol. 15: 69. 1977. Fig. 8.

Basionym: *Chloridium apiculatum* J.H. Mill., Giddens & A.A. Foster, Mycologia 49: 789. 1957.

≡ *Veronaea apiculata* (J.H. Mill., Giddens & A.A. Foster) M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 209. 1976.

[non *Rhinocladiella apiculata* Matsush., in Matsushima, *Icon. Microfung. Mats. lect.*: 122. 1975].

= *Rhinocladiella indica* Agarwal, Lloydia 32: 388. 1969.

[non *Chloridium indicum* Subram., Proc. Indian Acad. Sci., Sect. B, 42: 286. 1955].

In vitro: *Submerged hyphae* hyaline to subhyaline, thin-walled, 1–2.5 µm wide; *aerial hyphae* slightly darker, smooth-walled. *Conidiophores* generally arising at right angles from creeping aerial hyphae, straight, unbranched, thick-walled, dark brown, continuous or with 1–2(–3) additional thin septa, up to 100 µm long; intercalary cells 10–28 µm long. *Conidiogenous cells* integrated, terminal, smooth, thick-walled, golden-brown, straight, cylindrical, 25–37(–47) × 2–3.5 µm; proliferating sympodially, resulting in a straight rachis with conspicuous conidiogenous loci; scars prominent, crowded, slightly pigmented, less than 1 µm diam. *Conidia* solitary, obovate to obconical, pale brown, finely verrucose, (3–)5–5.5(–7.5) × (2–)2.5–3(–4) µm, hilum conspicuous, slightly pigmented, about 1 µm diam.

Cultural characteristics: Colonies on MEA reaching 35 mm diam after 14 d at 24 °C; minimum temperature for growth above 6 °C, optimum 24 °C, maximum 30 °C. Colonies raised, velvety, dense, with entire margin; surface olivaceous-green, reverse olivaceous-black, often with a diffusing citron-yellow pigment.

Specimens examined: **Pakistan**, Lahore, from soil, A. Kamal, CBS 400.76 = IMI 088021. **South Africa**, from preserved *Cucumis sativus* in 8-oxyquinoline sulphate, M.C. Papendorf, CBS 390.67; Potchefstroom, from *Aloe* sp., M.C. Papendorf, CBS 391.67. **U.S.A.**, Georgia, isolated from forest soil, CBS 156.59 = ATCC 13211 = IMI 100716 = QM 7716, **ex-type** culture.

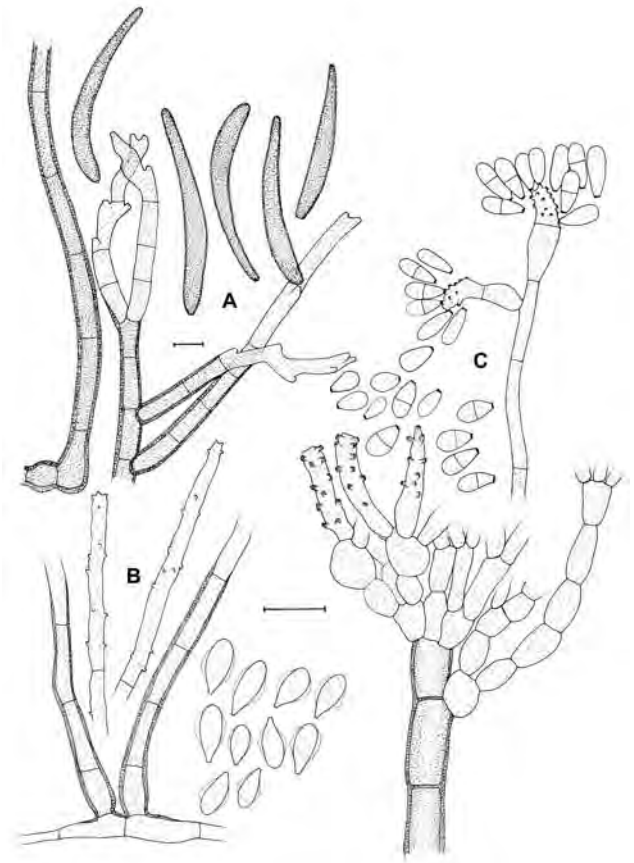

Fig. 7. A. *Periconiella arcuata* (CBS 113477). B. *Myrmecridium schulzeri* (CBS 325.74). C. *Thysanorea papuana* (CBS 212.96). Scale bars = 10 µm.

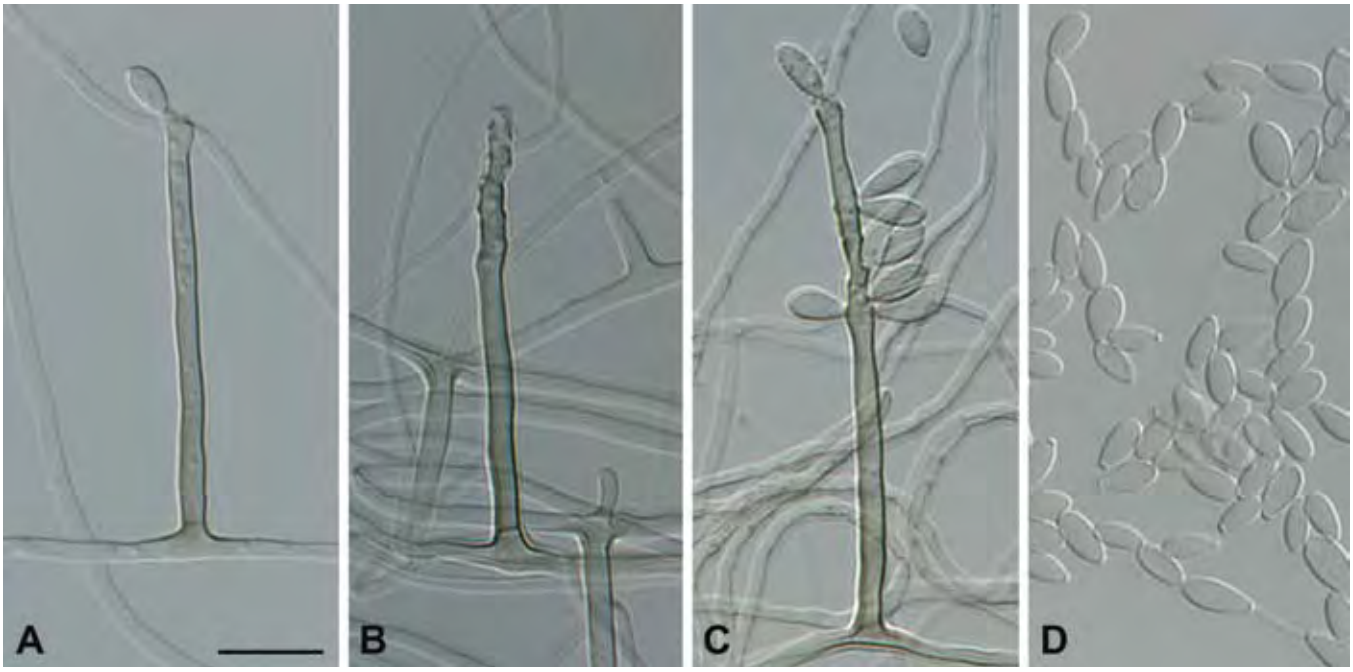

Fig. 8. *Ramichloridium apiculatum* (CBS 156.59). A–C. Macronematous conidiophores with sympodially proliferating conidiogenous cells, which give rise to a conidium-bearing rachis with crowded and prominent scars. D. Conidia. Scale bar = 10 µm.

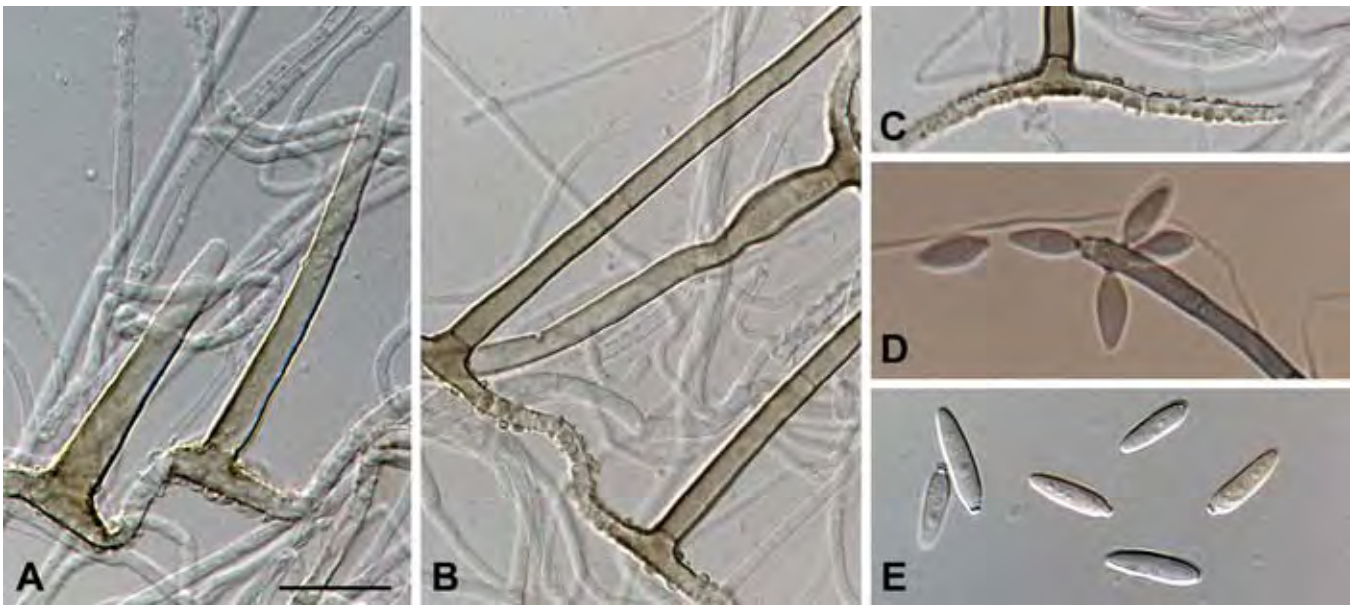

Fig. 9. *Ramichloridium australiense* (CBS 121710). A–C. Macronematous conidiophores with thick-walled and warted subtending hyphae. D. Sympodially proliferating conidiogenous cell, which results in a short rachis with darkened and slightly thickened scars. E. Conidia. Scale bar = 10 µm.

Ramichloridium australiense Arzanlou & Crous, **sp. nov.** MycoBank MB504548. Figs 9–10A.

Etymology: Named after its country of origin, Australia.

Ab aliis speciebus *Ramichloridii* conidiophoris ex hyphis verrucosis, crassitunicatis ortis distinguendum.

In vitro: *Submerged hyphae* hyaline, smooth, thin-walled, 1–2 µm wide; *aerial hyphae* pale brown, warted. *Conidiophores* arising vertically and clearly differentiated from creeping aerial hyphae, up to 400 µm tall, with several additional thin septa; intercalary cells, 8–40 × 2–5 µm, from the broadest part at the base tapering towards the apex, subhyaline, later becoming pale brown and warted in the lower part. Subtending hyphae thick-walled, warted. *Conidiogenous cells* integrated, terminal, 10–18 µm long, proliferating sympodially, giving rise to a short rachis with conspicuous conidiogenous loci; scars slightly thickened and darkened, about 1 µm diam. *Conidia* solitary, aseptate, thin-walled, smooth, subhyaline, subcylindrical to obclavate, (10–)12–15(–23) × 2.5–3 µm, with a truncate base and a slightly darkened and thickened hilum,1.5–2 µm diam, rarely fusing at the basal part.

Cultural characteristics: Colonies on MEA rather slow growing, reaching 8 mm diam after 14 d at 24 °C, with entire, smooth margin; mycelium flat, olivaceous-grey, becoming granular, with gelatinous droplets at the margin developing with aging; reverse pale olivaceous-grey.

Specimen examined: **Australia**, Queensland, Mount Lewis, Mount Lewis Road, 16°34'47.2" S, 145°19'7" E, 538 m alt., on *Musa banksii* leaf, Aug. 2006, P.W. Crous and B. Summerell, **holotype** CBS H-19928, culture ex-type CBS 121710.

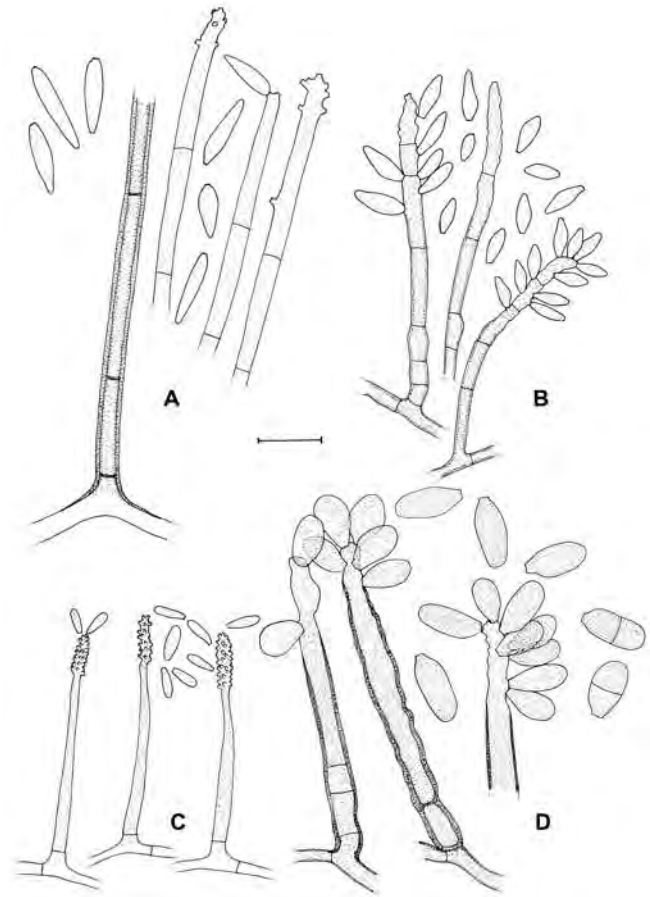

Fig. 10. A. *Ramichloridium australiense* (CBS 121710). B. *Ramichloridium brasilianum* (CBS 283.92). C. *Radulidium subulatum* (CBS 405.76). *D. Rhodoveronaea varioseptata* (CBS 431.88). Scale bar = 10 µm.

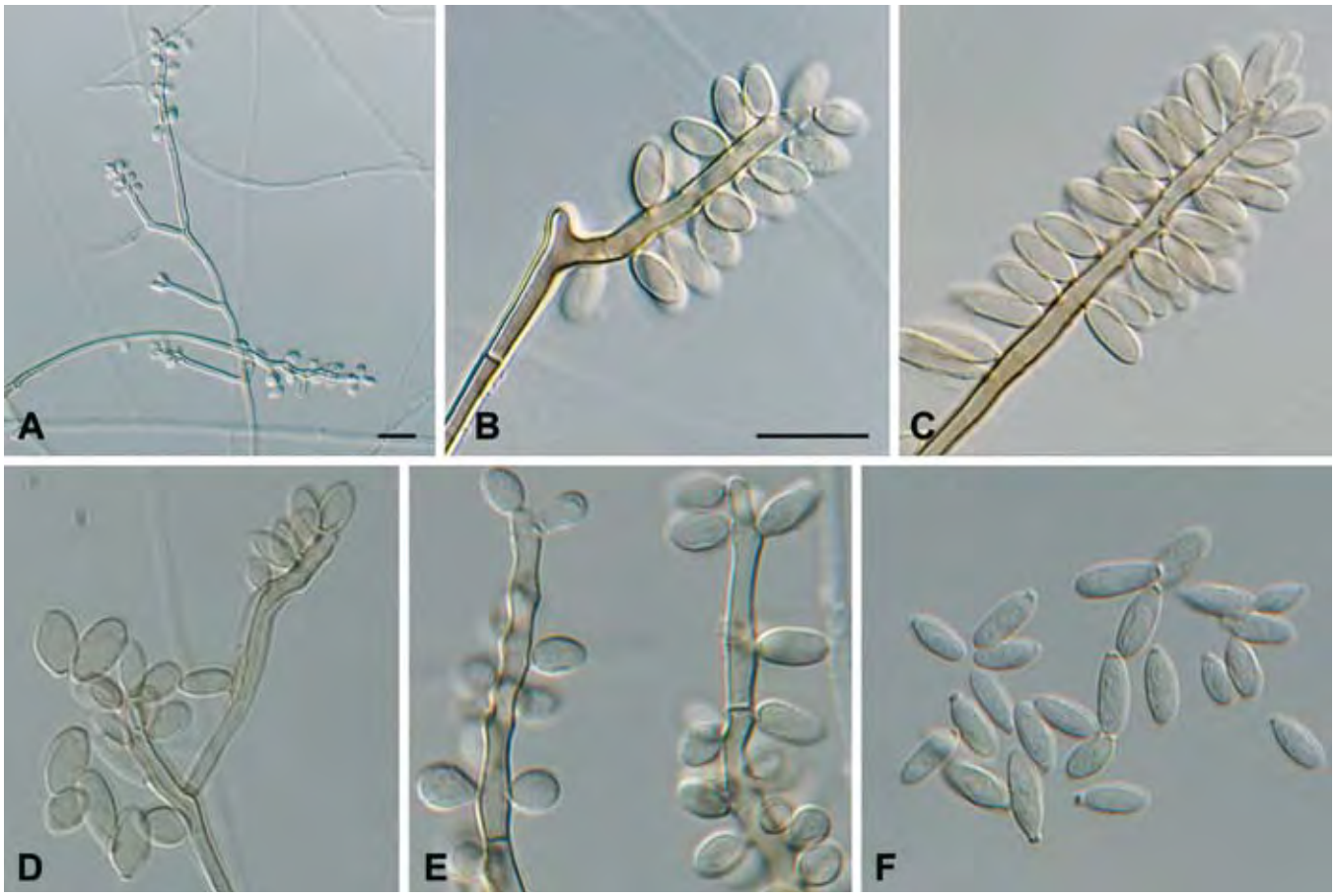

Fig. 11. *Ramichloridium musae* (CBS 365.36). A. Conidiophores with loose branches. B–D. Sympodially proliferating conidiogenous cells, resulting in a long conidium-bearing rachis. E. Rachis with hardly prominent, slightly darkened scars. F. Conidia. Scale bars = 10 µm.

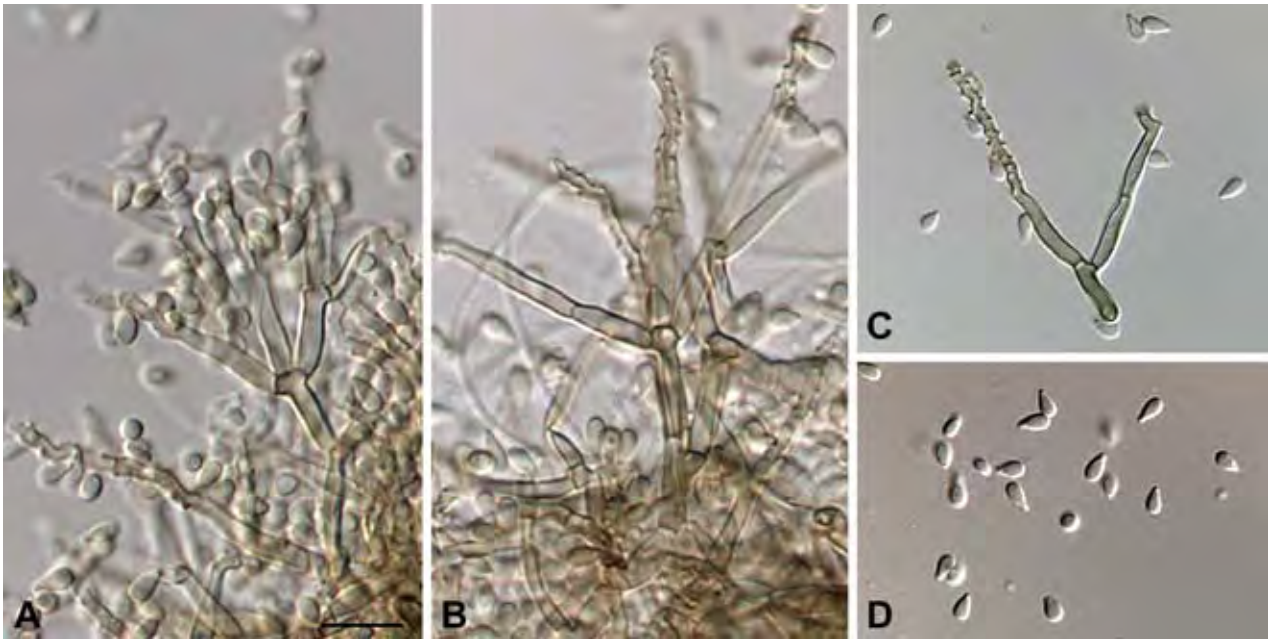

Fig. 12. *Ramichloridium biverticillatum* (CBS 335.36). A–B. Profusely branched and biverticillate conidiophores. C. Sympodially proliferating conidiogenous cells, which give rise to a conidium-bearing rachis with crowded, slightly pigmented and thickened scars. D. Conidia. Scale bar = 10 µm.

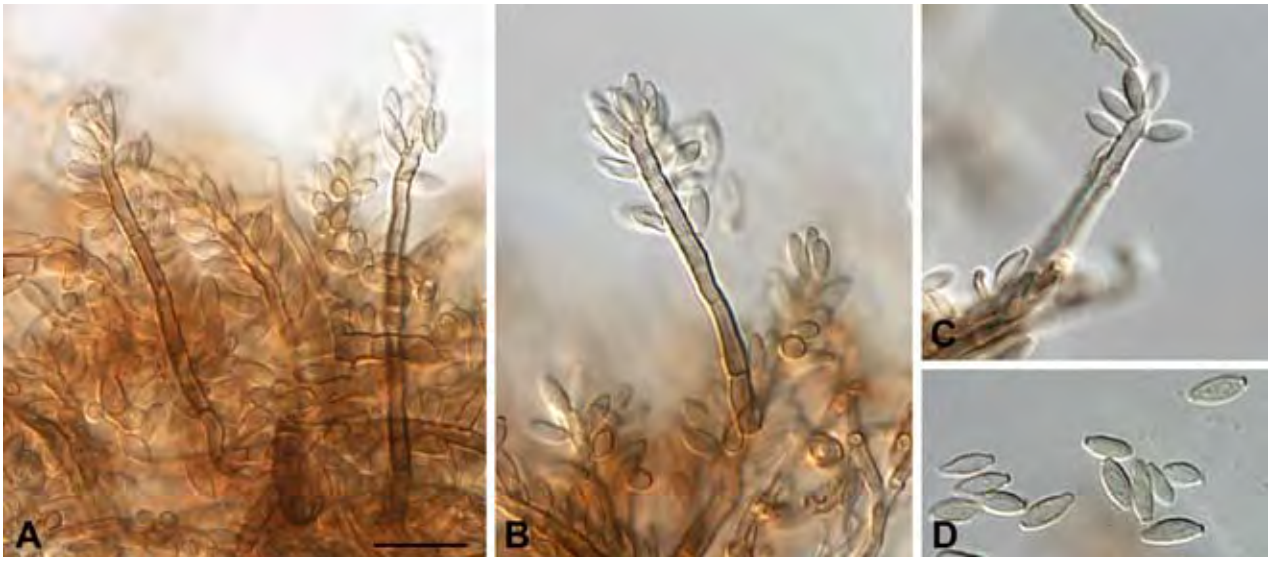

Fig. 13. *Ramichloridium brasilianum* (CBS 283.92). A–B. Macronematous conidiophores with sympodially proliferating conidiogenous cells, resulting in a conidium-bearing rachis. C. Rachis with crowded and slightly pigmented scars. D. Conidia. Scale bar = 10 µm.

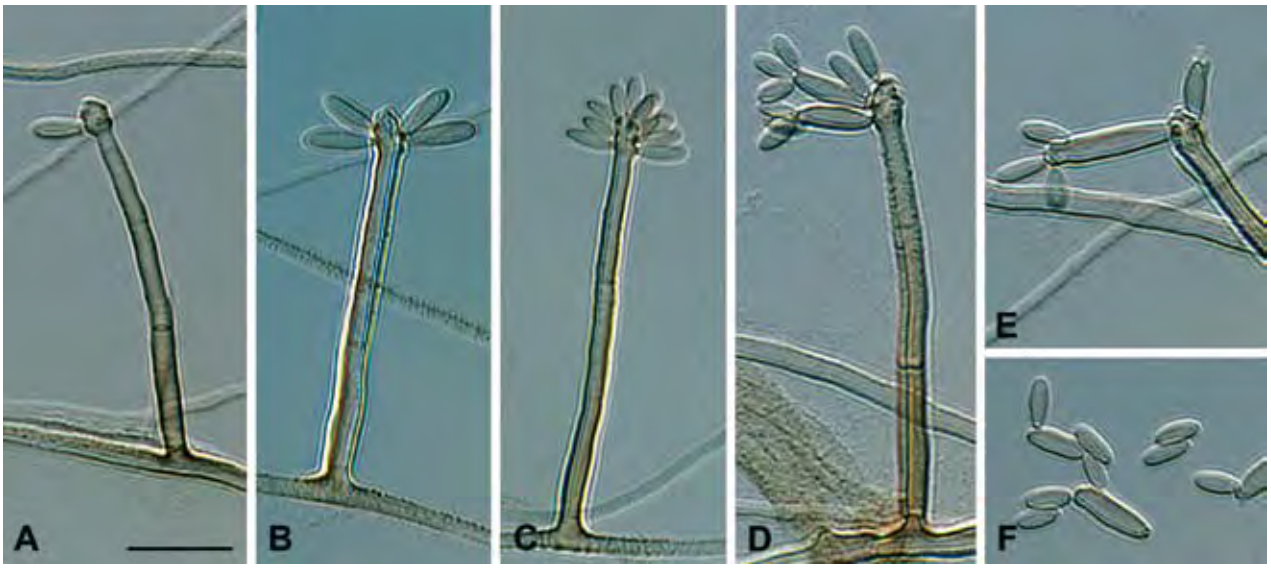

Fig. 14. *Ramichloridium cerophilum* (CBS 103.59). A–C. Conidial apparatus at different stages of development, resulting in macronematous conidiophores and sympodially proliferating conidiogenous cells. D–E. Formation of secondary conidia. F. Conidia. Scale bar = 10 µm.

Ramichloridium musae (Stahel ex M.B. Ellis) de Hoog, Stud. Mycol. 15: 62. 1977. Fig. 11.

Basionym: *Veronaea musae* Stahel ex M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 209. 1976.

≡ *Chloridium musae* Stahel, Trop. Agric., Trinidad 14: 43. 1937 (nom. inval. Art. 36).

Misapplied name: *Chloridium indicum* Subram., *sensu* Batista & Vital, Anais Soc. Biol. Pernambuco 15: 379. 1957.

In vitro: *Submerged hyphae* smooth, hyaline, thin-walled, 1–2 µm wide; *aerial hyphae* subhyaline, smooth. *Conidiophores* arising vertically and mostly sharply differentiated from creeping aerial hyphae, golden-brown; unbranched, rarely branched in the upper part, up to 250 µm tall, with up to 6 additional thin septa, cells 23–40 × 2–2.5 µm, basal cell occasionally inflated. *Conidiogenous cells* terminally integrated, cylindrical, variable in length, 10–40 µm long, golden-brown near the base, subhyaline to pale brown near the end, fertile part as wide as the basal part, later also becoming septate; rachis elongating sympodially, 2–2.5 µm wide, with hardly prominent, scattered, slightly pigmented scars, about 0.5 µm diam. *Conidia* solitary, aseptate, hyaline to subhyaline, ellipsoidal, (4–)7–8(–12) × 2–3 µm, smooth or verruculose, thin-walled, with slightly darkened hilum, about 1 µm diam.

Cultural characteristics: Colonies on MEA slow-growing, reaching 27 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium mostly submerged, some floccose to lanose aerial mycelium in the olivaceous-grey centre, becoming pale pinkish olivaceous towards the margin; reverse pale orange.

Specimens examined: **Cameroon**, from *Musa sapientum*, J.E. Heron, CBS 169.61 = ATCC 15681 = IMI 079492 = DAOM 84655 = MUCL 2689; from *Musa sapientum,* J. Brun, CBS 190.63 = MUCL 9557. **Surinam**, Paramaribo, from *Musa sapientum* leaf, G. Stahel, CBS 365.36 = JCM 6973 = MUCL 9556, **ex-type** strain of *Chloridium musae*; from *Musa sapientum,* G. Stahel, CBS 365.36; dried culture preserved as CBS H-19933.

Ramichloridium biverticillatum Arzanlou & Crous, **nom. nov.** MycoBank MB504549. Fig. 12.

Basionym: *Periconiella musae* Stahel ex M.B. Ellis, Mycol. Pap. 111: 5. 1967.

[non *Ramichloridium musae* (Stahel ex M.B. Ellis) de Hoog, 1977].

≡ *Ramichloridium musae* Stahel, Trop. Agric., Trinidad 14: 43. 1937 (nom. inval. Art. 36).

= *Ramichloridium musae* (Stahel ex M.B. Ellis) de Hoog, Stud. Mycol. 15: 62. 1977, *sensu* de Hoog, p.p.

Etymology: Named after its biverticillate conidiophores.

In vitro: *Submerged hyphae* smooth, hyaline, thin-walled, 1–2 µm wide; *aerial hyphae* subhyaline, smooth, slightly darker. *Conidiophores* arising vertically from creeping aerial hyphae, pale brown, profusely branched, biverticillate, with up to three levels of main branches; branches tapering distally, 2–3 µm wide at the base, approx. 2 µm wide in the upper part, up to 250 µm long. *Conidiogenous cells* terminally integrated, cylindrical, variable in length, 15–50 µm long, rachis straight or geniculate, pale brown, as wide as the basal part; elongating sympodially, forming a rachis with crowded, slightly darkened and thickened minute scars, less than 0.5 µm wide. *Conidia* solitary, aseptate, hyaline to subhyaline, dacryoid to pyriform, (2–)3–4(–6) × (1.5–)2(–2.5) µm, smooth, thin-walled, with an inconspicuous hilum.

Cultural characteristics: Colonies on MEA slow-growing, reaching 16 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin, rather compact, velvety; surface vinaceous-buff to olivaceous-buff; reverse buff.

Specimen examined: **Surinam**, from *Musa sapientum*, Aug. 1936, G. Stahel, CBS 335.36.

Notes: *Ramichloridium biverticillatum* is a new name based on *Periconiella musae*. The species is distinct from *R. musae* because of its profusely branched conidiophores, and conidia that are smaller (2–5 × 1.5–2.5 µm) than those of *R. musae* (5–11 × 2–3 µm).

Ramichloridium brasilianum Arzanlou & Crous, **sp. nov.** MycoBank MB504550. Figs 10B, 13.

Etymology: Named after its country of origin, Brazil.

A simili *Ramichloridio cerophilo* conidiis minoribus, ad 8 µm longis, et conidiis secundariis absentibus distinguendum.

In vitro: *Submerged hyphae* pale olivaceous, smooth or slightly rough, 1.5–2 µm wide; *aerial hyphae* olivaceous, smooth or rough, narrower and darker than the submerged hyphae. *Conidiophores* unbranched, arising vertically from creeping aerial hyphae, straight or flexuose, dark brown, with up to 10 additional septa, thick-walled, cylindrical, 2–2.5 µm wide and up to 70 µm long. *Conidiogenous cells* integrated, terminal, 10–30 µm long, proliferating sympodially, giving rise to a long, straight rachis with crowded, slightly darkened minute scars, about 0.5 µm diam. *Conidia* solitary, obovoid to fusiform with the widest part below the middle, thin-walled, verruculose, aseptate, pale brown, slightly rounded at the apex, truncate at the base, (4–)5–6(–8.5) × 2–2.5(–3) µm, with a slightly thickened and darkened hilum, 1–1.5 µm diam.

Cultural characteristics: Colonies on MEA slow-growing, reaching 6 mm diam after 14 d at 24 °C, velvety to hairy, colonies with entire margin, surface dark olivaceous-grey; black gelatinous exudate droplets produced on OA.

Specimen examined: **Brazil**, São Paulo, Peruibe, Jureia Ecological Reserve, forest soil, Jan. 1991, D. Attili, **holotype** CBS H-19929, culture ex-type CBS 283.92.

Ramichloridium cerophilum (Tubaki) de Hoog, Stud. Mycol. 15: 74. 1977. Fig. 14.

Basionym: *Acrotheca cerophila* Tubaki, J. Hattori Bot. Lab. 20: 143. 1958.

≡ *Cladosporium cerophilum* (Tubaki) Matsush., in Matsushima, *Icon. Microfung. Matsush. lect.* (Kobe): 34. 1975.

In vitro: *Submerged hyphae* pale olivaceous-brown, smooth or slightly rough, 1.5–3 µm wide; *aerial hyphae* olivaceous-brown, smooth or slightly rough, somewhat narrower and darker than the submerged hyphae. *Conidiophores* unbranched, arising vertically from creeping aerial hyphae, dark brown, thick-walled, smooth or verruculose, hardly tapering towards the apex, 2–3 µm wide, up to 50 µm long, with up to 3 additional septa. *Conidiogenous cells* integrated, terminal, proliferating sympodially, rachis short and straight, with crowded, prominent, pigmented unthickened scars, minute, approx. 0.5 µm diam. *Conidia* solitary, fusiform to clavate, thin-walled, smooth, 0(–1)-septate, subhyaline, (4–)6–7(–11) × (2–)2.5(–3) µm, with a conspicuous hilum, about 0.5 µm diam, slightly raised with an inconspicuous marginal frill, somehow resembling those of *Cladosporium*. Conidia sometimes producing 1–3(–4) short secondary conidia.

Cultural characteristics: Colonies on MEA rather slow-growing, reaching 12 mm diam after 14 d at 24 °C, velvety to hairy, with entire margin; surface dark olivaceous-grey, with black gelatinous exudate droplets on OA.

Specimen examined: **Japan**, isolated from *Sasa* sp., K. Tubaki, CBS 103.59, **ex-type**.

Fig. 15. *Ramichloridium indicum* (CBS 171.96). A–B. Macronematous conidiophores. C–E. Sympodially proliferating conidiogenous cells, resulting in a conidium-bearing rachis with pigmented and thickened scars. F. Conidia. Scale bar = 10 µm.

Fig. 16. *Ramichloridium strelitziae* (CBS 121711). A–C. Conidial apparatus at different stages of development, resulting in macronematous conidiophores and sympodially proliferating conidiogenous cells. D–E. Rachis with crowded, slightly pigmented, thickened, circular scars. F. Conidia. Scale bars = 10 µm.

Notes: Phylogenetically, this species together with *Ramichloridium apiculatum* and *R. musae* cluster within the *Mycosphaerellaceae* clade. *Ramichloridium cerophilum* can be distinguished from its relatives by the production of secondary conidia and its distinct conidial hila.

Ramichloridium indicum (Subram.) de Hoog, Stud. Mycol. 15: 70. 1977. Fig. 15.

Basionym: *Chloridium indicum* Subram., Proc. Indian Acad. Sci., Sect. B, 42: 286. 1955 [non *Rhinocladiella indica* Agarwal, Lloydia 32: 388. 1969].

≡ *Veronaea indica* (Subram.) M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 209. 1976.

= *Veronaea verrucosa* Geeson, Trans. Brit. Mycol. Soc. 64: 349. 1975.

In vitro: *Submerged hyphae* smooth, thin-walled, hyaline, 1–2.5 µm wide, with thin septa; *aerial hyphae* coarsely verrucose, olivaceous-green, rather thick-walled, 2–2.5 µm wide, with thin septa. *Conidiophores* arising vertically from creeping hyphae at right angles, straight, unbranched, thick-walled, smooth, dark brown, with up to 10 thin septa, up to 250 µm long, 2–4 µm wide, often with inflated basal cells. *Conidiogenous cells* terminally integrated, up to 165 µm long, smooth, dark brown, sympodially proliferating, rachis straight or flexuose, geniculate or nodose, subhyaline; scars thickened and darkened, clustered at nodes, approx. 0.5 µm diam. Microcyclic conidiation observed in culture. *Conidia* solitary, (0–)1-septate, not constricted at the septum, subhyaline to pale brown, smooth or coarsely verrucose, rather thin-walled, broadly ellipsoidal to globose, (5–)7–8(–10) × (4–)6–6.5(–9) µm, with truncate base; hilum conspicuous, slightly darkened, not thickened, about 1 µm diam.

Cultural characteristics: Colonies on MEA reaching 35 mm diam after 14 d at 24 °C. Colonies velvety, rather compact, slightly elevated, with entire, smooth, whitish margin, dark olivaceous-green in the central part.

Specimen examined: Living culture, Feb. 1996, L. Marvanová, CBS 171.96.

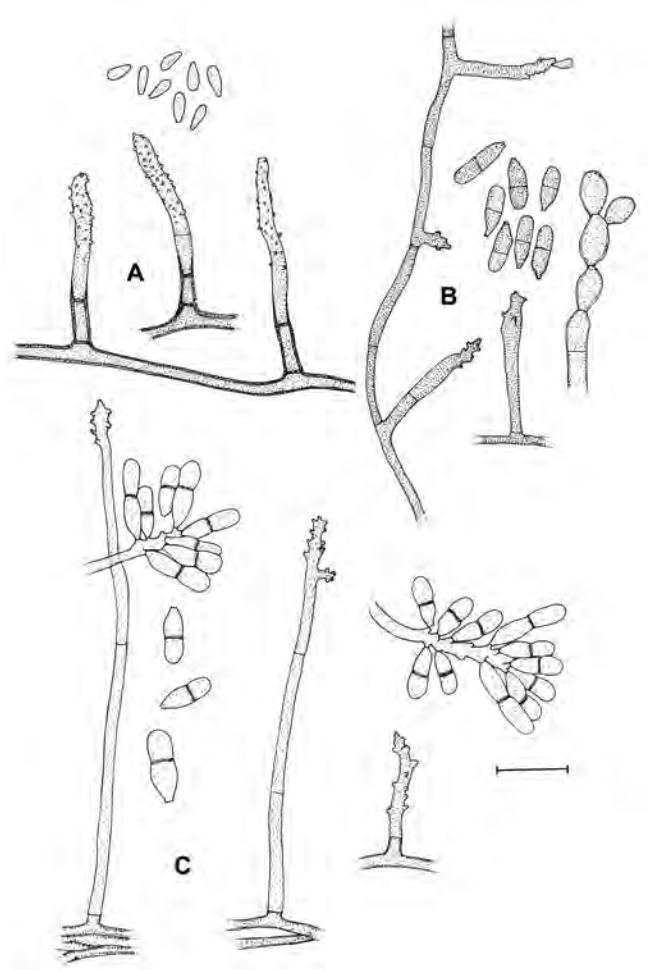

Fig. 17. A. *Ramichloridium strelitziae* (CBS 121711). B. *Veronaea japonica* (CBS 776.83). C. *Veronaeopsis simplex* (CBS 588.66). Scale bar = 10 µm.

Ramichloridium pini de Hoog & Rahman, Trans. Brit. Mycol. Soc. 81: 485. 1983.

Specimen examined: **U.K.**, Scotland, Old Aberdeen, branch of *Pinus contorta* (*Pinaceae*), 1982, M.A. Rahman, **ex-type** strain, CBS 461.82 = MUCL 28942.

Note: The culture examined (CBS 461.82) was sterile. For a full description see de Hoog *et al.* (1983).

Ramichloridium strelitziae Arzanlou, W. Gams & Crous, **sp. nov.** MycoBank MB504551. Figs 16–17A.

Etymology: Named after its host, *Strelitzia*.

Ab aliis speciebus *Ramichloridii* conidiophoris brevibus, ad 40 µm longis, et cicatricibus rotundis, paulo protrudentibus distinguendum.

In vitro: *Submerged hyphae* smooth, hyaline, thin-walled, 2–2.5 µm wide; *aerial hyphae* pale brown, verrucose. *Conidiophores* arising vertically from creeping aerial hyphae, clearly differentiated from the vegetative hyphae, subhyaline, later becoming pale brown, thick-walled, smooth or verruculose, with 1–3 additional septa; up to 40 µm long and 2 µm wide. *Conidiogenous cells* integrated, terminal, cylindrical, variable in length, 10–35 µm long, subhyaline, later turning pale brown, fertile part as wide as the basal part, proliferating sympodially, forming a straight rachis with slightly thickened and darkened, circular, somewhat protruding scars, approx. 0.5 µm diam. *Conidia* solitary, aseptate, smooth or verruculose, subhyaline, oblong, ellipsoidal to clavate, (3–)4–5(–5.5) × (1–)2(–2.5) µm, with truncate base and unthickened, non-pigmented hilum.

Cultural characteristics: Colonies on MEA slow-growing, reaching 5 mm diam after 14 d at 24 °C, with entire margin; aerial mycelium rather compact, raised, dense, olivaceous-grey; reverse olivaceous-black.

Specimen examined: **South Africa**, KwaZulu-Natal, Durban, near Réunion, on leaves of *Strelitzia nicolai*, 5 Feb. 2005, W. Gams & H. Glen, CBS-H 19776, **holotype**, culture ex-type CBS 121711.

Zasmidium Fr., *Summa Veg. Scand.* 2: 407. 1849.

In vitro: *Submerged hyphae* smooth, thin-walled, hyaline, with thin septa; *aerial hyphae* coarsely verrucose, olivaceous-green, thick-walled, with thin septa. *Conidiophores* not differentiated from vegetative hyphae, often reduced to conidiogenous

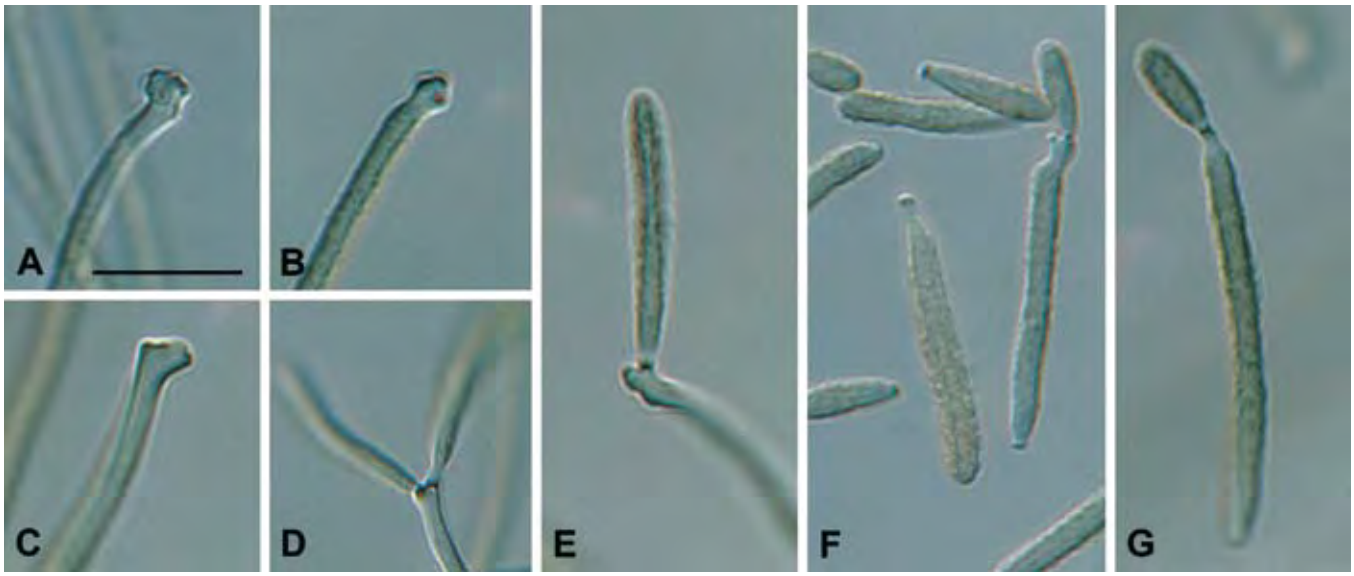

Fig. 18. *Zasmidium cellare* (CBS 146.36). A–D. Micronematous conidiophores with terminal, integrated conidiogenous cells. E. Conidiogenous cell with pigmented, thickened and refractive scars. F–G. Primary and secondary conidia. Scale bar = 10 µm.

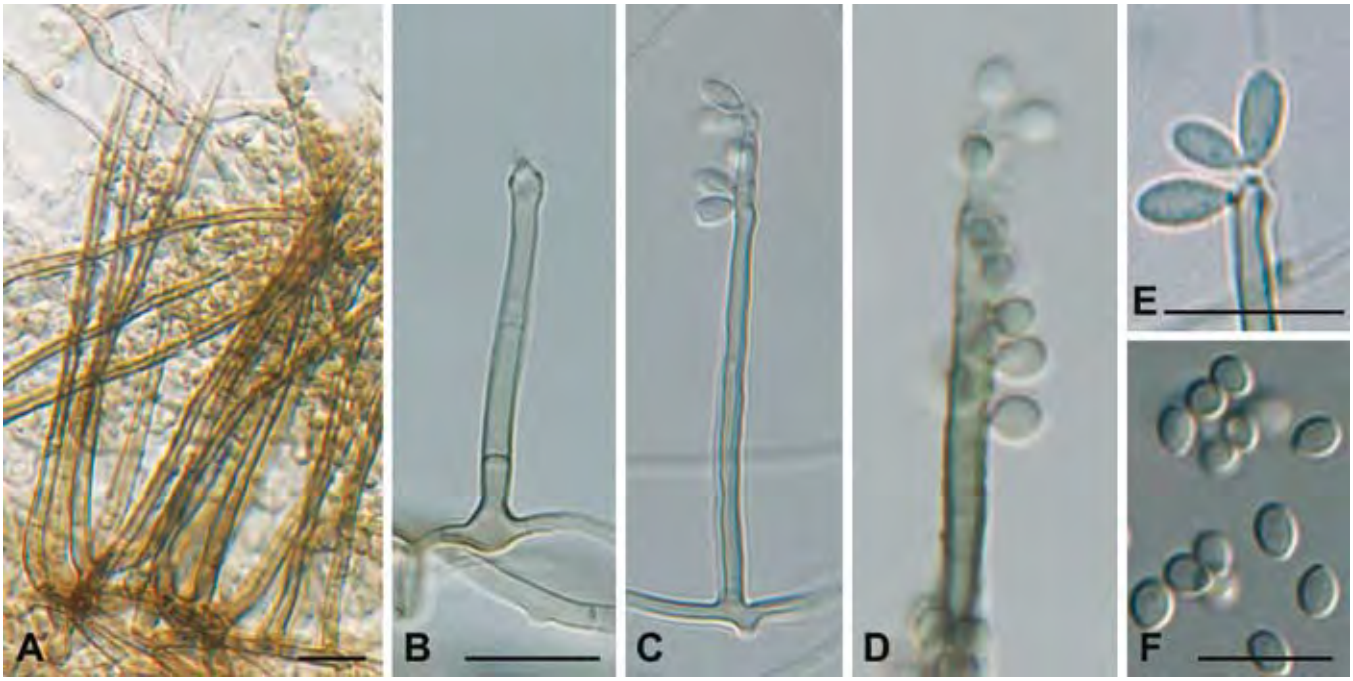

Fig. 19. *Rhinocladiella anceps* (CBS 181.65). A. Macronematous conidiophores. B–D. Conidial apparatus at different stages of development, resulting in semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. E. Conidiogenous loci. F. Conidia. Scale bars = 10 µm.

cells. *Conidiogenous cells* integrated, predominantly terminal, sometimes lateral, arising from aerial hyphae, cylindrical, pale brown; polyblastic, proliferating sympodially producing crowded, conspicuously pigmented, almost flat, darkened, somewhat refractive scars. *Conidia* in short chains, cylindrical to fusiform, verrucose, obovate to obconical, pale brown, base truncate, with a conspicuous, slightly pigmented, thickened and refractive hilum. *Primary conidia* sometimes larger, subhyaline, verrucose or smooth-walled, 0–4-septate, variable in length, fusiform to cylindrical; conidial secession schizolytic.

Type species: *Zasmidium cellare* (Pers. : Fr.) Fr., *Summa Veg. Scand*. 2: 407. 1849.

Zasmidium cellare (Pers. : Fr.) Fr., *Summa Veg. Scand*. 2: 407. 1849. Fig. 18.

Basionym: *Racodium cellare* Pers., Neues Mag. Bot. 1: 123. 1794.

≡ *Antennaria cellaris* (Pers. : Fr.) Fr., Syst. Mycol. 3: 229. 1829.

≡ *Cladosporium cellare* (Pers. : Fr.) Schanderl, Zentralbl. Bakteriol., 2. Abt., 94: 117. 1936.

≡ *Rhinocladiella cellaris* (Pers. : Fr.) M.B. Ellis, in Ellis, *Dematiaceous Hyphomycetes*: 248. 1971.

In vitro: *Submerged hyphae* smooth, thin-walled, hyaline, 2–3 µm wide, with thin septa; *aerial hyphae* coarsely verrucose, olivaceous-green, rather thick-walled, 2–2.5 µm wide, with thin septa. *Conidiophores* not differentiated from vegetative hyphae, often reduced to conidiogenous cells. *Conidiogenous cells* integrated, predominantly terminal, sometimes lateral, arising from aerial hyphae, cylindrical, 20–60 µm long and 2–2.5 µm wide, pale brown, proliferating sympodially producing crowded, conspicuously pigmented scars that are thickened and refractive, about 1 µm diam. *Conidia* cylindrical to fusiform, verrucose, obovate to obconical, pale brown, with truncate base, (6–)9–14(–27) × 2–2.5 µm, with a conspicuous, slightly pigmented, refractive hilum, approx. 1 µm diam. *Primary conidia* sometimes subhyaline, verrucose or smooth-walled, thin-walled, 0–1(–4)-septate, variable in length, fusiform to cylindrical.

Cultural characteristics: Colonies reaching 7 mm diam after 14 d at 24 °C. Colonies velvety, rather compact, slightly elevated with entire margin; surface dark olivaceous-green in the central part, margin smooth, whitish.

Specimen examined: Wall in wine cellar, Jun. 1936, H. Schanderl, ATCC 36951 = IFO 4862 = IMI 044943 = LCP 52.402 = LSHB BB274 = MUCL 10089 = CBS 146.36.

Notes: The name *Racodium* Fr., typified by *Ra. rupestre* Pers. : Fr., has been conserved over the older one by Persoon, with *Ra. cellare* as type species. De Hoog (1979) defended the use of *Zasmidium* in its place for the well-known wine-cellar fungus.

Morphologically *Zasmidium* resembles *Stenella* Syd., and both reside in the *Capnodiales*, though the type of *Stenella*, *S. araguata* Syd., clusters in the *Teratosphaeriaceae*, and the type of *Zasmidium*, *Z. cellare*, in the *Mycosphaerellaceae*. When accepting anamorph genera as polyphyletic within an order, preference would be given to the well-known name *Stenella* over the less known *Zasmidium*, even though the latter name is older. Further studies are required, however, to clarify if all stenella-like taxa should be accommodated in a single genus, *Stenella*. If this is indeed the case, a new combination for *Zasmidium cellare* will be proposed in *Stenella*, and the latter genus will have to be conserved over *Zasmidium*.

Chaetothyriales (*Herpotrichiellaceae*)

The four "*Ramichloridium*" species residing in the *Chaetothyriales* clade do not differ sufficiently in morphology to separate them from *Rhinocladiella* (type *Rh. atrovirens*). Because of the pale brown conidiophores, conidiogenous cells with crowded, slightly prominent scars and the occasional presence of an *Exophiala* J.W. Carmich. synanamorph, *Rhinocladiella* is a suitable genus to accommodate them. These four species chiefly differ from *Ramichloridium* in the morphology of their conidial apparatus, which is clearly differentiated from the vegetative hyphae. The appropriate combinations are therefore introduced for *Ramichloridium anceps*, *R. mackenziei*, *R. fasciculatum* and *R. basitonum*.

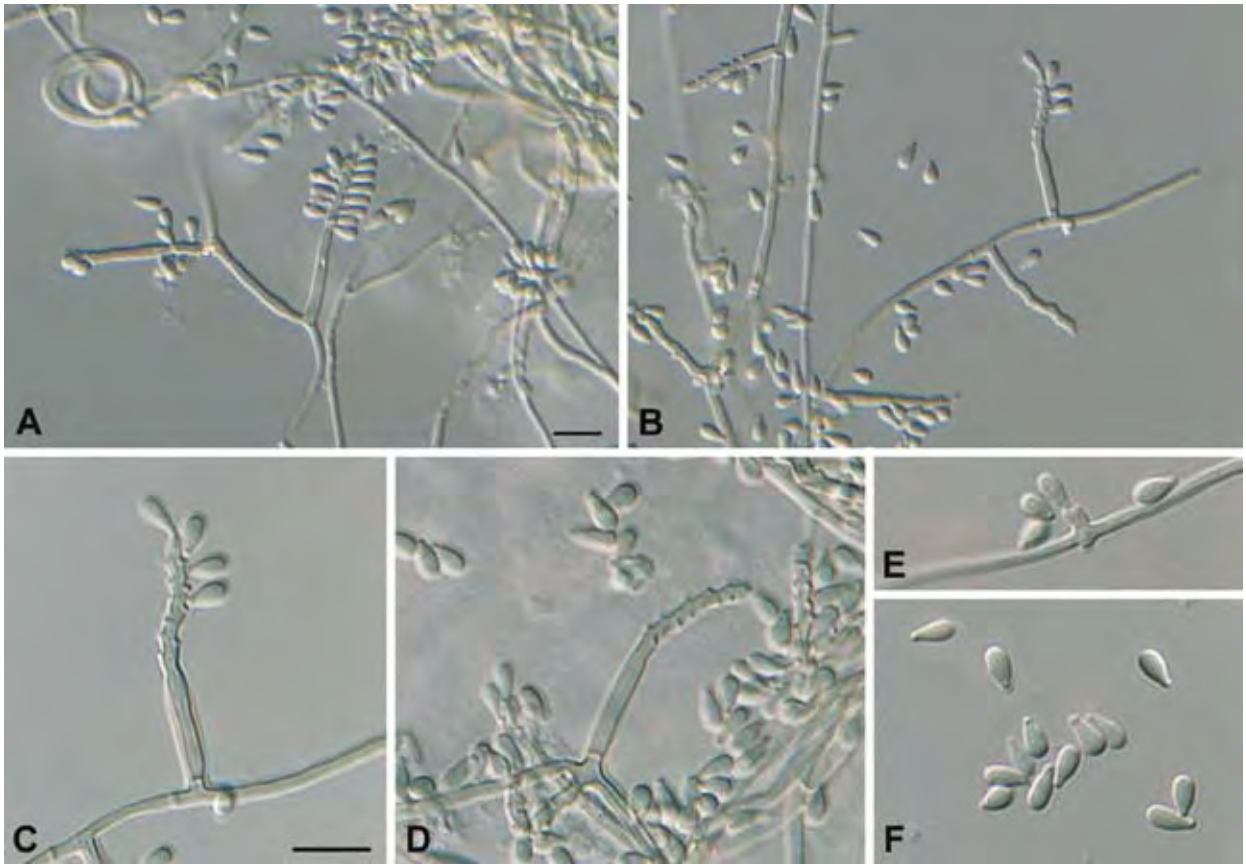

Fig. 20. *Rhinocladiella basitona* (CBS 101460). A–B. Semi-micronematous conidiophores with verticillate branching pattern. C–D. Sympodially proliferating conidiogenous cells, giving rise to a long rachis with slightly prominent, truncate conidium-bearing denticles. E. Intercalary conidiogenous cell. F. Conidia. Scale bars = 10 µm.

The genus *Veronaea* (type species: *V. botryosa*) also resides in the *Chaetothyriales* clade. *Veronaea* can be distinguished from *Rhinocladiella* by the absence of exophiala-type budding cells and its predominantly 1-septate conidia. Furthermore, the conidiogenous loci in *Veronaea* are rather flat, barely prominent.

Rhinocladiella Nannf., Svensk Skogsvårdsfören. Tidskr., Häfte 32: 461. 1934.

In vitro: *Colonies* dark olivaceous-brown, slow-growing, almost moist. *Submerged hyphae* hyaline to pale olivaceous, smooth; *aerial hyphae*, if present, more darkly pigmented. Exophiala-type budding cells usually present in culture. *Conidial apparatus* usually branched, olivaceous-brown, consisting of either slightly differentiated tips of ascending hyphae or septate, markedly differentiated conidiophores. *Conidiogenous cells* intercalary or terminal, polyblastic, cylindrical to acicular, with a sympodially proliferating, subdenticulate rachis; scars unthickened, non-pigmented to somewhat darkened-refractive. *Conidia* solitary, hyaline to subhyaline, aseptate, thin-walled, smooth, subglobose, with a slightly pigmented hilum; conidial secession schizolytic.

Type species: *Rh. atrovirens* Nannf., Svenska Skogsvårdsfören. Tidskr. 32: 461. 1934.

Rhinocladiella anceps (Sacc. & Ellis) S. Hughes, Canad. J. Bot. 36: 801. 1958. Fig. 19.

Basionym: *Sporotrichum anceps* Sacc. & Ellis, Michelia 2: 576. 1882.

= *Veronaea parvispora* M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 210. 1976.

Misapplied name: *Chloridium minus* Corda *sensu* Mangenot, Rev. Mycol. (Paris) 18: 137. 1953.

In vitro: *Submerged hyphae* subhyaline, smooth, thick-walled, 2–2.5 µm wide; *aerial hyphae* pale brown. Swollen germinating cells often present on MEA, giving rise to an *Exophiala* synanamorph. *Conidiophores* slightly differentiated from vegetative hyphae, arising from prostrate aerial hyphae, consisting of either unbranched or loosely branched stalks, thick-walled, golden to dark-brown, up to 350 µm tall, which may have up to 15 thin, additional septa, intercalary cells 9–14 µm long. *Conidiogenous cells* terminal, rarely lateral, cylindrical, occasionally intercalary, variable in length, smooth, golden to dark brown at the base, paler toward the apex, later becoming inconspicuously septate, fertile part as wide as the basal part, 15–40 × 1.5–2 µm; with crowded, slightly prominent, unpigmented, conidium-bearing denticles, about 0.5 µm diam. *Conidia* solitary, subhyaline, thin-walled, smooth, subglobose to ellipsoidal, 2.5–4 × 2–2.5 µm, with a less conspicuous, slightly darkened hilum, less than 0.5 µm diam.

Cultural characteristics: Colonies on MEA reaching 6–12 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium powdery, becoming hairy at centre; olivaceous-green to brown, reverse dark-olivaceous.

Specimens examined: **Canada**, Ontario, Campbellville, from soil under *Thuja plicata*, Apr. 1965, G. L. Barron, CBS H-7715 (isoneotype); CBS H-7716 (isoneotype); CBS H-7717 (isoneotype); CBS H-7718 (isoneotype); CBS H-7719 (isoneotype), **ex-type** strain, CBS 181.65 = ATCC 18655 = DAOM 84422 = IMI 134453 = MUCL 8233 = OAC 10215. **France**, from stem of *Fagus sylvatica*, 1953, F. Mangenot, CBS 157.54 = ATCC 15680= MUCL 1081= MUCL 7992 = MUCL 15756.

Notes: *Rhinocladiella anceps* (conidia 2.5–4 µm long) resembles *Rh. phaeophora* Veerkamp & W. Gams (1983) (conidia 5.5–6 µm long), but has shorter conidia.

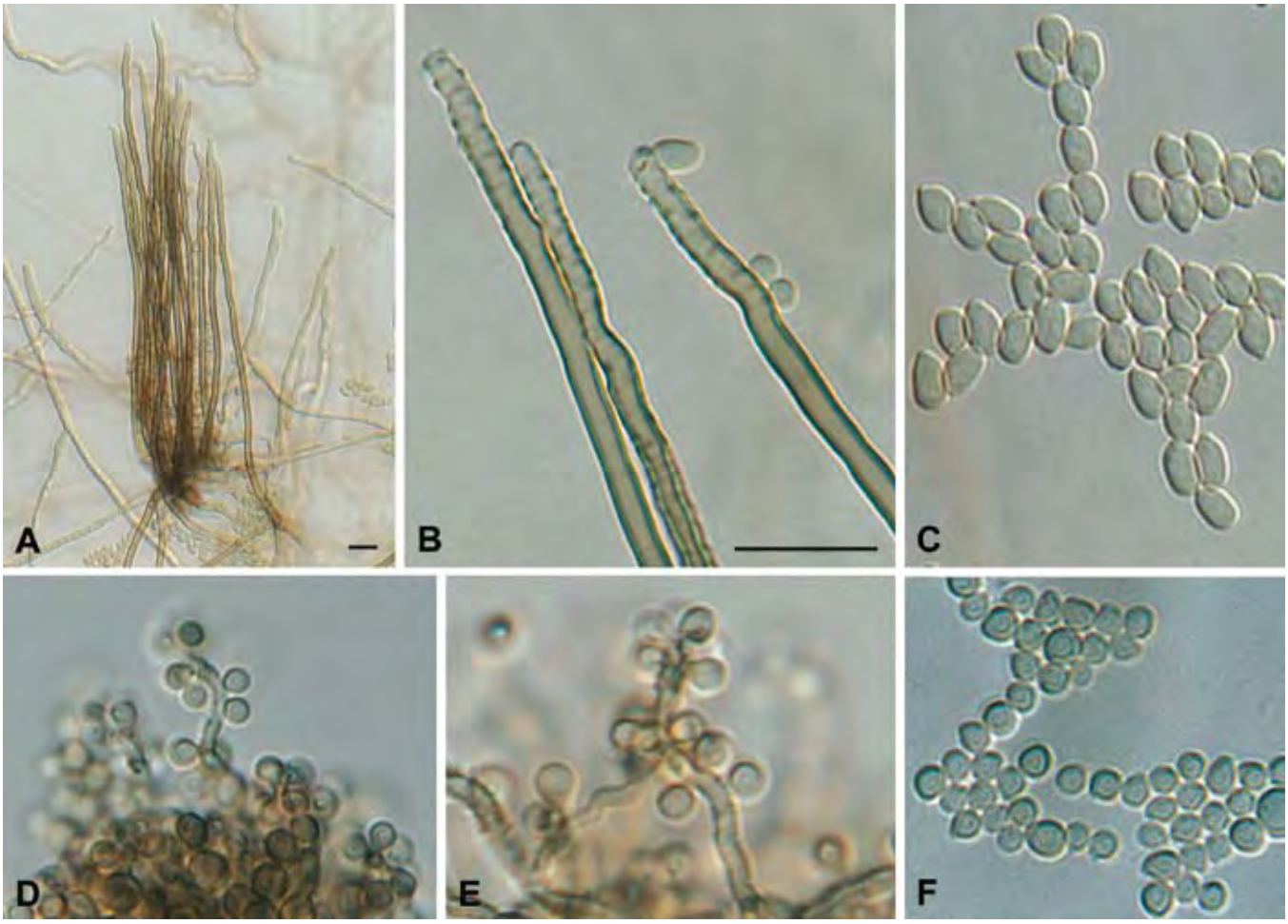

Fig. 21. *Rhinocladiella fasciculata* (CBS 132.86). A. Conidiophores. B. Sympodially proliferating conidiogenous cells, which give rise to a long rachis with slightly prominent, unthickened scars. C. Conidia. D–E. Synanamorph consisting of conidiogenous cells with percurrent proliferation. F. Conidia. Scale bars = 10 µm.

Rhinocladiella basitona (de Hoog) Arzanlou & Crous, **comb. nov.** MycoBank MB504552. Fig. 20.
Basionym: *Ramichloridium basitonum* de Hoog, J. Clin. Microbiol. 41: 4774. 2003.

In vitro: *Submerged hyphae* hyaline, smooth, thin-walled, 2 µm wide; *aerial hyphae* rather thick-walled, pale brown. *Conidiophores* slightly differentiated from vegetative hyphae, profusely and mostly verticillately branched, straight or flexuose, pale-brown, 2–2.5 µm wide. *Conidiogenous cells* terminal, variable in length, 10–100 µm long, pale brown, straight or geniculate, proliferating sympodially, giving rise to a long, 2–2.5 µm wide rachis, with slightly prominent, truncate conidium-bearing denticles, slightly darkened. *Conidia* solitary, hyaline, thin-walled, smooth, pyriform to clavate, with a round apex, and slightly truncate base, (1–)3–4(–5) × 1–2 µm, hilum conspicuous, slightly darkened and thickened, less than 0.5 µm diam.

Cultural characteristics: Colonies on MEA reaching 19 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium rather flat and slightly elevated in the centre, pale olivaceous-grey to olivaceous-grey; reverse olivaceous-black.

Specimen examined: **Japan**, Hamamatsu, from subcutaneous lesion with fistula on knee of 70-year-old male, Y. Suzuki, **ex-type** culture CBS 101460 = IFM 47593.

Rhinocladiella fasciculata (V. Rao & de Hoog) Arzanlou & Crous, **comb. nov.** MycoBank MB504553. Fig. 21.
Basionym: *Ramichloridium fasciculatum* V. Rao & de Hoog, Stud. Mycol. 28: 39. 1986.

In vitro: *Submerged hyphae* subhyaline, smooth, thick-walled, 2–2.5 µm wide; *aerial hyphae* pale brown. *Conidiophores* arising vertically from ascending hyphae in loose fascicles, unbranched or loosely branched at acute angles, cylindrical, smooth, brown and thick-walled at the base, up to 220 µm long and 2–3 µm wide, with 0–5 thin additional septa. *Conidiogenous cells* terminal, cylindrical, 30–100 µm long, thin-walled, smooth, pale brown, fertile part as wide as the basal part, up to 2 µm wide, proliferating sympodially, giving rise to a rachis with hardly prominent, slightly pigmented, not thickened scars, less than 0.5 µm diam. *Conidia* solitary, smooth, thin-walled, subhyaline, ellipsoidal, (2.5–)4–5(–6) × 2–3 µm, with truncate, slightly pigmented hilum, about 0.5 µm diam. *Synanamorph* forming on torulose hyphae originating from giant cells; compact heads of densely branched hyphae forming thin-walled, lateral, subglobose cells, on which conidiogenous cells are formed; conidiogenous cells proliferating percurrently, giving rise to tubular annellated zones with inconspicuous annellations, up to 12 µm long, 1–1.5 µm wide. *Conidia* smooth, thin-walled, aseptate, subhyaline, globose, 2–2.5 µm diam.

Cultural characteristics: Colonies on MEA reaching 8 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium velvety, becoming farinose in the centre due to abundant sporulation, olivaceous-green to brown, reverse dark olivaceous. Blackish droplets often produced at the centre, which contain masses of *Exophiala* conidia.

Specimen examined: **India**, Karnataka, Thirathahalli, isolated by V. Rao from decayed wood, **holotype** CBS-H 3866, culture ex-type CBS 132.86.

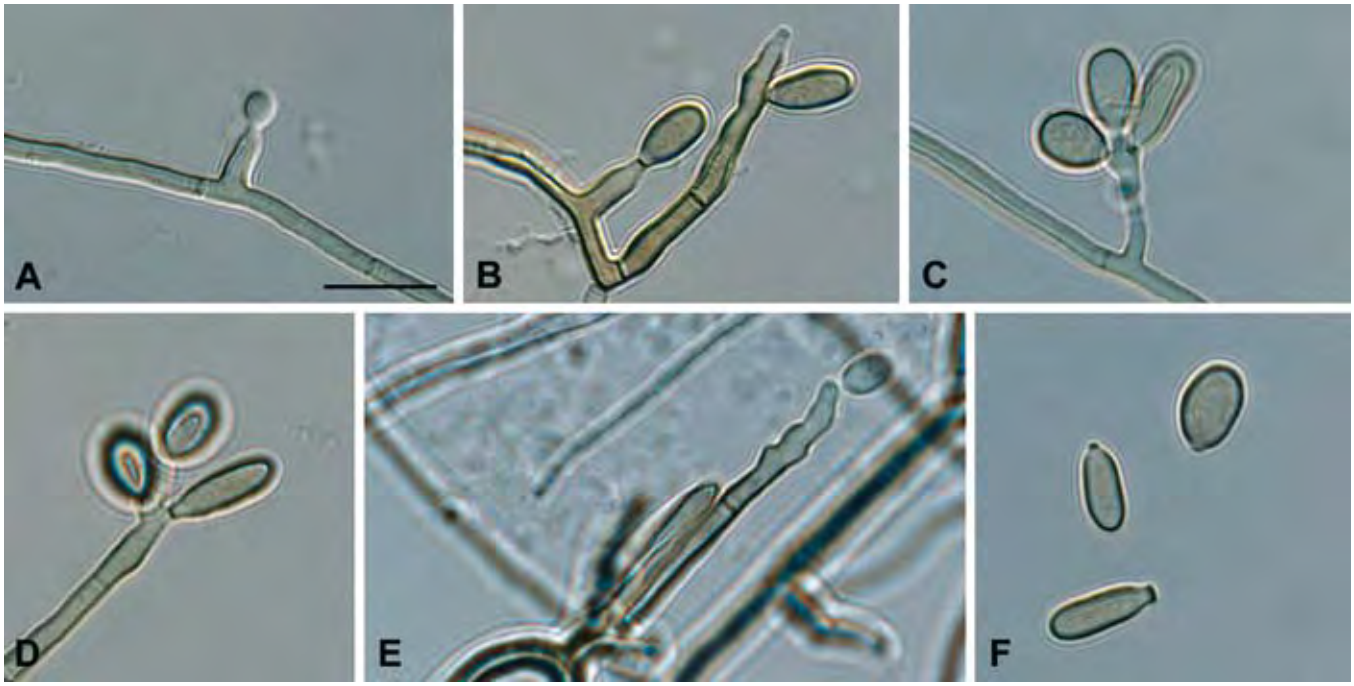

Fig. 22. *Rhinocladiella mackenziei* (CBS 368.92). A. Intercalary conidiogenous cell. B–E. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells, resulting in a rachis with slightly prominent, unthickened scars. F. Conidia. Scale bar = 10 µm.

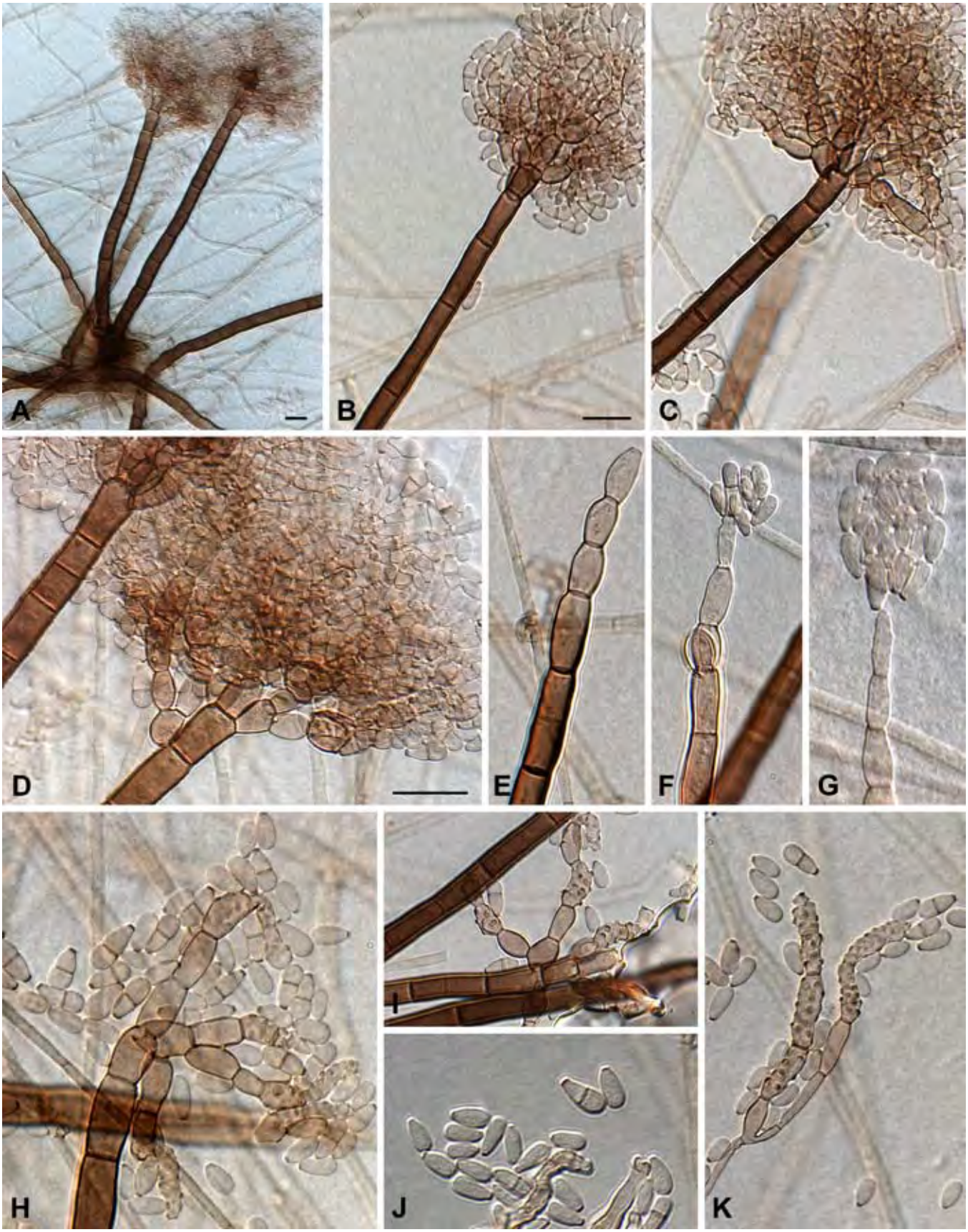

Fig. 24. *Thysanorea papuana* (CBS 212.96), periconiella-like synanamorph. A. Macronematous conidiophores. B–C. Conidiophores with dense apical branches. D. Branches with different levels of branchlets. E–I. Conidiogenous cells at different stages of development; sympodially proliferating conidiogenous cells give rise to a denticulate rachis. J–K. Conidia. Scale bars = 10 µm.

Fig. 23. (Page 78). *Thysanorea papuana* (CBS 212.96). A. Intercalary conidiogenous cell. B–I. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells, resulting in a rachis with prominent conidium bearing denticles. J–K. Microcyclic conidiation observed in slide cultures. L. Conidia. Scale bar = 10 µm.

Rhinocladiella mackenziei (C.K. Campb. & Al-Hedaithy) Arzanlou & Crous, **comb. nov.** MycoBank MB504554. Fig. 22.
Basionym: *Ramichloridium mackenziei* C.K. Campb. & Al-Hedaithy, J. Med. Veterin. Mycol. 31: 330. 1993.

In vitro: *Submerged hyphae* subhyaline, smooth, thin-walled, 2–3 µm wide; *aerial hyphae* pale brown, slightly narrower. *Conidiophores* slightly or not differentiated from vegetative hyphae, arising laterally from aerial hyphae, with one or two additional septa, often reduced to a discrete or intercalary conidiogenous cell, pale-brown, 10–25 × 2.5–3.5 µm. *Conidiogenous cells* terminal or intercalary, variable in length, 5–15 µm long and 3–5 µm wide, occasionally slightly wider than the basal part, pale brown, rachis with slightly prominent, unpigmented, non-thickened scars, about 0.5 µm diam. *Conidia* golden-brown, thin-walled, smooth, ellipsoidal to obovate, subcylindical, (5–)8–9(–12) × (2–)3–3.5(–5) µm, with darkened, inconspicously thickened, protuberant or truncate hilum, less than 1 µm diam.

Cultural characteristics: Colonies on MEA reaching 5 mm diam after 14 d at 24 °C, with entire, smooth, sharp margin; mycelium densely lanose and elevated in the centre, olivaceous-green to brown; reverse dark olivaceous.

Specimens examined: **Israel**, Haifa, isolated from brain abscess, CBS 368.92 = UTMB 3170; human brain abscess, E. Lefler, CBS 367.92 = NCPF 2738 = UTMB 3169. **Saudi Arabia**, from phaeohyphomycosis of the brain, S.S.A. Al-Hedaithy, **ex-type** strain, CBS 650.93 = MUCL 40057 = NCPF 2808; from brain abscess, Pakistani male who travelled to Saudi Arabia, CBS 102592 = NCPF 7460. **United Arab Emirates**, from fatal brain abscess, CBS 102590 = NCPF 2853.

Notes: Morphologically *Rhinocladiella mackenziei* is somewhat similar to *Pleurothecium obovoideum* (Matsush.) Arzanlou & Crous, which was originally isolated from dead wood. However, *P. obovoideum* has distinct conidiophores, and the ascending hyphae are thick-walled, and the denticles cylindrical, up to 1.5 µm long. In contrast, *Rh. mackenziei* has only slightly prominent denticles. *Rhinocladiella mackenziei* is a member of the *Chaetothyriales*, while *P. obovoideum* clusters in the *Chaetosphaeriales*.

Thysanorea Arzanlou, W. Gams & Crous, **gen. nov.** MycoBank MB504555.

Etymology: (Greek) *thysano* = brush, referring to the brush-like branching pattern, suffix derived from *Veronaea*.

Veronaeae similis sed conidiophoris partim *Periconiae* similibus dense ramosis distinguenda.

In vitro: *Submerged hyphae* subhyaline, smooth, thin-walled; *aerial hyphae* pale brown, smooth or verrucose. *Conidiophores* dimorphic; *micronematous conidiophores* slightly differentiated from vegetative hyphae, branched or simple, multiseptate. *Conidiogenous cells* terminal, polyblastic, variable in length, smooth, golden- to dark brown at the base, paler towards the apex, later sometimes inconspicuously septate; fertile part often wider than the basal part, clavate to doliiform, with crowded, more or less prominent conidium-bearing denticles, unpigmented, but slightly thickened. *Macronematous conidiophores* consisting of well-differentiated, thick-walled, dark brown stalks; apically repeatedly densely branched, forming a complex head, each branchlet giving rise to a conidium-bearing denticulate rachis with slightly pigmented, thickened scars. *Conidia* of both kinds of conidiophore formed singly, smooth, pale brown, obovoidal to pyriform, (0–)1-septate, with a truncate base and darkened hilum; conidial secession schizolytic.

Type species: *Thysanorea papuana* (Aptroot) Arzanlou, W. Gams & Crous, comb. nov.

Thysanorea papuana (Aptroot) Arzanlou, W. Gams & Crous, **comb. nov.** MycoBank MB504556. Figs 7C, 23–24.
Basionym: *Periconiella papuana* Aptroot, Nova Hedwigia 67: 491. 1998.

In vitro: *Submerged hyphae* subhyaline, smooth, thin-walled, 1.5–3 µm wide; *aerial hyphae* pale brown, smooth to verrucose, 1.5–2 µm wide. *Conidiophores* dimorphic; *micronematous conidiophores* slightly differentiated from vegetative hyphae, branched or simple, up to 6-septate. *Conidiogenous cells* terminal or intercalary, variable in length, 5–20 µm long, thin-walled, smooth, golden- to dark brown at the base, paler toward the apex, later sometimes becoming inconspicuously septate, fertile part wider than basal part, often clavate, with crowded, more or less prominent conidium-bearing denticles, about 1 µm diam, unpigmented but slightly thickened. *Conidia* solitary, subhyaline, thin-walled, smooth, cylindrical to pyriform, rounded at the apex and truncate at the base, pale brown, (0–)1-septate, (5–)7–8(–11) × (2–)3(–4) µm, with a truncate base and darkened hilum, 1 µm diam. *Macronematous conidiophores* present in old cultures after 1 mo of incubation, consisting of well-differentiated, thick-walled, dark brown stalks, up to 220 µm long, (4–)5–6(–7) µm wide, with up to 15 additional septa, often with inflated basal cells; apically densely branched, forming a complex head, with up to five levels of branchlets, 20–50 µm long, each branchlet giving rise to a denticulate conidium-bearing rachis; scars slightly pigmented, thickened, about 1 µm diam. *Conidia* solitary, thin-walled, smooth, pale brown, obovoidal to pyriform, (0–)1-septate, (4–)5–6(–8) × (2–)3(–4) µm, with a truncate base and darkened hilum, 1–2 µm diam.

Cultural characteristics: Colonies on MEA reaching 10 mm diam after 14 d at 24 °C, with entire, sharp margin; mycelium velvety, elevated, with colonies up to 2 mm high, surface olivaceous-grey to iron-grey; reverse greenish black.

Specimen examined: **Papua New Guinea**, Madang Province, foothill of Finisterre range, 40.8 km along road Madang-Lae, alt. 200 m, isolated from unknown stipe, 2 Nov. 1995, A. Aptroot, **holotype** CBS-H 6351, culture ex-type CBS 212.96.

Veronaea Cif. & Montemart., Atti Ist. Bot. Lab. Crittog. Univ. Pavia, sér. 5, 15: 68. 1957.

In vitro: *Colonies* velvety, pale olivaceous-brown, moderately fast-growing. *Submerged hyphae* hyaline to pale olivaceous, smooth; *aerial hyphae*, more darkly pigmented. Exophiala-type budding cells absent in culture. *Conidiophores* erect, straight or flexuose, unbranched or occasionally loosely branched, sometimes geniculate, smooth-walled, pale to medium- or olivaceous-brown. *Conidiogenous cells* terminally integrated, polyblastic, occasionally intercalary, cylindrical, pale brown, later often becoming septate, fertile part subhyaline, often as wide as the basal part, rachis with crowded, flat to slightly prominent, faintly pigmented, unthickened scars. *Conidia* solitary, smooth, cylindrical to pyriform, rounded at the apex and truncate at the base, pale brown, 1(–2)-septate; conidial secession schizolytic.

Type species: *Veronaea botryosa* Cif. & Montemart., Atti Ist. Bot. Lab. Crittog. Univ. Pavia, sér. 5, 15: 68. 1957.

Veronaea botryosa Cif. & Montemart., Atti Ist. Bot. Lab. Crittog. Univ. Pavia, sér. 5, 15: 68. 1957. Fig. 25.

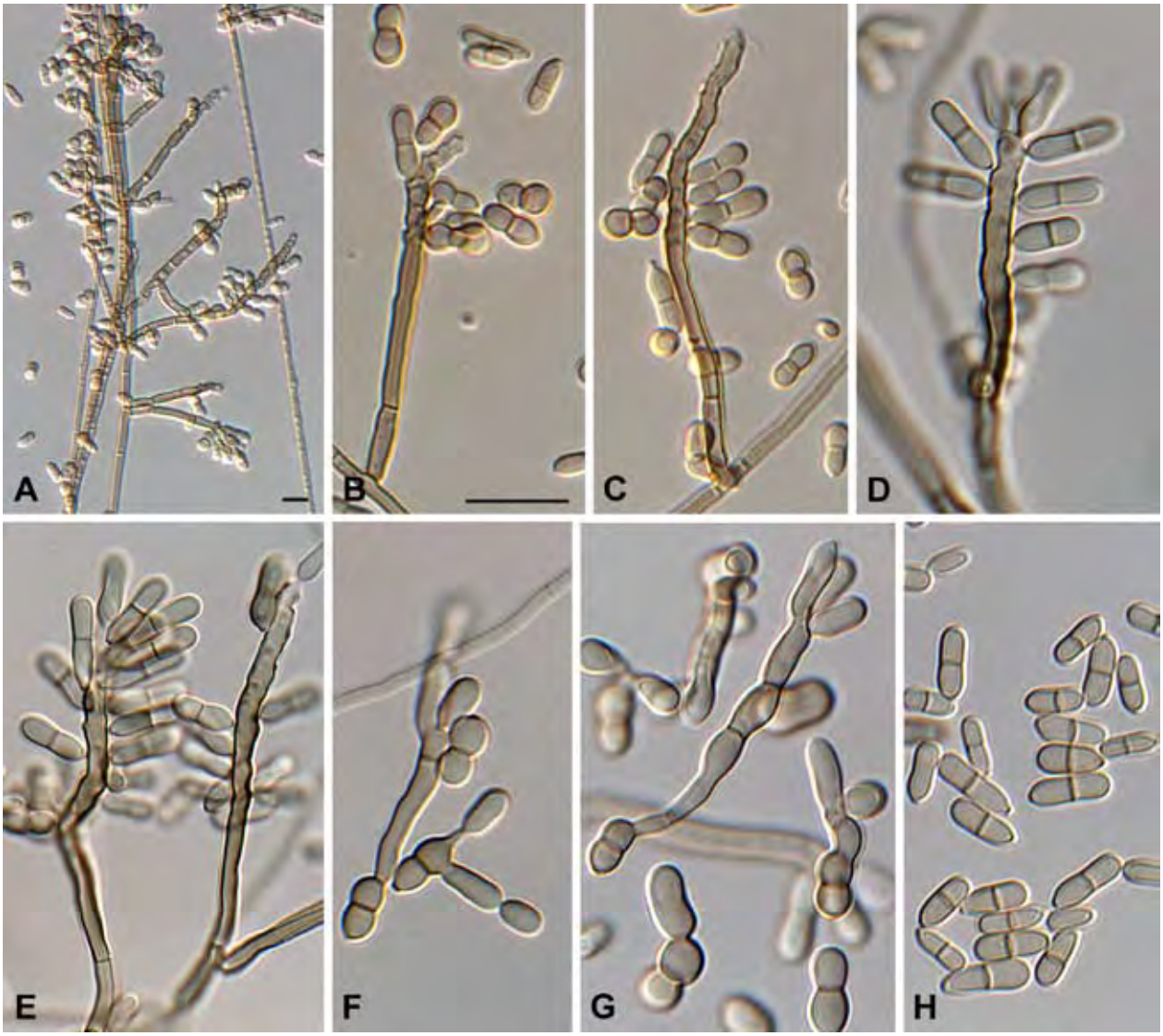

Fig. 25. *Veronaea botryosa* (CBS 254.57). A–C. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. D–E. Rachis with crowded and flat scars. F–G. Microcyclic conidiation. H. Conidia. Scale bars = 10 µm.

In vitro: *Submerged hyphae* hyaline to pale olivaceous, smooth; *aerial hyphae* more darkly pigmented. *Conidiophores* erect, straight or flexuose, unbranched or occasionally loosely branched, sometimes geniculate, smooth-walled, pale brown to olivaceous-brown, 2–3 µm wide and up to 200 µm long. *Conidiogenous cells* terminal, occasionally intercalary, cylindrical, 10–100 µm long, pale brown, later often becoming septate, fertile part subhyaline, often as wide as the basal part, rachis with crowded, flat to slightly prominent, faintly pigmented, unthickened scars. *Conidia* solitary, smooth, cylindrical to pyriform, (3–)6.5–8.5(–12) × (1.5–)2–2.5(–3) µm, rounded at the apex and truncate at the base, pale brown, 1(–2)-septate, with a faintly darkened, unthickened hilum, about 0.5 µm diam.

Cultural characteristics: Colonies on MEA reaching 30 mm diam after 14 d at 24 °C, with entire, sharp margin; mycelium velvety, slightly elevated in the centre, surface olivaceous-grey to greyish-brown; reverse greenish black.

Specimens examined: **India**, Ramgarh, about 38 km from Jaipur, isolated from goat dung, 1 Sep. 1963, B.C. Lodha, CBS 350.65 = IMI 115127 = MUCL 7972. **Italy**, Tuscany, Pisa, isolated from Sansa olive slag, 1954, O. Verona, **ex-type** strain, CBS 254.57 = IMI 070233 = MUCL 9821.

Veronaea compacta Papendorf, Bothalia 12: 119. 1976. Fig. 26.

In vitro: *Submerged hyphae* subhyaline, smooth, thin-walled, 1.5–3 µm wide; *aerial hyphae* rather thick-walled, pale brown. *Conidiophores* slightly differentiated from vegetative hyphae, lateral or occasionally terminal, often wider than the supporting hypha, up to 4 µm wide, unbranched or branched at acute angles, with 1–3 adititional septa, cells often inflated and flask-shaped, pale-brown, up to 60 µm long. *Conidiogenous cells* terminal, occasionally intercalary, variable in length, up to 10 µm long, pale brown, cylindrical to doliiform or flask-shaped, with hardly prominent denticles; scars flat, slightly pigmented, not thickened, about 0.5 µm diam. *Conidia* solitary, pale brown, smooth, thin-walled, ellipsoidal to ovoid, 0–1(–2)-septate, often constricted at the septa, (4–)6–7(–9) × 2–3 µm, with a round apex and truncate base; hilum prominent, slightly darkened, unthickened, about 0.5 µm diam.

Cultural characteristics: Colonies rather slow growing, reaching 15 mm diam on MEA after 14 d at 24 °C; surface velvety to lanose, slightly raised in the centre, pale grey to pale brownish grey; reverse dark grey.

Specimen examined: **South Africa**, soil, M.C. Papendorf, **ex-type** culture CBS 268.75.

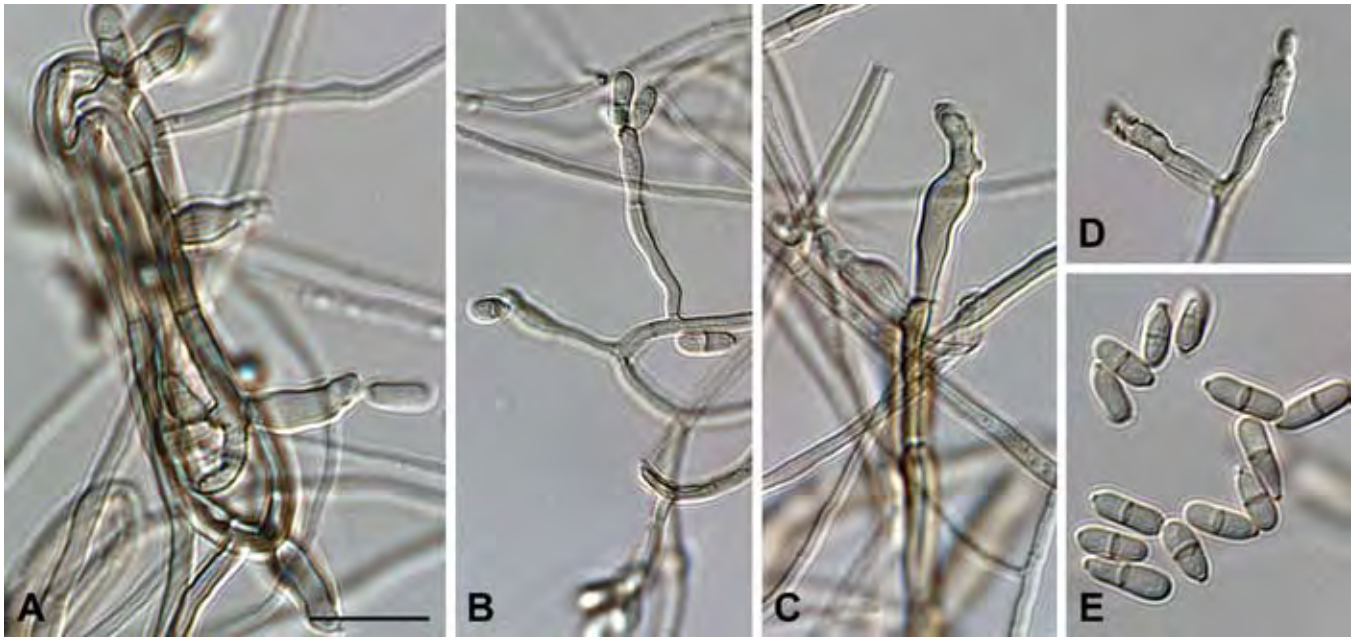

Fig. 26. *Veronaea compacta* (CBS 268.75). A–B. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. C–D. Rachis with hardly prominent denticles. E. Conidia. Scale bar = 10 µm.

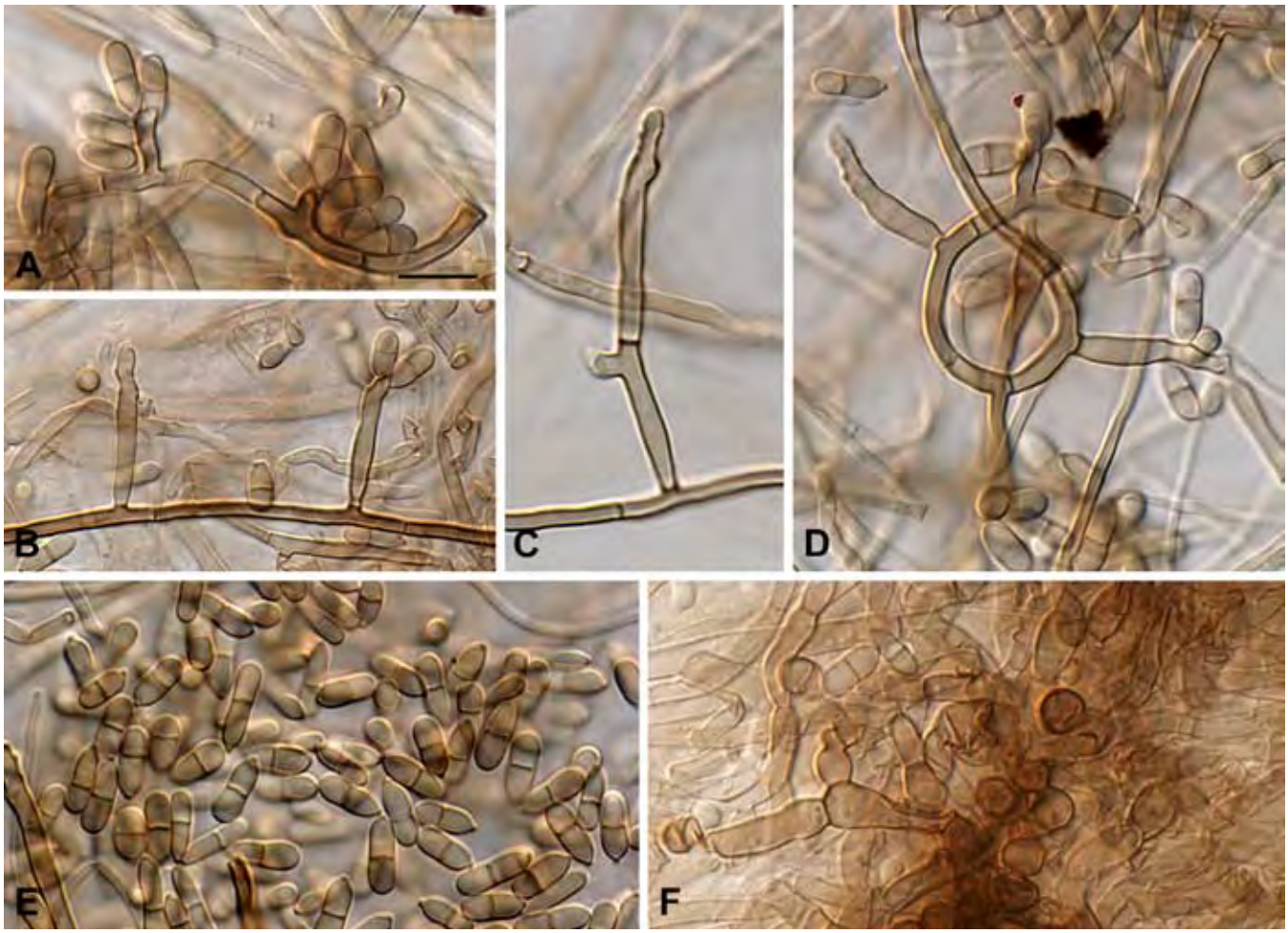

Fig. 27. *Veronaea japonica* (CBS 776.83). A. Intercalary conidiogenous cells. B–D. Semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. E. Conidia. F. Thick-walled, dark brown hyphal cells. Scale bar = 10 µm.

Veronaea japonica Arzanlou, W. Gams & Crous, **sp. nov.** MycoBank MB504557. Figs 17B, 27.

Etymology: Named after the country of origin, Japan.

Veronaeae compactae similis, sed cellulis inflatis, aggregatis, crassitunicatis, fuscis *in vitro* formatis distinguenda.

In vitro: *Submerged hyphae* subhyaline, smooth, thin-walled, 1.5–3 µm wide; *aerial hyphae* slightly narrower, pale brown; hyphal cells later becoming swollen, thick-walled, dark brown, often aggregated. *Conidiophores* slightly differentiated from aerial vegetative hyphae, lateral, or terminal, often wider than the supporting hypha, 2–3 µm wide, up to 65 µm long, unbranched or occasionally branched,

Fig. 28. *Pleurothecium obovoideum* (CBS 209.95). A–C. Conidial apparatus consisting of conidiophores with sympodially proliferating conidiogenous cells as seen in slide cultures of ca. 14 d. D. Short chain of conidia. E–G. Sympodially proliferating conidiogenous cells, resulting in a short rachis with subcylindrical to cylindrical denticles. H. Conidia. Scale bar = 10 µm.

pale brown, thin-walled, smooth, with 1–3 additional septa. *Conidiogenous cells* terminal, occasionally intercalary, variable in length, up to 15 µm long, pale brown, cylindrical to clavate, with hardly prominent denticles; scars flat, slightly pigmented, not thickened, about 0.5 µm diam. *Conidia* solitary, pale brown, smooth, thin-walled, ellipsoidal to ovoid, (0–)1-septate, often constricted at the septum, (6–)7–8(–10) × 2–2.5(–4) µm, with a round apex and truncate base; hilum unthickened but slightly darkened, about 1 µm diam.

Cultural characteristics: Colonies rather slow growing, reaching 7.5 mm diam on MEA after 14 d at 24 °C; surface velvety to lanose, slightly raised in the centre, olivaceous-brown, with entire margin; reverse dark-olivaceous.

Specimen examined: **Japan**, Kyoto, Daitokuji Temple, Kyoto, inside dead bamboo culm, Dec. 1983, W. Gams, **holotype** CBS-H 3490, ex-type culture CBS 776.83.

Note: This species is morphologically similar to *V. compacta* (Papendorf 1976), but can be distinguished based on the presence of dark brown, swollen hyphal cells in culture, which are absent in *V. compacta.*

Pleurothecium obovoideum clade (*Chaetosphaeriales*)

Ramichloridium obovoideum was regarded as similar to "*Ramichloridium*" (*Rhinocladiella*) *mackenziei* by some authors, and subsequently reduced to synonymy (Ur-Rahman *et al.* 1988). However, *R. obovoideum* clusters with *Carpoligna pleurothecii*, the teleomorph of *Pleurothecium* Höhn. Because it is also morphologically similar to other species of *Pleurothecium*, we herewith combine it into that genus.

Pleurothecium obovoideum (Matsush.) Arzanlou & Crous, **comb. nov.** MycoBank MB504558. Fig. 28.
Basionym: *Rhinocladiella obovoidea* Matsush., *Icones Microfung. Mats. lect.*: 123. 1975.
≡ *Ramichloridium obovoideum* (Matsush.) de Hoog, Stud. Mycol. 15: 73. 1977.

In vitro: *Submerged hyphae* smooth, hyaline, thin-walled, 1–2 µm wide; *aerial hyphae* hyaline to subhyaline, smooth. *Conidiophores* arising vertically from creeping hyphae, ascending hyphae thick-walled and dark brown; conidiophores 10–35 µm long, 1–2-septate, often reduced to a conidiogenous cell, unbranched, thick-walled, smooth, tapering towards the apex, pale brown. *Conidiogenous cells* integrated, cylindrical to ampulliform, 5–20 µm long, pale brown, elongating sympodially, with a short rachis giving rise to denticles, 1 µm long, slightly pigmented. *Conidia* aseptate, solitary or in short chains of up to 3, smooth, pale brown, ellipsoidal to obovate, (9–)11–12(–14.5) × (3–)4(–5) µm, smooth, thin-walled, with a more or less rounded apex, a truncate base and a slightly darkened, unthickened hilum, 1.5 µm diam.

Cultural characteristics: Colonies slow-growing, reaching 15 diam after 14 d at 24 °C, with entire, smooth margin; surface rather compact, mycelium mainly flat, submerged, some floccose to lanose aerial mycelium in the centre, buff; reverse honey.

Specimen examined: **Japan**, Kobe Municipal Arboretum, T. Matsushima, from dead leaf of *Pasania edulis*, CBS 209.95 = MFC 12477.

Incertae sedis (*Sordariomycetes*)

Ramichloridium schulzeri clade

Ramichloridium schulzeri, including its varieties, clusters near *Thyridium* Nitschke and the *Magnaporthaceae*, and is phylogenetically as well as morphologically distinct from the other genera in the *Ramichloridium* complex. To accommodate these taxa, a new genus is introduced below.

Myrmecridium Arzanlou, W. Gams & Crous, **gen. nov.** MycoBank MB504559.

Etymology: (Greek) *myrmekia* = wart, referring to the wart-like denticles on the rachis, suffix *-ridium* from *Chloridium*.

Genus ab allis generibus *Ramichloridii* similibus rachide recta longa, subhyalina, denticulis distantibus, verruciformibus praedita distinguendum.

In vitro: *Colonies* moderately fast-growing, flat, with mainly submerged mycelium, and entire margin, later becoming powdery to velvety, pale orange to orange. *Mycelium* rather compact, mainly submerged, in the centre velvety with fertile bundles of hyphae. *Conidiophores* arising vertically and clearly distinct from creeping hyphae, unbranched, straight or flexuose, brown, thick-walled. *Conidiogenous cells* terminally integrated, polyblastic, cylindrical, straight or flexuose, pale brown, sometimes secondarily septate, fertile part subhyaline, as wide as the basal part, with scattered pimple-shaped, apically pointed, unpigmented, conidium-bearing denticles. *Conidia* solitary, subhyaline, smooth or finely verrucose, rather thin-walled, with a wing-like gelatinous sheath, obovoidal or fusiform, tapering towards a narrowly truncate base with a slightly prominent, unpigmented hilum; conidial secession schizolytic.

Type species: *Myrmecridium schulzeri* (Sacc.) Arzanlou, W. Gams & Crous, comb. nov.

Notes: *Myrmecridium schulzeri* was fully described as *Acrotheca acuta* Grove by Hughes (1951). The author discussed several genera, none of which is suitable for the present fungus for various reasons as analysed by de Hoog (1977). Only *Gomphinaria* Preuss is not yet sufficiently documented. Our examination of *G. amoena* Preuss (B!) showed that this is an entirely different fungus, of which no fresh material is available to ascertain its position.

Myrmecridium can be distinguished from other ramichloridium-like fungi by having entirely hyaline vegetative hyphae, and widely scattered, pimple-shaped denticles on the long hyaline rachis. The conidial sheath is visible in lactic acid mounts with bright-field microscopy. The *Myrmecridium* clade consists of several subclusters, which are insufficiently resolved based on the ITS sequence data. However, two morphologically distinct varieties of *Myrmecridium* are treated here. The status of the other isolates in this clade will be dealt with in a future study incorporating more strains, and using a multi-gene phylogenetic approach.

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, **comb. nov.** MycoBank MB504560. var. ***schulzeri*** Figs 7B, 29.

Basionym: *Psilobotrys schulzeri* Sacc., Hedwigia 23: 126. 1884.

≡ *Chloridium schulzerii* (Sacc.) Sacc., Syll. Fung. 4: 322. 1886.

≡ *Rhinocladiella schulzeri* (Sacc.) Matsush., *Icon. Microfung. Mats. lect.* (Kobe): 124. 1975.

≡ *Ramichloridium schulzeri* (Sacc.) de Hoog, Stud. Mycol. 15: 64. 1977 var. *schulzeri*.

= *Acrotheca acuta* Grove, J. Bot., Lond. 54: 222. 1916.

≡ *Pleurophragmium acutum* (Grove) M.B. Ellis in Ellis, *More Dematiaceous Hyphomycetes*: 165. 1976.

= *Rhinotrichum multisporum* Doguet, Rev. Mycol., Suppl. Colon. 17: 78. 1953 (nom. inval. Art. 36) [non *Acrotheca multispora* (Preuss) Sacc., Syll. Fung. 4: 277. 1886].

[non *Acrothecium* (?) *multisporum* G. Arnaud, Bull. Trimestriel Soc. Mycol. France 69: 288. 1953 (nom. inval. Art. 36)].

[non *Acrothecium multisporum* G. Arnaud *sensu* Tubaki, J. Hattori Bot. Lab. 20: 145. 1958].

In vitro: *Submerged hyphae* hyaline, thin-walled, 1–2 µm wide; *aerial hyphae*, if present, pale olivaceous-brown. *Conidiophores* arising vertically from creeping aerial hyphae, unbranched, straight, reddish brown, thick-walled, septate, up to 250 µm tall, 2.5–3.5 µm wide, with 2–7 additional septa, basal cell often inflated, 3.5–5 µm wide. *Conidiogenous cells* integrated, cylindrical, variable in length, 15–110 µm long, subhyaline to pale brown, later becoming inconspicuously septate, fertile part subhyaline, as wide as the basal part, forming a straight rachis with scattered, pimple-shaped denticles less than 1 µm long and approx. 0.5 µm wide, apically pointed, unpigmented, slightly thickened scars. *Conidia* solitary, subhyaline, thin-walled, smooth or finely verrucose, surrounded by a wing-like, gelatinous conidial sheath, up to 0.5 µm thick, ellipsoid, obovoid or fusiform, (6–)9–10(–12) × 3–4 µm, tapering to a subtruncate base; hilum unpigmented, inconspicuous.

Cultural characteristics: Colonies reaching 29 mm diam after 14 d at 24 °C, pale orange to orange, with entire margin; mycelium flat, rather compact, later becoming farinose or powdery due to sporulation, which occurs in concentric zones when incubated on the laboratory bench.

Specimens examined: **Germany**, Kiel-Kitzeberg, from wheat-field soil, W. Gams, CBS 134.68 = ATCC 16310. **The Netherlands**, isolated from a man, bronchial secretion, A. Visser, CBS 156.63 = MUCL 1079; Lienden, isolated from *Triticum aestivum* root, C.L. de Graaff, CBS 325.74 = JCM 7234.

Myrmecridium schulzeri var. ***tritici*** (M.B. Ellis) Arzanlou, W. Gams & Crous, **comb. nov.** MycoBank MB504562.

Basionym: *Pleurophragmium tritici* M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 165. 1976.

≡ *Ramichloridium schulzeri* var. *tritici* (M.B. Ellis) de Hoog, Stud. Mycol. 15: 68. 1977.

Specimen examined: **Ireland**, Dublin, on wheat stem, Oct. 1960, J.J. Brady, **holotype** IMI 83291.

Notes: No reliable living culture is available of this variety. Based on a re-examination of the type specimen in this study, the variety appears sufficiently distinct from *Myrmecridium schulzeri* var. *schulzeri* based on the frequent production of septate conidia.

Myrmecridium flexuosum (de Hoog) Arzanlou, W. Gams & Crous, **comb. et stat. nov.** MycoBank MB504563. Fig. 30.

Basionym: *Ramichloridium schulzeri* var. *flexuosum* de Hoog, Stud. Mycol. 15: 67. 1977.

In vitro: *Submerged hyphae* hyaline, thin-walled, 1–2 µm wide. *Conidiophores* unbranched, flexuose, arising from creeping aerial

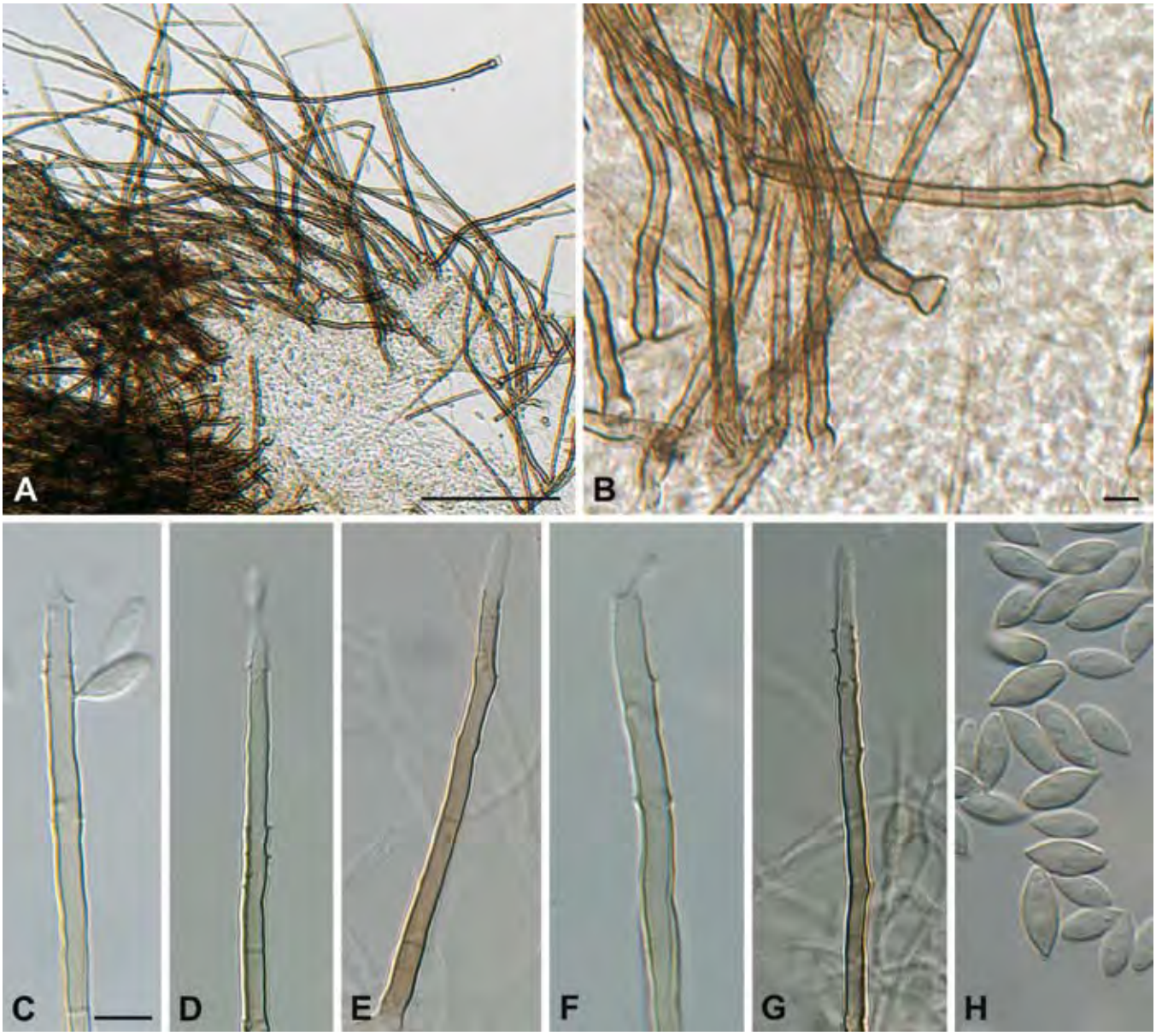

Fig. 29. *Myrmecridium schulzeri* (CBS 325.74). A. Macronematous conidiophores. B. Inflated basal cells visible in some conidiophores. C–E. Conidial apparatus at different stages of development, resulting in macronematous conidiophores and sympodially proliferating conidiogenous cells. F–G. Rachis with scattered, pimple-shaped denticles. H. Conidia. Scale bars: A =100 µm, B–H = 10 µm.

hyphae, pale brown, up to 250 µm tall, 3–3.5 µm wide, thick-walled, smooth, with up to 24 thin septa, delimiting 8–12 µm long cells. *Conidiogenous cells* integrated, elongating sympodially, cylindrical, 20–150 µm long, flexuose, brown at the base, subhyaline in the upper part, later becoming inconspicuously septate; rachis slightly flexuose, subhyaline, as wide as the basal part, thick-walled near the base, hyaline and thin-walled in the apical part, with scattered pimple-shaped, unpigmented, approx. 0.5 µm long denticles. *Conidia* solitary, subhyaline, thin-walled, finely verrucose, with a wing-like gelatinous sheath, approx. 0.5 µm wide, ellipsoid to obovoid, (5–)6–7(–9) × 3–4 µm; hilum slightly prominent, unpigmented, approx. 0.5 µm diam.

Cultural characteristics: Colonies reaching 40 mm diam after 14 d at 24 °C; mycelium submerged, flat, smooth; centrally orange, later becoming powdery to velvety and greyish brown due to sporulation, with sharp, smooth, entire margin; reverse yellowish orange.

Specimen examined: **Surinam**, isolated from soil, J.H. van Emden, ex-type culture CBS 398.76 = JCM 6968.

Note: This former variety is sufficiently distinguished from *M. schulzeri s. str.* by its flexuose conidiophores and conidia which lack an acuminate base, to be regarded as a separate species.

Ramichloridium torvi (Ellis & Everh.) de Hoog, Stud. Mycol. 15: 79. 1977.

≡ *Ramularia torvi* Ellis & Everh., Rep. Missouri Bot. Gard. 9: 119. 1898.
≡ *Hansfordia torvi* (Ellis & Everh.) Deighton & Piroz., Mycol. Pap. 101: 39. 1965.
= *Acladium biophilum* Cif., Sydowia 10: 164. 1956.
≡ *Hansfordia biophila* (Cif.) M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 199. 1976.

Specimen: **Jamaica**, Port Marant, Dec. 1890, on leaves of *Solanum torvum*, **holotype** of *Ramularia torvi* (NY) (specimen not examined).

Notes: According to the description and illustration of *R. torvi* provided by de Hoog (1977), this appears to be an additional species of *Myrmecridium*. Although it is morphologically similar to *M. flexuosum* in having a flexuose rachis, it differs from the other species of the genus by having smooth, clavate conidia. Fresh collections and cultures would be required to resolve its status.

Fig. 30. *Myrmecridium flexuosum* (CBS 398.76). A–C. Conidial apparatus at different stages of development, resulting in macronematous conidiophores with sympodially proliferating conidiogenous cells. D–H. Sympodially proliferating conidiogenous cells giving rise to a flexuose conidium-bearing rachis with pimple-shaped denticles. I. Conidia. Scale bar = 10 µm.

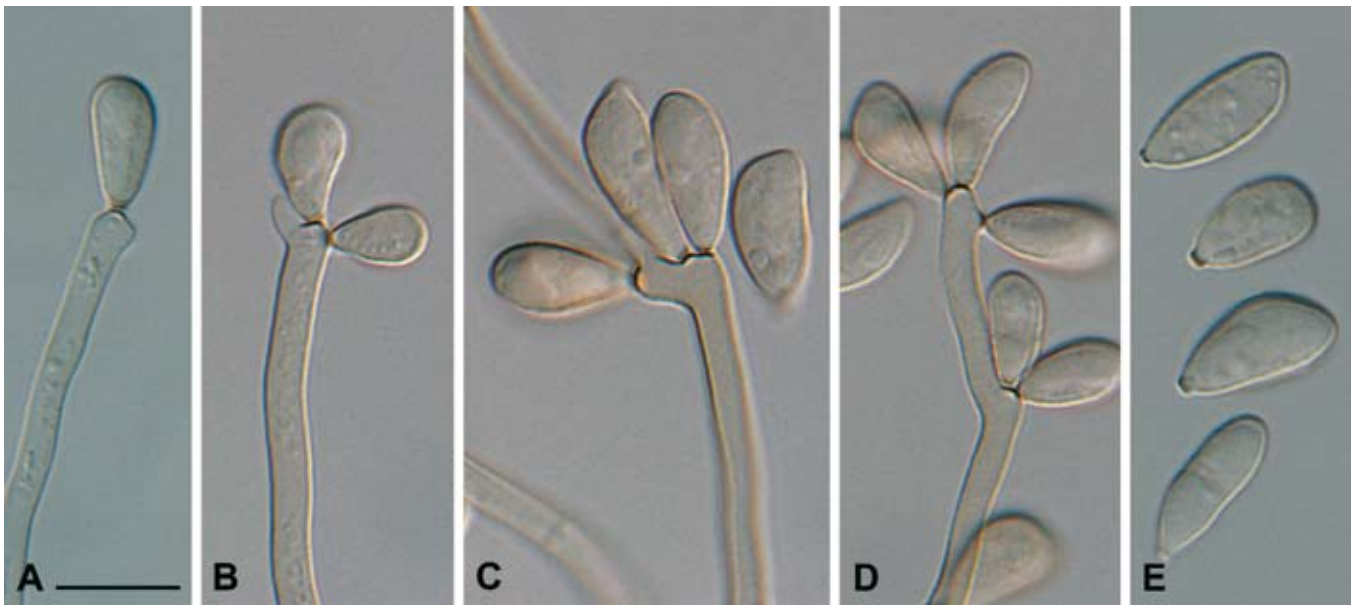

Fig. 31. *Pseudovirgaria hyperparasitica* (CBS 121739). A–D. Conidial apparatus at different stages of development; conidiogenous cells with geniculate proliferation. E. Conidia. Scale bar = 10 µm.

Pseudovirgaria H.D. Shin, U. Braun, Arzanlou & Crous, **gen. nov.** MycoBank MB504564.

Etymology: Named after its morphological similarity to *Virgaria*.

Hyphomycetes. Uredinicola. Coloniae *in vivo* pallide vel modice brunneae, ferrugineae vel cinnamomeae, *in vitro* lentissime crescentes, murinae. Mycelium immersum et praecipue externum, ex hyphis ramosis et cellulis conidiogenis integratis compositum, conidiophoris ab hyphis vegetativis vix distinguendis. Hyphae ramosae, septatae, leves, tenuitunicatae, hyalinae vel pallide brunneae. Cellulae conidiogenae integratae in hyphis repentibus, terminales et intercalares, polyblasticae, sympodialiter proliferentes, subcylindricae vel geniculatae, cicatricibus conspicuis, solitariis vel numerosis, dispersis vel aggregatis, subdenticulatis, prominentibus, umbonatis vel apicem versus paulo attenuatis, non inspissatis, non vel parce fuscatis-refringentibus. Conidia solitaria, holoblastica, plus minusve obovoidea, recta vel leniter curvata, asymmetrica, continua, hyalina, subhyalina vel pallidissime olivaceo-brunnea, hilo subconspicuo vel conspicuo, truncato vel rotundato, non inspissato, non vel lenissime fuscato-refringente; secessio schizolytica.

Hyperparasitic on uredosori of rust fungi. *Colonies in vivo* pale to medium brown, rusty or cinnamom, *in vitro* slow-growing, pale to dark mouse-grey. *Mycelium* immersed and mainly aerial, composed of branched hyphae with integrated conidiogenous cells, differentiation between vegetative hyphae and conidiophores barely possible. *Hyphae* branched, septate, smooth, thin-walled, hyaline to pale brown. *Conidiogenous cells* similarly hyaline to pale brown, integrated in creeping threads (hyphae), terminal and intercalary, polyblastic, proliferation sympodial, rachis subcylindrical to geniculate, conidiogenous loci (scars) conspicuous, solitary to numerous, scattered to aggregated, subdenticulate, bulging out, umbonate or slightly attenuated towards a rounded apex, wall unthickened, not to slightly darkened-refractive. *Conidia* solitary, formation holoblastic, more or less obovoid, straight to somewhat curved, asymmetrical, aseptate, hyaline, subhyaline to very pale olivaceous-brown, with more or less conspicuous hilum, truncate to rounded, unthickened, not or slightly darkened-refractive; conidial secession schizolytic.

Type species: *Pseudovirgaria hyperparasitica* H.D. Shin, U. Braun, Arzanlou & Crous, sp.nov.

Notes: Other ramichloridium-like isolates from various rust species form another unique clade, sister to *Radulidium subulatum* (de Hoog) Arzanlou, W. Gams & Crous and *Ra. epichloës* (Ellis & Dearn.) Arzanlou, W. Gams & Crous in the *Sordariomycetidae*. Although *Pseudovirgaria* is morphologically similar to *Virgaria* Nees, it has hyaline to pale brown hyphae, conidia and conidiogenous cells. The conidiogenous cells are integrated in creeping threads (hyphae), terminal and intercalary, and the proliferation is distinctly sympodial. The subdenticulate conidiogenous loci are scattered, solitary, at small shoulders of geniculate conidiogenous cells, caused by sympodial proliferation, or aggregated, forming slight swellings of the rachis, i.e., a typical raduliform rachis as in *Virgaria* is lacking. Furthermore, the conidiogenous loci of *Pseudovirgaria* are bulging, convex, slightly attenuated towards the rouded apex, in contrast to more cylindrical denticles in *Virgaria* (Ellis 1971). The scar type of *Pseudovirgaria* is peculiar due to its convex, papilla-like shape and reminiscent of conidiogenous loci in plant-pathogenic genera like *Neoovularia* U. Braun and *Pseudodidymaria* U. Braun (Braun 1998). The superficially similar genus *Veronaea* is quite distinct from *Pseudovirgaria* by having erect conidiophores with a typical rachis and crowded conidiogenous loci which are flat or only slightly prominent and darkened. *Pseudovirgaria* is characterised by its mycelium which is composed of branched hyphae with integrated, terminal and intercalary conidiogenous cells. A differentiation between branched hyphae and "branched conidiophores" is difficult and barely possible. It remains unclear if the "creeping threads" and terminal branches of hyphae are to be interpreted as "creeping conidiophores". In any case, the mycelium forms complex fertile branched hyphal structures in which individual conidiophores are barely discernable. These structures and difficulties in discerning individual conidiophores remind one of some species of *Pseudocercospora* Speg. and other cercosporoid genera with abundant superficial mycelium *in vivo*.

Pseudovirgaria hyperparasitica H.D. Shin, U. Braun, Arzanlou & Crous, **sp. nov.** MycoBank MB504565. Figs 6A, 31.

Etymology: Named after its hyperparasitic habit on rust fungi.

Hyphae 1.5–4 µm latae, tenuitunicatae, ≤ 0.5 µm crassae. Cellulae conidiogenae 15–50 × 2–5 µm, tenuitunicatae (≤ 0.5 µm), cicatricibus (0.5–)1.0(–1.5) µm diam, 0.5–1 µm altis. Conidia saepe obovoidea, interdum subclavata, 10–20 × 5–9 µm, apice rotundato vel paulo attenuato, basi truncata vel rotundata, hilo *ca* 1 µm diam.

In vivo: *Colonies* on rust sori, thin to moderately thick, loose, cobwebby, to dense, tomentose, pale to medium brown, rusty or cinnamon. *Mycelium* partly immersed in the sori, but mainly superficial, composed of a system of branched hyphae with integrated conidiogenous cells (fertile threads), distinction between conidiophores and vegetative hyphae difficult and barely possible. *Hyphae* 1.5–4 µm wide, hyaline, subhyaline to pale yellowish, greenish or very pale olivaceous, light brownish in mass, thin-walled (≤ 0.5 µm), smooth, pluriseptate, occasionally slightly constricted at the septa. *Conidiogenous cells* integrated in creeping fertile threads, terminal or intercalary, 15–50 µm long, 2–5 µm wide, subcylindrical to geniculate, subhyaline to very pale brownish, wall thin, ≤ 0.5 µm, smooth, proliferation sympodial, with a single to usually several conidiogenous loci per cell, often crowded, causing slight swellings, up to 6 µm wide, subdenticulate loci, formed by the slightly bulging wall, convex, slightly narrowed towards the rounded apex, (0.5–)1.0(–1.5) µm diam and 0.5–1 µm high, wall of the loci unthickened, not or slightly darkened-refractive, in surface view visible as minute circle (only rim visible and dark). *Conidia* solitary, obovoid, often slightly curved with ± unequal sides, 10–20 × 5–9 µm, aseptate, subhyaline, pale yellowish greenish to very pale olivaceous, wall ≤ 0.5 µm thick, smooth, apex slightly attenuated to usually broadly rounded, base rounded to somewhat attenuated towards a more or less conspicuous hilum, (0.5–)1(–1.5) µm diam, convex to truncate, unthickened, not to slightly darkened-refractive.

In vitro: *Submerged hyphae* hyaline to subhyaline, smooth; *aerial hyphae* smooth, subhyaline, up to 4 µm wide. *Conidiogenous cells* arising imperceptably from aerial vegetative hyphae, terminal, occasionally intercalary, holoblastic, proliferating sympodially in a geniculate pattern, with more or less long intervals between groups of scars; loci slightly darkened, unthickened, approx. 0.5 µm diam. *Conidia* hyaline to subhyaline, aseptate, ovoid, often somewhat curved, (10–)13–15(–17) × (5–)6–7(–8) µm, with truncate base and acutely rounded apex; hila unthickened, slightly darkened-refractive.

Cultural characteristics: Colonies on MEA rather slow-growing, reaching 11 mm diam after 14 d at 24 °C, pale to dark mouse-grey, velvety, compacted, with colonies being up to 1 mm high.

Specimens examined: **Korea**, Seoul, on uredosori of *Frommeëlla* sp., on *Duchesnea chrysantha*, 17 Sep. 2003, H.D. Shin, **paratype**, 4/10, CPC 10702–10703 = CBS 121735–121736, HAL 2053 F; Chunchon, on *Phragmidium griseum* on *Rubus crataegifolius*, 20 Jul. 2004, H.D. Shin, **paratype**, 2/8, HAL 2057 F; Suwon,

Fig. 32. *Radulidium subulatum* (CBS 405.76). A–B. Macronematous conidiophores with sympodially proliferating conidiogenous cells, resulting in a conidium-bearing rachis. C–D. Rachis with crowded, blunt conidium-bearing denticles. E. Conidia. Scale bar = 10 µm.

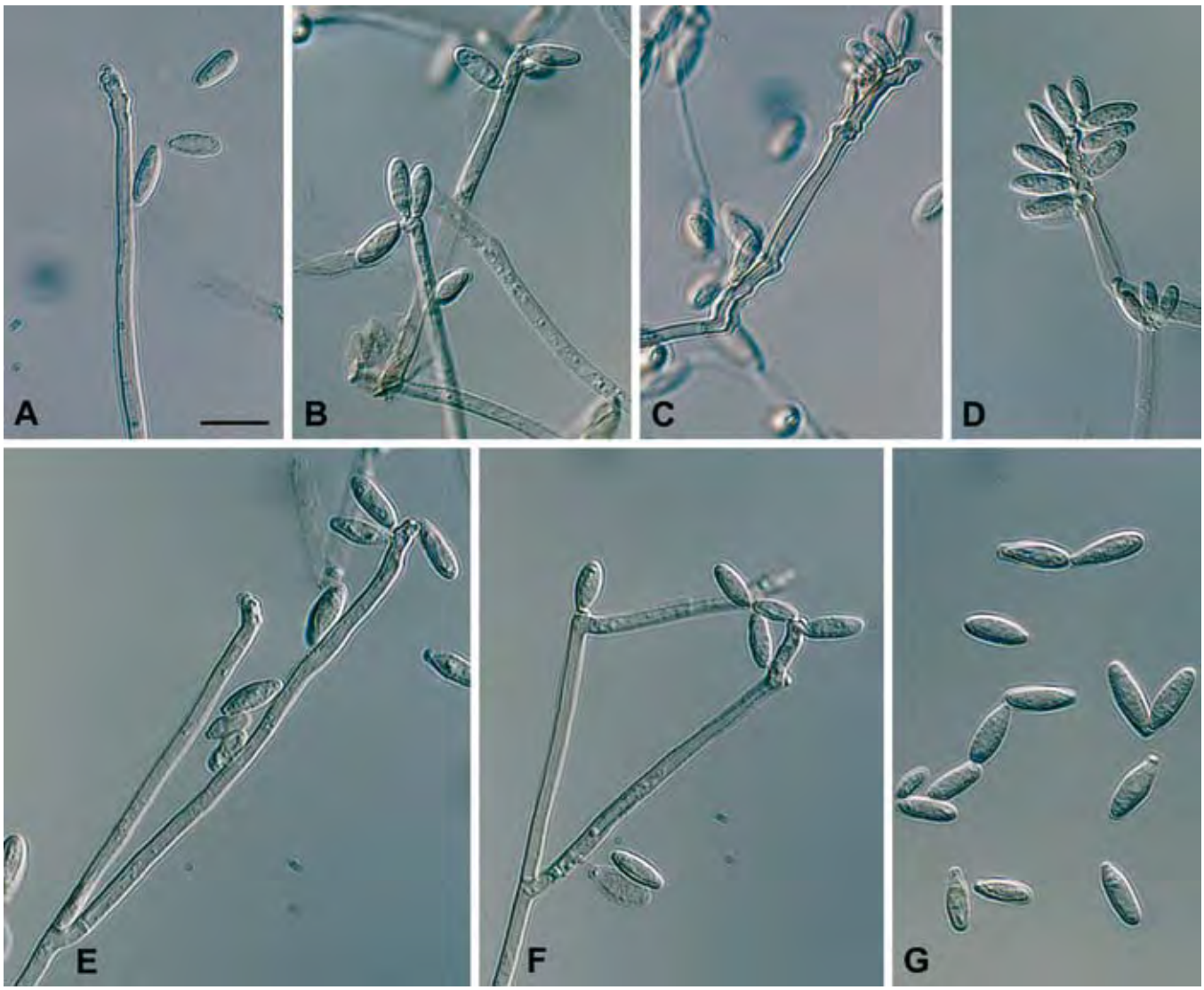

Fig. 33. *Radulidium epichloës* (CBS 361.63). A–C. Conidial apparatus at different stages of development, resulting in semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. D. Rachis with crowded, blunt conidium-bearing denticles. E–F. Conidiophores with acute branches in the lower part. G. Conidia. Scale bar = 10 µm.

on *Phragmidium pauciloculare* on *Rubus parvifolius*, 14 Oct. 2003, H.D. Shin, **paratype**, 23/10, HAL 2055 F; Hongchon, on *Phragmidium rosae-multiflorae* on *Rosa multiflora*, 11 Aug. 2004, H.D. Shin, **paratype**, 23/8, HAL 2056 F; Yangpyong, on *Phragmidium* sp. on *Rubus coreanus*, 30 Sep. 2003, H.D. Shin, **paratype**, 11/10-1, CPC 10704–10705 = CBS 121737–121738, HAL 2052 F, and the same locality, 23 Jul. 2004, HAL 2058 F; Chunchon, on *Pucciniastrum agrimoniae* on *Agrimonia pilosa*, 7 Oct. 2002, H.D. Shin, **holotype**, HAL 2054 F, culture ex-type CPC 10753–10755 = CBS 121739–121741.

Radulidium subulatum and *Ra. epichloës* clade

Ramichloridium subulatum and *R. epichloës* form a distinct, well-supported clade with uncertain affinity. This clade is morphologically distinct and a new genus is introduced below to accommodate it.

Radulidium Arzanlou, W. Gams & Crous, **gen. nov.** MycoBank MB504566.

Etymology: Latin *radula* = A flexible tongue-like organ in gastropods, referring to the radula-like denticles on the rachis.

Genus ab aliis generibus *Ramichloridii* similibus denticulis densissimis, prominentibus, hebetibus in rachide e cellula conidiogena aculeata orta distinguendum.

Type species: *Radulidium subulatum* (de Hoog) Arzanlou, W. Gams & Crous, comb. nov.

In vitro: *Colonies* fast-growing, velvety, floccose near the margin, centrally with fertile hyphal bundles up to 10 mm high, about 2 mm diam, with entire but vague margin; *mycelium* whitish, later becoming greyish brown. *Submerged hyphae* smooth, thin-walled. *Conidiophores* usually reduced to polyblastic conidiogenous cells arising from undifferentiated or slightly differentiated aerial hyphae, terminally integrated or lateral, rarely a branched conidiophore present, smooth, slightly thick-walled, pale brown, cylindrical to acicular, widest at the base and tapering towards the apex; apical part forming a pale brown, generally straight rachis, with crowded, prominent, blunt denticles, suggesting a gastropod radula; denticles 0.5–1 µm long, apically pale brown. *Conidia* solitary, subhyaline, thin- or slightly thick-walled, smooth or verrucose, obovoidal, fusiform to subcylindrical, base subtruncate and with a slightly prominent, conspicuously pigmented hilum; conidial secession schizolytic.

Notes: *Radulidium* can be distinguished from other ramichloridium-like fungi by its slightly differentiated conidiophores and prominent, blunt, very dense conidium-bearing denticles. Although the *Radulidium* clade consists of several subclusters that correlate with differences in morphology, the ITS sequence data appear insufficient to resolve this species complex. Therefore, only two species of *Radulidium* with clear morphological and molecular differences are treated here. The phylogenetic situation of other taxa in this clade will be treated in a further study employing a multi-gene approach.

Radulidium subulatum (de Hoog) Arzanlou, W. Gams & Crous, **comb. nov.** MycoBank MB504567. Figs 10C, 32.
Basionym: *Ramichloridium subulatum* de Hoog, Stud. Mycol. 15: 83. 1977.
Misapplied name: *Rhinocladiella elatior* Mangenot *sensu* dal Vesco & B. Peyronel, Allionia 14: 38. 1968.

In vitro: *Submerged hyphae* hyaline, thin-walled, 1–2.5 µm wide; *aerial hyphae* brownish. *Conidiogenous cells* arising laterally from vegetative hyphae, pale brown, smooth, thick-walled, sometimes without a basal septum, cylindrical to aculeate, tapering gradually towards the apex, widest at the base, 25–40 × 2–3 µm; proliferating sympodially, forming a pale brown rachis, with densely crowded, prominent, blunt conidium-bearing denticles, with pale brown apex. *Conidia* solitary, subhyaline, thin-walled, smooth, ellipsoidal to almost clavate, 5–7 × 1.5–2 µm, with a slightly pigmented, non-refractive hilum, about 1 µm diam.

Cultural characteristics: Colonies on MEA rather fast growing, reaching 50 mm diam after 14 d at 24 °C, with entire but vague margin, velvety, floccose near the margin, centrally with fertile hyphal bundles up to 10 mm high, about 2 mm diam; mycelium whitish, later becoming greyish brown; reverse grey, zonate.

Specimens examined: **Czech Republic**, on *Phragmites australis*, A. Samšiňáková, **ex-type** culture CBS 405.76; Opatovicky pond, from *Lasioptera arundinis* (gall midge) mycangia on *Phragmites australis*, M. Skuhravá, CBS 101010.

Radulidium epichloës (Ellis & Dearn.) Arzanlou, W. Gams & Crous, **comb. nov.** MycoBank MB504568. Fig. 33.
Basionym: *Botrytis epichloës* Ellis & Dearn., Canad. Record Sci. 9: 272. 1893.
≡ *Ramichloridium epichloës* (Ellis & Dearn.) de Hoog, Stud. Mycol. 15: 81. 1977.

In vitro: *Submerged hyphae* hyaline, thin-walled, 1–2.5 µm wide; *aerial hyphae* somewhat darker. *Conidiogenous cells* arising laterally or terminally from undifferentiated or slightly differentiated aerial hyphae, occasionally acutely branched in the lower part, smooth, thick-walled, pale brown, more or less cylindrical, later with thin septa, 25–47 µm long; proliferating sympodially, forming a rather short, pale brown, straight or somewhat geniculate rachis, with crowded, prominent, blunt denticles with pale brown apex. *Conidia* solitary, subhyaline, rather thin-walled, verruculose, obovoidal to fusiform, (4.5–)7–8(–11) × 2–3 µm, with a pigmented hilum, 1–1.5 µm diam.

Cultural characteristics: Colonies reaching 45 mm diam after 14 d at 24 °C, with smooth, rather vague, entire margin; velvety, centrally floccose and elevated up to 2 mm high; surface mycelium whitish, later becoming greyish brown; reverse pale ochraceous.

Specimen examined: **U.S.A.**, Cranberry Lake, Michigan, isolated from *Epichloë typhina* on *Glyceria striata*, G.L. Hennebert, CBS 361.63 = MUCL 3124; specimen in MUCL designated here as **epitype**.

Veronaea-like clade, allied to the *Annulatascaceae*

A veronaea-like isolate from *Bertia moriformis* clusters near the *Annulatascaceae*, and is morphologically distinct from other known anamorph genera in the *Ramichloridium* complex, and therefore a new genus is introduced to accommodate it.

Rhodoveronaea Arzanlou, W. Gams & Crous, **gen. nov.** MycoBank MB504569.

Etymology: (Greek) *rhodon* = the rose, referring to the red-brown conidiophores, suffix *-veronaea* from *Veronaea*.

Genus ab aliis generibus *Ramichloridii* similibus basi condiorum late truncata et marginata distinguenda.

In vitro: *Colonies* slow-growing, velvety, floccose; surface olivaceous-grey to dark olivaceous-green; reverse olivaceous-black. *Hyphae* smooth, thin-walled, pale olivaceous. *Conidiophores* arising vertically from creeping hyphae, straight or flexuose, simple, thick-walled, red-brown, with inflated basal cell. *Conidiogenous cells* terminally integrated, polyblastic, sympodial, smooth, thick-

Fig. 34. *Rhodoveronaea varioseptata* (CBS 431.88). A–D. Macronematous conidiophores with sympodially proliferating conidiogenous cells, resulting in conidium bearing rachis with slightly prominent conidium-bearing denticles. E–F. Conidia with minute marginal frill. Scale bar = 10 µm.

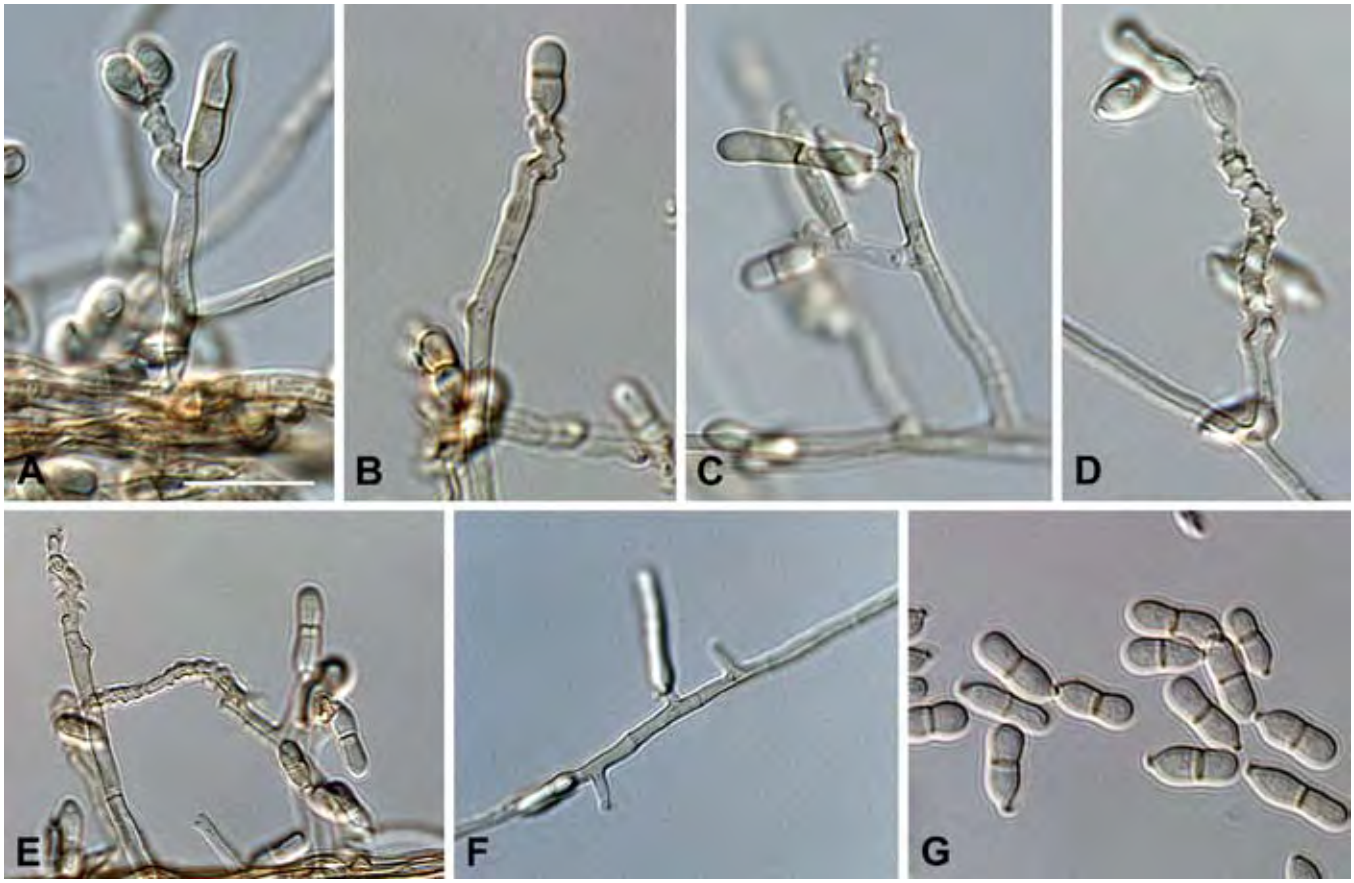

Fig. 35. *Veronaeopsis simplex* (CBS 588.66). A–C. Conidial apparatus at different stages of development, resulting in semi-micronematous conidiophores and sympodially proliferating conidiogenous cells. D–E. Rachis with crowded, prominent denticles. F. Intercalary conidiogenous cells. G. Conidia. Scale bar = 10 µm.

walled, pale brown, rachis straight, occasionally geniculate, with crowded, slightly prominent conidium-bearing denticles; denticles flat-tipped, slightly pigmented. *Conidia* solitary, pale brown, thin- or slightly thick-walled, smooth, ellipsoidal to obovoidal, 0–multi-septate, with a protruding base and a marginal basal frill; conidial secession schizolytic.

Type species: *Rhodoveronaea varioseptata* Arzanlou, W. Gams & Crous, sp. nov.

Notes: *Rhodoveronaea* differs from other ramichloridium-like fungi by the presence of a basal, marginal conidial frill, and variably septate conidia.

Rhodoveronaea varioseptata Arzanlou, W. Gams & Crous, **sp. nov.** MycoBank MB504570. Figs 10D, 34.

Etymology: Named for its variably septate conidia.

Hyphae 2–3 µm latae. Conidiophora ad 125 µm longa et 3–5 µm lata. Cellulae conidiogenae 30–70 µm longae et 3–5 µm latae. Conidia 0–2(–3)-septata, (8–)11–13(–15) × (2–)3–4(–6) µm.

In vitro: *Submerged hyphae* smooth, thin-walled, pale olivaceous, 2–3 µm wide; *aerial hyphae* smooth, brownish and slightly narrower. *Conidiophores* arising vertically from creeping hyphae, straight or flexuose, simple, smooth, thick-walled, red-brown, up to 125 µm long, 3–5 µm wide, often with inflated basal cell. *Conidiogenous cells* terminally integrated, smooth, thick-walled, pale brown at the base, paler towards the apex, straight, variable in length, 30–70 µm long and 3–5 µm wide, rachis straight, occasionally geniculate; slightly prominent conidium-bearing denticles, crowded, with slightly pigmented apex, about 1 µm diam. *Conidia* solitary, pale brown, thin- or slightly thick-walled, smooth, ellipsoid to obovoid, 0–2(–3)-septate, (8–)11–13(–15) × (2–)3–4(–6) µm with a protruding base, 1.5 µm wide, and marginal frill.

Cultural characteristics: Colonies reaching 12 mm diam after 14 d at 24 °C, velvety, floccose; surface olivaceous-grey to dark olivaceous-green; reverse olivaceous-black.

Specimen examined: **Germany**, Eifel, Berndorf, on *Bertia moriformis*, Sep. 1987, W. Gams, **holotype** CBS-H 19932, culture ex-type CBS 431.88.

Venturiaceae (*Pleosporales*)

The ex-type strain of *Veronaea simplex* (Papendorf 1969) did not cluster with the genus *Veronaea* (*Herpotrichiellaceae*), but is allied to the *Venturiaceae*. *Veronaea simplex* is distinct from species of *Fusicladium* Bonord. by having a well-developed rachis with densely aggregated scars. A new genus is thus introduced to accommodate this taxon.

Veronaeopsis Arzanlou & Crous, **gen. nov.** MycoBank MB504571.

Etymology: The suffix *-opsis* refers to its similarity with *Veronaea*.

Genus *Veronaeae* simile sed conidiophoris brevioribus (ad 60 µm longis) et rachide dense denticulata distinguendum.

In vitro: *Colonies* moderately fast-growing; surface velvety, floccose, greyish sepia to hazel, with smooth margin; reverse mouse-grey to dark mouse-grey. *Conidiophores* arising vertically from aerial hyphae, lateral or intercalary, simple or branched, occasionally reduced to conidiogenous cells, pale brown. *Conidiogenous cells* terminally integrated on simple or branched conidiophores, polyblastic, smooth, thin-walled, pale brown; rachis commonly straight, geniculate, with densely crowded, prominent denticles, and slightly pigmented scars. *Conidia* solitary, subhyaline to pale brown, thin- or slightly thick-walled, smooth, oblong-ellipsoidal to subcylindrical, (0–)1-septate, with a slightly darkened, thickened, hilum; conidial secession schizolytic.

Type species: *Veronaeopsis simplex* (Papendorf) Arzanlou & Crous, comb. nov.

Veronaeopsis simplex (Papendorf) Arzanlou & Crous, **comb. nov.** MycoBank MB504572. Figs 17C, 35.
Basionym: *Veronaea simplex* Papendorf, Trans. Brit. Mycol. Soc. 52: 486. 1969.

In vitro: *Submerged hyphae* smooth, thin-walled, pale brown; *aerial hyphae* aggregated in bundles. *Conidiophores* arising vertically from aerial hyphae, lateral or intercalary, simple or branched, occasionally reduced to conidiogenous cells, pale brown, rather short, up to 60 µm long, 1.5–2 µm wide. *Conidiogenous cells* terminally integrated in the conidiophores, smooth, thin-walled, pale brown, variable in length, 5–25 µm long, rachis generally straight or irregularly geniculate, with crowded, prominent denticles, about 0.5 µm long, flat-tipped, with slightly pigmented apex. *Conidia* solitary, subhyaline to pale brown, thin- or slightly thick-walled, smooth, oblong-ellipsoidal to subcylindrical, (0–)1-septate, slightly constricted at the septum, (6–)10–12(–15) × (2–)2.5–3(–4) µm; hilum slightly darkened and thickened, not refractive, about 1 µm diam.

Cultural characteristics: Colonies reaching 25 mm diam after 14 d at 24 °C; surface velvety, floccose, greyish sepia to hazel, with smooth margin; reverse mouse-grey to dark mouse-grey.

Specimen examined: **South Africa**, Potchefstroom, on leaf litter of *Acacia karroo*, 1966, M.C. Papendorf, **holotype**, CBS H-7810; culture ex-type CBS 588.66 = IMI 203547.

Notes: The presence of 1-septate conidia in *Veronaeopsis* overlaps with *Veronaea.* However, *Veronaeopsis* differs from *Veronaea* based on its conidiophore and conidiogenous cell morphology. *Veronaea* has much longer, macronematous conidiophores than *Veronaeopsis.* Furthermore, *Veronaea* has a more or less straight rachis, whereas in *Veronaeopsis* the rachis is often geniculate. The conidiogenous loci in *Veronaea* are less prominent, i.e., less denticle-like.

DISCUSSION

The present study was initiated chiefly to clarify the status of *Ramichloridium musae*, the causal organism of tropical speckle disease of banana (Jones 2000). Much confusion surrounded this name in the past, relating, respectively, to its validation, species and generic status. As was revealed in the present study, however, two species are involved in banana speckle disease, namely *R. musae* and *R. biverticillatum*. Even more surprising was the fact that *Ramichloridium* comprises anamorphs of *Mycosphaerella* Johanson (*Mycosphaerellaceae*), though no teleomorphs have thus far been conclusively linked to any species of *Ramichloridium*. By investigating the *Ramichloridium* generic complex as outlined by de Hoog (1977), another genus associated with leaf spots, namely *Periconiella*, was also shown to represent an anamorph of *Mycosphaerella*. Although no teleomorph connections have been proven for ramichloridium-like taxa, de Hoog *et al.* (1983) refer to the type specimen of *Wentiomyces javanicus* Koord. (*Pseudoperisporiaceae*), on the type specimen of which (PC) some ramichloridium-like conidiophores were seen. Without fresh material and an anamorph-teleomorph connection proven in culture, however, this matter cannot be investigated further. It is interesting to note, however, that *Wentiomyces* Koord. shows a strong resemblance to *Mycosphaerella*, except for the external perithecial appendages.

The genus *Mycosphaerella* is presently one of the largest genera of ascomycetes, containing close to 3 000 names (Aptroot 2006), to which approximately 30 anamorph genera have already been linked (Crous *et al*. 2006a, b, 2007). By adding two additional anamorph genera, the *Mycosphaerella* complex appears to be expanding

even further, though some taxa have been shown to reside in other families in the *Capnodiales*, such as *Davidiella* Crous & U. Braun (*Davidiellaceae*) and *Teratosphaeria* (*Teratosphaeriaceae*) (Braun *et al.* 2003, Crous *et al.* 2007, Schubert *et al.* 2007 – this volume).

Another family, which proved to accommodate several ramichloridium-like taxa, is the *Herpotrichiellaceae* (*Chaetothyriales*). Members of the *Chaetothyriales* are regularly encountered as causal agents of human mycoses (Haase *et al.* 1999, de Hoog *et al.* 2003), whereas species of the *Capnodiales* are common plant pathogens, or chiefly associated with plants. Species in the *Chaetothyriales* have consistently melanized thalli, which is a factor enabling them to invade humans, and cause a wide diversity of mycoses, such as chromoblastomycosis, mycetoma, brain infection and subcutaneous phaeohyphomycosis (de Hoog *et al.* 2003). The only known teleomorph connection in this genus is *Capronia* Sacc. (Untereiner & Naveau 1999).

Rhinocladiella and *Veronaea* were in the past frequently confused with the genus *Ramichloridium*. However, *Rhinocladiella*, as well as *Veronaea* and *Thysanorea*, were shown to cluster in the *Chaetothyriales,* while *Ramichloridium* clusters in the *Capnodiales*. *Rhinocladiella mackenziei,* which causes severe cerebral phaeohyphomycosis in humans (Sutton *et al.* 1998), has in the past been confused with *Pleurothecium obovoideum* (Ur-Rahman *et al.* 1988). Data presented here reveal, however, that although morphologically similar, these species are phylogenetically separate, with *P. obovoideum* belonging to the *Sordariales*, where it clusters with sexual species of *Carpoligna* F.A. Fernández & Huhndorf that have *Pleurothecium* anamorphs (Fernández *et al.* 1999).

In addition to the genera clustering in the *Capnodiales* and *Chaetothyriales*, several ramichloridium-like genera are newly introduced to accommodate species that cluster elsewhere in the ascomycetes, namely *Pseudovirgaria*, *Radulidium* and *Myrmecridium*, *Veronaeopsis*, and *Rhodoveronaea*. Although the ecological role of these taxa is much less known than that of taxa in the *Capnodiales* and *Chaetothyriales*, some exhibit an interesting ecology. For instance, the fungicolous habit of *Pseudovirgaria*, as well as some species in *Radulidium*, which are found on various rust species, suggests that these genera should be screened further to establish if they have any potential biocontrol properties. Furthermore, these two genera share a common ancestor, and further work is required to determine whether speciation was shaped by co-evolution with the rusts. A further species of "*Veronaea*" that might belong to *Pseudovirgaria* is *Veronaea harunganae* (Hansf.) M.B. Ellis, which is known to occur on *Hemileia harunganae* Cummins on *Harungana* in Tanzania and Uganda (Ellis 1976). The latter species, however, is presently not known from culture, and needs to be recollected to facilitate further study.

The genera distinguished here represent homogeneous clades in the phylogenetic analysis. Only the species of *Rhinocladiella* are dispersed among others morphologically classified in *Exophiala* or other genera.

By integrating the phylogenetic data generated here with the various morphological data sets, we were able to resolve eight clades for taxa formerly regarded as representative of the *Ramichloridium* complex. According to the phylogeny inferred from 28S rDNA sequence data, the genera *Ramichloridium* and *Periconiella* were heterogeneous, requiring the introduction of several novel genera. Although the present 11 odd genera can still be distinguished based on their morphology, it is unlikely that morphological identifications without the supplement of molecular data would in the future be able to accurately identify all the novel isolates that undoubtably await description. The integration of morphology with phylogenetic data not only helps to resolve generic affinities, but it also assists in discriminating between the various cryptic species that surround many of these well-known names that are presently freely used in the literature. To that end it is interesting to note that for the majority of the taxa studied here, the ITS domain (Table 1) provided good species resolution. However, more genes will have to be screened in future studies aimed at characterising some of the species complexes where the ITS domain provided insufficient phylogenetic signal (data not shown) to resolve all of the observed morphological species.

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Cladosporium leaf-blotch and stem rot of *Paeonia* spp. caused by *Dichocladosporium chlorocephalum* gen. nov.

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Abstract: *Cladosporium chlorocephalum* (= *C. paeoniae*) is a common, widespread leaf-spotting hyphomycete of peony (*Paeonia* spp.), characterised by having dimorphic conidiophores. During the season, one stage of this fungus causes distinct, necrotic leaf-blotch symptoms on living leaves of *Paeonia* spp. In late autumn, winter or after overwintering, a second morphologically distinct conidiophore type occurs on dead, blackish, rotting stems. Conspecificity of the two morphs, previously proposed on the basis of observations in culture, was supported by DNA sequence data from the ITS and LSU gene regions, using cultures obtained from leaf-blotch symptoms on living leaves, as well as from dead stems of *Paeonia* spp. Sequence data were identical, indicating a single species with two morphs. On account of its distinct conidiogenous loci and conidial hila, as well as its sequence-based phylogenetic position separate from the *Davidiella/Cladosporium* clade, the peony fungus has to be excluded from *Cladosporium s. str.*, but still belongs to the *Davidiellaceae* (*Capnodiales*). The leaf-blotching (cladosporioid) morph of this fungus morphologically resembles species of *Fusicladium*, but differs in having dimorphic fruiting, and is phylogenetically distant from the *Venturiaceae*. The macronematous (periconioid) morph resembles *Metulocladosporiella* (*Chaetothyriales*), but lacks rhizoid conidiophore hyphae, and has 0–5-septate conidia. Hence, *C. chlorocephalum* is assigned to the new genus *Dichocladosporium*.

Taxonomic novelties: *Dichocladosporium* K. Schub., U. Braun & Crous, gen. nov., *Dichocladosporium chlorocephalum* (Fresen.) K. Schub., U. Braun & Crous, comb. nov.
Key words: Anamorphic fungi, *Cladosporium chlorocephalum*, *C. paeoniae*, hyphomycetes, new genus, phylogeny, taxonomy.

INTRODUCTION

Fresenius (1850) described *Periconia chlorocephala* Fresen. from Germany on dead stems of *Paeonia* sp. Mason & Ellis (1953) examined this species *in vitro* and *in vivo* and stated that it only occurred on dead stems of *Paeonia* spp. They described, illustrated and discussed this species in detail, and reallocated it to the genus *Cladosporium* Link.

A second, cladosporioid hyphomycete on *Paeonia* spp., *Cladosporium paeoniae* Pass., was collected by Passerini on living leaves of *P. albiflora* (as *P. edulis*) in Italy, and distributed in Thümen, Herbarium mycologicum oeconomicum, Fasc. IX, No. 416 (1876), together with the first valid description, which was repeated by Passerini (1876). Later, Passerini collected this fungus on *Paeonia officinalis* at Parma in Italy and distributed it in Thümen, Mycotheca universalis, No. 670 (1877). Saccardo (1882) listed a collection of this species on *Paeonia anomala* from Russia, Siberia, which he later described as *Cladosporium paeoniae* var. *paeoniae-anomalae* Sacc. (Saccardo 1886). A first examination of *C. paeoniae* in culture was accomplished by Meuli (1937), followed by a treatment *in vitro* by de Vries (1952). Mason & Ellis (1953) described and illustrated in their treatment of *C. chlorocephalum* macroconidiophores, agreeing with those of the original diagnosis and illustration of *Periconia chlorocephala*, as well as semi-macronematous conidiophores concurring with those of *C. paeoniae*, although no mention was made of the latter name. McKemy & Morgan-Jones (1991) carried out comprehensive studies on *Cladosporium* on *Paeonia* spp. *in vitro* and *in vivo*, including detailed discussions of the history of the taxa concerned, taxonomic implications and comprehensive descriptions and illustrations. They concluded that *Cladosporium paeoniae*, found in culture together with *C. chlorocephalum*, was a semi-macronematous form (synanamorph) of the latter species, and reduced *C. paeoniae* to synonymy with the latter species.

In the present study, re-examination and reassessment of morphological characters, conidiogenesis, and DNA sequence data of the ITS and 28S nrDNA were used to confirm the identity of *Cladosporium chlorocephalum* (the periconioid morph) and *C. paeoniae* (the cladosporioid morph), and clarify their relation to *Cladosporium s. str.* (*Davidiellaceae*) (*emend.* David 1997, Braun *et al.* 2003).

MATERIALS AND METHODS

Isolates

Single-conidial isolates were obtained from symptomatic leaves and dead stems, and cultured as detailed in Crous (1998). Cultural characteristics and morphology of isolates (Table 1) were recorded from plates containing either 2 % potato-dextrose agar (PDA) or synthetic nutrient-poor agar (SNA) (Gams *et al*. 2007). Plates were incubated at 25 °C under continuous near-UV light to promote sporulation.

DNA isolation, amplification and sequencing

Fungal colonies were established on agar plates, and genomic DNA was isolated following the protocol of Lee & Taylor (1990). The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White *et al*. 1990), LR0R (Rehner & Samuels 1994), LR3R (www.biology.duke.edu/fungi/mycolab/primers.htm) and LR16 (Moncalvo *et al.* 1993) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous *et al*. (2006b). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Morphology

Morphological examinations were made from herbarium samples, fresh symptomatic leaves and stems, as well as cultures sporulating on SNA. Structures were mounted in water or Shear's solution (Dhingra & Sinclair 1985), and 30 measurements at × 1 000 magnification were made of each structure under an Olympus BX 50 microscope (Hamburg, Germany). The 95 % confidence levels were determined and the extremes of spore measurements given in parentheses. Scanning electron microscopic examinations were conducted at the Institute of Zoology, Martin-Luther-University, Halle (Saale), Germany, using a Hitachi S-2400. Samples were coated with a thin layer of gold applied with a sputter coater SCD 004 (200 s in an argon atmosphere of 20 mA, 30 mm distant from the electrode). Colony colours were noted after 2 wk growth on PDA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures studied were deposited in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1). Taxonomic novelties were lodged with MycoBank (www.MycoBank.org).

RESULTS

DNA phylogeny

Amplification products of approximately 1 700 bases were obtained for the isolates listed in Table 1. The ITS region of the sequences was used to obtain additional sequences from GenBank which were added to the alignment. The manually adjusted alignment contained 26 sequences (including the two outgroup sequences) and 518 characters including alignment gaps. Of the 518 characters used in the phylogenetic analysis, 226 were parsimony-informative, 33 were variable and parsimony-uninformative, and 259 were constant. Neighbour-joining analysis using three substitution models on the sequence data yielded trees supporting the same clades but with a different arrangement at the deeper nodes. These nodes were supported poorly in the bootstrap analyses (the highest value observed for one of these nodes was 64 %; data not shown).

Two equally most parsimonious trees (TL = 585 steps; CI = 0.761; RI = 0.902; RC = 0.686), one of which is shown in Fig. 1, was obtained from the parsimony analysis of the ITS region. The *Dichocladosporium* K. Schub., U. Braun & Crous isolates formed a well-supported clade (100 % bootstrap support), distinct from clades containing species of *Davidiella* Crous & U. Braun, *Mycosphaerella* Johanson and *Teratosphaeria* Syd. & P. Syd. This placement was also supported by analyses of the first part of the 28S rRNA gene (see Crous *et al.* 2007 – this volume).

Taxonomy

Because conidia formed holoblastically in simple or branched acropetal chains, *Cladosporium chlorocephalum* and *C. paeoniae* coincided with previous concepts of *Cladosporium s. lat.* (Braun *et al.* 2003, Schubert 2005), belonging to a wide assemblage of genera classified by Kiffer & Morelet (1999) as "*Acroblastosporae*". Previous studies conducted *in vitro* concluded that *Cladosporium chlorocephalum* and *C. paeoniae* represent two developmental stages (morphs) of a single species, a result confirmed here by DNA sequence analyses. A detailed analysis of conidiogenesis, structure of the conidiogenous loci and conidial hila, and a comparison with *Cladosporium s. str.*, typified by *C. herbarum* (Pers. : Fr.) Link, revealed obvious differences: The conidiogenous loci and conidial hila of *C. chlorocephalum* are quite distinct from those of *Cladosporium s. str.* by being denticulate or subdenticulate, apically broadly truncate, unthickened or slightly thickened, but somewhat darkened-refractive. The scars in *Cladosporium s. str.* are, however, characteristically coronate, i.e., with a central convex dome surrounded by a raised periclinal rim (David 1997, Braun *et al.* 2003, Schubert 2005). Hence, the peony fungus has to be excluded from *Cladosporium s. str.* A comparison with phaeoblastic hyphomycetous genera revealed a close similarity of this fungus with the genus *Metulocladosporiella* Crous, Schroers, J.Z. Groenew., U. Braun & K. Schub. recently introduced for the Cladosporium speckle disease of banana (Crous *et al.* 2006a). Both fungi have dimorphic fruiting, pigmented macronematous conidiophores often with distinct basal swellings and densely branched terminal heads composed of short branchlets and ramoconidia, denticulate or subdenticulate unthickened, but somewhat darkened-refractive conidiogenous loci, as well as phaeoblastic conidia, formed in simple or branched acropetal chains. The semi-macronematous leaf-blotching morph is close to and barely distinguishable from *Fusicladium* Bonord. However, unlike *Metulocladosporiella*, the peony fungus does not form rhizoid hyphae at the base of conidiophore swellings and the conidia are amero- to phragmosporous [0–5-septate *versus* 0(–1)-septate in *Metulocladosporiella*]. Furthermore, the peony fungus neither clusters within the *Chaetothyriales* (with *Metulocladosporiella*) nor within the *Venturiaceae* (with *Fusicladium*), but clusters basal to the *Davidiellaceae* (see also Crous *et al.* 2007 – this volume). Hence, we propose to place *C. chlorocephalum* in the new genus *Dichocladosporium*.

Dichocladosporium K. Schub., U. Braun & Crous, **gen. nov.** MycoBank MB504428. Figs 2–5.

Etymology: *dicha* in Greek = twofold.

Differt a *Metulocladosporiella* conidiophoris cum cellulis basalibus saepe inflatis, sed sine hyphis rhizoidibus, conidiis amero- ad phragmosporis (0–5-septatis).

Type species: Dichocladosporium chlorocephalum (Fresen.) K. Schub., U. Braun & Crous, comb. nov.

Dichocladosporium chlorocephalum (Fresen.) K. Schub., U. Braun & Crous, **comb. nov.** MycoBank MB504429. Figs 2–5.

Basionym: *Periconia chlorocephala* Fresen., Beiträge zur Mykologie 1: 21. 1850.

≡ *Haplographium chlorocephalum* (Fresen.) Grove, Sci. Gossip 21: 198. 1885.

≡ *Graphiopsis chlorocephala* (Fresen.) Trail, Scott. Naturalist (Perth) 10: 75. 1889.

≡ *Cladosporium chlorocephalum* (Fresen.) E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 123. 1953.

= *Cladosporium paeoniae* Pass., in Thümen, Herb. Mycol. Oecon., Fasc. IX, No. 416. 1876, and in Just´s Bot. Jahresber. 4: 235. 1876.

= *Periconia ellipsospora* Penz. & Sacc., Atti Reale Ist. Veneto Sci. Lett. Arti, Ser. 6, 2: 596. 1884.

= *Cladosporium paeoniae* var. *paeoniae-anomalae* Sacc., Syll. Fung. 4: 351. 1886.

= *Haplographium chlorocephalum* var. *ovalisporum* Ferraris, Fl. Ital. Cryptog., Hyphales: 875. 1914.

Descriptions: Mason & Ellis (1953: 123–126), McKemy & Morgan-Jones (1991: 140–144), Schubert (2005: 216).

Illustrations: Fresenius (1850: Pl. IV, figs 10–15), Mason & Ellis (1953: 124–125, figs 42–43), McKemy & Morgan-Jones (1991: 137, fig. 1; 141, fig. 2; 139, pl. 1; 143, pl. 2), Schubert (2005: 217, fig. 113; 275, pl. 34).

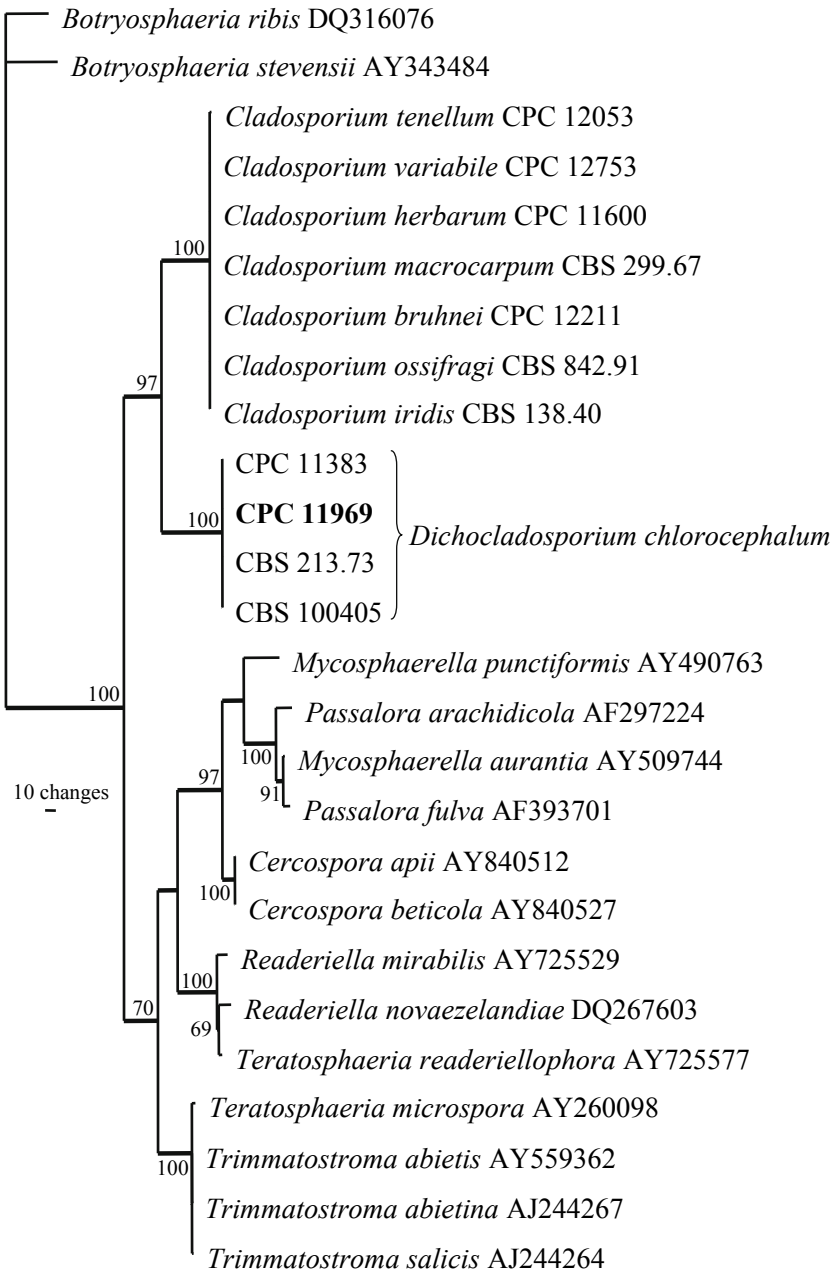

Fig. 1. One of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches. The tree was rooted to two *Botryosphaeria* species.

Characters of the cladosporioid morph: *Leaf-blotch symptoms* on living leaves amphigenous, variable in shape and size, subcircular-oval to irregular, broad, oblong to expanded, up to 30 mm long and 20 mm wide, at times covering the entire leaf surface, forming olivaceous-brown to blackish brown patches, rarely violet-brown, margin usually indefinite, attacked areas turning dry with age, also occurring on young, green stems. *Colonies* amphigenous, punctiform to effuse, loose to dense, caespitose, brown, villose. *Mycelium* immersed, subcuticular to intraepidermal; hyphae sparingly branched, 4–7(–10) µm wide, septate, sometimes with swellings and constrictions, swollen cells up to 13 µm diam, subhyaline to pale brown, smooth, walls thickened, hyphae sometimes aggregated; *in vitro* mycelium at first mainly immersed, later also superficial, branched, 1–5(–7) µm wide, pluriseptate, often constricted at septa and with swellings and constrictions, therefore irregular in outline, smooth to verruculose or irregularly rough-walled, loosely verruculose with distinct large warts. *Semi-macronematous conidiophores* formed on leaf-blotches solitary or in small, loose groups, arising from internal hyphae or swollen hyphal cells, erumpent through the cuticle, occasionally emerging through stomata, erect, straight to somewhat flexuous, oblong-cylindrical, usually unbranched or occasionally branched, 13–80 (–120) × (4–)5–8(–10) µm, slightly attenuated towards the apex, septate, septa often dense, unconstricted, pale to medium brown, sometimes paler towards the apex, smooth, thick-walled, wall often with two distinct layers, often somewhat inflated at the very base, up to 14 µm diam, occasionally proliferating enteroblastically; *in vitro* conidiophores arising laterally from plagiotropous hyphae or terminally from ascending hyphae, the latter usually appearing more filiform than those arising laterally from plagiotropous hyphae, erect, straight to slightly flexuous, cylindrical-oblong, not geniculate, usually unbranched, rarely with a short lateral prolongation near the apex, 18–60(–100) × 3–6 µm, slightly attenuated towards the apex, septate, pale to medium brown or olivaceous-brown, smooth to asperulate, walls somewhat thickened. *Conidiogenous cells* integrated, terminal or intercalary, subcylindrical, 7–45 µm

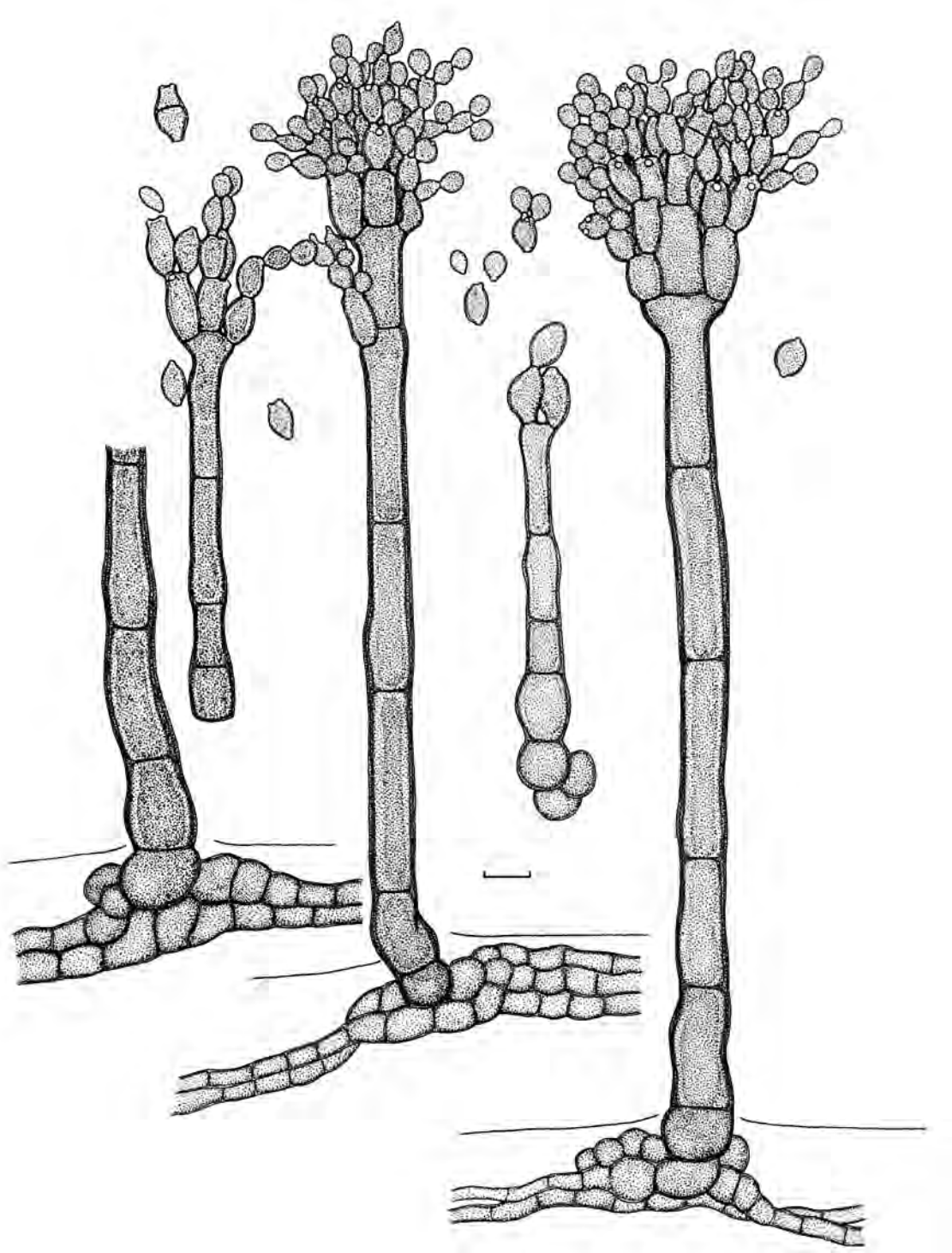

Fig. 2. *Dichocladosporium chlorocephalum* (HAL 1924 F), periconioid, stem rotting morph. Conidiophores and conidia. Scale bar = 10 µm.

Table 1. Isolates subjected to DNA analysis and morphological examination.

Species	Accession number[1]	Host	Country	Collector	GenBank accession number
Dichocladosporium chlorocephalum	CBS 213.73; IMI 048108a	*Paeonia* sp.	United Kingdom	F. Rilstone	EU009455
	CBS 100405	*Paeonia* sp.	New Zealand	M. Braithwaite	EU009456
	CBS 121522; CPC 11383	*Paeonia delavayi*	Germany	K. Schubert	EU009457
	CBS 121523*; CPC 11969	*Paeonia officinalis*	Germany	K. Schubert	EU009458

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.

*Ex-type cultures.

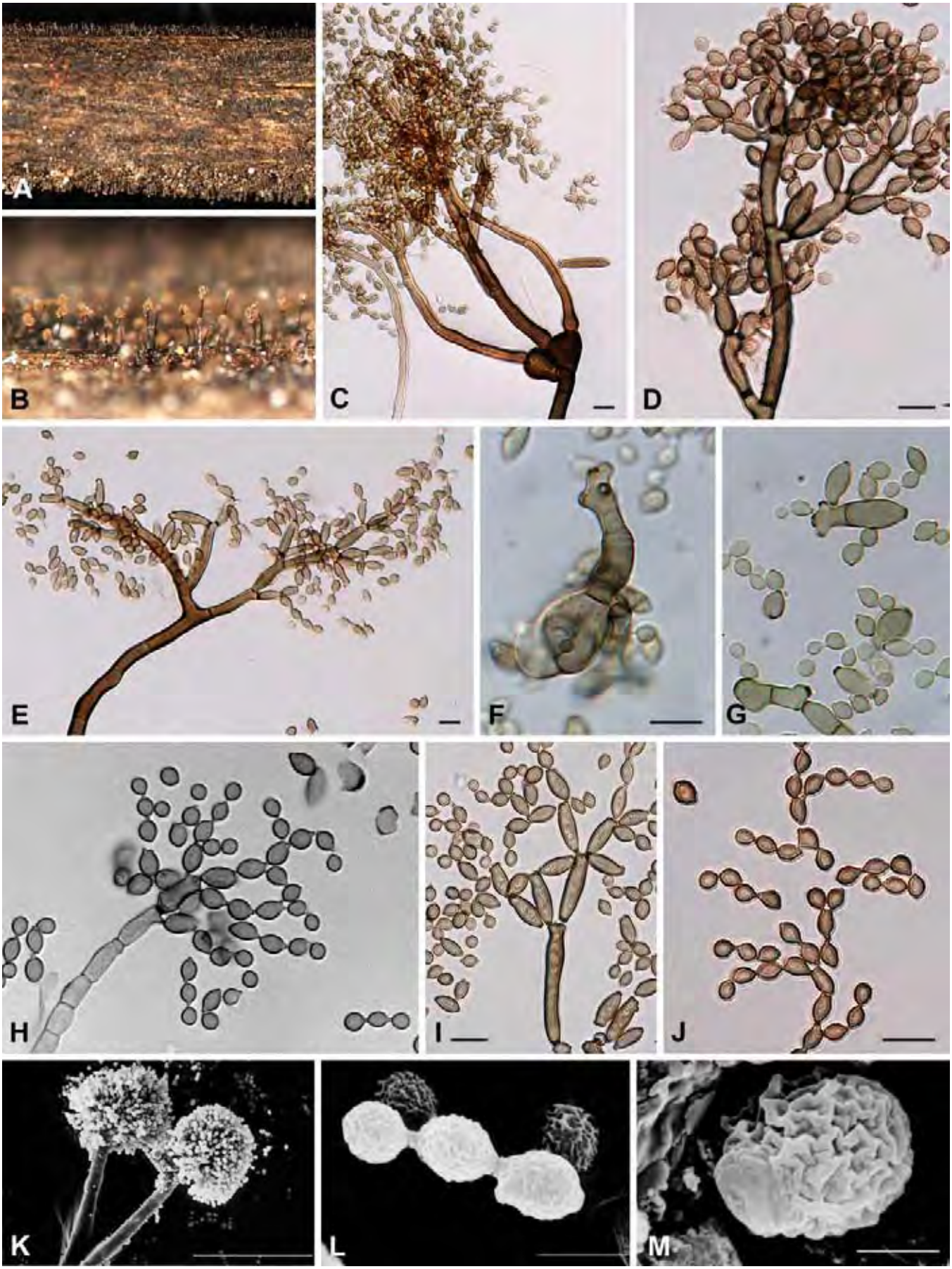

Fig. 3. *Dichocladosporium chlorocephalum* (CBS 121523 = CPC 11969). A–B. Symptoms of the periconioid, stem rotting morph. C–E, H. Macroconidiophores and conidia. F–G. Semi-macronematous conidiophores and conidia. I–J. Ramoconidia and conidia. K–M. Scanning electron microscopic photographs. K. Conidiophores. L. Conidial chain. M. Single conidium showing the surface ornamentation and scar structure. Scale bars: C–J, L = 10 µm; K = 100 µm; M = 2 µm.

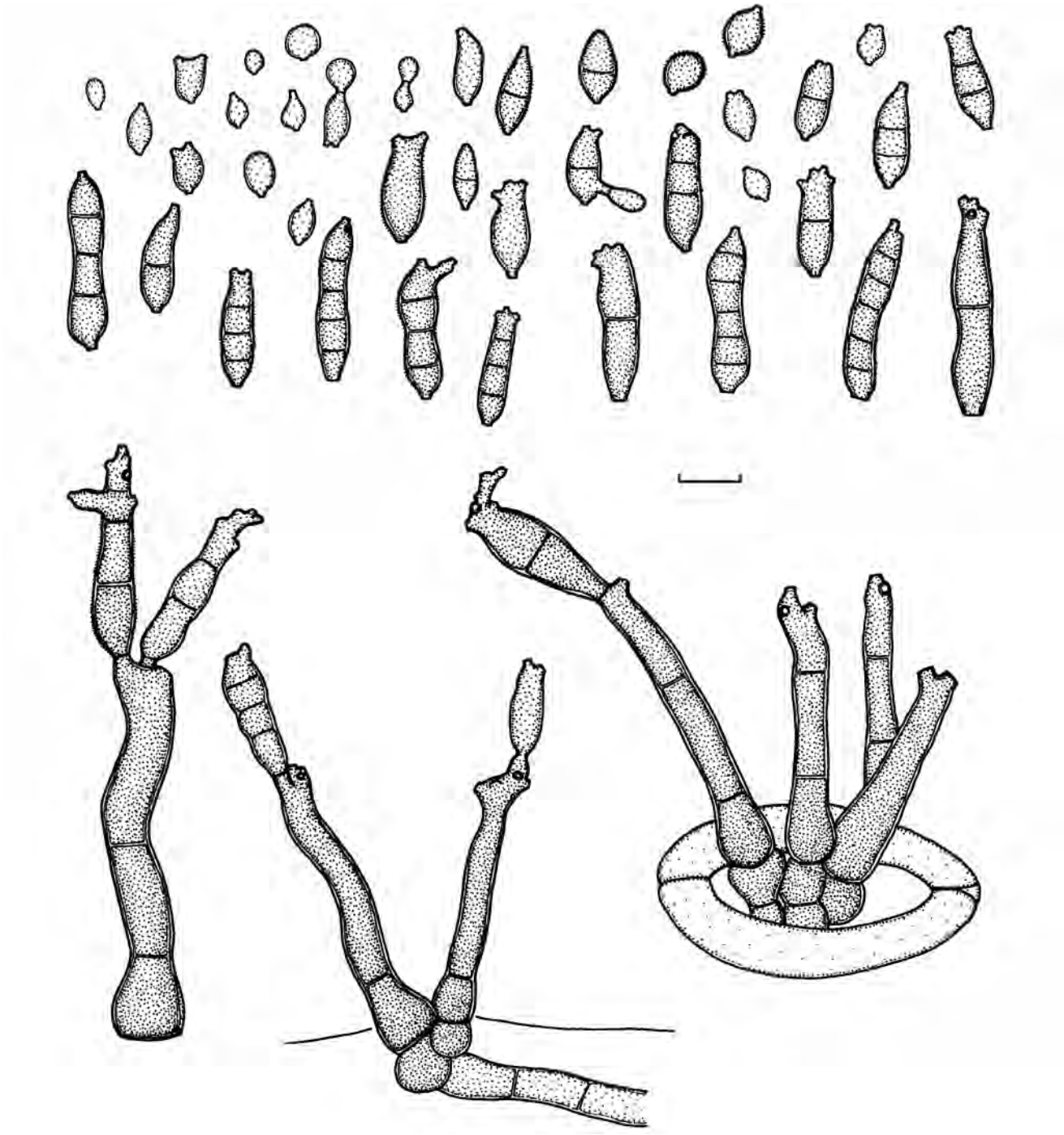

Fig. 4. *Dichocladosporium chlorocephalum* (HAL 2011 F), cladosporioid, leaf-spotting morph. Conidiophores and conidia. Scale bar = 10 µm.

long, proliferation sympodial, with one to several conidiogenous loci, subdenticulate or denticulate, protuberant, terminally broadly truncate, 1.5–3 µm wide, unthickened or almost so, somewhat darkened-refractive. *Conidia* catenate, in simple or branched chains, polymorphous, small conidia globose, subglobose, broadly obovoid, 3–9 × 3–5 µm, aseptate, pale to medium brown, smooth, intercalary conidia limoniform, ellipsoid-fusiform, oblong, 5–23 × 3.5–6.5 µm, 0–2-septate, medium brown, smooth to minutely verruculose or irregularly rough-walled, large conidia ellipsoid, oblong-cylindrical, ampulliform, 22–45(–52) × (4.5–)5–8 µm, 0–5-septate, medium brown, smooth to minutely verruculose or irregularly rough-walled, walls somewhat thickened, hila truncate, 1–3 µm wide, unthickened or almost so, somewhat darkened-refractive; occasionally with microcyclic conidiogenesis; *in vitro* numerous, polymorphous, catenate, in loosely branched chains, small conidia globose, subglobose, or obovoid, 3–8 × 3–4 µm, aseptate, intercalary conidia limoniform to ellipsoid-fusiform, 9–18 × 3.5–4.5 µm, 0–1-septate, large conidia ellipsoid to cylindrical-oblong, 14–30(–38) × 3–6(–7) µm, 0–3-septate, pale to medium brown, asperulate, minutely verruculose to irregularly rough-walled, walls thickened, hila usually short denticle-like, protuberant, truncate, in smaller conidia 0.5–1.8 µm wide, in larger conidia (1.5–)2–3 µm wide, unthickened or almost so but usually darkened-refractive; with occasional microcyclic conidiogenesis.

Characters of the periconioid morph: *Macronematous conidiophores* formed on faded or dead stems in late autumn, winter or after overwintering; colonies at first visible as reddish brown streaks, later turning olivaceous-brown to black, sometimes linear, sometimes encircling the stems, often occupying large stem segments, effuse, densely caespitose, velvety. *Mycelium* immersed, subcuticular

to intraepidermal; hyphae at first sparsely branched, 3–7 µm wide, septate, not constricted at the septa, becoming swollen and wider, up to 11 µm wide, often branched, pale to medium olivaceous-brown, walls thickened, forming loose to dense hyphal aggregations; *in vitro* mycelium immersed to superficial, loosely branched, 2–6(–7) µm wide, pluriseptate, usually without swellings and constrictions, subhyaline to medium brown or olivaceous-brown, almost smooth to asperulate or irregularly rough-walled, in older colonies on PDA up to 10 µm wide, sometimes single hyphal cells distinctly swollen, up to 16(–20) µm wide, mainly at the base of conidiophores, sometimes covered by a slime coat or enveloped in a polysaccharide-like layer. *Stromata* well-developed, large and expanded, up to about 50–320 µm in length, 15–30 µm deep, composed of a single to several layers of swollen pale to medium brown stromatic cells, 5–18 µm diam, thick-walled. *Conidiophores* solitary or in loose groups, arising from swollen hyphal cells or stromata, erumpent through the cuticle, erect, straight, rigid to slightly flexuous, 150–680 µm long, composed of a subcylindrical stipe, 13–24 µm wide at the base, slightly attenuated towards the apex, 5–15 µm just below the branched head, pluriseptate, not constricted at the septa, young conidiophores pale medium olivaceous-brown, later medium to usually dark brown, sometimes slightly paler at the distal end, smooth or almost so, often appearing somewhat granular, roughened, walls distinctly thickened, 1.5–3(–4) µm wide; apex with a roughly subglobose to ovoid head, about 35–70 µm diam, composed of dense branchlets and ramoconidia, primary branchlets close to the apex and below the first and sometimes second and third septa, solitary, in pairs or small verticils, appressed against the stipe or somewhat divergent, subcylindrical to ellipsoid-oval, aseptate, rarely 1-septate, pale olivaceous to dark brown, 10–20 × 5–8.5 µm; *in vitro* conidiophores initially micro- and semimacronematous, then progressively macronematous as colonies age, arising laterally from plagiotropous hyphae or terminally from ascending hyphae, sometimes also from swollen hyphal cells; micronematous conidiophores filiform, narrowly cylindrical-oblong, unbranched, up to 150 µm long, 2–3.5 µm wide, septate, septa often appear to be darkened, pale to pale medium olivaceous-brown, asperulate, walls slightly thickened; semi-macronematous conidiophores often resembling those formed by the leaf-blotching (cladosporioid) morph on the natural host, subcylindrical to cylindrical-oblong, straight to slightly flexuous, unbranched, rarely branched, (10–)15–120 × 3–5(–6) µm, slightly attenuated towards the apex, septate, medium brown, minutely verruculose to irregularly rough-walled, walls more or less thickened; macronematous conidiophores formed in older cultures on SNA, PDA and also MEA (according to McKemy & Morgan-Jones 1991), but more prominent on PDA and MEA, resembling those formed by the stem-rotting morph (i.e., the periconioid morph, in planta), consisting of a long unbranched stipe and a subglobose head, but in culture the heads are often more loosely branched than on the natural substratum, not always forming a compact head, up to 580 µm long, 5–13 µm wide, attenuated towards the apex, 4–8 µm just below the branched upper part, somewhat swollen at the base, septate, medium to very dark brown, minutely verruculose, walls distinctly thickened, two distinct wall layers visible, 1–2 µm thick. *Conidiogenous cells* holoblastic, integrated, terminal, intercalary or even discrete, ellipsoid to cylindrical or doliiform, subdenticulate, proliferation sympodial, multilocal, conidiogenous loci truncate, flat, unthickened, 1–3 µm wide, somewhat darkened-refractive; in culture conidiogenous loci appearing to be somewhat thickened and distinctly darkened-refractive, 1–2.5(–3) µm wide. *Conidia* catenate, in long, branched chains, straight, subglobose, aseptate, 3.5–7 µm diam, or ellipsoid-ovoid, 6–15 × 4–9 µm, 0(–1)-septate, pale olivaceous to olivaceous-brown, smooth to verruculose (under the light microscope), hila flat, truncate, unthickened, (0.5–)1–2(–2.5) µm wide, not darkened, but somewhat refractive; *in vitro* conidia numerous, catenate, formed in long, branched chains, small conidia globose to subglobose, (2–)3–7 × (2–)3–4 µm, aseptate, intercalary ones ellipsoid-ovoid, 6–16 × 3.5–5 µm, 0(–1)-septate, *secondary ramoconidia* ellipsoid to cylindrical-oblong, (13–)15–34(–47) × (3–)4–6(–7) µm, 0–2-septate, sometimes slightly constricted at the septa, medium olivaceous-brown, verruculose or irregularly rough-walled, walls slightly to distinctly thickened, hila more or less protuberant, subdenticulate to denticulate, in small and intercalary conidia 0.5–1(–1.5) µm, in secondary ramoconidia 1–2.5(–3) µm, unthickened or somewhat thickened, darkened-refractive; occasional microcyclic conidiogenesis.

Cultural characteristics: Colonies on PDA at first whitish or smoke grey, reverse smoke-grey to olivaceous-grey, with age smoke-grey to olivaceous or olivaceous-grey, sometimes even dark mouse-grey, reverse iron-grey to dark mouse-grey or black, felty; margin white to smoke-grey, narrow to more or less broad, regular to slightly undulate, glabrous to somewhat feathery; aerial mycelium at first mainly in the colony centre, with age abundantly formed, covering almost the whole colony, whitish, smoke-grey to olivaceous, felty; growth low convex to raised; numerous small exudates formed, sometimes becoming prominent; fertile.

Specimens examined: **Czechoslovakia**, Bohemia, Turnau, on leaves of *Paeonia arborea*, 15 Sep. 1905, J.E. Kabát, Kabát & Bubák, Fungi Imperf. Exs. 396, B 70-6669. **France**, on dead stems of *Paeonia* sp., 1901, ex Herbario Musei Parisiensis, ex herb. Magnus, exs. Desmazières, Pl. Crypt. N. France, Ed. 2, Ser. 1, 1621, HBG, as "*Periconia atra*"; Chailly-en-Biere, Seine-et-Marne, Feuilleaubois, on stems of *P. officinalis*, 27 Mar. 1881, Roumeguère, Fungi Sel. Gall. Exs. 1803, HBG, as "*Periconia atra*". **Germany**, Baden-Würtemberg, Kreis Tübingen, Drusslingen, on leaves of *P. officinalis*, Jun. 1935, Raabe, B 70-6670; Bayern, Freising, on leaves of *P. officinalis*, Sep. 1918, Prof. Dr. J.E. Weiß, Herbarium pathologicum, B 70-6663; Brandenburg, Schloßpark zu Tamsel, on leaves of *P. officinalis*, 15 Aug. 1924, P. Vogel, Sydow, Mycoth. Germ. 2447, M-57751, PH; Triglitz, on leaves of *P. officinalis*, 3 Oct. 1909, Jaap, B 70-6668; Hessen, Frankfurt am Main, botanical garden, on leaves of *P. potaninii*, 7 Oct. 2004, R. Kirschner, HAL, RoKi 2222; Kreis Kassel, Hofgeismar, Garten von Prof. Grupe, on leaves of *P. officinalis*, 3 Sep. 1947, Schulz, B 70-6658; Mecklenburg-Vorpommern, Rostock, neuer botanischer Garten, on leaves of *P. corallina* (= *P. mascula*), 27 Aug. 1950, Becker, B 70-6662; Nordrhein-Westfalen, Duisburg, Dinslake, private garden, on leaves of *P. anomala*, 9 Aug. 2005, N. Ale-Agha, HAL 2014 F; Hamborn, botanical garden, on leaves of *P. obovata*, 10 Aug. 2005, N. Ale-Agha, HAL 2017 F; Essen, botanical garden of the university of Essen, on leaves of *P. mlokosewitschii*, 10 Aug. 2005, N. Ale-Agha, HAL 2013 F; on leaves of *P. officinalis* and *P. suffruticosa*, 11 Aug. 2005, N. Ale-Agha, HAL 2016, 2017 F; Sachsen, Königstein, in Gärten, verbreitet, on leaves of *P. officinalis*, Aug. 1896, W. Krieger, Krieger, Fungi Saxon. Exs. 1545, M-57749; Aug., Sep. 1896, 1915, W. Krieger, Krieger, Schädliche Pilze, B 70-6666, 70-6667; Sachsen-Anhalt, Halle (Saale), Botanical Garden, on leaves of *P. delavayi*, 22 Jun. 2004, K. Schubert, HAL 2011 F, culture deposited at the CBS, CBS 121522 = CPC 11383; on leaves of *P. officinalis*, 22 Jun. 2004, K. Schubert, HAL 2012 F; on stems of *P. officinalis*, 16 Mar. 2005, K. Schubert, **neotype of *Dichocladosporium chlorocephalum* designated here** HAL 1924 F, isoneotype CBS-H 19869, culture ex-neotype CBS 121523 = CPC 11969; on dead stems of *Paeonia* sp., Jan. 1873, G. Winter, Rabenhorst, Fungi Eur. Exs. 1661, HBG, as "*Periconia chlorocephala*"; Thüringen, Fürstlicher Park zu Sondershausen, on leaves of *P. arborea*, 20 Aug. 1903, G. Oertel, Sydow, Mycoth. Germ. 196, PH. **Italy**, Thümen, Herb. Mycol. Oecon. 416, on living leaves of *Paeonia lactiflora* [= *P. edulis*] (M-57753), **lectotype of *"Cladosporium paeoniae"* designated here**; *isolectotypes*: Thümen, Herb. Mycol. Oecon. 416; Padova, on leaves of *P. officinalis*, Aug. 1902, P.A. Saccardo, Saccardo, Mycoth. Ital. 1186, B 70-6660, SIENA; Parma, on leaves of *P. officinalis*, Jul. 1876, Prof. Passerini, Thümen, Mycoth. Univ. 670, B 70-6654, 70-6655, M-57752; Pavia, botanical garden, on leaves of *P. officinalis*, summer 1889, Briosi & Cavara, Fung. Paras. Piante Colt. Utili Ess. 78, M-57748; F. Cavara, Roumeguère, Fungi Sel. Gall. Exs. 5193, mixed infection with *Cladosporium herbarum*, B 70-6656; Siena, Hort. Bot., on leaves of *Paeonia* sp., Nov. 1899, SIENA. **Latvia**, prov. Vidzeme, Kreis Riga, Riga, in a garden, on leaves of *P. foemina* [= *P. officinalis*], 28 Aug. 1936, J. Smarods, Fungi Lat. Exs. 799, M-57747. **New Zealand**, isolated from

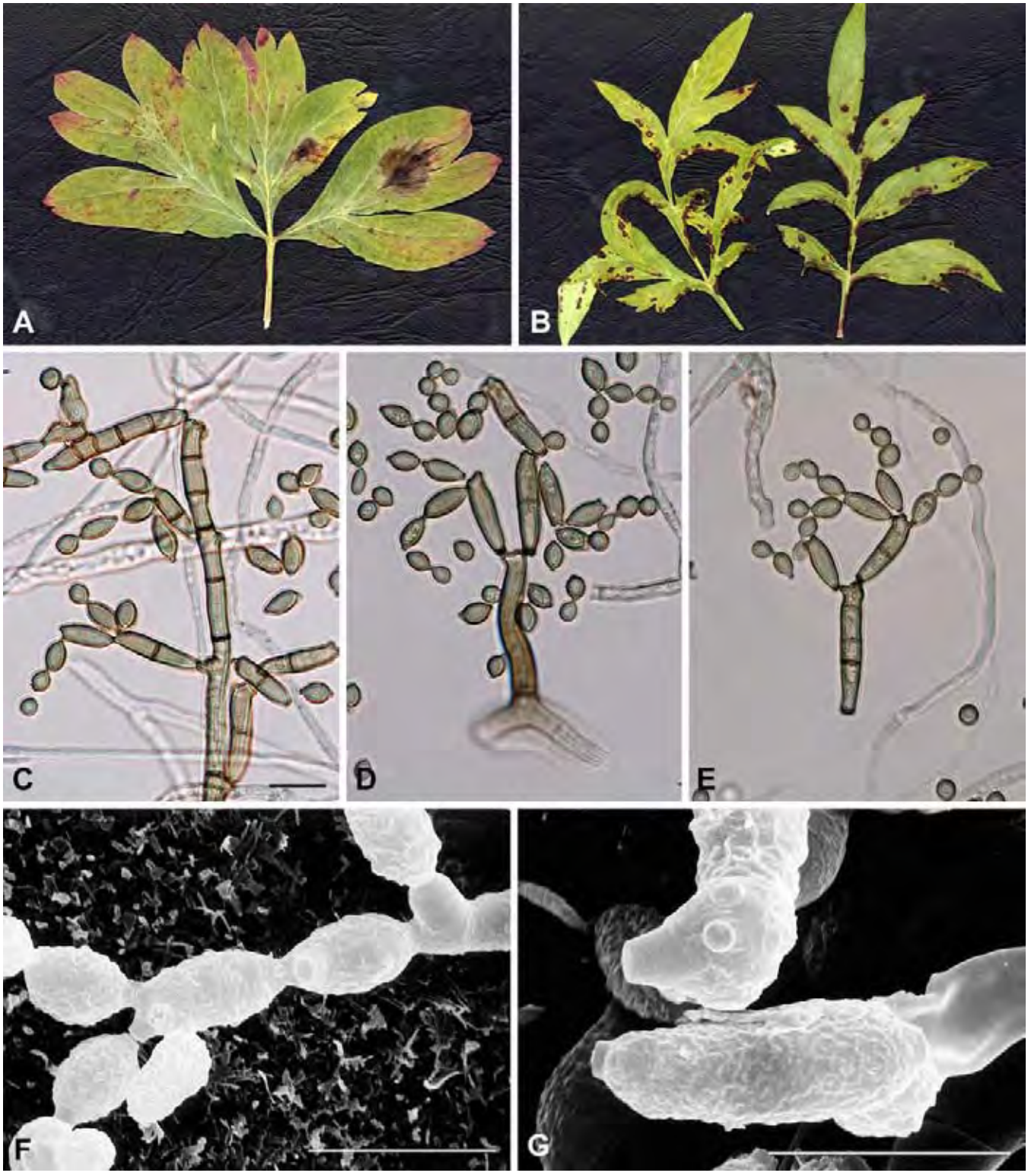

Fig. 5. *Dichocladosporium chlorocephalum* (CBS 121522 = CPC 11383). A–B. Symptoms on leaves of *Paeonia officinalis* and *P. delavayi* caused by the cladosporioid, leaf spotting morph. C–D. Conidiophores and conidia. E. Ramoconidia and conidia. F–G. Scanning electron microscopic photographs. F. Conidial chain still attached to a conidiophore. G. Conidia showing surface ornamentation and scar structure. Scale bars: C–E, G = 10 µm; F = 5 µm.

red leaf and stem lesions on *Paeonia* sp., M. Braithwaite, CBS 100405. **Romania**, Râmnicu-Vâlcea, distr. Vâlcea, Oltenia, on leaves of *P. officinalis*, 17 Aug. 1930, Tr. Săvulescu & C. Sandu, Săvulescu, Herb. Mycol. Roman. 298, M-57742. **U.K.**, England, Cornwall, Lambounce Hill, Perranzubuloe, isolated from dead stems of *Paeonia* sp., isol. F. Rilstone, CBS 213.73 = IMI 048108a. **U.S.A.**, on leaves of *Paeonia* sp., Sep. 1878, Ellis, N. Amer. Fungi 543, B 70-6659, M-57744, PH; Illinois, Cobden, on leaves of *Paeonia* sp., 8 Aug. 1882, F.S. Earle, No. 91, B 70-6657; Kansas, Topeka, on leaves of *P. officinalis*, 7 Jul. 1922, C.F. Menninger, US Dept. Agric., Pathol. Mycol. Coll. 60085, B 70-6661, F; Montana, Columbia, on leaves of *P. officinalis*, Aug. 1886, B.T. Galloway, Ellis & Everh., N. Amer. Fungi Ser. II, 1991, PH; on leaves of *Paeonia* sp., 18 Oct. 1931, W.E. Maneval, F.

Host range and distribution: On *Paeonia anomala, P. arborea, P. delavayi, P. hybrida, P. lactiflora, P. mascula, P. mlokosewitschii, P. moutan, P. obovata, P. obovata* var. *willmotiae, P. officinalis, P. potaninii, P. suffruticosa, Paeonia* spp. (*Paeoniaceae*), Asia (Armenia, China, Georgia, Kazakhstan, Russia), Europe (Belgium,

Czechoslovakia, Denmark, France, Germany, Italy, Latvia, Moldova, Poland, Romania, Switzerland, U.K., Ukraine), North America (Canada, U.S.A.), New Zealand.

Notes: Type material of *Periconia chlorocephala* is not preserved in the herbarium of G. Fresenius at FR (Forschungsinstitut Senkenberg, Frankfurt a. M., Germany). Hence, a new specimen collected in the Botanical Garden of the Martin-Luther-University Halle (Saale), Germany, is proposed to serve as neotype. A culture derived from this collection is deposited at the CBS, Utrecht, the Netherlands as ex-neotype culture. A leaf-blotch sample, also collected in the Botanical Garden at Halle (Saale), from which we also derived a living culture, is designated as representative of the synanamorph, *Cladosporium paeoniae*. Both cultures have been used to generate DNA sequence data.

The two stages (morphs) of this fungus are usually ecologically and seasonally separated, but sometimes conidiophores of the leaf-blotching (cladosporioid) morph also occur on dead stems of peony intermixed with the macronematous conidiophores of the periconioid morph. In culture conidiophore and conidial width tends to be narrower than on the natural substratum, and the conidia are not as frequently septate.

DISCUSSION

Cultural studies by ourselves and McKemy & Morgan-Jones (1991), and molecular sequence analyses documented herein clearly demonstrate that *Cladosporium chlorocephalum*, occurring on necrotic stems, and *C. paeoniae*, causing leaf-blotch symptoms on living leaves of *Paeonia* spp., are two synanamorphs of a single species, which has to be excluded from *Cladosporium s. str.* since the conidiogenous loci are quite distinct from the characteristically coronate scars in the latter genus and because ITS sequences indicate clear separation from *Cladosporium s. str*.

Analysis of the morphology and conidiogenesis showed that the macronematous stage of this fungus (*C. chlorocephalum*, the periconioid morph) closely resembles *Metulocladosporiella*, recently introduced for the Cladosporium speckle disease of banana. There are, however, some differences. In *Metulocladosporiella musae* (E.W. Mason) Crous *et al.*, the type species, micronematous conidiophores occur *in vitro* and *in vivo*, and macronematous conidiophores occur on leaf-spots, whereas in *C. chlorocephalum* the semi-macronematous conidiophores usually accompany leaf-blotch symptoms on living leaves and the macronematous conidiophores occur in saprobic growth on old necrotic stems. Rhizoid hyphae arising from the swollen basal cells of the macronematous conidiophores are characteristic for *M. musae*, but lacking in *C. chlorocephalum*, and the conidia in the latter species are 0–5-septate, but only 0(–1)-septate in *M. musae*. The semi-macronematous, leaf-blotching stage (the cladosporioid morph) is barely distinguishable from the present concept of *Fusicladium*, which includes species with catenate conidia (Schubert *et al.* 2003). However, the peony fungus does not cluster within the *Venturiaceae*. Since *C. chlorocephalum* clusters apart of the *Chaetothyriales*, the clade to which *Metulocladosporiella* belongs, the differences observed here seem to be sufficient to place this fungus in a new genus (also see Crous *et al.* 2007 – this volume). Crous *et al.* (2006a) discussed differences between *Metulocladosporiella* and allied dematiaceous hyphomycete genera and provided a key to the latter genus and morphologically similar genera. Using this key, attempts to determine the macronematous morph of *Cladosporium chlorocephalum* lead to *Metulocladosporiella*. Differences between morphologically similar genera have been discussed in the paper by Crous *et al.* (2006a) and are also valid for the new genus *Dichocladosporium*. *Parapericoniella* U. Braun, Heuchert & K. Schub., a fungicolous genus recently introduced to accommodate *Cladosporium asterinae* Deighton, is also morphologically similar in having apically, densely branched conidiophores and truncate, unthickened conidiogenous loci and hila, but is quite distinct in not having micronematous conidiophores (Heuchert *et al.* 2005).

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Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics

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Abstract: The *Cladosporium herbarum* complex comprises five species for which *Davidiella* teleomorphs are known. *Cladosporium herbarum s. str.* (*D. tassiana*), *C. macrocarpum* (*D. macrocarpa*) and *C. bruhnei* (*D. allicina*) are distinguishable by having conidia of different width, and by teleomorph characters. *Davidiella variabile* is introduced as teleomorph of *C. variabile*, a homothallic species occurring on *Spinacia*, and *D. macrospora* is known to be the teleomorph of *C. iridis* on *Iris* spp. The *C. herbarum* complex combines low molecular distance with a high degree of clonal or inbreeding diversity. Entities differ from each other by multilocus sequence data and by phenetic differences, and thus can be interpreted to represent individual taxa. Isolates of the *C. herbarum* complex that were formerly associated with opportunistic human infections, cluster with *C. bruhnei*. Several species are newly described from hypersaline water, namely *C. ramotenellum*, *C. tenellum*, *C. subinflatum*, and *C. herbaroides*. *Cladosporium pseudiridis* collected from *Iris* sp. in New Zealand, is also a member of this species complex and shown to be distinct from *C. iridis* that occurs on this host elsewhere in the world. A further new species from New Zealand is *C. sinuosum* on *Fuchsia excorticata*. *Cladosporium antarcticum* is newly described from a lichen, *Caloplaca regalis*, collected in Antarctica, and *C. subtilissimum* from grape berries in the U.S.A., while the new combination *C. ossifragi*, the oldest valid name of the *Cladosporium* known from *Narthecium* in Europe, is proposed. Standard protocols and media are herewith proposed to facilitate future morphological examination of *Cladosporium* spp. in culture, and neotypes or epitypes are proposed for all species treated.

Taxonomic novelties: *Cladosporium antarcticum* K. Schub., Crous & U. Braun, sp. nov., *C. herbaroides* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. ossifragi* (Rostr.) U. Braun & K. Schub., comb. nov., *C. pseudiridis* K. Schub., C.F. Hill, Crous & U. Braun, sp. nov., *C. ramotenellum* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. sinuosum* K. Schub., C.F. Hill, Crous & U. Braun, sp. nov., *C. subinflatum* K. Schub., Zalar, Crous & U. Braun, sp. nov., *C. subtilissimum* K. Schub., Dugan, Crous & U. Braun, sp. nov., *C. tenellum* K. Schub., Zalar, Crous & U. Braun sp. nov., *Davidiella macrocarpa* Crous, K. Schub. & U. Braun, sp. nov., *D. variabile* Crous, K. Schub. & U. Braun, sp. nov.
Key words: Clonality, *Davidiella*, homothallism, new species, phylogeny, recombination, taxonomy.

INTRODUCTION

Cladosporium herbarum (Pers. : Fr.) Link, type species of the genus *Cladosporium* Link, is one of the most common environmental fungi to be isolated worldwide. It abundantly occurs on fading or dead leaves of herbaceous and woody plants, as secondary invader on necrotic leaf spots, and has frequently been isolated from air (Samson *et al.* 2000), soil (Domsch *et al.* 1980), foodstuffs, paints, textiles, humans (de Hoog *et al.* 2000) and numerous other substrates. It is also known to occur on old carpophores of mushrooms and other fungi (Heuchert *et al.* 2005) and to be a common endophyte (Riesen & Sieber 1985, Brown *et al.* 1998, El-Morsy 2000), especially in temperate regions. Under favourable climatic conditions *C. herbarum* also germinates and grows as an epiphyte on the surface of green, healthy leaves (Schubert 2005).

Persoon (1794) introduced *C. herbarum* as *Dematium herbarum* Pers., which was later reclassified by Link (1809) as *Acladium herbarum* (Pers.) Link. In 1816, Link included *C. herbarum* together with three additional species in his newly described genus *Cladosporium*. Clements & Shear (1931) proposed *C. herbarum* as lectotype species of the latter genus, a decision followed by de Vries (1952) and Hughes (1958). Several authors provided detailed treatments of *C. herbarum* (de Vries 1952, Ellis 1971, Domsch *et al.* 1980, Prasil & de Hoog 1988), and there are literally thousands of records of this species in the literature. McKemy & Morgan-Jones (1991) and Ho *et al.* (1999) examined *C. herbarum* in culture and published detailed descriptions of its features *in vitro*.

Cladosporium macrocarpum Preuss, a second component within the herbarum complex, has hitherto been known and treated as an allied, but morphologically distinct species on the basis of its wider and somewhat larger, frequently 2–3-septate, more regularly verrucose conidia, shorter conidial chains and more pronounced prolongations of the conidiophores. Dugan & Roberts (1994) carried out examinations of morphological and reproductive aspects of both species, and in so doing demonstrated a morphological continuum between *C. macrocarpum* and *C. herbarum*, concluding that the name *herbarum* should have preference. Therefore, Ho *et al.* (1999) introduced the new combination *C. herbarum* var. *macrocarpum* (Preuss) M.H.M. Ho & Dugan. Although transitional forms have been discussed to occur between the two species, several authors still prefer to retain *C. macrocarpum* as a separate species.

In an attempt to elucidate the species within the *C. herbarum* complex, therefore, a multilocus DNA sequence typing approach was used, employing five genes, namely the internal transcribed spacers of the rDNA genes (ITS), actin, calmodulin, translation elongation factor 1-α, and histone H3. These data were supplemented with morphological examinations under standardised conditions, using light and scanning electron microscopy, as well as cultural characteristics and growth studies.

MATERIAL AND METHODS

Isolates

Isolates included in this study were obtained from the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands, or were freshly isolated from a range of different substrates. Single-conidial and ascospore isolates were obtained using the techniques as explained in Crous (1998) for species of *Mycosphaerella* Johanson and its anamorphs. Isolates

were inoculated onto 2 % potato-dextrose agar (PDA), synthetic nutrient-poor agar (SNA), 2 % malt extract agar (MEA) and oatmeal agar (OA) (Gams *et al.* 2007), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. All cultures obtained in this study are maintained in the culture collection of the CBS (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org).

DNA isolation, amplification and sequence analysis

Fungal colonies were established on agar plates, and genomic DNA was isolated as described in Gams *et al*. (2007). Partial gene sequences were determined as described by Crous *et al*. (2006) for actin (ACT), calmodulin (CAL), translation elongation factor 1-alpha (EF), histone H3 (HIS) and part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer, the 5.8S rRNA gene, the second internal transcribed spacer and the 5' end of the 28S rRNA gene (LSU). The nucleotide sequences were generated using both PCR primers to ensure good quality sequences over the entire length of the amplicon. Subsequent sequence alignment and phylogenetic analysis followed the methods of Crous *et al*. (2006). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as new character states. Sequence data were deposited in GenBank (Table 1) and the alignment and tree in TreeBASE (www.treebase.org).

Data analysis

The number of entities in the dataset of 79 strains was inferred with STRUCTURE v. 2.2 software (Pritchard *et al*. 2000, Falush *et al*. 2003) using an UPGMA tree of data of the ACT gene compared with CAL, EF and HIS with the exclusion of the nearly invariant ITS region. For this analysis group indications were derived from a tree produced with MRAIC (Nylander 2004). The length of the burn-in period was set to 1 000 000, number of MCMC repeats after burn-in 10 000, with admixture ancestry and allele frequencies correlated models, assuming that all groups diverged from a recent ancestral population and that allele frequencies are due to drift. Uniform prior for ALPHA was set to 1.0 (default) and allele frequencies with λ set to 1.0 (default). The numbers of MCMC repetitions after burn-in were set as 10 000 and 100 000. The number of clusters (K) in STRUCTURE was assumed from 5 to 7. Population differentiation F_{ST} (index: θ) was calculated with 1–6 runs using the same software. The null hypothesis for this analysis is no population differentiation. When observed theta (θ) is significantly different from those of random data sets ($p < 0.05$), population differentiation is considered.

Association of multilocus genotypes was screened with the multilocus option in BIONUMERICS v. 4.5. To test for reproductive mode in each population, the standardised index of association (I^S_A; Haubold *et al*. 1998) was calculated with START2 software (Jolley *et al*. 2001). The null hypothesis for this analysis is complete panmixia. The values of I^S_A were compared between observed and randomised datasets. The hypothesis would be rejected when $p < 0.05$. Mean genetic diversity (H) and diversities of individual loci were calculated with LIAN v. 3.5 (Haubold & Hudson 2000). Degrees of recombination or horizontal gene transfer were also visualised using SPLITSTREE v. 4.8 software (Huson & Bryant 2006). Split decomposition was carried out with default settings, i.e., character transformation using uncorrected (observed, "P") distances, splits transformation using "equal angle", and optimise boxes iteration set to 2.

Morphology

As the present study represents the first in a series dealing with *Cladosporium* spp. and their *Davidiella* Crous & U. Braun teleomorphs in culture, a specific, standardised protocol was established by which all species complexes will be treated in future.

Morphology of the anamorph: Microscopic observations were made from colonies cultivated for 7 d under continuous near-ultraviolet light at 25 °C on SNA. Preparations were mounted in Shear's solution (Gams *et al*. 2007). To study conidial development and branching patterns, squares of transparent adhesive tape (Titan Ultra Clear Tape, Conglom Inc., Toronto, Canada) were placed on conidiophores growing in the zone between the colony margin and 2 cm inwards, and mounted between two drops of Shear's solution under a glass coverslip. Different types of conidia are formed by *Cladosporium* species for which different terms need to be adopted. **Ramoconidia** are conidia with usually more than one (mostly 2 or 3) conidial hilum, which typically accumulate at the tip of these conidia. Conidiogenous cells with more than one conidiogenous locus are first formed as apical parts of conidiophores. Such apical

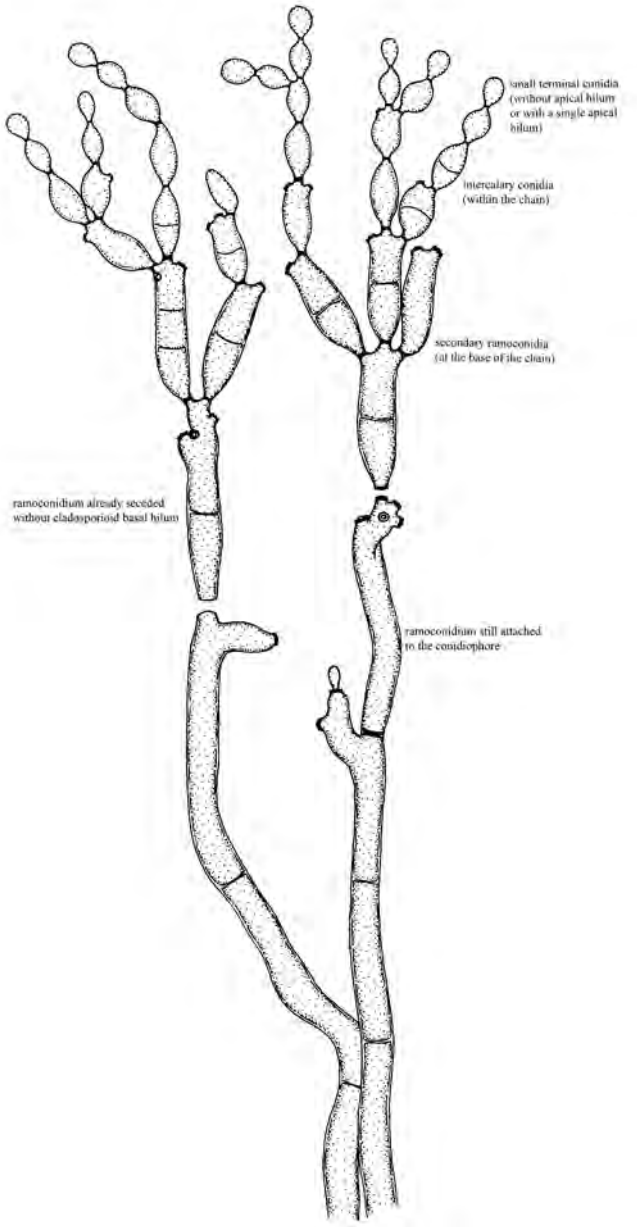

Fig. 1. *Cladosporium* conidiophore with ramoconidia, secondary ramoconidia, intercalary conidia, and small, terminal conidia. Scale bar = 10 µm. K. Schubert del.

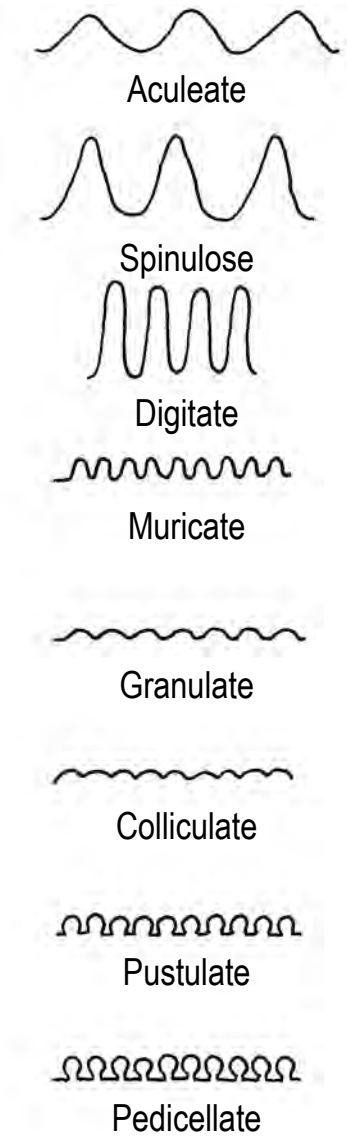

Fig. 2. Terms used to describe conidium wall ornamentation under the cryo-electron microscope. Adapted from David (1997).

parts of conidiophores are called **ramoconidia** if they secede at a septum from the conidiophore (Kirk *et al.* 2001). The septum at which the ramoconidium secedes often appears to be somewhat refractive or darkened. Ramoconidia are characterised by having a truncate, undifferentiated base (thus they lack a differentiated, coronate basal hilum formed in the context of conidiogenesis) and they can be very long, aseptate to sometimes multi-septate. Although they were formed initially as part of the conidiophore, they function as propagules. Only few of the species known until now have the ability to form true ramoconidia. **Secondary ramoconidia** also have more than one distal conidial hilum but they always derive from a conidiogenous locus of an earlier formed cell, which can be either a conidiogenous cell or a ramoconidium. Secondary ramoconidia are often shorter but somewhat wider than ramoconidia; they are often septate, and typically have a narrowed base with a coronate hilum (Fig. 1). Conidia in *Cladosporium* are cells with a coronate basal hilum, which is formed in the context of conidiogenesis and with either a single (when formed as intercalary units in unbranched parts of chains) or without any distal conidial hilum (when formed at the tip of conidial chains). For the first, the term "**intercalary conidium**" and for the latter, "**small terminal conidium**" is used. Intercalary conidia typically are larger and more pigmented and have a more differentiated surface ornamentation than the small terminal conidia. In older literature true ramoconidia were often cited as "ramoconidia *s. str.*", whereas secondary ramoconidia have been referred to as "ramoconidia *s. lat.*"

Morphology of the teleomorph: Teleomorphs were induced by inoculating plates of 2 % tap water agar onto which autoclaved stem pieces of *Urtica dioica* (European stinging nettle) were placed. Inoculated plates were incubated on the laboratory bench for 7 d, after that period they were further incubated at 10 °C in the dark for 1–2 mo to stimulate teleomorph development. Wherever possible, 30 measurements (× 1 000 magnification) were made of conidia and ascospores, with the extremes of spore measurements given in parentheses. Cultural characteristics: Colonies were cultivated on PDA, MEA and OA plates for 14 d at 25 °C in the dark, after which the surface and reverse colours were rated using the charts of Rayner (1970). Linear growth was determined on MEA, PDA and OA plates by inoculating three plates per isolate for each medium, and incubating them for 14 d at 25 ºC, after that period colony diameters were determined.

Low-temperature scanning electron microscopy

Isolates of *Cladosporium* spp. were grown on SNA with 30 g agar/L for 3–4 d at room temperature under black light. Relevant parts of the small colonies with conidiophores and conidia were selected under a binocular, excised with a surgical blade as small agar (3 × 3 mm) blocks, and transferred to a copper cup for snap-freezing in nitrogen slush. Agar blocks were glued to the copper surface with frozen tissue medium (KP-Cryoblock, Klinipath, Duiven, Netherlands) mixed with 1 part colloidal graphite (Agar Scientific, Stansted, U.K.). Samples were examined in a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with an Oxford CT1500 Cryostation for cryo-electron microscopy (cryoSEM). Electron micrographs were acquired from uncoated frozen samples, or after sputter-coating by means of a gold/palladium target for 3 times during 30 s (Fig. 2). Micrographs of uncoated samples were taken at an acceleration voltage of 3 kV, and consisted out of 30 averaged fast scans (SCAN 2 mode), and at 5 kV in case of the coated samples (PHOTO mode).

RESULTS

Phylogeny and differentiation

The manually adjusted alignment contained 80 sequences (including the outgroup sequence) and the five loci were represented by a total of 1 516 characters including alignment gaps which were used in the analysis. Of the 1 516 characters, 369 were parsimony-informative, 259 were variable and parsimony-uninformative, and 888 were constant.

Forty equally most parsimonious trees (TL = 1 933 steps; CI = 0.569; RI = 0.786; RC = 0.447), one of which is shown in Fig. 3, were obtained from the parsimony analysis of the combined genes. Neighbour-joining analysis using three substitution models (uncorrected "p", Kimura 2-parameter and HKY85) on the sequence data yielded trees with identical topologies. These differed from the tree presented in Fig. 3 with regard to the placement of *C. macrocarpum* strain CPC 12054 which was placed as a sister branch to the *C. bruhnei* Linder clade in the distance analyses (results not shown) because it shares an identical CAL sequence. All cryptic species consisting of multiple strains are clustering in well-supported clades with bootstrap support values ranging from 71 % (*C. herbarum*) to 100 % [*e.g. C. ramotenellum* K. Schub.,

Table 1. Isolates subjected to DNA sequence analyses and morphological examinations.

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank numbers[2] (ITS, EF, ACT, CAL, HIS)
Cladosporium antarcticum	—	CBS 690.92* (ex-type)	*Caloplaca regalis*	Antarctica	C. Möller	EF679334, EF679405, EF679484, EF679560, EF679636
Cladosporium bruhnei	*Davidiella allicina*	CBS 134.31 = ATCC 11283	—	Germany	—	EF679335, EF679406, EF679485, EF679561, EF679637
		CBS 157.82	*Quercus robur*	Belgium	—	EF679336, EF679407, EF679486, EF679562, EF679638
		CBS 159.54 = ATCC 36948	Man, skin	The Netherlands	—	EF679337, EF679408, EF679487, EF679563, EF679639
		CBS 161.55	Man, sputum	The Netherlands	—	EF679338, EF679409, EF679488, EF679564, EF679640
		CBS 177.71	*Thuja* tincture	The Netherlands	—	EF679339, EF679410, EF679489, EF679565, EF679641
		CBS 188.54 = ATCC 11290 = IMI 049638 = CPC 3686	—	—	—	AY251077, EF679411, EF679490, EF679566, EF679642
		CBS 366.80	Man, skin	The Netherlands	—	EF679340, EF679412, EF679491, EF679567, EF679643
		CBS 399.80	Man, skin	The Netherlands	—	AJ244227, EF679413, EF679492, EF679568, EF679644
		CBS 521.68	Air	The Netherlands	—	EF679341, EF679414, EF679493, EF679569, EF679645
		CBS 572.78	*Polyporus radiatus*	Russia	V.K. Melnik	DQ289799, EF679415, DQ289866, DQ289831, EF679646
		CBS 813.71	*Polygonatum odoratum*	Czech Republic	—	EF679342, EF679416, EF679494, EF679570, EF679647
		CBS 110024	Industrial water	Germany, Nordrhein-Westfalen	—	EF679343, EF679417, EF679495, EF679571, EF679648
		CBS 115683 = ATCC 66670 = CPC 5101	CCA-treated Douglas-fire pole	U.S.A., New York	C.J. Wang	AY361959, EF679418, AY752193, AY752224, AY752255
		CBS 121624* = CPC 12211 (neotype)	*Hordeum vulgare*	Belgium	J.Z. Groenewald	EF679350, EF679425, EF679502, EF679578, EF679655
		CPC 11386	*Tilia cordata*	Germany, Sachsen-Anhalt	K. Schubert	EF679344, EF679419, EF679496, EF679572, EF679649
		CPC 11840	*Ourisia macrophylla*	New Zealand	A. Blouin	EF679345, EF679420, EF679497, EF679573, EF679650
		CPC 12042 = EXF-389	Hypersaline water from salterns	Slovenia	P. Zalar	EF679346, EF679421, EF679498, EF679574, EF679651
		CPC 12045 = EXF-594	Hypersaline water from salterns	Spain	P. Zalar	EF679347, EF679422, EF679499, EF679575, EF679652
		CPC 12046 = EXF-680	Air conditioning system	Slovenia	P. Zalar	EF679348, EF679423, EF679500, EF679576, EF679653
		CPC 12139	*Hordeum vulgare*	The Netherlands	—	EF679349, EF679424, EF679501, EF679577, EF679654
		CPC 12212	*Hordeum vulgare*	Belgium	J.Z. Groenewald	EF679351, EF679426, EF679503, EF679579, EF679656
		CPC 12921	*Eucalyptus* sp.	Australia	—	EF679352, EF679427, EF679504, EF679580, EF679657
Cladosporium cladosporioides complex	—	CBS 673.69	Air	The Netherlands	—	EF679353, EF679428, EF679505, EF679581, EF679658
	Davidiella sp.	CBS 109082	*Silene maritima*	United Kingdom	A. Aptroot	EF679354, EF679429, EF679506, EF679582, EF679659
	—	CPC 11606	*Musa* sp.	India	M. Arzanlou	EF679355, EF679430, EF679507, EF679583, EF679660
	—	CPC 11609	*Musa* sp.	India	M. Arzanlou	EF679356, EF679431, EF679508, EF679584, EF679661
Cladosporium herbaroides	—	CBS 121626* = CPC 12052 = EXF-1733 (ex-type)	Hypersaline water from salterns	Israel	P. Zalar	EF679357, EF679432, EF679509, EF679585, EF679662
Cladosporium herbarum	*Davidiella tassiana*	CBS 111.82	*Arctostaphylos uva-ursi*	Switzerland	E. Müller	AJ238469, EF679433, EF679510, EF679586, EF679663
		CBS 300.49	*Biscutella laevigata*	Switzerland	J.A. von Arx	EF679358, EF679434, EF679511, EF679587, EF679664
		CBS 121621* = CPC 12177 (epitype)	*Hordeum vulgare*	The Netherlands	—	EF679363, EF679440, EF679516, EF679592, EF679670
		CPC 11600	*Delphinium barbeyi*	U.S.A., Colorado	A. Ramalay	DQ289800, EF679435, DQ289867, DQ289832, EF679665
		CPC 11601	*Delphinium barbeyi*	U.S.A., Colorado	A. Ramalay	EF679359, EF679436, EF679512, EF679588, EF679666
		CPC 11602	*Delphinium barbeyi*	U.S.A., Colorado	A. Ramalay	EF679360, EF679437, EF679513, EF679589, EF679667
		CPC 11603	*Delphinium barbeyi*	U.S.A., Colorado	A. Ramalay	EF679361, EF679438, EF679514, EF679590, EF679668

		CPC 11604	*Delphinium barbeyi*	U.S.A., Colorado	A. Ramalay	EF679362, EF679439, EF679515, EF679591, EF679669
		CPC 12178	*Hordeum vulgare*	The Netherlands	—	EF679364, EF679441, EF679517, EF679593, EF679671
		CPC 12179	*Hordeum vulgare*	The Netherlands	—	EF679365, EF679442, EF679518, EF679594, EF679672
		CPC 12180	*Hordeum vulgare*	The Netherlands	—	EF679366, EF679443, EF679519, EF679595, EF679673
		CPC 12181	*Hordeum vulgare*	The Netherlands	—	EF679367, EF679444, EF679520, EF679596, EF679674
		CPC 12183	*Hordeum vulgare*	The Netherlands	—	EF679368, EF679445, EF679521, EF679597, EF679675
Cladosporium iridis	*Davidiella macrospora*	CBS 107.20	*Iris* sp.	—	—	EF679369, EF679446, EF679522, EF679598, EF679676
		CBS 138.40* (epitype)	*Iris* sp.	The Netherlands	—	EF679370, EF679447, EF679523, EF679599, EF679677
Cladosporium macrocarpum	*Davidiella macrocarpa*	CBS 175.82	Water	Romania	—	EF679371, EF679448, EF679524, EF679600, EF679678
		CBS 223.31 = ATCC 11287	*Mycosphaerella tulasnei*	—	—	AF222830, EF679449, EF679525, EF679601, EF679679
		CBS 299.67	*Triticum aestivum*	Turkey	—	EF679372, EF679450, EF679526, EF679602, EF679680
		CBS 121811* = CPC 12755 (neotype)	*Spinacia oleracea*	U.S.A.	—	EF679376, EF679454, EF679530, EF679606, EF679684
		CPC 11817	*Corylus* sp.	U.S.A.	—	EF679373, EF679451, EF679527, EF679603, EF679681
		CPC 12054 = EXF-2287	Hypersaline water from salterns	Slovenia	P. Zalar	EF679374, EF679452, EF679528, EF679604, EF679682
		CBS H-19855 = CPC 12752 = CBS 121623	*Spinacia oleracea*	U.S.A.	—	EF679375, EF679453, EF679529, EF679605, EF679683
		CPC 12756	*Spinacia oleracea*	U.S.A.	—	EF679377, EF679455, EF679531, EF679607, EF679685
		CPC 12757	*Spinacia oleracea*	U.S.A.	—	EF679378, EF679456, EF679532, EF679608, EF679686
		CPC 12758	*Spinacia oleracea*	U.S.A.	—	EF679379, EF679457, EF679533, EF679609, EF679687
		CPC 12759	*Spinacia oleracea*	U.S.A.	—	EF679380, EF679458, EF679534, EF679610, EF679688
Cladosporium ossifragi	—	CBS 842.91* (epitype)	*Narthecium ossifragum*	Norway	M. di Menna	EF679381, EF679459, EF679535, EF679611, EF679689
		CBS 843.91	*Narthecium ossifragum*	Norway	M. di Menna	EF679382, EF679460, EF679536, EF679612, EF679690
Cladosporium pseudiridis	—	CBS 116463* = LYN 1065 = ICMP 15579 (ex-type)	*Iris* sp.	New Zealand	C.F. Hill	EF679383, EF679461, EF679537, EF679613, EF679691
Cladosporium ramotenellum	—	CBS 121628* = CPC 12043 = EXF-454 (ex-type)	Hypersaline water from salterns	Slovenia	P. Zalar	EF679384, EF679462, EF679538, EF679614, EF679692
		CPC 12047 = EXF-967	Air conditioning system	Slovenia	P. Zalar	EF679385, EF679463, EF679539, EF679615, EF679693
Cladosporium sinuosum	—	CBS 121629* = CPC 11839 = ICMP 15819 (ex-type)	*Fuchsia excorticata*	New Zealand	A. Blouin	EF679386, EF679464, EF679540, EF679616, EF679694
Cladosporium spinulosum	—	CBS 102044	Hypersaline water from salterns	Slovenia	S. Soujak	EF679387, EF679465, EF679541, EF679617, EF679695
		CBS 119907* = CPC 12040 = EXF-334 (ex-type)	Hypersaline water from salterns	Slovenia	P. Zalar	EF679388, EF679466, EF679542, EF679618, EF679696
Cladosporium subinflatum	—	CBS 121630* = CPC 12041 = EXF-343 (ex-type)	Hypersaline water from salterns	Slovenia	P. Zalar	EF679389, EF679467, EF679543, EF679619, EF679697
Cladosporium sp.	—	CBS 172.52 = ATCC 11320	*Carya illinoensis*	U.S.A.	—	EF679390, EF679468, EF679544, EF679620, EF679698
		CBS 113741	Grape berry	U.S.A.	—	EF679391, EF679469, EF679545, EF679621, EF679699
		CBS 113742	Grape berry	U.S.A.	—	EF679392, EF679470, EF679546, EF679622, EF679700
		CBS 113744	Grape bud	U.S.A.	—	EF679393, EF679471, EF679547, EF679623, EF679701
		CPC 12484	*Pinus ponderosa*	Argentina	A. Greslebin	EF679394, EF679472, EF679548, EF679624, EF679702
		CPC 12485	*Pinus ponderosa*	Argentina	A. Greslebin	EF679395, EF679473, EF679549, EF679625, EF679703
Cladosporium subtilissimum	—	CBS 113753	Bing cherry fruits	U.S.A.	—	EF679396, EF679474, EF679550, EF679626, EF679704
		CBS 113754*	Grape berry	U.S.A.	—	EF679397, EF679475, EF679551, EF679627, EF679705
		CPC 12044 = EXF-462	Hypersaline water from salterns	Slovenia	P. Zalar	EF679398, EF679476, EF679552, EF679628, EF679706
Cladosporium tenellum	—	CBS 121634* = CPC 12053 = EXF-1735 (ex-type)	Hypersaline water from salterns	Israel	P. Zalar	EF679401, EF679479, EF679555, EF679631, EF679709

Table 1. (Continued).

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank numbers[2] (ITS, EF, ACT, CAL, HIS)
		CPC 11813	*Phyllactinia* sp. on *Corylus* sp.	U.S.A.	D. Glawe	EF679399, EF679477, EF679553, EF679629, EF679707
		CPC 12051 = EXF-1083	Hypersaline water from salterns	Israel	P. Zalar	EF679400, EF679478, EF679554, EF679630, EF679708
Cladosporium variabile	*Davidiella variabile*	CBS 121636* = CPC 12751 (epitype)	*Spinacia oleracea*	U.S.A.	—	EF679402, EF679480, EF679556, EF679632, EF679710
		CPC 12753	*Spinacia oleracea*	U.S.A.	—	EF679403, EF679481, EF679557, EF679633, EF679711
—	*Davidiella* sp.	CBS 289.49	*Allium schoenoprasum*	Switzerland	E. Müller	AY152552, EF679482, EF679558, EF679634, EF679712
		CBS 290.49	*Trisetum distichophyllum*	Switzerland	E. Müller	EF679404, EF679483, EF679559, EF679635, EF679713

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; EXF: Extremophilic Fungi Culture Collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.

[2]ACT: partial actin gene, CAL: partial calmodulin gene, EF: partial elongation factor 1-alpha gene, HIS: partial histone H3 gene, ITS: internal transcribed spacer region.

*Ex-type cultures.

Zalar, Crous & U. Braun and *C. ossifragi* (Rostr.) U. Braun & K. Schub.]. The intraspecific variation in the *C. bruhnei* clade is due to genetic variation present in the sequence data of all loci except for ITS, those in the *C. macrocarpum* clade in all loci except for ITS and ACT, and those in the *C. herbarum* clade in all loci except for ITS and CAL (data not shown). However, none of the variation for these species could be linked to host specificity or morphological differences. In general, ITS data did not provide any resolution within the *C. herbarum* complex, whereas EF data provided species clades with very little intraspecific variation and ACT, CAL and HIS revealed increasing intraspecific variation (ACT the least and HIS the most).

The mean genetic diversity (H) of the entire data set excluding the nearly invariant ITS region was 0.9307, with little difference between genes (ACT = 0.9257, CAL = 0.9289, EF = 0.9322, HIS = 0.9361). The loci showed different numbers of alleles (ACT: 22, CAL: 16, EF: 21, HIS: 20, ITS: 6). Differentiation of entities when calculated with STRUCTURE software using the admixture/correlated model showed highest value with K = 6. At this value F_{ST} varied between 0.1362 and 0.3381. Linkage disequilibrium calculated using the standardised index of association (I^S_A) for the entire dataset (observed variance V_o = 0.5602, expected variance V_e = 0.2576) was 0.3914 (P = 0.0001), consistent with a small amount of recombination that did not destroy the linkage between alleles. Only few groups appeared to be separated for all alleles; degrees of gene flow are indicated in Fig. 4. SPLITSTREE software produced unresolved star-shaped structures for all genes, without any sign of reticulation (Fig. 5).

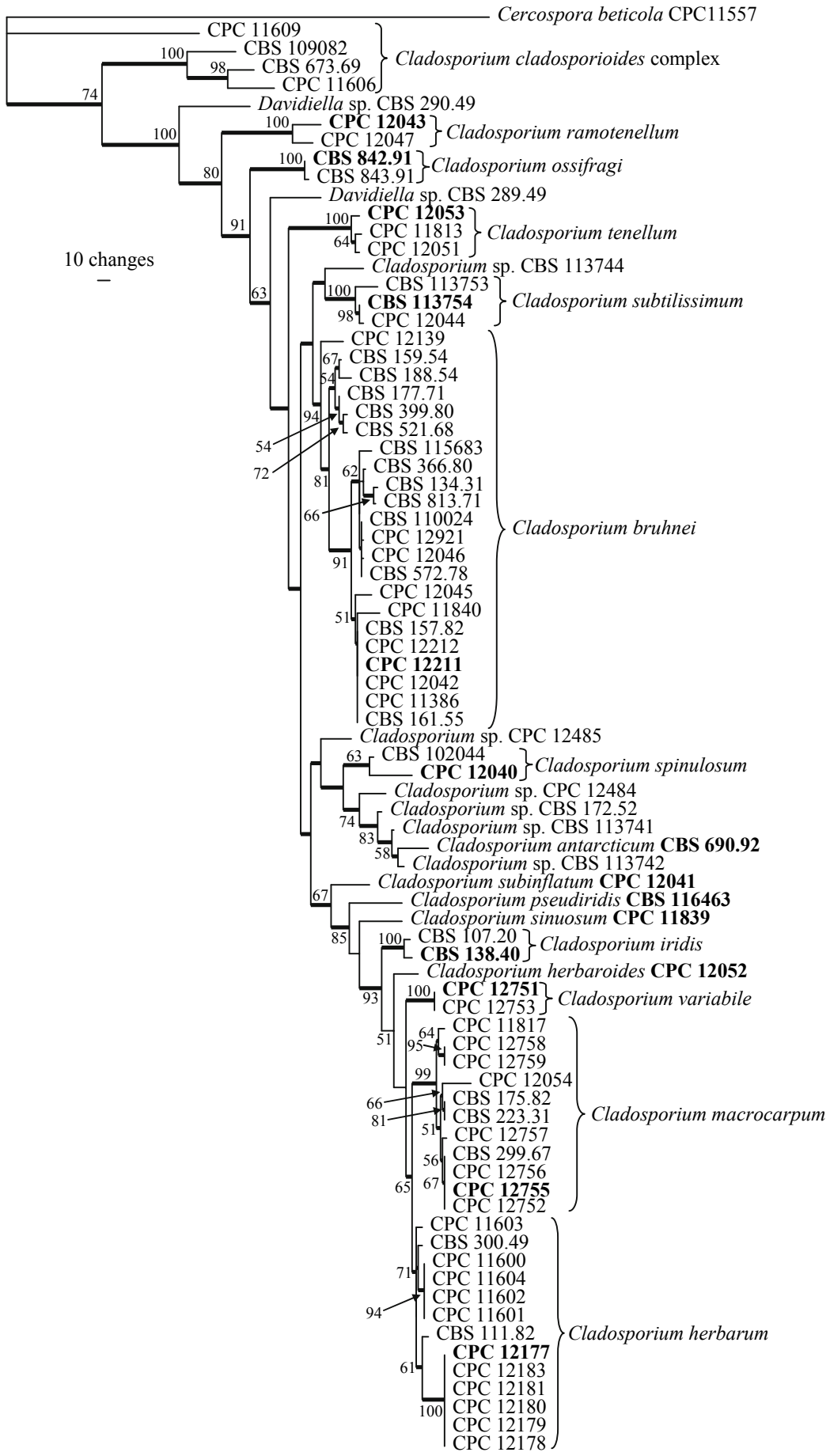

Fig. 3. One of 40 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined sequence alignment (ITS, ACT, CAL, EF, HIS). The scale bar shows ten changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and strain numbers in bold represent ex-type sequences. The tree was rooted to sequences of *Cercospora beticola* strain CPC 11557 (GenBank accession numbers AY840527, AY840458, AY840425, AY840494, AY840392, respectively).

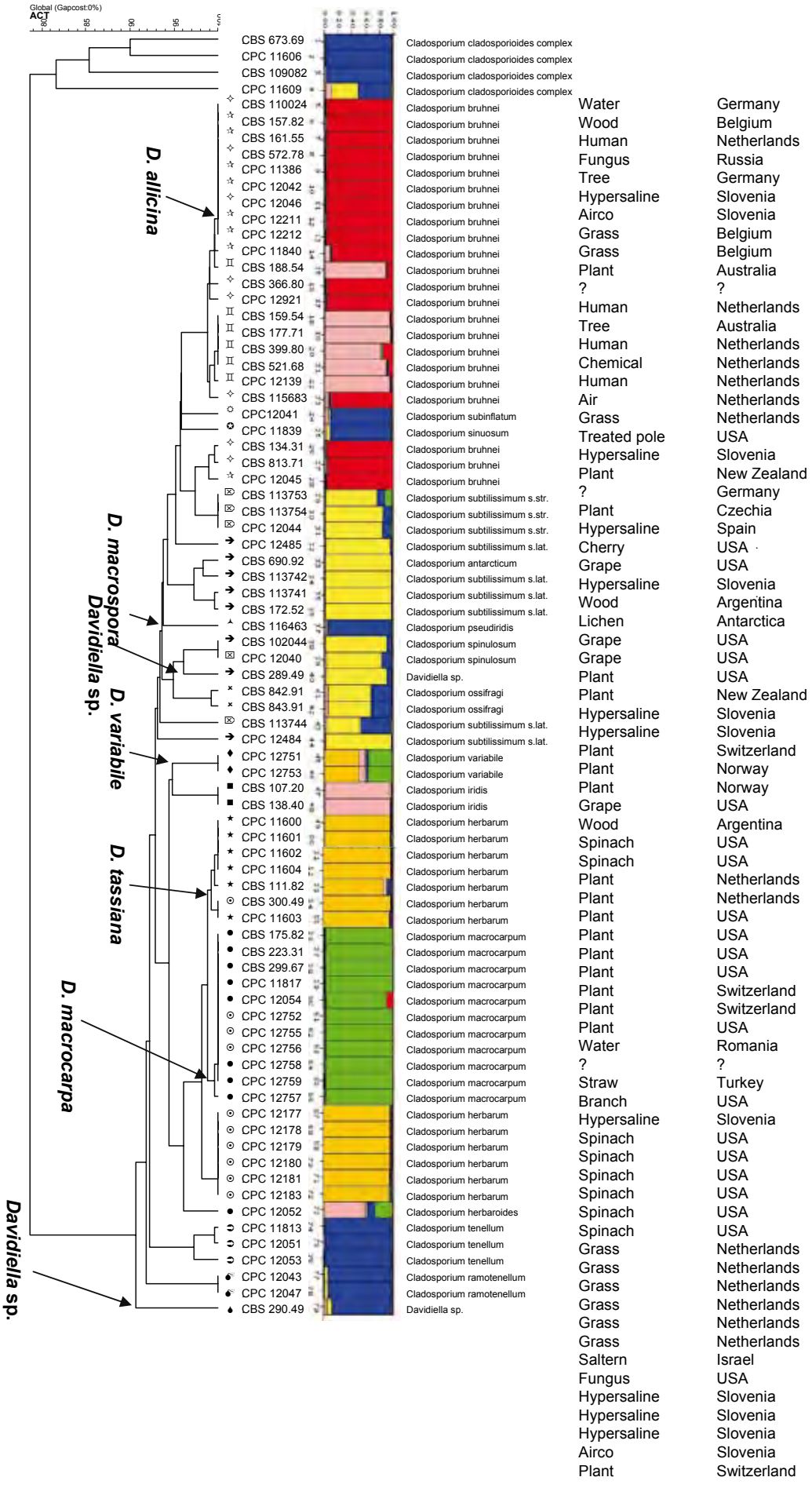

Fig. 4. Distance tree of the *Cladosporium herbarum* complex based on ACT sequence data generated with UPGMA, showing STRUCTURE analysis at K = 6 under admixture model with correlated allele frequencies. Group indications (18) are taken from a tree based on EF sequences with AIC under the HKYG model.

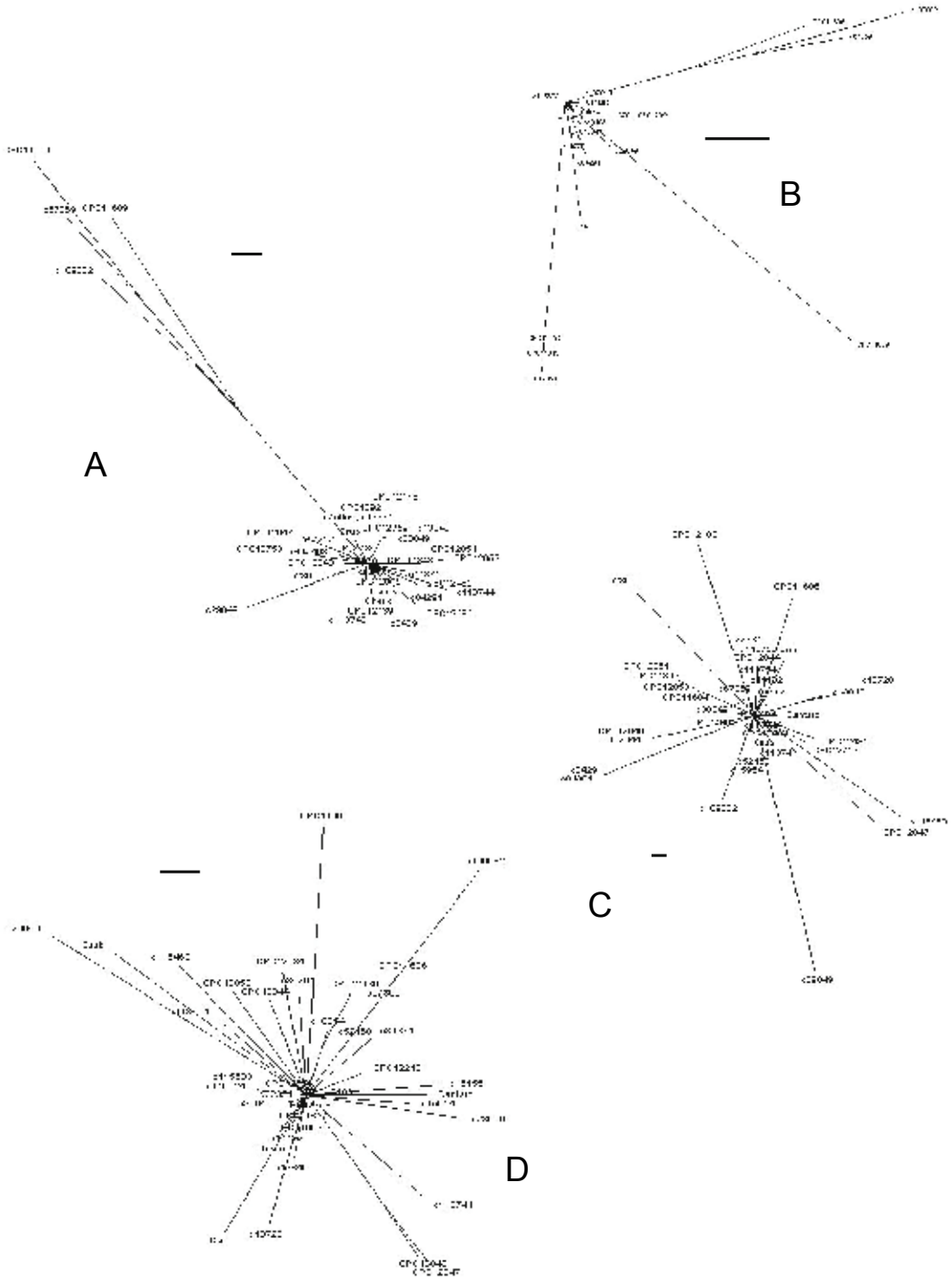

Fig. 5. Split decomposition of the *Cladosporium herbarum* complex using SplitsTree of 16–22 unique alleles obtained from 79 *Cladosporium* isolates for four loci. The star-like structures suggest clonal development. A = ACT, B = CAL, C = HIS, D = EF. Scale bars = 0.01 nucleotide substitutions per site.

Taxonomy

Key to the *Cladosporium* species treated

Morphological features used in the key to distinguish the species treated in this study were determined after 7 d growth at 25 ºC on SNA using light microscopy, and cultural characteristics after 14 d incubation on PDA.

1. Conidia usually smooth, rarely minutely verruculose ..... ***C. cladosporioides*** (species complex)
1. Conidia with different surface ornamentation, minutely to distinctly verruculose, verrucose to echinulate or spiny 2

2. Conidiophores uniform, macronematous; conidia solitary, sometimes formed in short unbranched chains 3
2. Conidiophores both macronematous and micronematous; conidia always catenate, usually formed in branched chains 5

3. Conidiophores due to geniculations often growing zigzag-like, (4–)5–7 µm wide; conidia 9–21 × (5–)6–8 µm, 0–1-septate; conidiogenous loci and conidial hila 1.2–2(–2.2) µm diam ..... ***C. sinuosum***
3. Conidiophores not growing zigzag-like, wider, 6–11 µm; conidia very large and wide, 15–75(–87) × (7–)10–19(–21) µm, often with more septa; conidiogenous loci and hila wider, (2–)2.5–4 µm diam 4

4. Conidia (18–)30–75(–87) × (7–)10–16(–18) µm, (0–)2–6(–7)-septate, walls thickened, especially in older conidia, up to 1 µm thick ..... ***C. iridis***
4. Conidia shorter and wider, 15–55 × (9–)11–19(–21) µm, 0–3-septate, walls distinctly thickened, up to 2 µm, usually appearing zonate ..... ***C. pseudiridis***

5(2) Macronematous conidiophores nodulose or nodose with conidiogenous loci usually confined to swellings 6
5. Macronematous conidiophores non-nodulose or only occasionally subnodulose due to geniculate proliferation, but conidiogenous loci not confined to swellings 11

6. Macronematous conidiophores 3–6 µm wide, swellings 5–11 µm wide 7
6. Macronematous conidiophores somewhat narrower, (1.5–)2.5–5 µm wide, swellings 3–8 µm wide 8

7. Aerial mycelium twisted; conidial septa often distinctly darkened, becoming sinuous with age, apex and base of the conidia often appear to be distinctly darkened; slower growing in culture (29 mm after 14 d on PDA) ..... ***C. variabile***
7. Aerial mycelium not twisted; conidial septa as well as apex and base not distinctly darkened, septa not sinuous with age; faster growing in culture (on average 38 mm after 14 d on PDA) ..... ***C. macrocarpum***

8. Macronematous conidiophores (1.5–)2.5–4.5(–5.5) µm wide, swellings 3–6.5 µm wide; conidia 4–17(–22) µm long, ornamentation variable, but usually densely echinulate, spines up to 0.8 µm long ..... ***C. subinflatum***
8. Macronematous conidiophores slightly wider, 3–5 µm, swellings (4–)5–8(–9) µm wide; conidia longer, up to 25(–35) µm, ornamentation minutely verruculose to verrucose, but not echinulate or spiny 9

9. Conidia formed by macronematous conidiophores 3–33 × (2–)3–6(–7) µm, with age becoming wider, (3.5–)5–9(–11) µm, darker and more thick-walled ..... ***C. herbaroides***
9. Conidia formed by macronematous conidiophores not becoming wider and darker with age, usually up to 7 µm wide 10

10. Conidiophores usually with small head-like swellings, sometimes also with a second intercalary nodule; small terminal conidia 4–9 × 2.5–3.5 µm, secondary ramoconidia and occasionally formed ramoconidia 10–24(–31) × 3–5(–7) µm ..... ***C. bruhnei***
10. Conidiophores with a single or often numerous swellings in short succession giving the stalk a knotty/gnarled appearance; conidia wider, small terminal conidia 4–10 × 3–5(–6) µm, intercalary conidia 6–16 × 4–6 µm, secondary ramoconidia 12–25(–35) × (3–)5–7(–9) µm ..... ***C. herbarum***

11(5) Small terminal and intercalary conidia 4–15 × 3–5 µm, secondary ramoconidia 16–36(–40) × (4–)5–8 µm, 0–3(–4)-septate, ramoconidia absent ..... ***C. ossifragi***
11. Small terminal conidia, ramoconidia and secondary ramoconidia distinctly narrower, 2–5(–6) µm wide, 0–2(–3)-septate 12

12. Mycelium dimorphic, narrow hyphae 1–3 µm wide, hyaline to subhyaline, thin-walled, hyphae of the second type wider, 3.5–8(–9) µm, pale to dark greyish olivaceous or olivaceous-brown, thick-walled, sometimes even two-layered, 1(–1.5) µm thick, hyphae appearing consistently enveloped in polysaccharide-like material or covered by a slime coat; conidiophores usually several times slightly to distinctly geniculate towards the apex, with numerous conidiogenous loci crowded towards the apex, up to 14 per conidiogenous cell ..... ***C. antarcticum***
12. Mycelium not dimorphic, neither enveloped in polysaccharide-like material nor covered by a slime coat; conidiophores usually not geniculate, occasionally only slightly so 13

13. Conidial ornamentation distinctly echinulate, spiny (baculate, digitate or capitate under SEM), spines 0.5–1.3 µm long, loose to moderately dense, conidial hila usually situated on small peg-like prolongations or denticles ..... ***C. spinulosum***
13. Conidial ornamentation different, minutely verruculose to verruculose, conidial hila not situated on peg-like prolongations 14

14. Small terminal conidia narrowly obovoid, limoniform or fusiform, but neither globose nor subglobose; conidiogenous loci and conidial hila 0.5–2(–2.5) µm diam ..... ***C. subtilissimum*** (species complex)
14. Numerous small globose or subglobose terminal conidia formed, also ovoid or limoniform; conidiogenous loci and conidial hila somewhat smaller, 0.5–1.5(–2) µm diam 15

15. Conidiophores usually with numerous conidiogenous loci forming sympodial clusters of pronounced scars at the apex, sometimes up to 10 or even more denticulate loci; conidia 3–20(–28) × 2.5–5(–6) µm, 0–1(–2)-septate, often with several apically crowded hila, up to 7(–9) ..... ***C. tenellum***
15. Conidiophores usually only with few conidiogenous loci, mostly 1–3; conidia longer and narrower, 2.5–35 × 2–4(–5) µm, 0–3-septate, usually with up to three distal conidial hila ..... ***C. ramotenellum***

Key to the *Davidiella* species treated

1. Ascospores frequently wider than 7 µm when mounted in Shear's solution or lactic acid, apical cell obtusely rounded 2
1. Ascospores not wider than 7 µm when mounted in Shear's solution or lactic acid, apical cell acutely rounded, ascospores (20–)25–27 (–30) × (5.5–)6–7 µm ..... ***D. allicina***

2. Pseudoparaphyses prominent; asci frequently >95 µm; ascospores (22–)23–26(–28) × (6–)6.5–7(–8) µm ..... ***D. macrocarpa***
2. Pseudoparaphyses mostly absent in older ascomata; asci <95 µm 3

3. Ascospores (22–)26–30(–35) × (7–)7.5–8(–9) µm; asci wider than 18 µm ..... ***D. variabile***
3. Ascospores (17–)20–23(–25) × (6–)7(–8) µm; asci not wider than 18 µm ..... ***D. tassiana***

Generic concept of the teleomorph

The introduction of the teleomorph genus *Davidiella* was mainly based on phylogenetic studies within the *Mycosphaerellaceae* (Braun *et al.* 2003), where it could be demonstrated that "*Mycosphaerella*" species with *Cladosporium* anamorphs formed a sister clade to *Mycosphaerella* (Crous *et al*. 2000, 2001). Braun *et al*. (2003) transferred five species to *Davidiella* based on prior established anamorph-teleomorph connections, though no details were provided pertaining to morphological differences between *Davidiella* and *Mycosphaerella*. Aptroot (2006) transferred several additional species to *Davidiella*, and distinguished them from true *Mycosphaerella* species by the presence of distinct, irregular cellular inclusions (lumina) in their ascospores. Furthermore, Schoch *et al.* (2006) placed *Davidiella* in a separate family (*Davidiellaceae*) in the *Capnodiales*. During the course of the present study, several fresh specimens of *Davidiella* spp. were collected or induced in culture, making it possible to circumscribe the genus as follows:

Davidiella Crous & U. Braun, Mycol. Progr. 2: 8. 2003, **emend.**

Ascomata pseudothecial, black to red-brown, globose, inconspicuous and immersed beneath stomata to superficial, situated on a reduced stroma, with 1(–3) short, periphysate ostiolar necks; periphysoids frequently growing down into cavity; wall consisting of 3–6 layers of *textura angularis*. *Asci* fasciculate, short-stalked or not, bitunicate, subsessile, obovoid to broadly ellipsoid or subcylindrical, straight to slightly curved, 8-spored. *Pseudoparaphyses* frequently present in mature ascomata, hyaline, septate, subcylindrical. *Ascospores* bi- to multiseriate, hyaline, obovoid to ellipsoid-fusiform, with irregular luminar inclusions, mostly thick-walled, straight to slightly curved; frequently becoming brown and verruculose in asci; at times covered in mucoid sheath. *Cladosporium* anamorph usually produced in culture, but not in all taxa.

Type species: *Davidiella tassiana* (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8. 2003.

Description of *Cladosporium* species

Based on morphological examinations (David 1997) and phylogenetic studies employing DNA sequence data (Crous *et al.* 2000, 2001, 2007 – this volume, Braun *et al.* 2003), the generic concept of the genus *Cladosporium* has been stabilised. *Cladosporium* is confined to *Davidiella* (*Davidiellaceae*, *Capnodiales*) anamorphs with coronate conidiogenous loci and conidial hila consisting of a central convex dome and a raised periclinal rim.

Cladosporium antarcticum K. Schub., Crous & U. Braun, **sp. nov.** MycoBank MB504573. Figs 6–8.

Etymology: Refers to Antarctica, where the fungus was collected.

Differt a Cladosporio licheniphilo conidiophoris saepe non-ramosis, frequentibus geniculatis, angustioribus, (2–)3–4.5 µm, conidiis longioribus et angustioribus, 4–30 × 2.5–5 µm, 0–3-septatis, verruculosis vel verrucosis.

Mycelium immersed and superficial, dimorphic, branched, often with short lateral outgrowths, narrow hyphae 1–3 µm wide, hyaline to subhyaline, thin-walled, hyphae of the second type wider, 3.5–8(–9) µm, pluriseptate, often somewhat constricted at the septa, sometimes swollen, pale to dark greyish olivaceous or olivaceous-brown, smooth or verruculose, thick-walled, sometimes even two-layered (two distinct wall layers visible), 1(–1.5) µm thick, hyphae appearing consistently enveloped in polysaccharide-like material or covered by a slime coat. *Conidiophores* micronematous and macronematous, solitary or in loose groups, arising from plagiotropous or ascending hyphae, terminally or usually laterally. *Macronematous conidiophores* erect to somewhat decumbent, straight to somewhat flexuous or bent, cylindrical, once or several times slightly to distinctly geniculate towards the apex due to sympodial proliferation, unbranched or once branched, up to 120 µm long, 3–4.5 µm wide, sometimes slightly attenuated towards the apex, pluriseptate, up to eight septa, occasionally slightly constricted at the septa, pale to medium or even dark olivaceous-brown or greyish brown, paler towards apices, smooth to somewhat rough-walled, walls thickened but thinner-walled towards apices, sometimes slightly swollen at the base, up to 6 µm wide. *Conidiogenous cells* integrated, terminal and intercalary, once or several times slightly to distinctly geniculate, 10–33 µm long, proliferation sympodial, with several or numerous conidiogenous loci, at first terminal, later turning to one side of the stalk and situated on small lateral shoulders, up to 14 per cell, protuberant, denticulate, 1–1.5(–2) µm diam, thickened and darkened-refractive. *Micronematous conidiophores* as short lateral, peg-like outgrowths with a single apical scar or somewhat longer, occasionally once

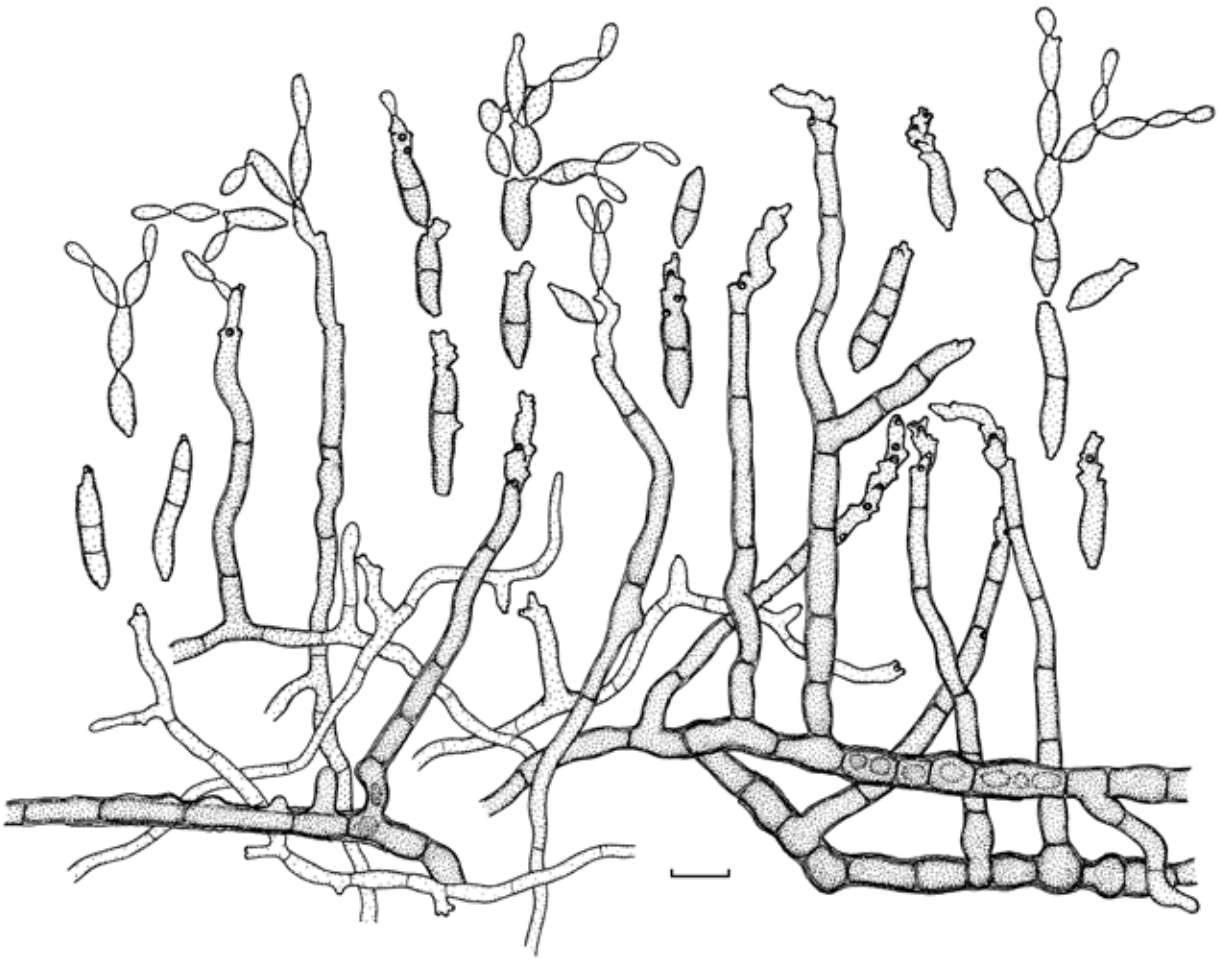

Fig. 6. *Cladosporium antarcticum* (CBS 690.92). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del.*

geniculate with several conidiogenous loci at the apex, 2–22 × 2–3 µm, pale greyish olivaceous, loci denticulate. *Ramoconidia* occasionally occurring, cylindrical, up to 30 µm long, 4–5 µm wide, 0–1-septate, concolorous with the tips of conidiophores, with a broadly truncate, unthickened and not darkened base, without dome and rim, 2.5 µm wide. *Conidia* catenate, in branched chains, straight, small terminal conidia obovoid, limoniform or narrowly ellipsoid, 4–14 × 2.5–4 µm [av. ± SD, 8.5 (± 3.3) × 3.5 (± 0.6)], 0(–1)-septate, secondary ramoconidia ellipsoid to cylindrical, often with several or numerous conidial hila crowded at the distal end, up to 12, 13–30 × 4–5 µm [av. ± SD, 20.1 (± 5.8) × 4.3 (± 0.5) µm], 0–3-septate, sometimes slightly constricted at the median septum, pale olivaceous-brown or greyish brown, minutely verruculose to verrucose (granulate under SEM), walls more or less thickened, rounded or slightly attenuated towards apex and base, hila protuberant, denticulate, 0.8–1.5(–2) µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring.

Cultural characteristics: Colonies on PDA attaining 9 mm diam after 14 d at 25 ºC, greenish olivaceous to grey-olivaceous, at the margin becoming dull green, reverse with a pale olivaceous-grey centre and a broad olivaceous-black margin, margin narrow, regular, entire edge, white, feathery, aerial mycelium sparse but colonies appearing felty, growth flat with somewhat elevated colony centre, prominent exudates not formed, sporulation dense, covering almost the whole colony. Colonies on MEA attaining 12 mm diam after 14 d at 25 ºC, olivaceous-grey to iron-grey, iron-grey reverse, velvety to powdery, aerial mycelium sparse, sporulation profuse. Colonies on OA attaining 4 mm after 14 d at 25 ºC, olivaceous-grey, aerial mycelium sparse, diffuse, growth flat, without prominent exudates, sporulating.

Specimen examined: **Antarctica**, King George, Arctowski, isolated from the lichen *Caloplaca regalis* (*Teloschistaceae*), C. Möller, No. 32/12, 1991, CBS-H 19857, **holotype**, isotype HAL 2024 F, culture ex-type CBS 690.92.

Substrate and distribution: On the lichen *Caloplaca regalis*; Antarctica.

Notes: This is the second genuine lichenicolous species of the genus *Cladosporium*. *Cladosporium licheniphilum* Heuchert & U. Braun, occurring on apothecia of *Pertusaria alpina* in Russia, is quite distinct from *C. antarcticum* by having subcylindrical or only slightly geniculate-sinuous, wider conidiophores, 5–8 µm, with numerous characteristic terminal branches and much shorter, 0–1-septate, smooth conidia, 3.5–13 × 3–7 µm (Heuchert & Braun 2006). *Cladosporium lichenicola* Linds. was invalidly published and *C. arthoniae* M.S. Christ. & D. Hawksw. as well as *C. lichenum* Keissl. are to be excluded from the genus *Cladosporium* since they do not possess the typical cladosporioid scar structure but inconspicuous, unthickened conidiogenous loci and conidial hila (Hawksworth 1979, Heuchert *et al.* 2005). The fungicolous species *C. uredinicola* Speg. and the foliicolous species *C. alneum* Pass. ex K. Schub. and *C. psoraleae* M.B. Ellis are morphologically superficially similar. However, *C. uredinicola*, a widespread fungus on rust fungi, downy mildews and powdery mildew fungi, differs in having somewhat longer and wider, smooth conidia, 3–39 × 2–6.5(–8) µm, and wider conidiogenous loci and conidial hila, 0.5–3

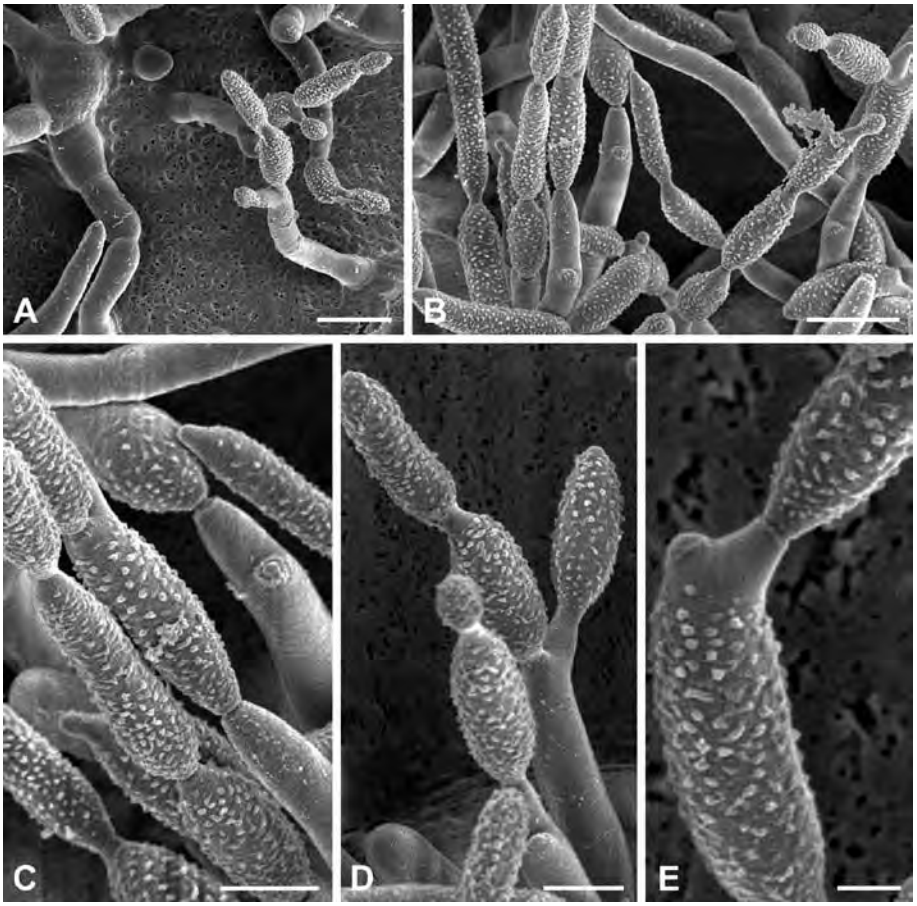

Fig. 7. *Cladosporium antarcticum* (CBS 690.92). A. Overview of the growth pattern on SNA. Note the very large bulbous cells formed at the base of different conidiophores. Other conidiophores sprout from the agar surface. B. Overview of conidiophores and conidia. Note the large distance of the scars on the conidiophore and the different stages of conidial formation on the tips of other conidia. The long secondary ramoconidia are also visible, and sparse aerial hyphae. C. Detail of B with details of the ornamentation and scars. The absence of ornamentation at the apical (spore-forming) end of the secondary ramoconidium is clearly visible. D–E. Tubular structures on coniophore (D) and secondary ramoconidium (E). Scale bars: A–B = 10 µm, C–D = 5 µm, E = 2 µm.

Fig. 8. *Cladosporium antarcticum* (CBS 690.92). A–B. Macronematous conidiophores. C, G. Mycelium enveloped by a polysaccharide-like layer. D, F. Conidia. E. Micronematous conidiophore. H. Ramoconidium with numerous distal scars. Scale bars = 10 µm.

µm (Heuchert *et al.* 2005); *C. alneum*, which causes leaf spots on *Alnus glutinosa*, possesses longer and wider conidiophores, 25–260 × (2–)3–7(–8.5) µm, and somewhat shorter, smooth conidia (Schubert 2005, Schubert *et al.* 2006); and *C. psoraleae*, known from Myanmar on *Psoralea corylifolia*, can easily be distinguished from *C. antarcticum* by its smooth and wider conidia, 3.5–7 µm, and wider conidiogenous loci and conidial hila, 1–3 µm diam (Ellis 1972, Schubert 2005).

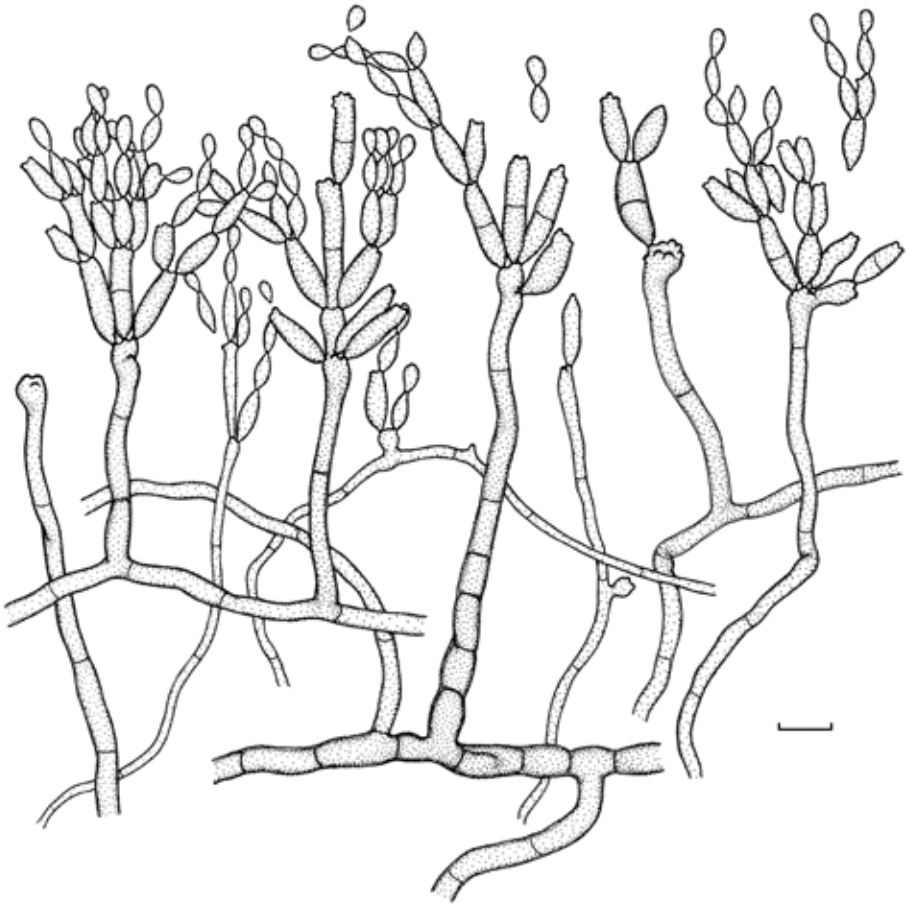

Fig. 9. *Cladosporium bruhnei* (CPC 12211). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del.*

Cladosporium bruhnei Linder, Bull. Natl. Mus. Canada 97: 259. 1947. Figs 9–12.

≡ *Hormodendrum hordei* Bruhne, in W. Zopf, Beitr. Physiol. Morph. nied. Org. 4: 1. 1894, non *C. hordei* Pass., 1887.

≡ *Cladosporium herbarum* (Pers.: Fr.) Link var. (δ) *cerealium* Sacc. f. *hordei* (Bruhne) Ferraris, Flora Ital. Crypt., Pars I, Fungi, Fasc.13: 882. 1914.

≡ *Cladosporium hordei* (Bruhne) Pidopl., Gribnaja Flora Grubych Kormov: 268. 1953, *nom. illeg.*, homonym, non *C. hordei* Pass., 1887.

Teleomorph: ***Davidiella allicina*** (Fr. : Fr.) Crous & Aptroot, in Aptroot, *Mycosphaerella* and its anamorphs: 2. Conspectus of *Mycosphaerella*. CBS Biodiversity Ser. 5: 30. 2006.

Basionym: *Sphaeria allicina* Fr., Kongl. Vetensk. Acad. Handl. 38: 247. 1817, sactioned by Fr., Syst. Mycol. 2: 437. 1823.

≡ *Sphaerella allicina* (Fr. : Fr.) Auersw., in Gonn. & Rabenh., Mycol. Europaea 5–6: 19. 1869.

Ascomata pseudothecial, black, superficial, situated on a small stroma, globose, up to 250 µm diam; ostioles periphysate, with apical periphysoids present; wall consisting of 3–6 layers of reddish brown *textura angularis*. *Asci* fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 65–90 × 16–25 µm; with pseudoparenchymatal cells of the hamathecium persistent. *Ascospores* tri- to multiseriate, overlapping, hyaline, with irregular lumina, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse basal end, and acutely rounded apical end, widest near the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, (20–)25–27(–30) × (5.5–)6–7 µm.

Mycelium superficial, hyphae branched, 1.5–8 µm wide, pluriseptate, broader hyphae usually slightly constricted at the septa and somewhat swollen, hyaline to subhyaline, almost smooth to somewhat verruculose or irregularly rough-walled, sometimes appearing to have a slime coat, walls unthickened. *Conidiophores* macronematous, sometimes also micronematous, arising as lateral or terminal branches from plagiotropous or ascending hyphae, erect, straight to more or less flexuous, sometimes geniculate, nodulose, usually with small head-like swellings, sometimes also with intercalary nodules, sometimes swellings protruding and elongated to one side, unbranched, occasionally branched, (7–)20–330 µm, sometimes even longer, (2–)3–5 µm wide, swellings (4–)5–8 µm wide, pluriseptate, not constricted at the septa, septa sometimes not very conspicuous, subhyaline to pale brown or pale olivaceous, smooth or somewhat verruculose, walls unthickened or almost so, more thickened with age. *Conidiogenous cells* integrated, usually terminal, cylindrical with a terminal head-like swelling, sometimes with a second swelling, 15–40 µm long, proliferation sympodial, with few conidiogenous loci confined to swellings, up to five per swelling, loci protuberant, conspicuous, 1–2 µm diam, thickened and darkened-refractive. *Conidia* catenate, formed in branched chains, straight to slightly curved, small terminal conidia subglobose, ovoid to obovoid or somewhat limoniform, 4–9 × 2.5–3.5 µm [av. ± SD, 6.5 (± 1.5) × 3.1 (± 0.5) µm], aseptate; secondary ramoconidia and occasionally formed ramoconidia ellipsoid to subcylindrical or cylindrical, 10–24(–31) × 3–5(–7) µm [av. ± SD, 16.1 (± 4.1) × 4.1 (± 0.8) µm], rarely up to 40 µm long, 0–1(–3)-septate, very rarely 5-septate, subhyaline to pale brown or pale olivaceous, minutely verruculose to verrucose (mostly granulate with some muricate projections under SEM), walls unthickened or almost so, apex rounded or slightly attenuated towards apex and base, hila protuberant, conspicuous, 1–2 µm wide, up to 1 µm high, thickened and darkened-refractive; microcyclic conidiogenesis occurring.

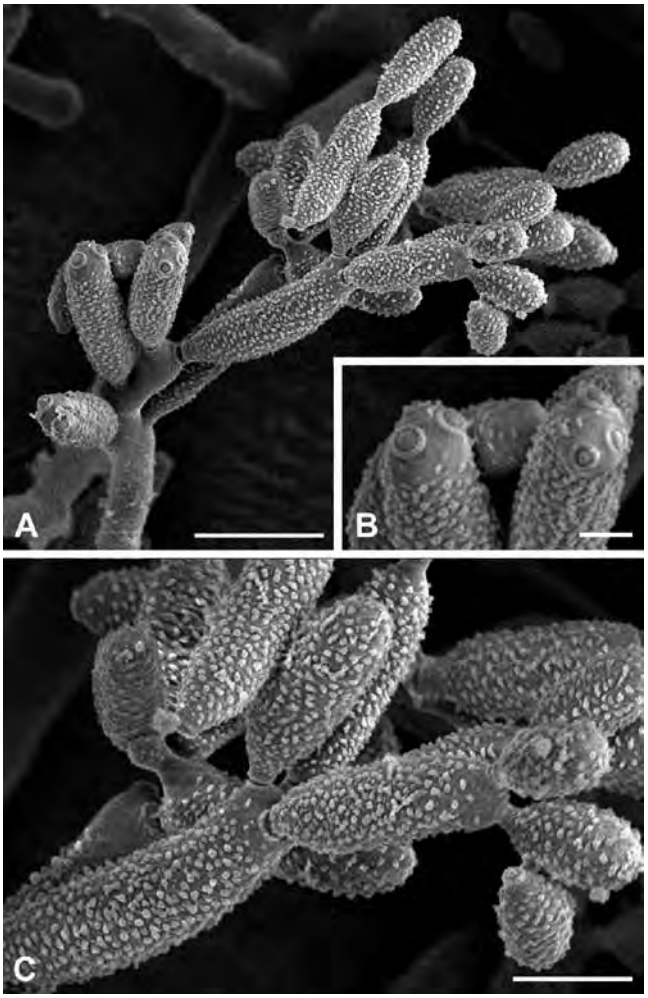

Fig. 10. *Cladosporium bruhnei* (CPC 12211). A. Conidiophore with characteristic long secondary ramoconidium and complex conidiophore. B. Detail of hila on secondary ramoconidia. C. Details of prominent ornamentation on conidia. Scale bars: A = 10 µm, B = 2 µm, C = 5 µm.

Cultural characteristics: Colonies on PDA reaching 22–32 mm diam after 14 d at 25 ºC, olivaceous-grey to iron-grey, sometimes whitish, smoke-grey to pale olivaceous due to abundant aerial mycelium covering almost the whole colony, with age collapsing becoming olivaceous-grey, occasionally zonate, velvety to floccose, margin narrow, entire edge, white, glabrous to somewhat feathery, aerial mycelium sparse to abundant, white, fluffy, growth regular, flat to low convex, sometimes forming few exudates in the colony centre, sporulating. Colonies on MEA reaching 21–32 mm diam after 14 d at 25 ºC, grey-olivaceous, olivaceous-grey to dull green or iron-grey, sometimes whitish to pale smoke-grey due to abundant aerial mycelium, olivaceous-grey to iron-grey reverse, velvety, margin narrow, entire edge to slightly undulate, white, radially furrowed, glabrous to slightly feathery, aerial mycelium sparse to abundant, mainly in the centre, white, fluffy, growth convex to raised, radially furrowed, distinctly wrinkled in the colony centre, without prominent exudates, sporulating. Colonies on OA reaching 20–32 mm diam after 14 d at 25 ºC, smoke-grey, grey-olivaceous to olivaceous-grey, greenish black or iron-grey reverse, margin narrow, entire edge, colourless to white, glabrous, aerial mycelium sparse to abundant, dark smoke-grey, diffuse, high, later collapsed, felty, growth flat, without prominent exudates, sporulation profuse.

Specimens examined: *Sine loco et dato*, CBS 188.54 = ATCC 11290 = IMI 049638. **Australia**, N.S.W., Barrington Tops National Park, isolated from leaves of *Eucalyptus stellulata* (*Myrtaceae*), 3 Jan. 2006, B. Summerell, CPC 12921. **Belgium**, isolated from *Quercus robur* (*Fagaceae*), CBS 157.82; Kampenhout, isolated from *Hordeum vulgare* (*Poaceae*), 26 June 2005, J.Z. Groenewald, CBS-H 19856, **neotype designated here** of *C. bruhnei*, isoneotype HAL 2023 F, cultures ex-type CBS 121624 = CPC 12211, CPC 12212. **Czech Republic**, Lisen, isolated from *Polygonatum odoratum* (*Liliaceae*), CBS 813.71, albino mutant of CBS 812.71. **Germany**, CBS 134.31 = ATCC 11283 = IMI 049632; Nordrhein-Westfalen, Mühlheim an der Ruhr, isolated from industrial water, IWW 727, CBS 110024; Sachsen-Anhalt, Halle (Saale), Robert-Franz-Ring, isolated from leaves of *Tilia cordata* (*Tiliaceae*), 2004, K. Schubert, CPC 11386. **Netherlands**, isolated from air, CBS 521.68; isolated from *Hordeum vulgare*, 1 Jan. 2005, P.W. Crous, CPC 12139; isolated from man, skin, CBS 159.54 = ATCC 36948; Amsterdam, isolated from Thuja tincture, CBS 177.71; Geleen, St. Barbara Ziekenhuis, isolated from man, skin, CBS 366.80, CBS 399.80; isolated from man, sputum, Aug. 1955, CBS 161.55. **New Zealand**, Otago, Lake Harris, isolated from *Ourisia macrophylla* (*Scrophulariaceae*), 30 Jan. 2005, A. Blouin, Hill 1135, CPC 11840. **Russia**, Moscow region, isolated from *Polyporus radiatus* (*Polyporaceae*), Oct. 1978, CBS 572.78 = VKM F-405. **Slovenia**, Ljubljana, isolated from an air conditioning system, 2004, M. Butala, EXF-680 = CPC 12046; Sečovlje, isolated from hypersaline water from salterns (reserve pond), 2005, P. Zalar, EXF-389 = CPC 12042. **Spain**, Ebro Delta, isolated from hypersaline water from salterns (crystallisation pond), 2004, P. Zalar, EXF-594 = CPC 12045. **Sweden**, Skåne, on tip blight of living leaves of *Allium* sp. (*Alliaceae*), Fr. no. F-09810, UPS-FRIES, **holotype** of *Davidiella allicina*. **U.S.A.**, New York, Geneva, isolated from CCA-treated Douglas-fir pole, CBS 115683 = ATCC 66670 = CPC 5101.

Substrate and distribution: Living and decaying plant material, man, air, hypersaline and industrial water; widespread.

Literature: Saccardo (1899: 1076), Linder (1947: 289).

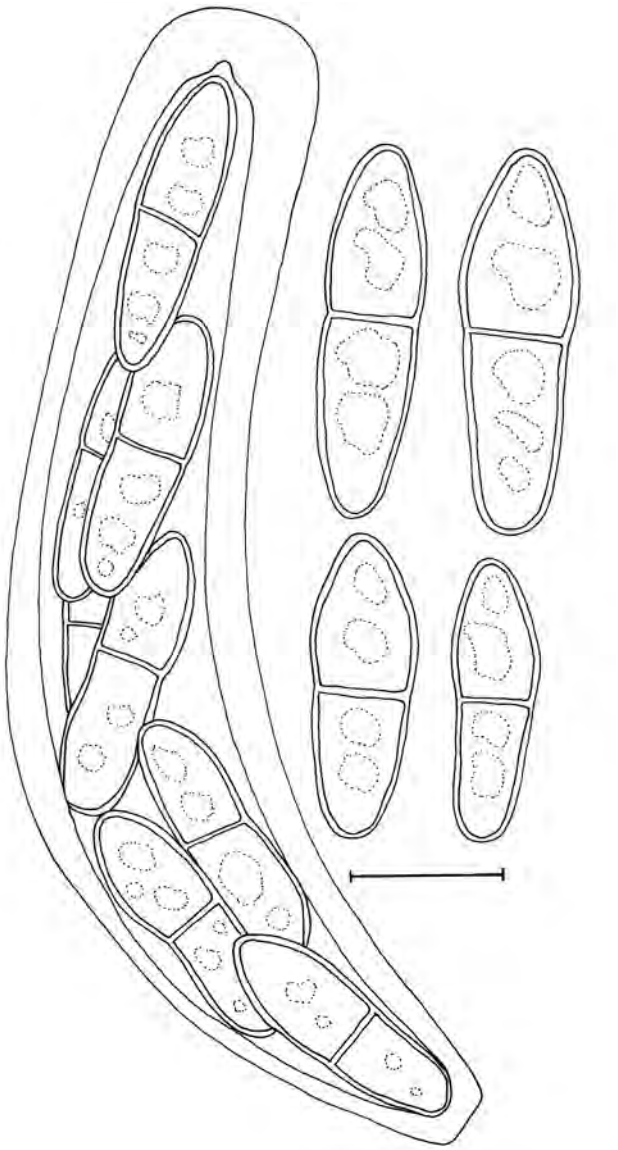

Fig. 11. *Davidiella allicina* (F-09810, UPS-FRIES, holotype). Ascus and ascospores. Scale bar = 10 µm. P.W. Crous *del*.

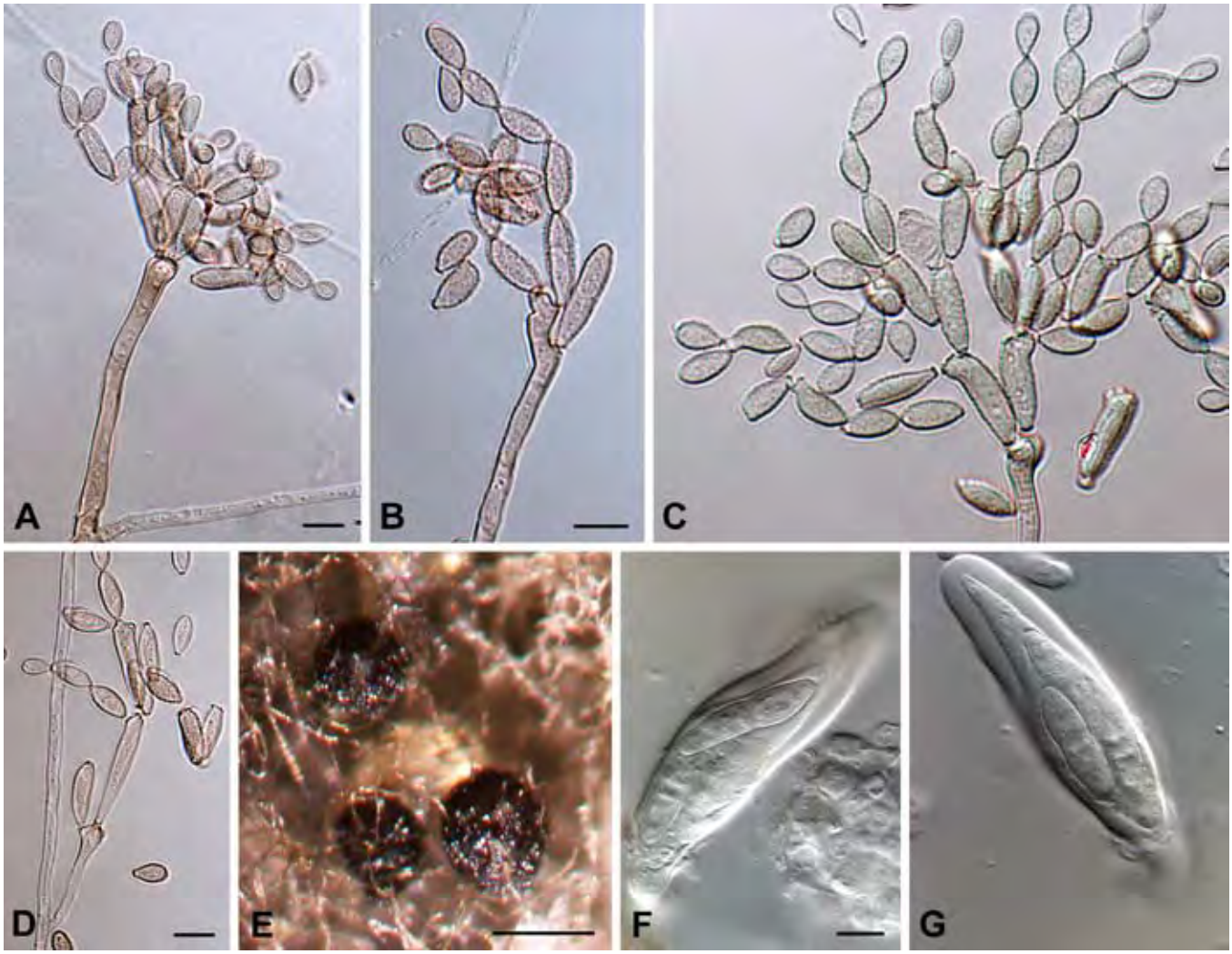

Fig. 12. *Cladosporium bruhnei* (CPC 12211) and its teleomorph *Davidiella allicina*. A–B. Macronematous conidiophores. C. Conidial chains. D. Micronematous conidiophore. E. Ascomata of the teleomorph formed on the host. F–G. Asci. Scale bars: A–B, D, F = 10 µm, E = 200 µm.

Notes: *Cladosporium bruhnei* proved to be an additional component of the herbarum complex. The species resembles *C. herbarum s. str.* as already stated by Linder (1947), but possesses consistently narrower conidia, usually 2.5–5 µm wide, and the conidiophores often form only a single apical swelling. The species was described by Bruhne (*l.c.*) as *Hormodendrum hordei* from Germany but type material could not be located. Linder (1947) examined No. 1481a-5 (Canada, N. Quebec, Sugluk, on *Elymus arenarius* var. *villosus*, 31 Jul. 1936, E. Meyer), presumably in the National Museum, and stated that this specimen agreed well with the description and illustration given by Bruhne (*l.c.*). Although the species occurs on numerous substrates and is widely distributed, it has not yet been recognised as a distinct species since it has probably been interpreted as a narrow variant of *C. herbarum*.

Based on morphology and DNA sequence data, the CBS strain CBS 177.71 chosen by Prasil & de Hoog (1988) as representative living strain of *C. herbarum*, rather clusters together with isolates of *C. bruhnei*. The strain CBS 813.71 is an albino mutant of the latter species as it does not appear to contain colour pigment. Furthermore, all isolates from humans treated until now as *C. herbarum* proved to be conspecific with the narrow-spored *C. bruhnei*.

Although *Davidiella tassiana* (ascospores 17–25 × 6–8.5 µm, RO) was treated as synonymous to *D. allicina* (ascospores 20–27 × 6–7 µm, UPS) in Aptroot (2006), they differ in apical ascospore taper, with ascospores of *D. allicina* being acutely rounded, while those of *D. tassiana* are obtusely rounded. The same ascospore taper was also observed in the teleomorph of *C. bruhnei*, and thus the name *D. allicina* is herewith linked to *C. bruhnei*, which is distinct from *C. herbarum*, having *D. tassiana* as teleomorph.

Cladosporium herbaroides K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504574. Figs 13–15.

Etymology: Refers to its morphological similarity to *Cladosporium herbarum*.

Differt a Cladosporio herbaro conidiis polymorphis, 3–33 × (2–)3–6(–7) µm, postremo latioribus, (3.5–)5–9(–11) µm, fuscis et crassitunicatis; et a Cladosporio macrocarpo conidiophoris leniter angustioribus, 3–5 µm latis, nodulis angustioribus, 5–8 µm latis.

Mycelium branched, (1–)2–8 µm wide, septate, often with small swellings and constrictions, subhyaline to pale brown or pale olivaceous-brown, smooth or almost so to somewhat verruculose, walls unthickened or almost so. *Conidiophores* macronematous and micronematous, arising lateral from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to slightly flexuous, often geniculate, nodulose, with unilateral or multilateral swellings, often numerous swellings in short succession giving them a gnarled appearance, often forming somewhat protruding or prolonged lateral swellings or a branch-

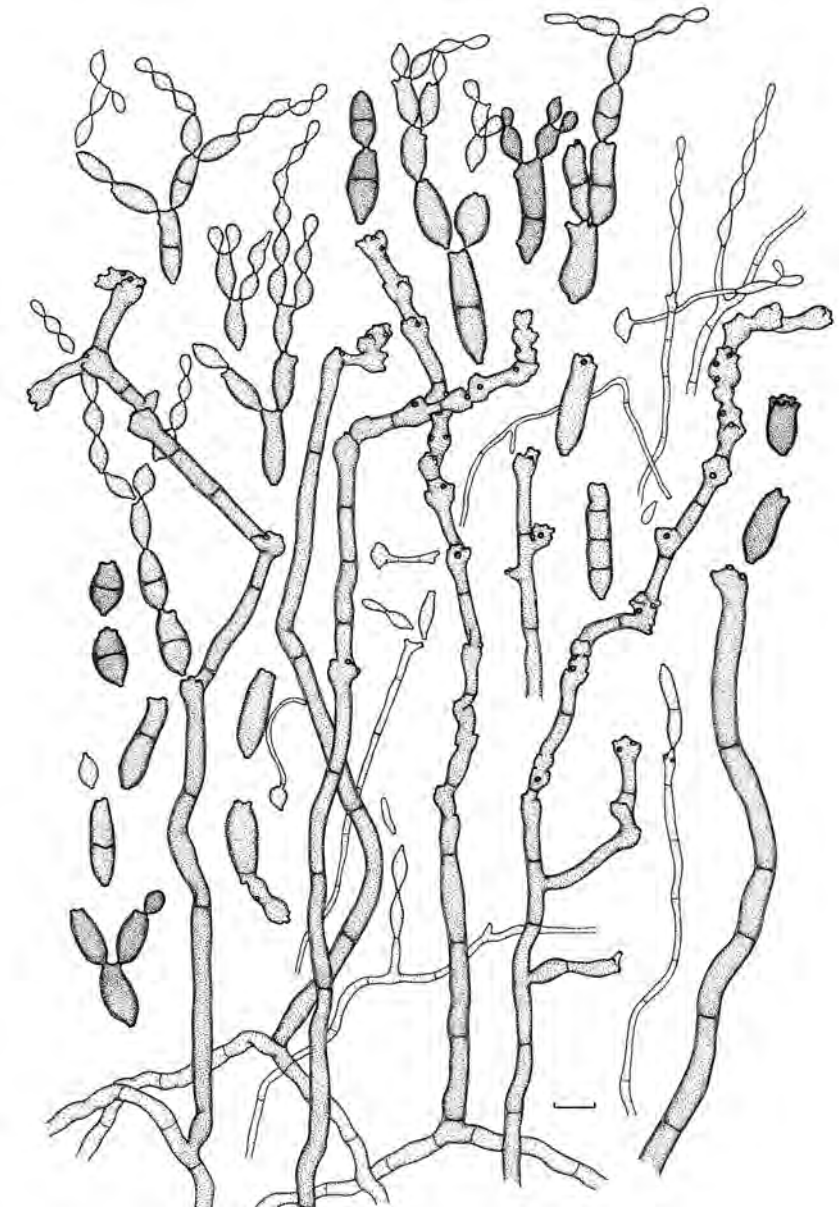

Fig. 13. *Cladosporium herbaroides* (CPC 12052). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

like prolongation below the terminal swelling (due to sympodial proliferation), unbranched or sometimes branched, 30–230 µm long or even longer, 3–5 µm wide, swellings 5–8 µm wide, septate, not constricted at septa, pale to medium olivaceous-brown, smooth or almost so, walls slightly thickened. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, usually nodulose to nodose forming distinct swellings, sometimes geniculate, 15–55 µm long, with numerous conidiogenous loci usually confined to swellings or situated on small lateral shoulders, sometimes on the top of short peg-like prolongations or denticles, loci protuberant, 1–2 µm diam, thickened and darkened-refractive. *Micronematous conidiophores* much shorter, narrower, paler, neither nodulose nor geniculate, arising laterally from plagiotropous hyphae, often only as short lateral denticles or branchlets of hyphae, erect, straight, conical to cylindrical, unbranched, 3–65 × 2–3 µm, mostly aseptate, sometimes up to five septa, subhyaline, smooth, walls unthickened. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, conidiogenous loci solitary or sometimes as sympodial clusters of pronounced denticles, protuberant, 1–1.5 µm diam, thickened and somewhat darkened-refractive. *Conidia* polymorphous, two main morphological types recognisable, formed by the two different types of conidiophores, conidia formed by macronematous conidiophores catenate, in branched chains, straight to slightly curved, subglobose, obovoid, limoniform, ellipsoid to cylindrical, 3–33 × (2–)3–6(–7) µm [av. ± SD, 14.5 (± 7.9) × 5.2 (± 1.2) µm], 0–2(–3)-septate, sometimes slightly constricted at septa, septa median or somewhat in the lower half, pale to medium olivaceous-brown, verruculose to verrucose (granulate under SEM), walls slightly thickened, with up to three rarely four distal scars, with age becoming medium or even dark brown (chocolate brown), wider and more thick-walled, 5.5–33 × (3.5–)5–9(–11) µm [av. ± SD, 14.4 (± 6.9) × 7.2 (± 1.9) µm], walls up to 1 µm thick, hila protuberant, 0.8–2(–2.5) µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring. *Conidia* formed by micronematous conidiophores paler and narrower, mostly formed in unbranched chains, sometimes in branched chains with up to three distal hila, straight to slightly curved, limoniform, narrowly fusiform, almost filiform to subcylindrical, 10–26(–35) × 2–3.5 µm [av. ± SD, 15.6 (± 6.2) × 2.9 (± 0.5) µm], 0–1(–3)-septate, subhyaline to pale brown, almost smooth to minutely verruculose, walls unthickened, hila protuberant, 1–1.5 µm diam, thickened and somewhat darkened-refractive.

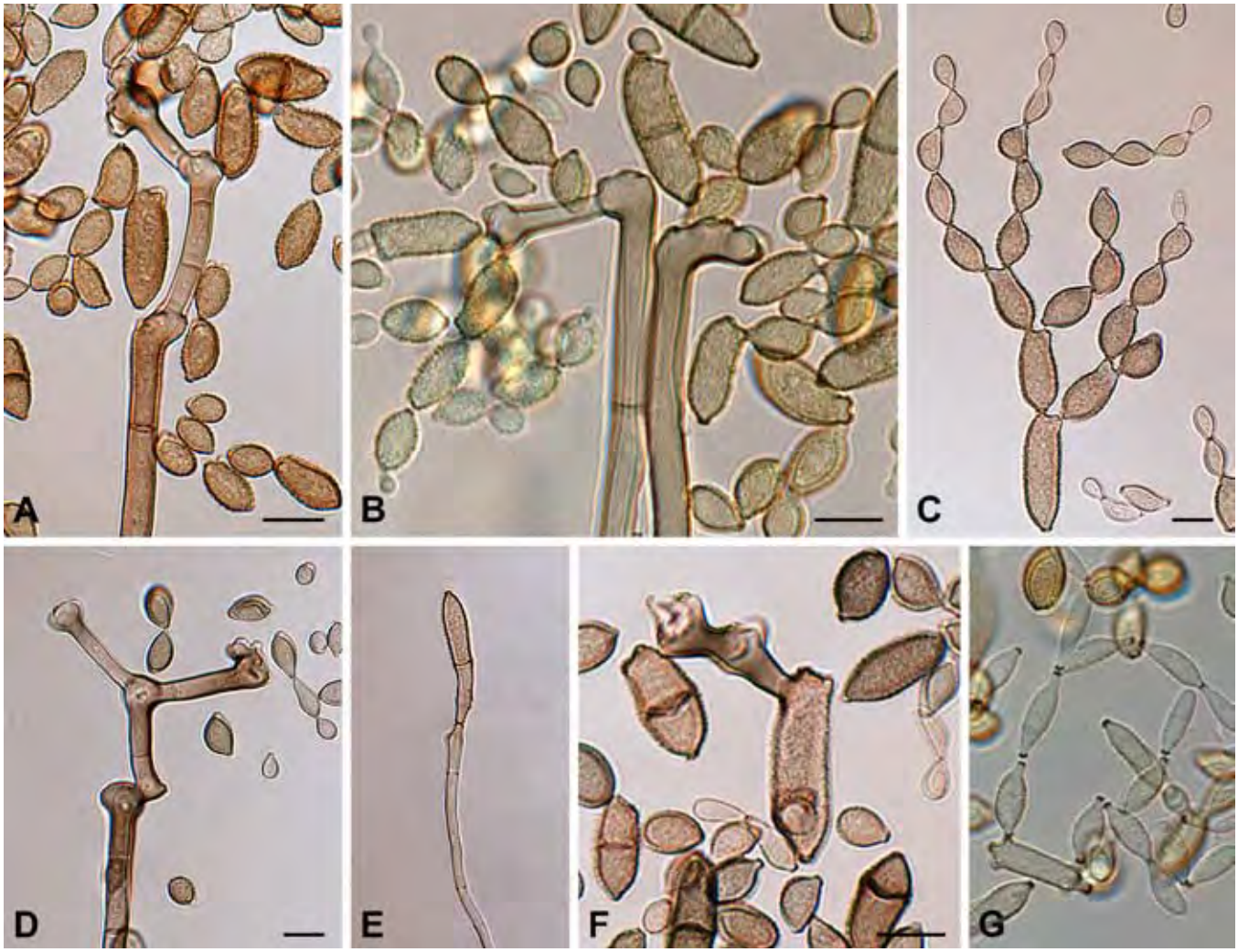

Fig. 14. *Cladosporium herbaroides* (CPC 12052). A–B, D. Macronematous conidiophores. C. Conidial chain. E. Micronematous conidiophore. F. Microcyclic conidiogenesis. G. Conidia formed by micronematous conidiophores. Scale bars = 10 µm.

Cultural characteristics: Colonies on PDA attaining 23 mm diam after 14 d at 25 ºC, grey-olivaceous to olivaceous, olivaceous-grey reverse, velvety, margin regular, entire edge, narrow, feathery, aerial mycelium abundantly formed, loose, with age covering large parts of the colony, woolly, growth flat with somewhat elevated colony centre, folded, regular, deep into the agar, with few prominent exudates, sporulation profuse. Colonies on MEA attaining 24 mm diam after 14 d at 25 ºC, grey- to greenish olivaceous, olivaceous-grey or iron-grey reverse, velvety to powdery, margin narrow, colourless, entire edge, somewhat feathery, aerial mycelium pale olivaceous-grey, sparse, growth convex, radially furrowed, folded in the colony centre, without prominent exudates, sporulating. Colonies on OA attaining 23 mm diam after 14 d at 25 ºC, grey-olivaceous, margin more or less regular, entire edge, colourless, somewhat feathery, aerial mycelium whitish to smoke grey, at first sparse, later more abundantly formed, growth flat, without exudates, sporulation profuse.

Specimen examined: **Israel**, from hypersaline water of Eilat salterns, 2004, coll. N. Gunde-Cimerman, isol. M. Ota, CBS-H 19858, **holotype**, isotype HAL 2025 F, culture ex-type CBS 121626 = EXF-1733 = CPC 12052.

Substrate and distribution: Hypersaline water; Israel.

Notes: *Cladosporium herbaroides* is morphologically similar to *C. herbarum* but differs in having somewhat longer conidia becoming wider, darker and even more thick-walled with age [at first conidia 3–33 × (2–)3–6(–7) µm, with age (3.5–)5–9(–11) µm wide]. Besides that, the species often produces a second conidial type formed on micronematous conidiophores, giving rise to unbranched conidial chains which are almost filiform, limoniform, narrowly fusiform to subcylindrical, much narrower and paler than the ones formed by macronematous conidiophores, 10–26(–35) × 2–3.5 µm. In *C. herbarum*, conidia formed by micronematous conidiophores do not occur as frequently as in *C. herbaroides*, and differ in being often clavate and somewhat wider, up to 4(–5) µm wide. *Cladosporium macrocarpum* is easily distinguishable by having somewhat wider conidiophores (3–)4–6 µm, with distinctly wider swellings, 5–10 µm wide, and the conidia are usually (3–)5–9(–10) µm wide.

Cladosporium herbarum (Pers. : Fr.) Link, Ges. Naturf. Freunde Berlin Mag. Neuesten Entdeck. Gesammten Naturk. 7: 37. 1816: Fr., Syst. mycol. 3(2): 370. 1832. Figs 16–19.

Basionym: *Dematium herbarum* Pers., Ann. Bot. (Usteri) 11: 32. 1794: Fr., Syst. mycol. 3(2): 370. 1832.

= *Dematium epiphyllum* var. (β) *chionanthi* Pers., Mycol. eur. 1: 16. 1822, **syn. nov.**

For additional synonyms see Dugan *et al*. (2004), Schubert (2005).

Teleomorph: ***Davidiella tassiana*** (De Not.) Crous & U. Braun, Mycol. Progr. 2: 8. 2003.

Basionym: *Sphaerella tassiana* De Not., Sferiacei Italici 1: 87. 1863.

≡ *Mycosphaerella tassiana* (De Not.) Johanson, Öfvers. Förh. Kongl. Svenska Vetensk.-Akad. 41: 167. 1884.

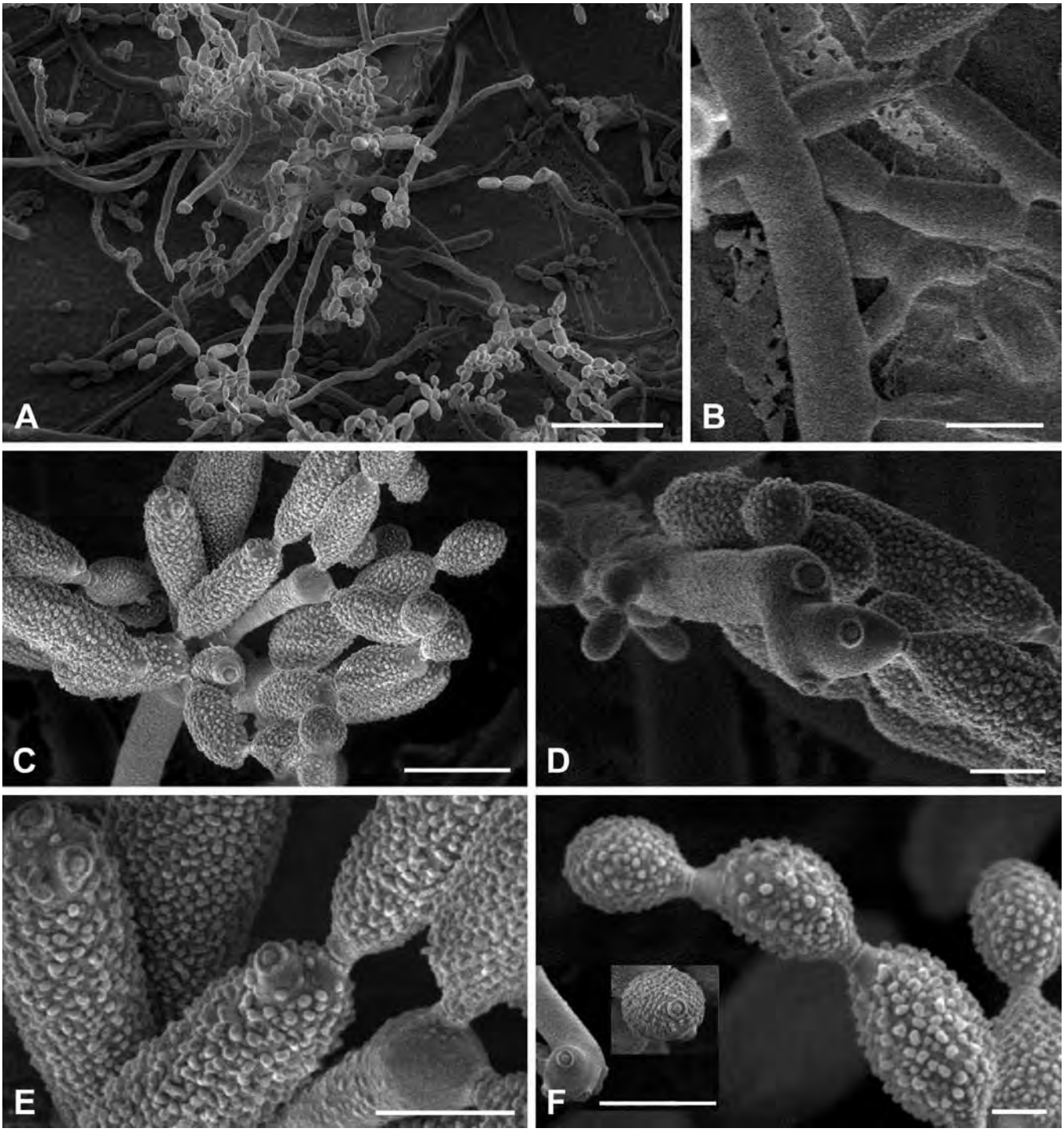

Fig. 15. *Cladosporium herbaroides* (CPC 12052). A. Overview of the growth characteristics of this fungus. Broad hyphae run over the surface of the agar, and possibly give rise to conidiophore branches. The conidiophores of this fungus can be rather long, resembling aerial hyphae. Clusters of conidia are clearly visible in this micrograph. B. The very wide surface hyphae can anastomose. C. Conidiophore with secondary ramoconidia and conidia. Note the variation in scar size. D. A very elaborate, complex conidiophore with different scars of variable size, one being more than 2 µm wide! E. Details of secondary ramoconidia and hila. Note the rather strong ornamentation in which smaller "particles" are between larger ones. F. Three conidia in a row. Note the scar formation in the chain and the reduction of the size of the cells throughout the spore-chain. The inset shows the resemblance of the scars on a conidiophore and on a secondary ramoconidium. Scale bars: A = 50 µm, B–C, F (inset) = 10 µm, D–E = 5 µm, F = 2 µm.

Ascomata pseudothecial, black, globose, erumpent to superficial, up to 200 µm diam, with 1(–3) short, periphysate ostiolar necks; wall consisting of 3–6 layers of medium red-brown *textura angularis*. *Asci* fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 65–85 × 13–17 µm. *Pseudoparaphyses* absent in host material, but remnants observed when studied in culture, hyaline, septate, subcylindrical, anastomosing, 3–4 µm wide. *Ascospores* tri- to multiseriate, overlapping, hyaline, with irregular luminar inclusions, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest near middle of apical cell, medianly 1-septate, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards the lower end, (17–)20–23(–25) × (6–)7(–8) µm; becoming brown and verruculose in asci. Ascospores germinating after 24 h on MEA from both ends, with spore body becoming prominently constricted at the septum, but not distorting, up to 7 µm wide, hyaline to pale brown and appearing somewhat verruculose, enclosed in a mucoid sheath, with germ tubes being irregular, somewhat nodular.

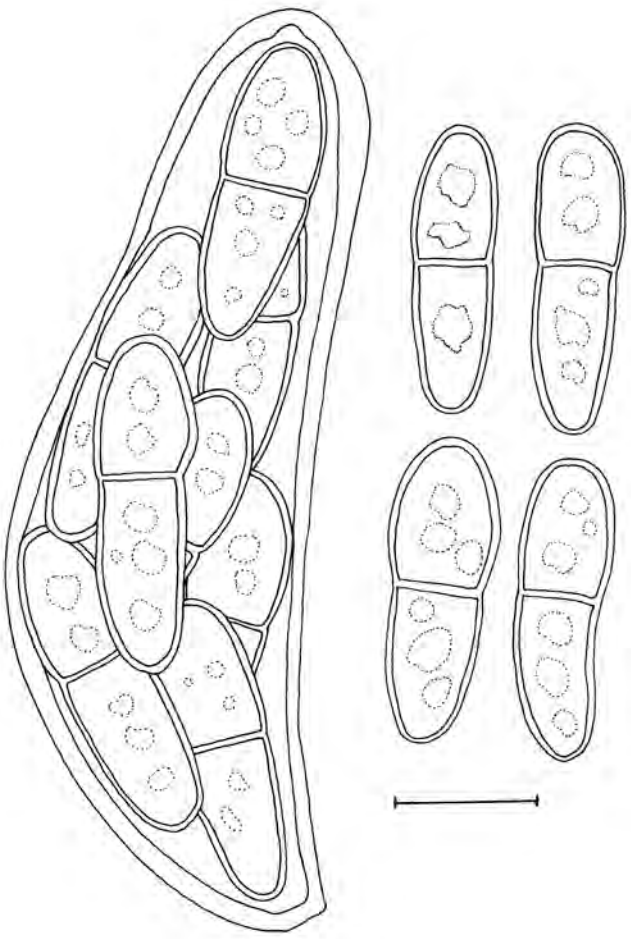

Fig. 16. *Davidiella tassiana* (RO, holotype). Ascus and ascospores. Scale bar = 10 µm. P.W. Crous *del*.

Mycelium superficial, loosely branched, (0.5–)1–5 µm wide, septate, sometimes constricted at septa, hyaline, subhyaline to pale brown, smooth or almost so to verruculose or irregularly rough-walled, sometimes appearing irregular in outline due to small swellings and constrictions, walls unthickened to somewhat thickened, cell lumen appearing to be granular. *Conidiophores* both macro- and micronematous, arising laterally from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to flexuous, somewhat geniculate-sinuous, nodulose to nodose with unilateral or multilateral swellings, with a single to numerous swellings in short succession giving the stalk a knotty/gnarled appearance, unbranched or occasionally branched, up to three times, sometimes with a lateral branch-like proliferation below or at the apex, 10–320 × 3.5–5 µm, swellings 5–8(–9) µm wide, pluriseptate, septa sometimes constricted when formed after a node, pale to medium brown, older ones almost dark brown, paler towards the apex, smooth or minutely verruculose, walls thickened, sometimes even two-layered. *Conidiogenous cells* integrated, terminal or intercalary, nodulose to nodose, with a single or up to five swellings per cell, 10–24 µm long, proliferation sympodial, with several conidiogenous loci confined to swellings, mostly situated on small lateral shoulders, more or less protuberant, broadly truncate to slightly convex, 1.5–2.5 µm diam, thickened and somewhat darkened-refractive. *Micronematous conidiophores* hardly distinguishable from hyphae, sometimes only as short lateral outgrowth with a single apical scar, short, conical to almost filiform or narrowly cylindrical, non-nodulose, not geniculate, unbranched, 5–120 × 1.5–3(–4) µm, pluriseptate, not constricted at septa, cells usually very short, 5–15 µm long, subhyaline to pale brown, almost smooth to minutely verruculose or irregularly rough-walled, sometimes forming clavate conidia, up to 33 µm long, 0–2-septate. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, narrowly cylindrical or filiform, with a single or two loci. *Conidia* catenate, in unbranched or loosely branched chains with branching mostly occurring in the lower part of the chain, straight to slightly curved, small terminal conidia without distal hilum obovoid, 4–10 × 3–5(–6) µm [av. ± SD, 7.8 (± 1.9) × 4.7 (± 0.9) µm], aseptate, intercalary conidia with a single or sometimes up to three distal hila limoniform, ellipsoid to subcylindrical, 6–16 × 4–6 µm [av. ± SD, 12.4 (± 1.6) × 5.3 (± 0.6) µm], 0–1-septate, *secondary ramoconidia* with up to four distal hila, ellipsoid to cylindrical-oblong, 12–25(–35) × (3–)5–7(–9) µm [av. ± SD, 18.8 (± 4.5) × 6.2 (± 0.9) µm], 0–1(–2)-septate, rarely with up to three septa, sometimes distinctly constricted at the septum, septum median or somewhat in the upper or lower half, pale greyish brown or brown to medium brown or greyish brown, minutely verruculose to verrucose, walls slightly to distinctly thickened, guttulate to somewhat granular, usually only slightly attenuated towards apex and base, apex obtuse or slightly truncate, towards the base sometimes distinctly attenuated with hila situated on short stalk-like prolongations, hila slightly to distinctly protuberant, truncate to slightly convex, (0.8–)1–2.5(–3) µm wide, 0.5–1 µm high, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring, conidia forming micro- and macronematous secondary conidiophores.

Cultural characteristics: Colonies on PDA reaching 19–37 mm diam after 14 d at 25 ºC, grey-olivaceous to olivaceous-grey, whitish to smoke-grey or pale olivaceous-grey due to abundant aerial mycelium, velvety, reverse olivaceous-grey or iron-grey, margin almost colourless, regular, entire edge, glabrous to feathery, aerial mycelium abundant mainly in the colony centre, dense, felty, woolly, sometimes becoming somewhat reddish brown, fawn coloured, growth regular, flat to low convex with an elevated colony centre, sometimes forming few large prominent exudates, sporulation profuse. Colonies on MEA reaching 17–37 mm diam after 14 d at 25 ºC, smoke-grey to pale olivaceous-grey towards margin, olivaceous-grey to iron-grey reverse, velvety, margin white, entire edge to slightly undulate, aerial mycelium abundant, dense, fluffy to felty, growth low convex or raised, radially furrowed, folded and wrinkled in the colony centre, without prominent exudates but sporulating. Colonies on OA reaching 12–28 mm diam after 14 d at 25 ºC, olivaceous-grey to iron-grey, due to abundant aerial mycelium pale olivaceous-grey, olivaceous-grey reverse, margin narrow, more or less undulate, white, aerial mycelium white, loose to dense, high, fluffy to felty, covering large parts of the colony, growth flat to low convex, without prominent exudates, sporulating.

Specimens examined: *Sine loco*, *sine dato*, L 910.225-733, **lectotype** of *C. herbarum*, selected by Prasil & de Hoog, 1988. *Sine loco*, on leaves of *Chionanthus* sp. (*Oleaceae*), L 910.255-872 = L-0115833, **holotype** of *Dematium epiphyllum* var. (β) *chionanthi*. **Netherlands**, Wageningen, isolated from *Hordeum vulgare* (*Poaceae*), 2005, P.W. Crous, CBS-H 19853, **epitype designated here** of *C. herbarum* and *D. tassiana*, isoepitype HAL 2022 F, ex-type cultures, CPC 12177 = CBS 121621, CPC 12178–12179, 12181, 12183. **Italy**, on upper and lower surface of dead leaves of *Carex nigra* ["*fusca*"] (*Cyperaceae*), Tassi no. 862, RO, **holotype** of *Davidiella tassiana*. **U.S.A.**, Colorado, San Juan Co., above Little Molas Lake, isolated from stems of *Delphinium barbeyi* (*Ranunculaceae*), 12 Sep. 2004, A. Ramaley, CBS-H 19868 (teleomorph), single ascospore isolates, CBS 121622 = CPC 11600, CPC 11601–11604.

Substrate and distribution: On fading and decaying plant material, on living leaves (phylloplane fungus), as secondary invader, as an endophyte, isolated from air, soil, foodstuffs, paints, textiles and numerous other materials; cosmopolitan.

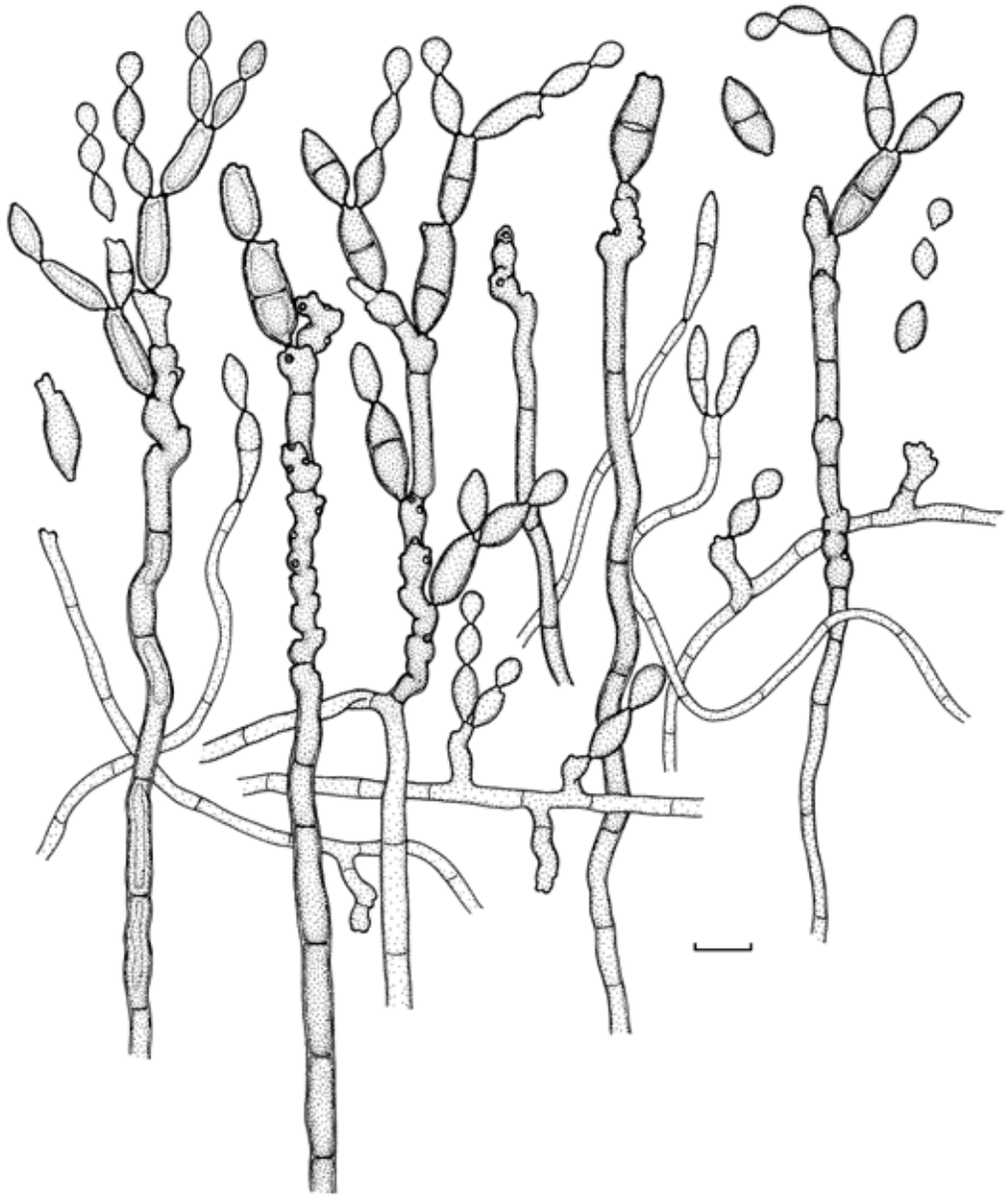

Fig. 17. *Cladosporium herbarum* (CPC 11600). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

Literature: de Vries (1952: 71), Hughes (1958: 750), Ellis (1971: 313), Domsch *et al.* (1980: 204), Sivanesan (1984: 225), Ellis & Ellis (1985: 290, 468, 1988: 168), Prasil & de Hoog (1988), Wang & Zabel (1990: 202), McKemy & Morgan-Jones (1991), Dugan & Roberts (1994), David (1997: 59), Ho *et al.* (1999: 129), de Hoog *et al.* (2000: 587), Samson *et al.* (2000: 110), Samson *et al.* (2001).

Notes: De Vries (1952) incorrectly selected a specimen of Link's herbarium at herb. B as lectotype. Prasil & de Hoog (1988) discussed this typification and designated one of Persoon's original specimens as lectotype in which *C. herbarum* could be recognised. The latter material, which is in poor condition, could be re-examined within the course of these investigations and showed conidia agreeing with the current species concept of *C. herbarum* being (6–)9.5–14.5(–21) × (5–)6–7(–8) µm. Since the identity of the strain CBS 177.71 chosen by Prasil & de Hoog (1988) as representative living strain of *C. herbarum* could not be corroborated, an epitype with a living ex-epitype culture is designated. The holotype specimen of *D. tassiana* (RO) is morphologically similar to that observed on the epitype of *C. herbarum*, having ascospores which are (17–)21–23(–25) × (6–)7–8(–8.5) µm, turning brown and verruculose in asci with age. However, no hamathecial remnants were observed in ascomata *in vivo*.

The connection to the teleomorph *D. tassiana* could be confirmed, which is in agreement with the findings of von Arx (1950) and Barr (1958). Ascospore isolates formed the typical *C. herbarum* anamorph in culture, and these anamorph cultures developed some immature fruiting bodies within the agar. When inoculated onto water agar plates with nettle stems, numerous ascomata with viable ascospores were formed in culture.

Cladosporium iridis (Fautrey & Roum.) G.A. de Vries, *Contr. Knowl. Genus Cladosporium*: 49. 1952. Figs 20–21.

Basionym: *Scolicotrichum iridis* Fautrey & Roum., Rev. Mycol. (Toulouse) 13: 82. 1891.

≡ *Heterosporium iridis* (Fautrey & Roum.) J.E. Jacques, Contr. Inst. Bot. Univ. Montréal 39: 18. 1941.

For additional synonyms see Dugan *et al*. (2004).

Teleomorph: ***Davidiella macrospora*** (Kleb.) Crous & U. Braun, Mycol. Progr. 2: 10. 2003.

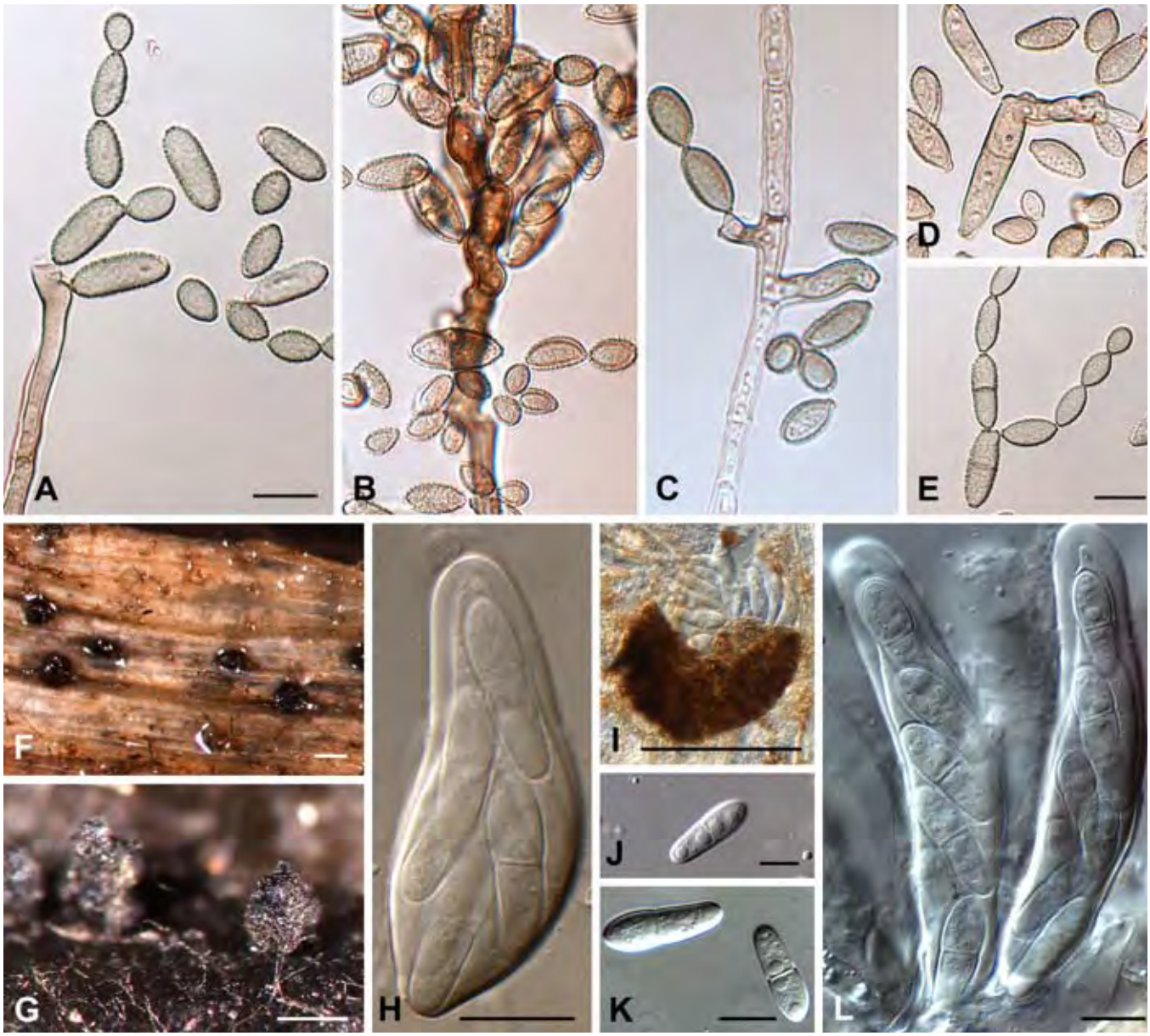

Fig. 18. *Cladosporium herbarum* (CPC 11600) and its teleomorph *Davidiella tassiana* (from the host and CPC 12181). A–B. Macronematous conidiophores. C. Micronematous conidiophore. D. Microcyclic conidiogenesis. E. Conidial chain. F. Ascomata on the leaf. G. Ascomata formed in culture on nettle stems. H–I. Asci on the host. J–K. Ascospores in culture. L. Asci in culture. Scale bars: A, E, H, J–L = 10 µm, F–G, I = 200 µm.

Basionym: *Didymellina macrospora* Kleb., Ber. Deutsch. Bot. Ges. 42: 60, 1924. 1925.

≡ *Mycosphaerella macrospora* (Kleb.) Jørst., Meld. Stat. Plantepatol. Inst. 1: 20. 1945.

Mycelium branched, 2–8 µm wide, septate, not constricted at the septa, hyaline to pale brown, smooth, walls slightly thickened, sometimes guttulate. *Conidiophores* very long, usually terminally arising from ascending hyphae, erect to subdecumbent, slightly to distinctly flexuous, geniculate-sinuous, usually several times, subnodulose due to geniculate, sympodial proliferation forming swollen lateral shoulders, unbranched, rarely branched, up to 720 µm long, 6–11 µm wide, swellings 8–11(–14) µm wide, pluriseptate, often very regularly septate, not constricted at the septa, pale to medium olivaceous-brown, somewhat paler towards the apex, smooth to minutely verruculose, walls only slightly thickened. *Conidiogenous cells* integrated, terminal as well as intercalary, cylindrical-oblong, 15–55 µm long, proliferation percurrent to sympodial, usually with a single geniculation forming laterally swollen shoulders often below a septum, conidiogenous loci confined to swellings, usually one locus per swelling, rarely two, protuberant, (2–)2.5–4 µm diam, somewhat thickened and darkened-refractive. *Conidia* solitary, sometimes in short, unbranched chains, straight to curved, young conidia pyriform to subcylindrical, connection between conidiophore and conidium being rather broad, subhyaline to pale olivaceous-brown, walls slightly thickened, then enlarging and becoming more thick-walled, cylindrical-oblong, soleiform with age, both ends rounded, usually with a slightly to distinctly bulbous base, visible from a very early stage, but broadest part often towards the apex not at the base, (18–)30–75(–87) × (7–)10–16(–18) µm [av. ± SD, 53.3 (± 17.8) × 12.6 (± 2.2) µm], (0–)2–6(–7)-septate, usually not constricted at the septa, rarely slightly constricted, septa often becoming sinuous with age, pale to medium olivaceous-brown, sometimes darker, verrucose to echinulate, walls thickened, especially in older conidia, up to 1 µm thick, hila protuberant, often stalk-like or conically prolonged, up to 2 µm long, (2–)2.5–3.5(–4) µm diam, with age becoming more sessile, sometimes just visible as a thickened plate just below the outer wall layer, especially in distal scars of branched conidia, periclinal rim often distinctly visible, hila somewhat thickened and darkened-refractive; microcyclic conidiogenesis not observed.

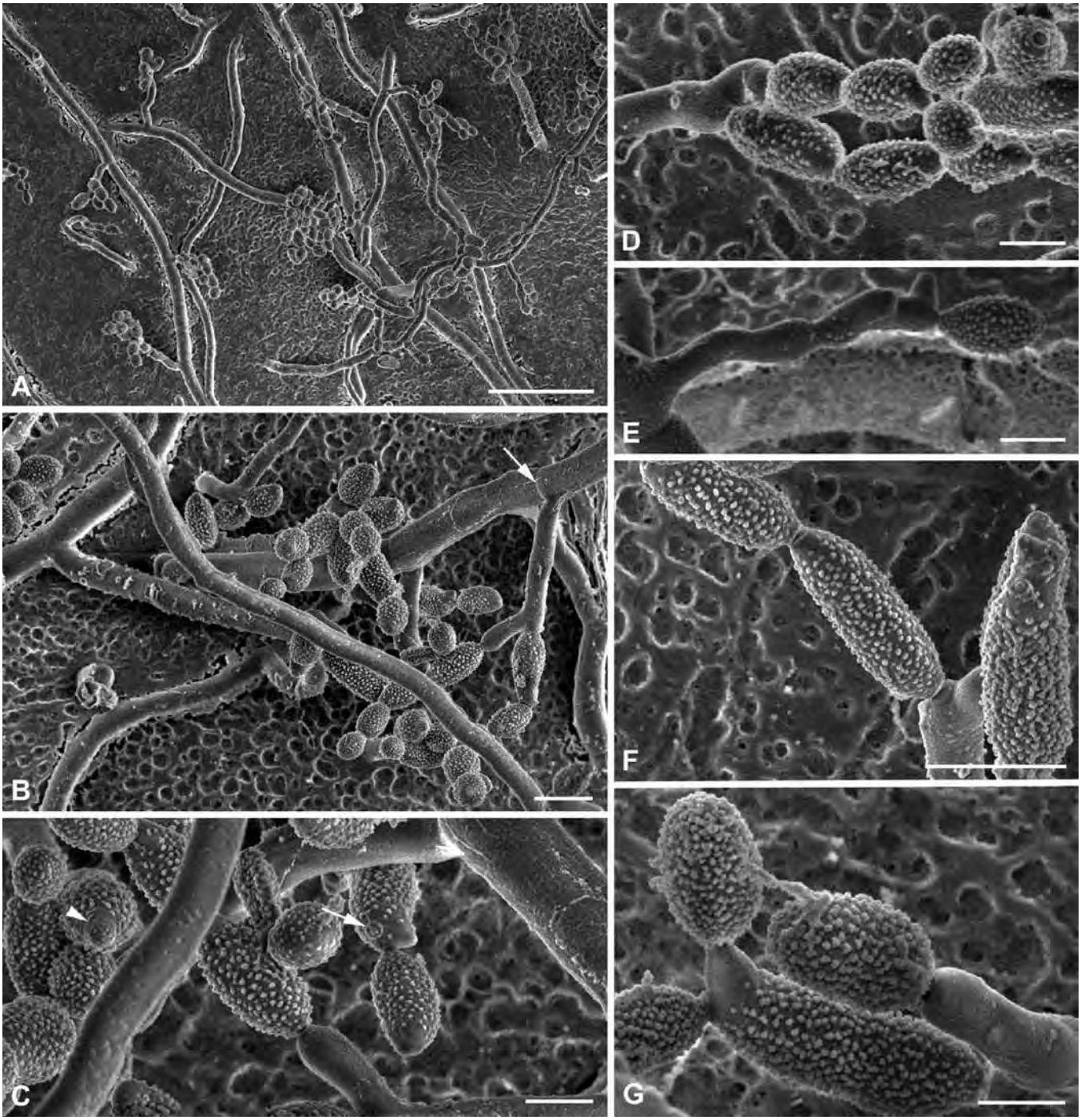

Fig. 19. *Cladosporium herbarum* (CPC 11600). A. Overview of hyphal growth and conidiophore formation of a colony on SNA. Conidiophores are often formed on very wide (approx. 10 µm), septate hyphae that often grow near the agar surface. B. A more detailed view on colony organisation reveals the ornamented conidia. Note the septum near the conidiophore (arrow). C. Detail of spore ornamentation and hila on a secondary ramoconidium (arrow). Ornamentation is visible during early stages of spore formation (arrow). D. Structure of the conidiophore, illustrating the complex morphology of the spore-forming apparatus. In addition, secondary ramoconidia, conidia, and a hilum on the conidium are visible. E. Complex structure of the spore-forming apparatus. F. Details of secondary ramoconidia with complex scar-pattern on the right cell. G. Details of a secondary ramoconidium giving rise to conidia. Note the lack of ornamentation at the location of spore formation. Scale bars: A = 50 µm, B, F = 10 µm, C–E, G = 5 µm.

Cultural characteristics: Colonies on PDA reaching 19–23 mm diam after 14 d at 25 ºC, pale greenish olivaceous, smoke-grey to olivaceous-grey due to abundant aerial mycelium, greenish olivaceous to olivaceous reverse, margin broad, regular, entire edge to slightly undulate, feathery, aerial mycelium abundantly formed, felty, fluffy, covering large parts of the colony, mainly in the central parts, high, growth low convex with a somewhat raised colony centre. Colonies on MEA reaching 9–23 mm diam after 14 d at 25 ºC, pale olivaceous-grey to olivaceous-grey, olivaceous-grey reverse, felty, margin slightly undulate, white, somewhat raised, aerial mycelium abundant, loose, diffuse, high, growth low convex, radially furrowed, slightly folded. Colonies on OA reaching 10–19 mm diam after 14 d at 25 ºC, olivaceous, margin broad, undulate, white, aerial mycelium white, very high, loose, diffuse, hairy, growth flat, due to the mycelium low convex, without prominent exudates and sporulating on all media.

Specimens examined: Isolated from *Iris* sp. (*Iridaceae*), CBS 107.20. **France**, Cote d'Or, Jardin de Noidan, on leaves of *Iris germanica*, Jul. 1880, F. Fautrey, Roumeguère, Fungi Sel. Gall. Exs. No. 5689, PC, **lectotype** of *C. iridis*, selected by David, 1997; K, isolectotype. **Netherlands**, Boterenbrood, isolated from leaves of *Iris* sp., Aug. 1940, CBS-H 19859, **epitype designated here** of *C. iridis*, culture ex-epitype CBS 138.40.

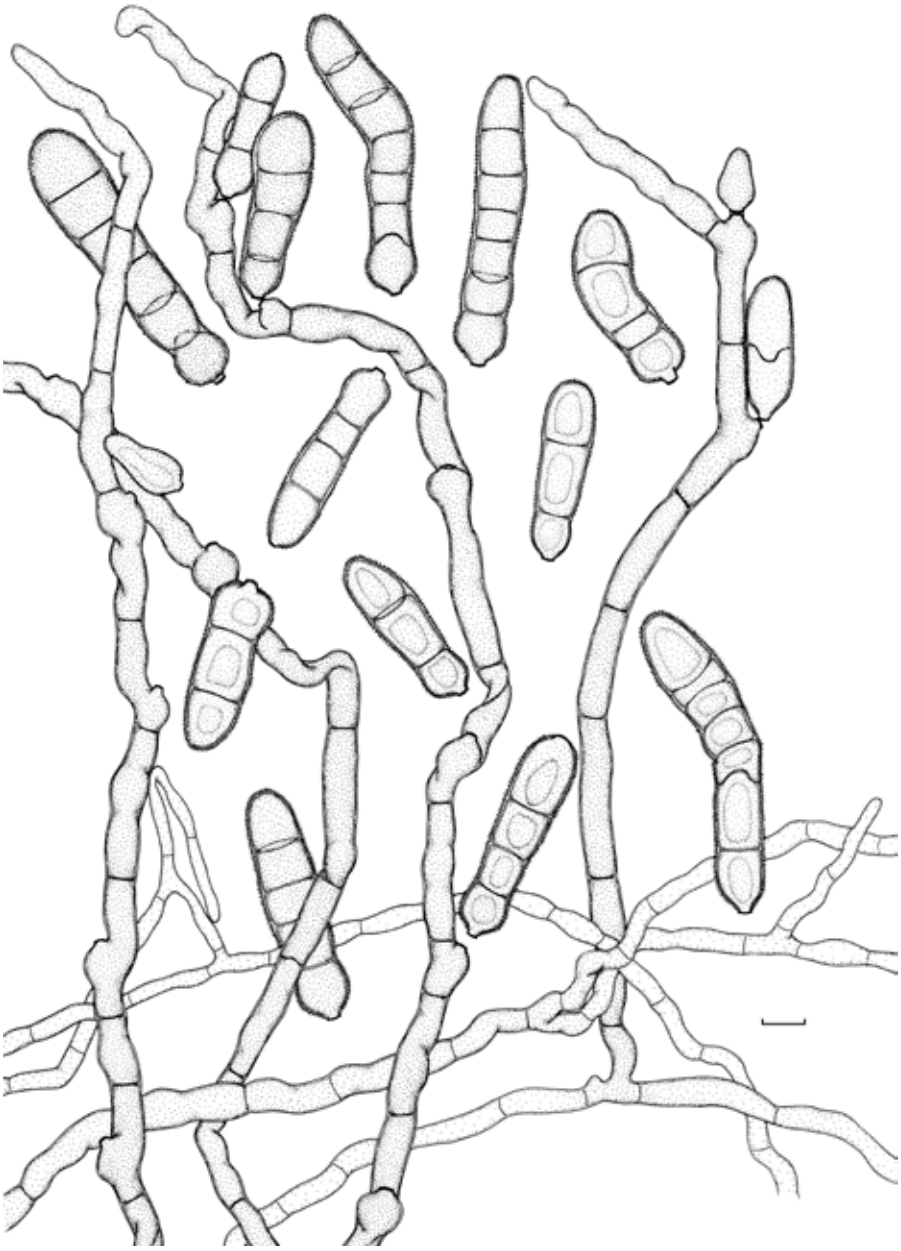

Fig. 20. *Cladosporium iridis* (CBS 138.40). Conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

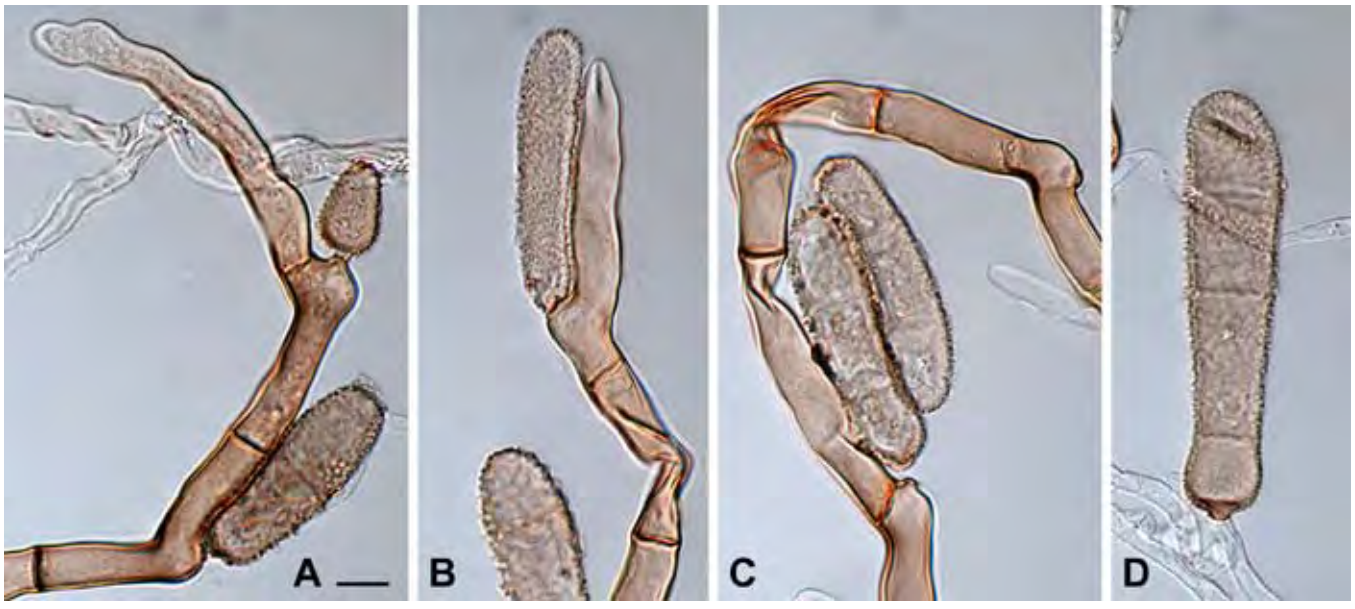

Fig. 21. *Cladosporium iridis* (teleomorph *Davidiella macrospora*) (CBS 138.40). A–C. Conidiophores with conidia. D. Conidium. Scale bar = 10 µm.

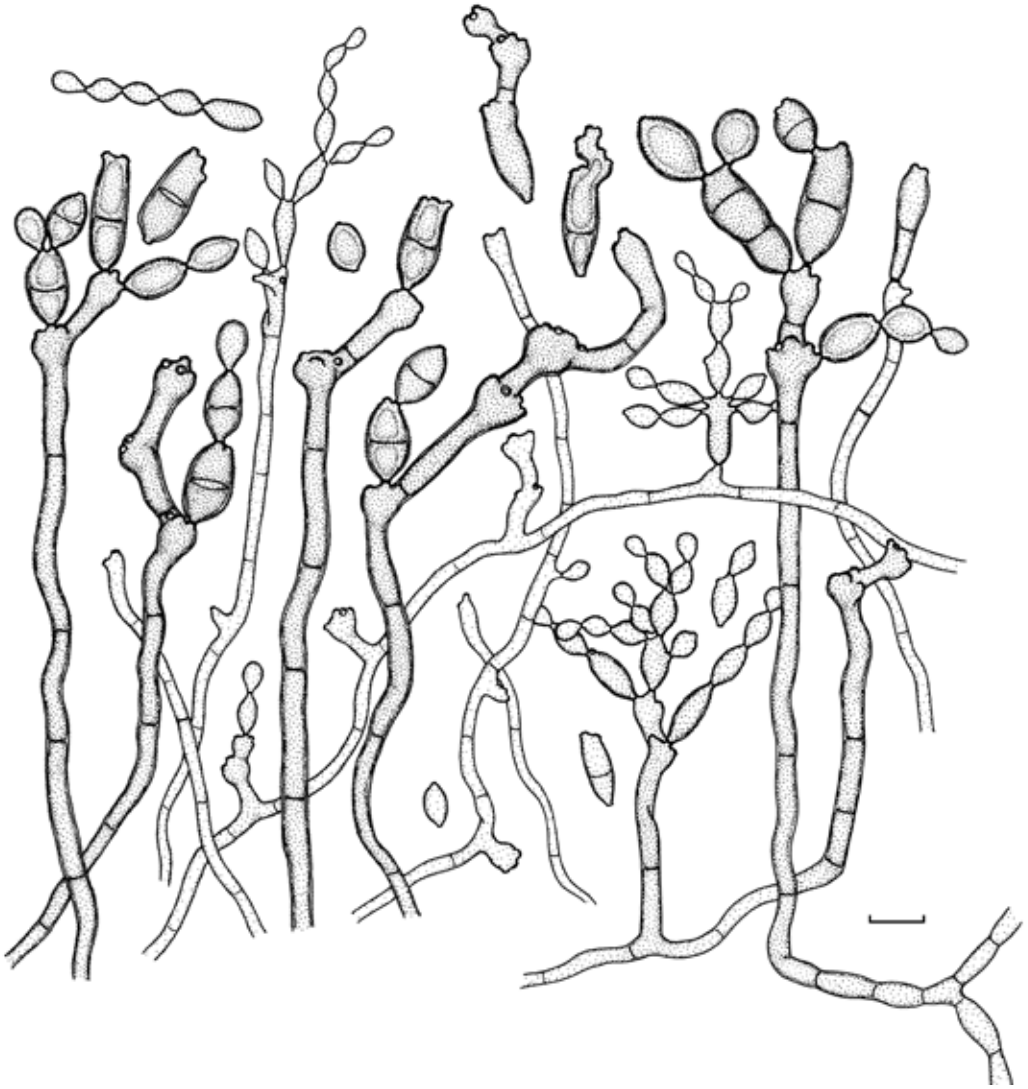

Fig. 22. *Cladosporium macrocarpum* (CBS 299.67). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

Substrates and distribution: Leaf spot and blotch of *Iris* spp. including *I. crocea*, *I. florentina*, *I. foetidissima*, *I. germanica*, *I. gueldenstaedtiana*, *I. kamaonensis*, *I. pallida*, *I. plicata* (= *I. swertii* Hort.), *I. pseudacorus*, *I. pumila*, *I. spuria* ssp. *halophila*, and other species, also on *Belacamanda chinensis* (= *Gemmingia chinensis*), *Hemerocallis fulva*, *Gladiolus gandavensis*; Africa (Algeria, Morocco, South Africa, Zambia, Zimbabwe), Asia (Armenia, Azerbaijan, China, Georgia, India, Iran, Israel, Japan, Kazakhstan, Kirgizstan, Korea, Russia, Turkey, Turkmenistan, Uzbekistan), Australasia (Australia, New Zealand), Europe (Austria, Belgium, Belorussia, Cyprus, Czech Republic, Denmark, Estonia, France, Germany, Great Britain, Greece, Italy, Latvia, Lithuania, Malta, Moldavia, Montenegro, Netherlands, Norway, Poland, Romania, Russia, Serbia, Spain, Sweden, Ukraine), North America (Canada, U.S.A.), Central & South America (Argentina, Chile, Jamaica, Panama, Uruguay).

Literature: Ellis (1971: 312), Ellis & Waller (1974), Sivanesan (1984: 222), McKemy & Morgan-Jones (1990), David (1997: 43), Shin *et al.* (1999).

Notes: The description of the morphological parameters in culture is based on the isolate sporulating on PDA, since sporulation on SNA was not observed. The conidiophores and conidia *in vivo* are usually wider than in culture [conidiophores (6–)9–15(–17) µm wide, conidia (11–)15–23(–28) µm].

Cladosporium macrocarpum Preuss, in Sturm, Deutsch. Fl. 3(26): 27. 1848. Figs 22–25.

≡ *Cladosporium herbarum* var. *macrocarpum* (Preuss) M.H.M. Ho & Dugan, in Ho *et al*., Mycotaxon 72: 131. 1999.

= *Dematium herbarum* var. (β) *brassicae* Pers., Syn. meth. fung. 2: 699. 1801, **syn. nov.**

= *Dematium graminum* Pers., Mycol. eur. 1: 16. 1822, **syn. nov.**

= *Dematium vulgare* var. (δ) *typharum* Pers., Mycol. eur. 1: 14. 1822, **syn. nov.**

= *Dematium vulgare* var. (β) *foliorum* Pers., Mycol. eur. 1: 14. 1822, **syn. nov.**

For additional synonyms see Dugan *et al*. (2004), Schubert (2005).

Teleomorph: ***Davidiella macrocarpa*** Crous, K. Schub. & U. Braun, **sp. nov.** MycoBank MB504582.

Davidiellae tassianae similis, sed pseudoparaphysibus prominentibus et ascosporis maioribus, (22–)23–26(–28) × (6–)6.5–7(–8) µm.

Ascomata superficial on a small stroma, black, up to 200 µm diam, globose, separate, but developing with 1–3 necks with age; ostioles consisting of pale brown to subhyaline cells, periphysate, with periphysoids growing into the cavity; wall consisting of 3–6 layers of medium brown *textura angularis*. *Pseudoparaphyses* present, hyaline, subcylindrical, septate, anastomosing, 3–4 µm diam; hamathecial cells persistent in cavity. *Asci* fasciculate, bitunicate, subsessile, broadly ellipsoid with a long tapered stalk, straight to curved, 8-spored, 70–110 × 15–20 µm. *Ascospores* tri- to multiseriate, overlapping, hyaline, guttulate, irregular lumina

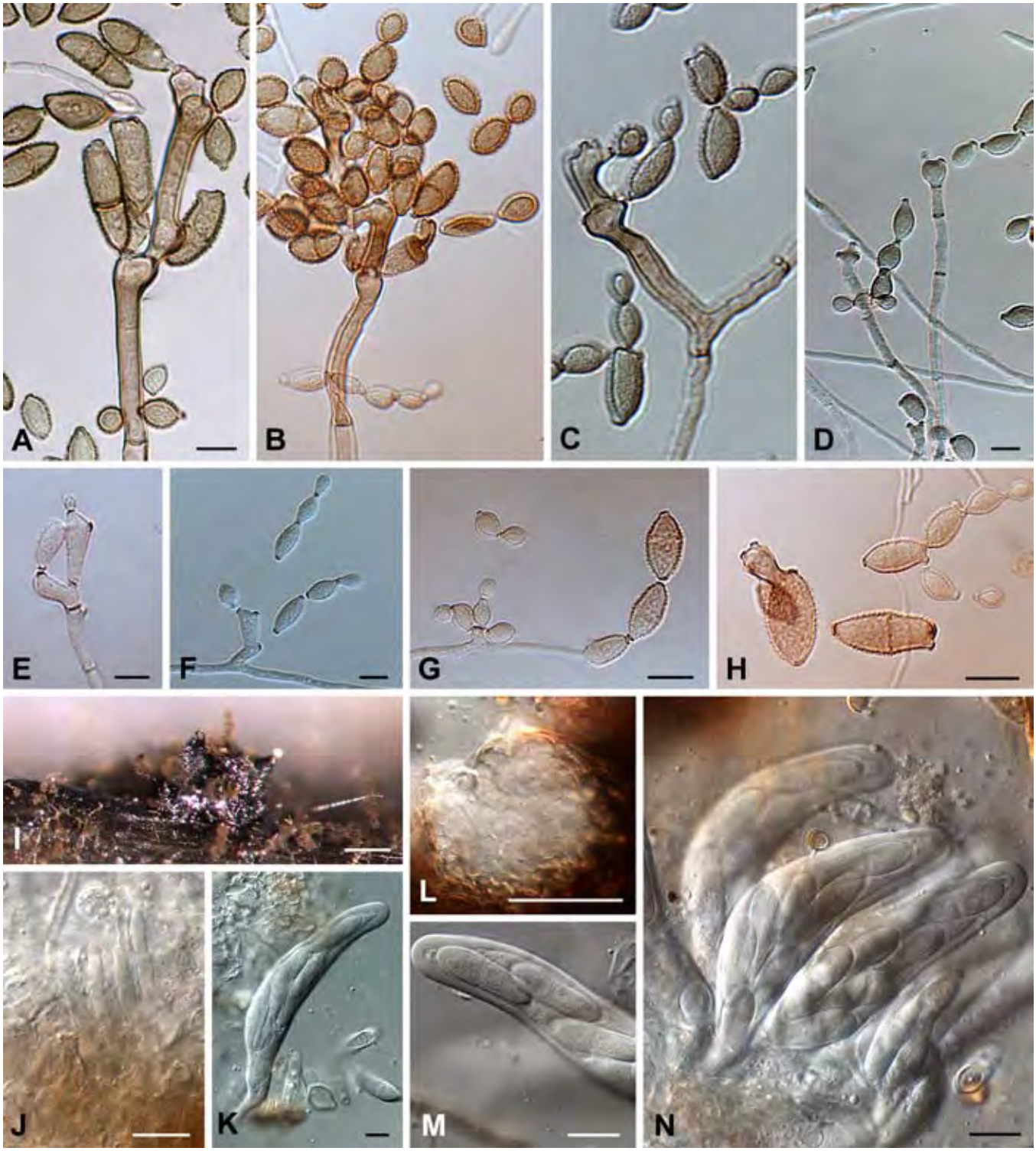

Fig. 23. *Cladosporium macrocarpum* (CBS 299.67) and its teleomorph *Davidiella macrocarpa* (CPC 12755). A–C. Macronematous conidiophores and conidia. D–G. Micronematous conidiophores. H. Microcyclic conidiogenesis. I. Ascomata formed on nettle stems in culture. J. Periphyses. K, M–N. Asci. L. Ostiole. Scale bars: A, D–H, J–N = 10 µm, I = 200 µm.

rarely observed, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest in the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, tapering towards both ends, but more prominently towards lower end, (22–)23–26(–28) × (6–)6.5–7(–8) µm; mucoid sheath rarely observed, mostly absent.

Mycelium unbranched or loosely branched, 1–4.5(–5) µm wide, septate, sometimes slightly constricted at septa, hyaline to pale brown, smooth to minutely verruculose, walls unthickened or slightly thickened. *Conidiophores* micronematous and macronematous, solitary, arising terminally from plagiotropous hyphae or terminally from ascending hyphae. *Macronematous conidiophores* erect, straight to somewhat flexuous, cylindrical-oblong, nodulose to nodose, with a single apical or usually several swellings either somewhat distinct from each other or often in short succession giving conidiophores a knotty appearance, swellings sometimes laterally elongated or formed at the top of a branch-like outgrowth below the apical swelling, sometimes distinctly geniculate, unbranched, sometimes branched, 12–260 × (3–)4–6 µm, swellings 5–10 µm wide, pluriseptate, sometimes slightly constricted at septa, pale to medium brown or olivaceous-brown, somewhat paler at apices,

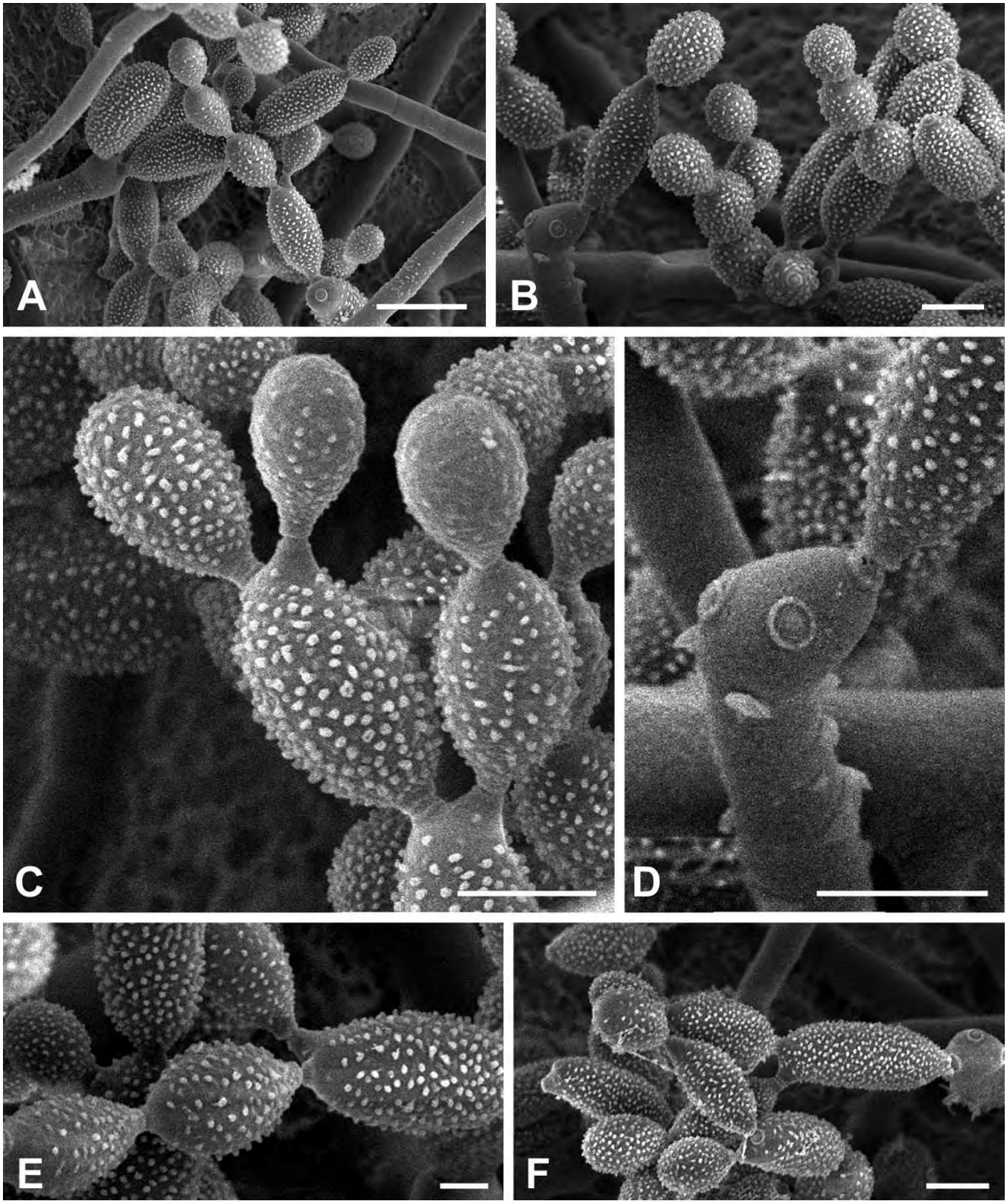

Fig. 24. *Cladosporium macrocarpum* (CBS 299.67). A. Survey of a conidiophore that forms several secondary ramoconidia and conidia. Several aerial hyphae are also visible in this picture. B. Conidiophore with broadly ellipsoid secondary ramoconidia and obovoid conidia. Note the different scars on the conidiophore at the lower left. C. Ellipsoid or obovoid conidia with notable areas of scar formation. The ornamentation is relatively widely distributed over the body of the cell and similar to *C. variabile*. D. Detail of a conidiophore (see B) with scars. Note the relatively shallow rings of the scars. E. Details of conidia and a secondary ramoconidium. F. Conidiophore with a secondary ramoconidium and conidia. Note the hila on several spores and the lack of ornamentation at the site where spores are formed. Scale bars: A–C, = 10 µm, D, F = 5 µm, E = 2 µm.

smooth to minutely verruculose or verruculose, walls somewhat thickened, sometimes even two-layered. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, nodulose with lateral shoulders or nodose with swellings round about the stalk, with conidiogenous loci confined to swellings, 12–37 µm long, with up to 12 loci per cell, usually with up to six, loci conspicuous, protuberant, (1–)1.5–2 µm diam, somewhat thickened and darkened-refractive. *Micronematous conidiophores* almost indistinguishable from hyphae, straight, narrowly filiform, non-nodulose or with a single or few swellings, mostly with small head-like swollen apices, usually

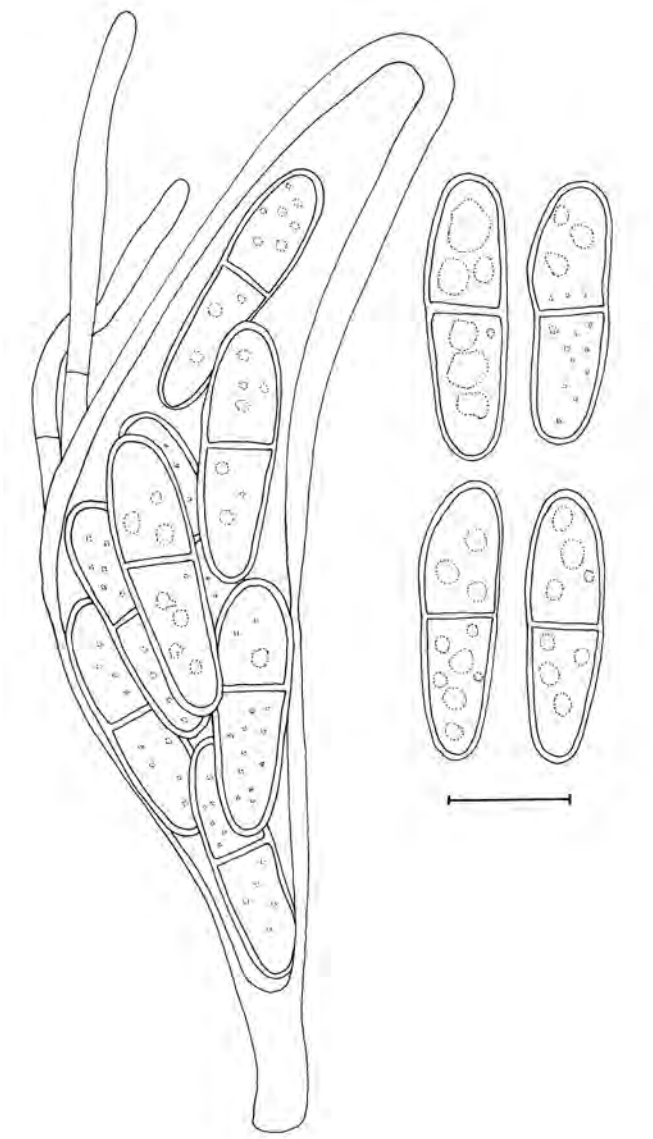

Fig. 25. *Davidiella macrocarpa* (CPC 12755). Ascus and ascospores. Scale bar = 10 µm. P.W. Crous *del*.

only few micrometer long, 1.5–3 µm wide, aseptate or with only few septa, subhyaline, smooth or almost so, walls unthickened, with a single or only few conidiogenous loci, narrow, 0.8–1.2 µm diam, thickened and somewhat darkened-refractive. *Conidia* catenate, in branched chains, small terminal conidia subglobose, obovoid, oval, limoniform, 4–11 × (3–)4–6 µm [av. ± SD, 7.6 (± 1.9) × 5.0 (± 0.8) µm], aseptate, intercalary conidia broadly ovoid-ellipsoid, 10–17 × (4.5–)5–9 µm [av. ± SD, 12.7 (± 2.1) × 6.8 (± 0.8) µm], 0–1-septate; secondary ramoconidia broadly ellipsoid to subcylindrical, 14–25(–30) × (5–)6–9(–10) µm [av. ± SD, 19.4 (± 3.5) × 7.6 (± 1.0) µm], 0–2(–3)-septate, sometimes slightly constricted at the septa, septa somewhat sinuous with age, pale brown to medium olivaceous-brown or brown, sometimes even dark brown, verruculose to echinulate (muricate under SEM), walls thickened, up to 1 µm thick, mostly broadly rounded at apex and base, sometimes attenuated, sometimes guttulate by oil drops, with up to three apical hila, mostly 1–2, hila sessile (apparently somewhat immersed) to somewhat protuberant, 1–2(–2.5) µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring with conidia forming secondary micro- and macronematous conidiophores, conidia often germinating with long hyphae. *Conidia* formed by micronematous conidiophores usually smaller, narrower and paler, catenate, in short unbranched or branched chains, subglobose, obovoid to limoniform, ellipsoid or fusiform, 2.5–16 × 1.5–5 µm, 0(–1)-septate, few longer conidia subcylindrical to clavate, up to 37(–43) µm long, 0–2(–3)-septate, occasionally with up to four septa, sometimes slightly constricted at the septa, subhyaline to pale brown, almost smooth to minutely verruculose, walls unthickened, hila 0.8–1.2 µm diam, thickened and darkened-refractive.

Cultural characteristics: Colonies on PDA reaching 30–43 mm in diam after 14 d at 25 ºC, dark dull green to olivaceous-grey, olivaceous-grey, dark olivaceous- to iron-grey reverse, pulvinate, velvety, sometimes somewhat zonate, paler zones towards the margin, margin regular, entire edge, almost colourless to white, glabrous to feathery, aerial mycelium sparse to more abundant in the colony centre or covering large areas of the colony, hairy, fluffy or felty, whitish to smoke-grey, sometimes becoming reddish, livid red to vinaceous, growth flat, regular, sometimes forming few prominent exudates, exudates sometimes slightly reddish, sporulation profuse with two kinds of conidiophores, low and high. Colonies on MEA reaching 31–50 mm in diam after 14 d at 25 ºC, grey-olivaceous to olivaceous-grey or iron-grey, sometimes pale olivaceous-grey to whitish due to abundant aerial mycelium, olivaceous-grey or iron-grey reverse, velvety or powdery, margin narrow, entire edge, colourless to white, glabrous, aerial mycelium sparse to abundant, hairy or felty, growth regular, flat to low convex, radially furrowed, without prominent exudates, sporulation profuse. Colonies on OA reaching 29–40 mm in diam after 14 d at 25 ºC, grey-olivaceous, olivaceous-grey to dark smoke-grey, olivaceous-black or iron grey reverse, margin entire edge, narrow, colourless or white, glabrous, aerial mycelium sparse, mainly in the colony centre, felty, white to smoke-grey or grey-olivaceous, felty, growth flat, regular, without exudates, sporulating.

Specimens examined: *Sine loco et dato*, L 910.255-723 = L-0115836, **lectotype designated here** of *Dematium graminum*. *Sine loco*, on dead stems of *Brassica* sp. (*Brassicaceae*), No. 601, L 910.255-716 = L-0115849, **holotype** of *D. herbarum* var. (β) *brassicae*. *Sine loco*, on leaves of *Iris* (*Iridaceae*), *Quercus* (*Fagaceae*), *Brassica* etc., L 910.255-736 = L-0115871, **holotype** of *D. vulgare* var. (β) *foliorum*, isotype L 910.255-718 = L-0115872. *Sine loco et dato*, L 910.255-698 = L-0115852, **lectotype designated here** for *D. vulgare* var. (δ) *typharum*. Isolated from "*Mycosphaerella tulasnei*", CBS 223.32 = ATCC 11287 = IMI 049635. **Romania**, isolated from water, CBS 175.82. **Slovenia**, Sečovlje, isolated from hypersaline water from salterns (precrystalisation pond), 2004, P. Zalar, EXF-2287 = CPC 12054. **Turkey**, Ankara, Tekeli, isolated from *Triticum aestivum* (*Poaceae*), isol. S. Tahsin, ident. A.C. Stolk, CBS 299.67. **U.S.A.**, Seattle, University of Washington Campus, 47.6263530, -122.3331440, isolated from cleistothecia of *Phyllactinia guttata* (*Erysiphaceae*) on leaves of *Corylus* sp. (*Corylaceae*), 16 Sep. 2004, D. Glawe, CPC 11817; Washington, isolated from *Spinacia oleracea* (*Chenopodiaceae*), 1 Jan. 2003, L. DuToit, CBS-H 19855, **neotype designated here** for *C. macrocarpum*, and **holotype** of *D. macrocarpa*, isoneotype HAL 2020 F, isotype HAL 2021 F, culture ex-type CPC 12752, 12756–12759, CPC 12755 = CBS 121623.

Substrate and distribution: Decaying plant material, human, hypersaline water, water; widespread.

Literature: de Vries (1952: 76), Ellis (1971: 315), Domsch *et al.* (1980: 208), Ellis & Ellis (1985: 290, 468), Matsushima (1985: 5), McKemy & Morgan-Jones (1991), Dugan & Roberts (1994), David (1997: 71), Samson *et al.* (2000: 112).

Notes: In the absence of Preuss's type material (not preserved) de Vries (1952) "lectotypified" *C. macrocarpum* by a specimen in Saccardo's herbarum (Herb. Myc. P.A. Saccardo no. 419, PAD). This material, subsequently distributed in Mycotheca Italica no. 1396, should correctly be regarded as neotype (David 1997). A single collection of Saccardo's Mycotheca Italica no. 1396 from herb. HBG, which can be considered as isoneotype material,

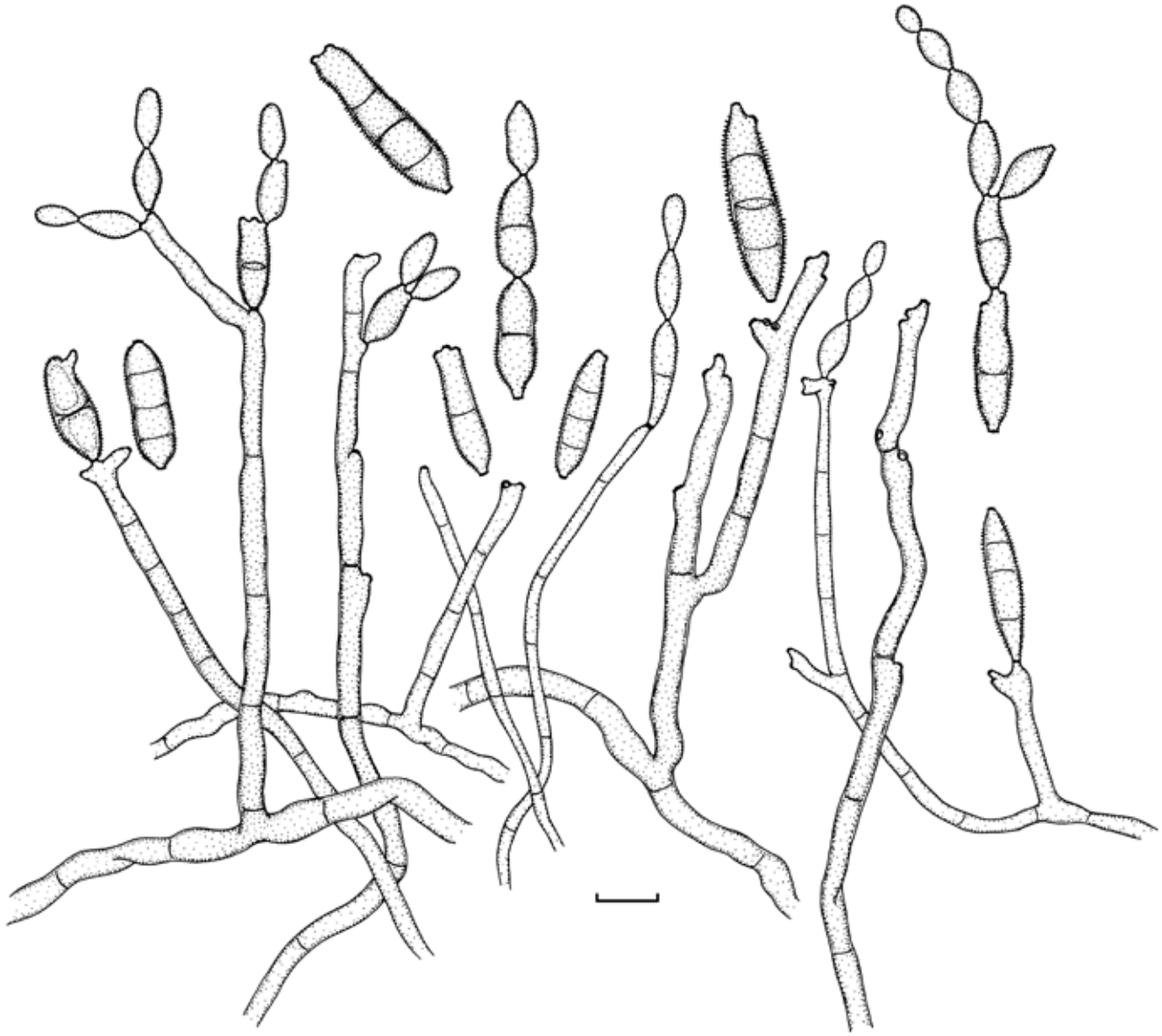

Fig. 26. *Cladosporium ossifragi* (CBS 842.91). Conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

was re-examined and proved to rather agree with the species concept of *C. herbarum s. str*. The conidia were formed in simple, rarely branched chains, 6–26 × (4–)5.5–8(–9) µm, 0–3-septate, almost smooth or minutely to densely verruculose or verrucose (Schubert 2005). However, since de Vries' "lectotypification" was incorrect according to the code (ICBN, Art. 9.2, 9.17), a neotype is designated.

The delimitation of *C. macrocarpum* as a morphologically distinct species from *C. herbarum* has been controversially discussed by several authors (McKemy & Morgan-Jones 1991, Dugan & Robert 1994, Ho *et al.* 1999). Based on molecular as well morphological studies, it can be shown that *C. macrocarpum* is a well-defined species distinguishable from *C. herbarum s. str.* by forming conidiophores with wider nodes, 5–10 µm, wider and more frequently septate conidia [small terminal conidia 4–11 × (3–)4–6 µm versus 4–10 × 3–5(–6) µm in *C. herbarum*, intercalary conidia 10–17 × (4.5–)5–9 µm versus 6–16 × 4–6 µm in *C. herbarum*, secondary ramoconidia 14–25(–30) × (5–)6–9(–10) µm versus 12–25(–35) × (3–)5–7(–9) µm in *C. herbarum*] and by being connected to *Davidiella macrocarpa*. On natural substrates the conidiophores are usually somewhat wider than in culture, 4–8(–10) µm wide, and also the conidia can be somewhat wider, sometimes up to 13(–15) µm.

Cladosporium graminum, described by Persoon (1822), as well as *C. brunneum* and *C. gracile*, introduced by Corda (1837), are older synonyms of *C. macrocarpum* and, according to the code, would have priority. However, since *C. macrocarpum* is a well established, currently used name with numerous records in literature, a proposal to conserve the name against these older names is in preparation for formal publication in *Taxon*.

A characteristic difference between ascomata of *C. macrocarpum* in comparison to those of *C. herbarum*, are the smaller, globose pseudothecia, asci with longer stalks, prominence of pseudoparaphyses, and rather inconspicuous luminar ascospore inclusions.

Cladosporium ossifragi (Rostr.) U. Braun & K. Schub., **comb. nov.** MycoBank MB504575. Figs 26–28.

Basionym: *Napicladium ossifragi* Rostr., Bot. Færöes 1: 316. 1901.

≡ *Heterosporium ossifragi* (Rostr.) Lind, Dan. fung.: 531. 1913.

= *Heterosporium magnusianum* Jaap, Schriften Naturwiss. Vereins Schleswig-Holstein 12: 346. 1902.

≡ *Cladosporium magnusianum* (Jaap) M.B. Ellis in Ellis, More Dematiaceous Hyphomycetes: 337. 1976.

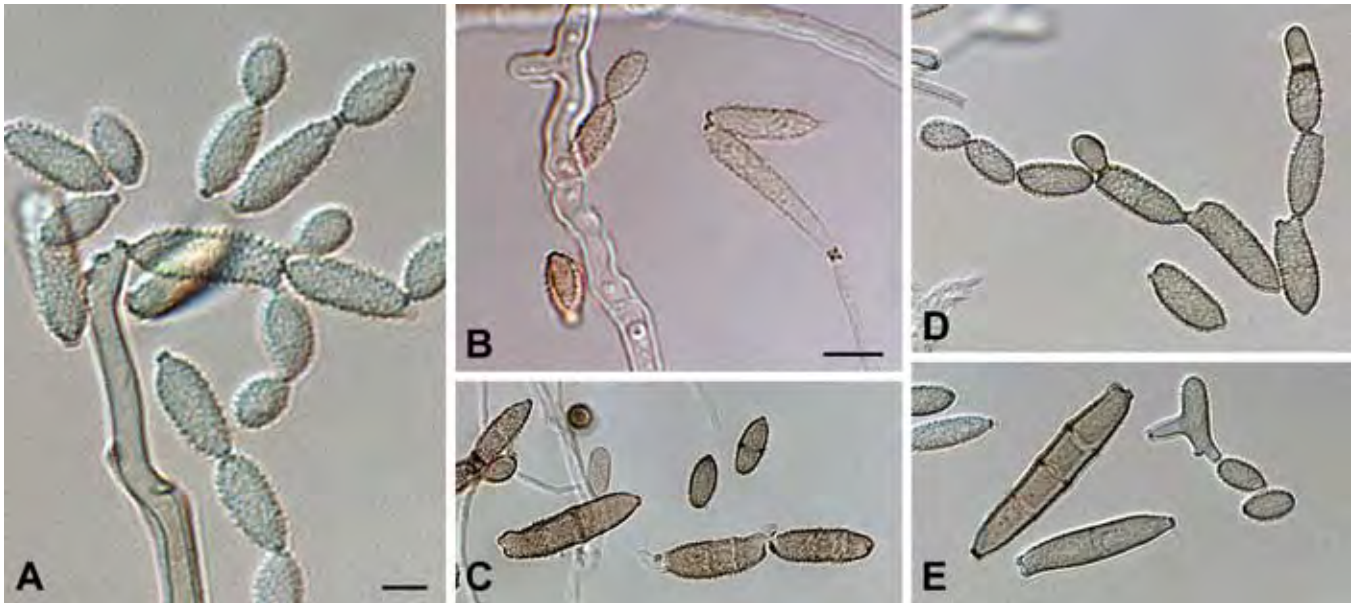

Fig. 27. *Cladosporium ossifragi* (CBS 842.91). A. Macronematous conidiophore. B. Micronematous conidiophore. C–D. Conidia. E. Conidia and microcyclic conidiogenesis. Scale bars = 10 µm.

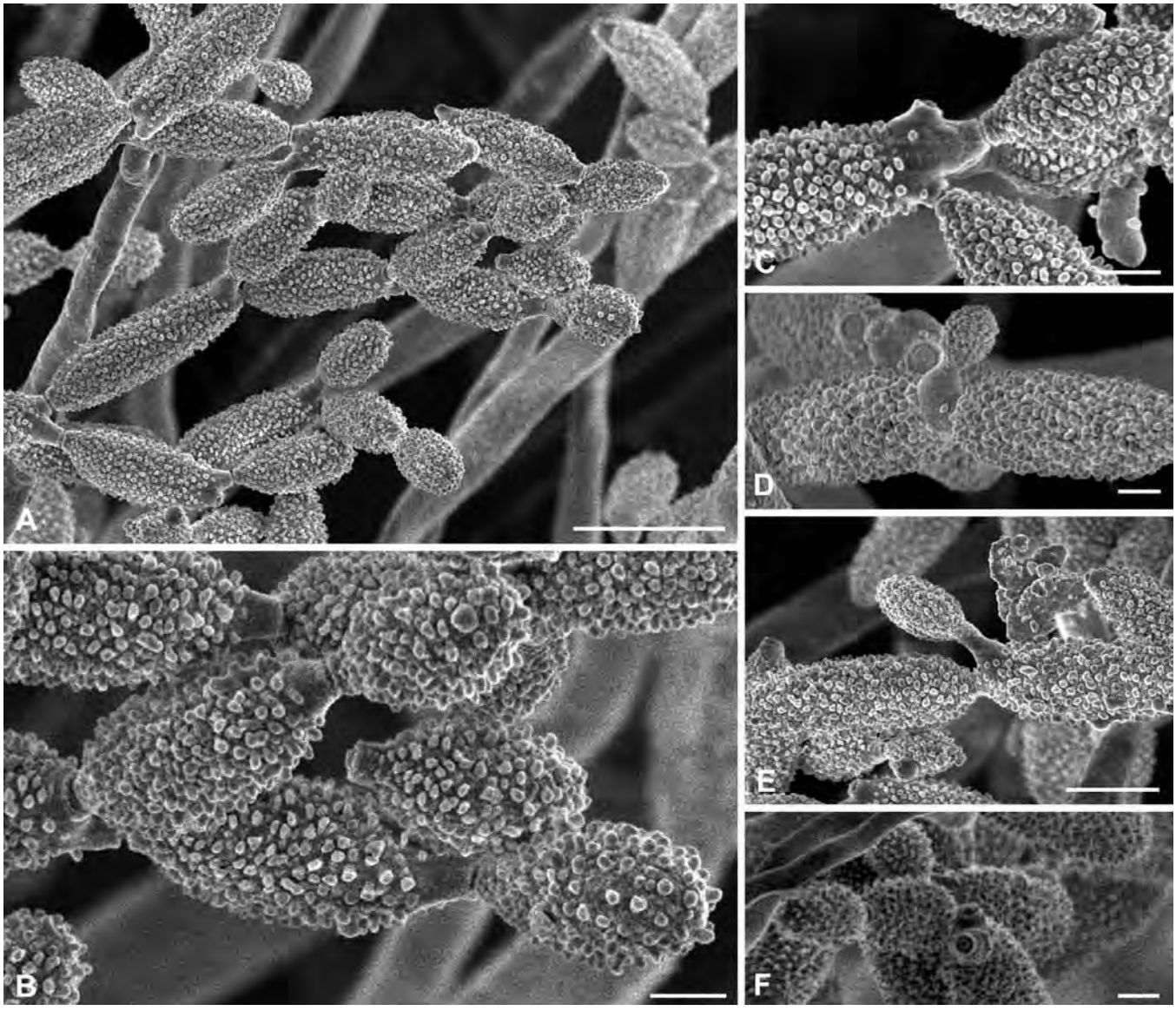

Fig. 28. *Cladosporium ossifragi* (CBS 842.91). A. Survey on different secondary ramoconidia and conidia. B. Details of conidia and hila. Note the very pronounced ornamentation and the absence of ornamentation near the site of spore formation. C. Detail of the end of a secondary ramoconidium with pronounced hila. D. Formation of a new conidium. Note the broad scar behind it (> 1 µm). E. Formation of a new conidium from a smooth-walled stalk. F. Hila on a secondary ramoconidium. This micrograph is from the sample before coating with gold-palladium and shows similar features as the sample after sputter coating. Scale bars: A = 10 µm, B–D, F = 2 µm, E = 5 µm.

Mycelium abundantly formed, twisted, often somewhat aggregated, forming ropes, branched, 1–5 µm wide, septate, often irregularly swollen and constricted, hyaline or subhyaline to pale brown, smooth, walls unthickened or only slightly thickened. *Conidiophores* macronematous and micronematous, arising from plagiotropous hyphae, terminally or laterally, erect to subdecumbent, more or less straight to flexuous, cylindrical, sometimes geniculate, subnodulose with loci often situated on small lateral shoulders, unbranched, sometimes branched, often very long, up to 350 µm long, 3–4.5(–5) µm wide, pluriseptate, shorter ones aseptate, not constricted at septa, pale to pale medium brown, paler towards apices, sometimes subhyaline, smooth to minutely verruculose, especially towards apices, walls somewhat thickened, up to 0.5 µm, sometimes appearing two-layered. *Conidiogenous cells* integrated, terminal as well as intercalary, cylindrical, sometimes geniculate, subnodulose, 5–31 µm long, proliferation sympodial, with few loci (1–3) per cell, loci usually confined to small lateral shoulders, protuberant, conspicuous, short cylindrical, 1–2 µm wide, up to 1 µm high, somewhat thickened, darkened-refractive. *Conidia* catenate, in short, unbranched or branched chains, straight, small terminal and intercalary conidia subglobose, obovoid to ellipsoid, 4–15 × 3–5 µm [av. ± SD, 9.3 (± 3.7) × 4.0 (± 0.7) µm], 0–1-septate, not constricted at the septa, pale brown, hila 0.8–1 µm diam, secondary ramoconidia cylindrical, sometimes ellipsoid or subfusiform, 16–36(–40) × (4–)5–8 µm [av. ± SD, 26.6 (± 7.4) × 6.0 (± 1.2) µm], (0–)1–3(–4)-septate [*in vivo* wider, (6–)7–9(–11) µm, and with up to five, rarely seven septa], not constricted at the septa, septa sometimes slightly sinuous, pale brown to pale medium brown, densely verruculose, verrucose to echinulate (densely muricate under SEM), walls unthickened to somewhat thickened, rounded or somewhat attenuated at apex and base, hila protuberant, conspicuous, sometimes situated on short, small prolongations, 1–2.5 µm diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occasionally occurring.

Cultural characteristics: Colonies on PDA reaching 53 mm diam after 14 d at 25 ºC, greenish olivaceous, grey-olivaceous to olivaceous-grey or iron-grey, appearing somewhat zonate, dull green to olivaceous-black reverse, margin colourless, regular, entire edge, aerial mycelium abundantly formed, covering at first the colony centre later most of the surface, dense, high, growth flat with elevated colony centre, somewhat folded. Colonies on MEA reaching 54 mm diam after 14 d at 25 ºC, pale olivaceous-grey to olivaceous-grey in the centre, iron-grey reverse, velvety, margin colourless to white, entire edge, radially furrowed, aerial mycelium abundantly formed, fluffy to felty, growth flat with somewhat raised, folded colony centre. Colonies on OA attaining 52 mm diam after 14 d at 25 ºC, olivaceous-grey to iron-grey, iron-grey to greenish black reverse, margin white, entire edge, aerial mycelium diffuse, loose, growth flat, prominent exudates absent, sporulation profuse on all media.

Specimens examined: **Denmark**, Undallslund, on leaves of *Narthecium ossifragum* (*Melanthiaceae*), 13 Sep. 1885, E. Rostrup, CP, **neotype designated here** of *C. ossifragi*; Tønder, Rømø near Twismark, 19 Aug. 1911, H. Sydow, Sydow, Mycoth. Germ. 1047, M. **Germany**, Hamburg, Eppendorfer Moor, on leaves of *Narthecium ossifragum*, 12 Sep. 1897, O. Jaap, HBG, **lectotype selected here** of *C. magnusianum*; 4 Sep. 1903, O. Jaap, Jaap, Fungi Sel. Exs. 49, M; Wernerwald near Cuxhaven, Aug. 1927, A. Ludwig, Petrak, Mycoth. Gen. 146, M. **Norway**, Bjerkreim County, isolated from leaves of *Narthecium ossifragum*, M. di Menna, CBS-H 19860, **epitype designated here** of *C. ossifragi*, culture ex-epitype CBS 842.91 = ATCC 200946; Møre og Romsdal County, isolated from leaves of *Narthecium ossifragum*, M. di Menna, CBS 843.91.

Substrate and distribution: Causing leaf spots on *Narthecium ossifragum*; Europe (Austria, Denmark, Germany, Great Britain, Ireland, Norway).

Literature: Ellis & Ellis (1985: 390), David (1995a; 1997: 85–86, 88), Ho *et al.* (1999: 132).

Notes: Type material of *Napicladium ossifragi* is not preserved in Rostrup's herbarium (on *Narthecium ossifragum*, Faeroe Islands, Viderö, Viderejde and Österö, Svinaa, *sine dato*, *leg.* Ostenfeld & Harz). However, other authentic collections seen and examined by Rostrup are deposited at CP. Lind (1913) re-examined these samples, synonymised *N. ossifragi* with *H. magnusianum* and correctly introduced the combination *H. ossifragi*. Nevertheless, the correct oldest name for this fungus has been ignored by most authors. David (1997), who clearly stated that *N. ossifragi* is the earliest name for this species, preferred to use the name *C. magnusianum* because the typification of Rostrup's name was still uncertain. Despite the lacking type material, there is no doubt about the correct identity of *N. ossifragi* since authentic material of this species, examined by and deposited in Rostrup's herbarium (CP), is preserved. Therefore, there is no reason to reject the oldest valid name for this species. The original collection of *C. magnusianum* cited by Jaap (1902) (on leaves of *Narthecium ossifragum*, Denmark, Tønder, Rømø, peatbog by Twismark, Jul.–Aug. 1901, Jaap), but not designated as type, is not preserved (David 1997). It is neither deposited at B, HBG nor S. However, in the protologue Jaap (1902) also referred to material of this species found near Hamburg, which is, hence, syntype material available for lectotypification.

Cladosporium pseudiridis K. Schub., C.F. Hill, Crous & U. Braun, **sp. nov.** MycoBank MB504576. Figs 29–30.

Etymology: Epithet derived from its similar morphology to *Cladosporium iridis*.

Differt a Cladosporio iridis conidiis 0–3-septatis, brevioribus et latioribus, 15–55 × (9–)11–19(–21) µm.

Mycelium sparingly branched, 2–7 µm wide, septate, not constricted at the septa, subhyaline to pale brown, smooth or almost so, walls somewhat thickened, guttulate or protoplasm appearing granular, sometimes enveloped by a slime coat. *Conidiophores* arising mostly terminally from ascending hyphae, sometimes also laterally from plagiotropous hyphae, erect, more or less straight, broadly cylindrical-oblong, once or several times slightly to distinctly geniculate-sinuous, forming more or less pronounced lateral shoulders, nodulose, unbranched, 100–320(–500) × 7–11 µm, swellings 10–14 µm wide, becoming narrower and paler towards the apex, septate, not constricted at the septa, septa mainly basal, apical cell often very long, pale to medium olivaceous-brown, subhyaline at the apex, smooth or almost so, sometimes minutely verruculose, walls usually distinctly thickened, sometimes even two-layered, up to 1(–2) µm thick, protoplasm granular, often clearly contrasting from the outer wall. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical-oblong, slightly to distinctly geniculate-sinuous, nodulose with conidiogenous loci confined to swellings or lateral shoulders, 30–110 µm long, proliferation percurrent to sympodial, with a single or three, sometimes up to five geniculations per cell, usually only a single locus per swelling, protuberant, very prominent, short cylindrical, peg-like, clearly composed of a dome and surrounding rim, dome often higher than the periclinal rim, broad, somewhat paler than rim, conically narrowed, (2–)2.5–4 µm wide, up to 2 µm high, thickened and darkened-refractive.

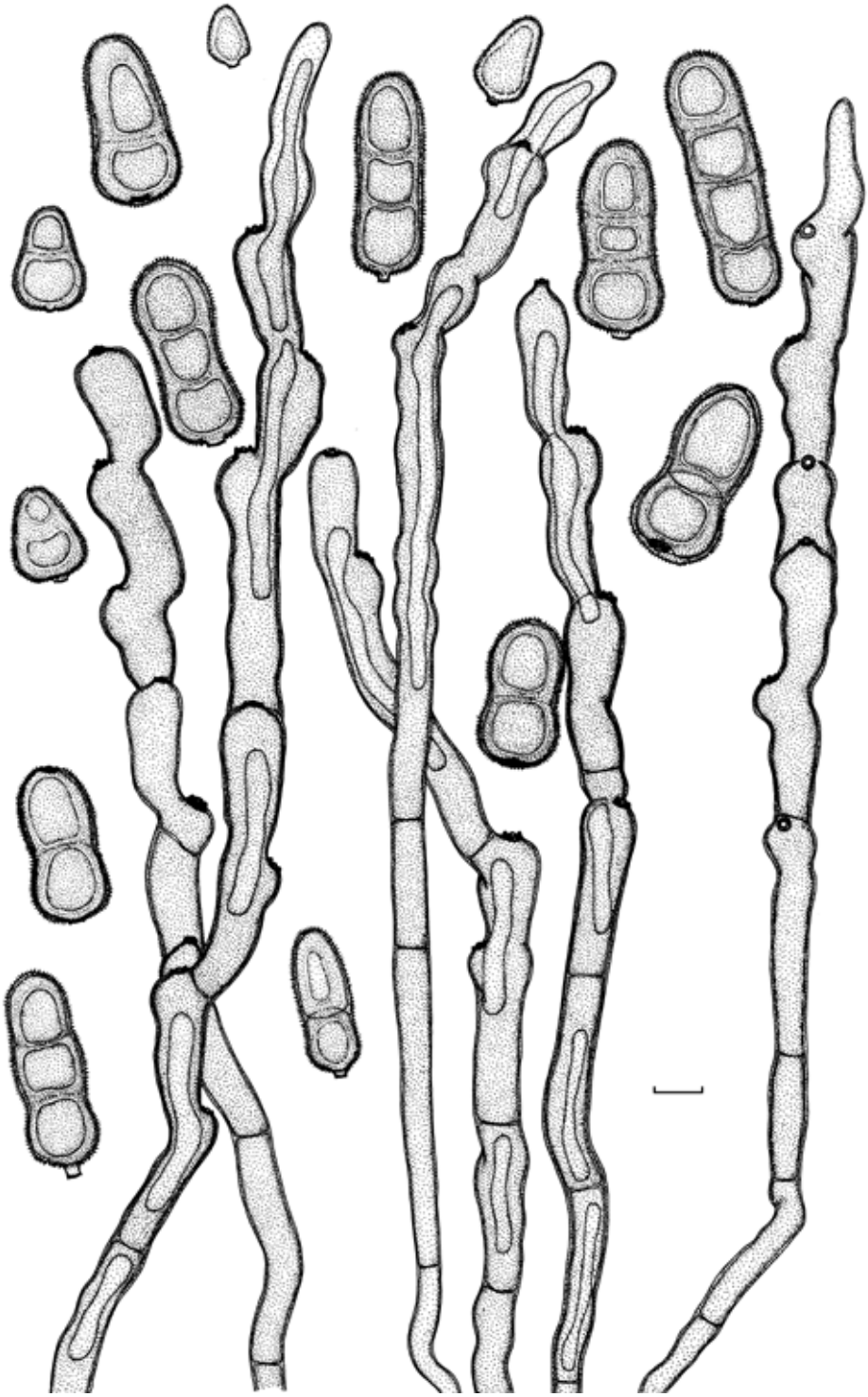

Fig. 29. *Cladosporium pseudiridis* (CBS 116463). Conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

Conidia solitary, sometimes in short unbranched chains of two or three, straight to slightly curved, young conidia small, 0–1-septate, broadly ovoid to pyriform, 15–26 × (9–)11–16(–18) µm [av. ± SD, 19.2 (± 4.3) × 14.2 (± 3) µm], first septum somewhat in the upper half, the upper cell is much smaller but gradually extending as the conidium matures, mature conidia 1–3-septate, broadly pyriform, cylindrical-oblong or soleiform, usually with a distinctly bulbous base, 30–55 × 12–19(–21) µm [av. ± SD, 41.5 (± 6.8) × 17.1 (± 2.1) µm], broadest part of conidia usually at the bulbous base, mostly attenuated towards the basal septum, septa becoming sinuous with age, pale to medium olivaceous-brown or brown, usually echinulate, sometimes coarsely verrucose, walls distinctly thickened, up to 2 µm thick, often appearing layered with a large lumen in the centre of the cell, broadly rounded to flattened at apex and base, hila often very prominent, often peg-like elongated, up to 3 µm long, with age becoming less prominent, visible as a thickened flat plate just below the outer echinulate wall layer, slightly raised towards the middle, 2–3.5 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis not observed.

Cultural characteristics: Colonies on PDA attaining 6 mm diam after 14 d at 25 ºC, whitish, smoke-grey to pale olivaceous-grey due to abundant aerial mycelium, olivaceous-black reverse, margin narrow, white, more or less crenate, aerial mycelium zonate, fluffy, covering most of the colony, mainly in the colony centre, growth

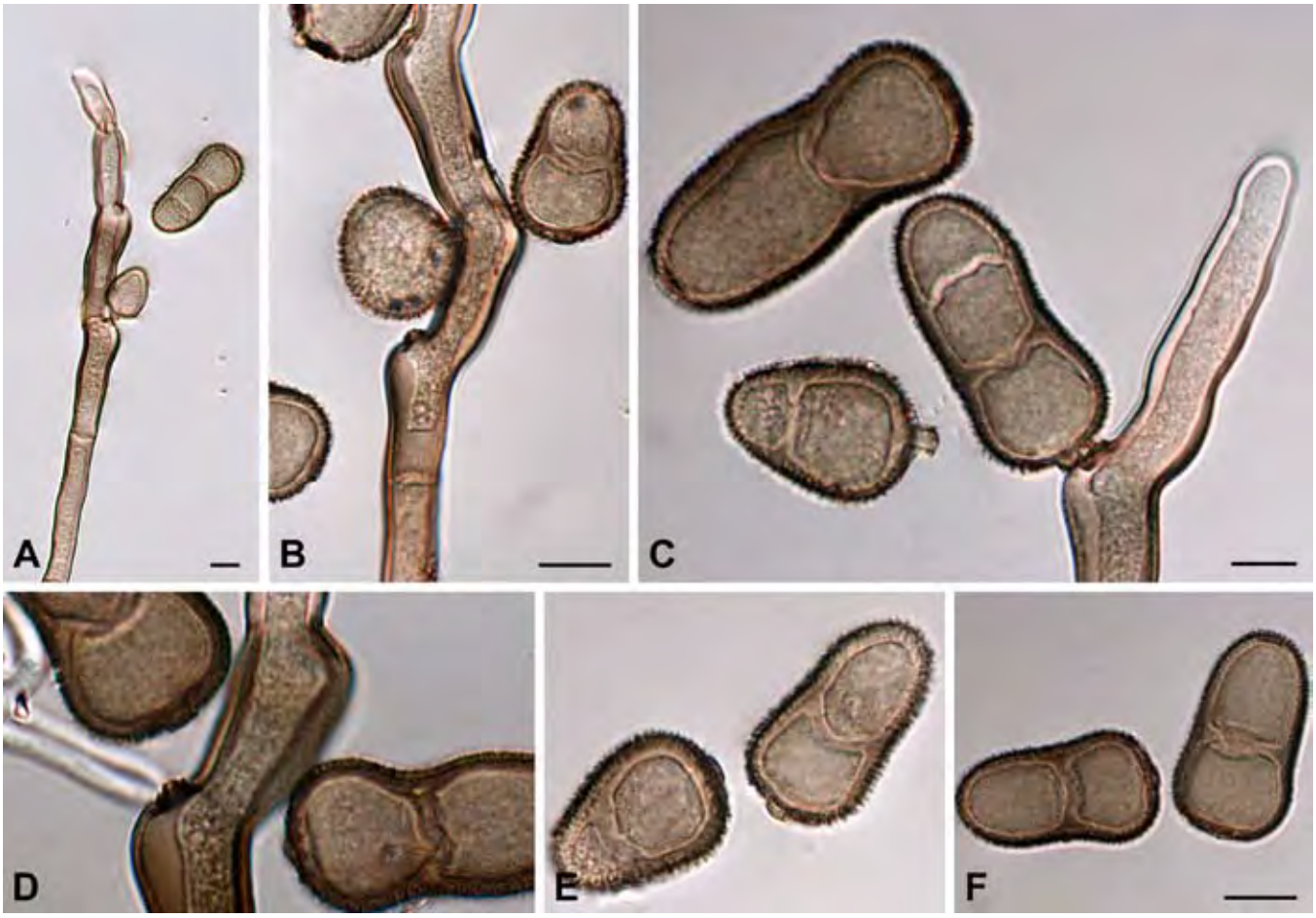

Fig. 30. *Cladosporium pseudiridis* (CBS 116463). A–C. Conidiophores and conidia. D. Part of a conidiogenous cell showing a protuberant cladosporioid conidiogenous locus. E–F. Conidia. Scale bars = 10 µm.

convex to raised, deep into the agar, with age few large prominent exudates formed, sparingly sporulating. Colonies on MEA attaining 7 mm diam after 14 d at 25 ºC, olivaceous-grey, pale olivaceous-grey to pale rosy-buff due to abundant aerial mycelium covering almost the whole colony, iron-grey reverse, margin colourless or white, broad, regular, more or less glabrous, aerial mycelium fluffy, dense, high, growth convex to umbonate, sometimes with elevated colony centre, prominent exudates lacking, sporulation sparse. Colonies on OA attaining 8 mm diam after 14 d at 25 ºC, white, pale buff to pale olivaceous-grey in the centre, margin grey-olivaceous, olivaceous- to iron-grey reverse, margin entire edge or somewhat undulate, somewhat feathery, growth raised with a somewhat depressed centre forming an elevated outer rim, without prominent exudates, sporulation more abundant.

Specimen examined: **New Zealand**, Auckland, Mt. Albert, Carrington Road, Unitec Campus, isolated from large leaf lesions on *Iris* sp. (*Iridaceae*), 15 Aug. 2004, C.F. Hill, CBS-H 19861, **holotype**, culture ex-type CBS 116463 = LYN 1065 = ICMP 15579.

Substrate and distribution: On living leaves of *Iris* sp.; New Zealand.

Notes: *Cladosporium pseudiridis* closely resembles *C. iridis*, a common and widespread species causing leaf spots on numerous *Iris* spp. and a few additional hosts of the host family *Iridaceae*, but the latter species is easily distinguishable by having longer and narrower, more frequently septate conidia, (18–)30–75(–87) × (7–)10–16(–18) µm, (0–)2–6(–7)-septate.

It is unlikely that *C. pseudiridis* is of New Zealand origin since the genus *Iris* is not indigenous to New Zealand. All *Iris* species that are found in this country have been introduced, mainly for horticultural purposes. The species is, therefore, probably more common than indicated above. However, within the course of the recent monographic studies in the genus *Cladosporium* numerous herbarium specimens, mainly of European origin, have been examined and proved to be correctly identified agreeing with the species concept of *C. iridis*. Additional collections and cultures are necessary to determine its distribution.

Cladosporium ramotenellum K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504577. Figs 31–33.

Etymology: Refers to the morphological similarity with *Cladosporium tenellum*.

Differt a Cladosporio cladosporioide conidiophoris et conidiis leniter angustioribus, 2–4(–5) µm latis, conidiis 0–2(–3)-septatis, semper verruculosis; et a Cladosporio tenello locis conidiogenis non numerosis et non aggregatos ad apicem, conidiis longioribus et angustioribus, 2.5–35 × 2–4(–5) µm, 0–3-septatis.

Mycelium unbranched or only sparingly branched, 1.5–4 µm wide, septate, without swellings and constrictions, hyaline or subhyaline, smooth, sometimes irregularly rough-walled, walls unthickened. *Conidiophores* solitary, macronematous and micronematous, arising as lateral branches of plagiotropous hyphae or terminally from ascending hyphae, erect, straight or slightly flexuous, cylindrical, neither geniculate nor nodulose, without head-like swollen apices or intercalary swellings, unbranched, sometimes

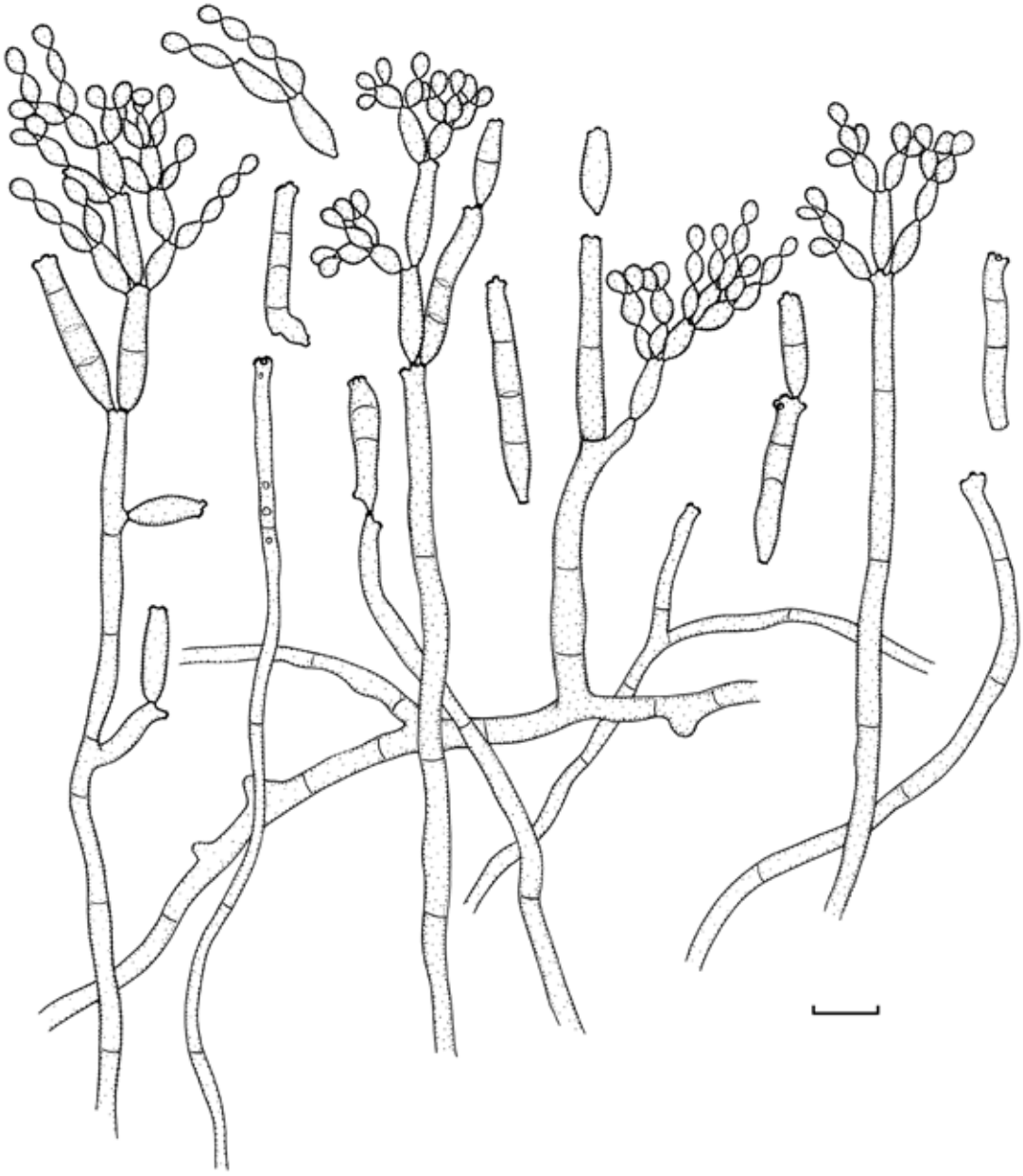

Fig. 31. *Cladosporium ramotenellum* (CPC 12043). Conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del.*

branched, branches often only as short lateral prolongations, mainly formed below a septum, 14–110 × 2–4 µm, septate, not constricted at the septa, subhyaline to pale olivaceous or brown, smooth to minutely verruculose, walls unthickened, sometimes guttulate. *Conidiogenous cells* integrated, terminal, sometimes also intercalary, cylindrical, not geniculate, non-nodulose, 10–28(–50) µm long, proliferation sympodial, with few conidiogenous loci, mostly 1–3, loci sometimes situated on small lateral prolongations, protuberant, 0.5–1.5(–2) µm diam, thickened and somewhat darkened-refractive. *Ramoconidia* formed, cylindrical-oblong, up to 47 µm long, 2–4 µm wide, 0–1-septate, rarely up to 4-septate, subhyaline to very pale olivaceous, smooth or almost so, with a broadly truncate base, without any dome and raised rim, 2–3 µm wide, not thickened but somewhat refractive. *Conidia* numerous, polymorphous, catenate, in branched chains, straight, sometimes slightly curved, small terminal conidia numerous, globose, subglobose or ovoid, obovoid or limoniform, 2.5–7 × 2–4(–4.5) µm [av. ± SD, 5.1 (± 1.3) × 3.1 (± 0.6) µm], aseptate, without distal hilum or with a single apical scar, intercalary conidia ellipsoid to subcylindrical, 8–15 × 3–4(–4.5) µm [av. ± SD, 11.5 (± 2.4) × 3.6 (± 0.5) µm], 0–1-septate; secondary ramoconidia subcylindrical to cylindrical-oblong, 17–35 × 3–4(–5) µm [av. ± SD, 22.5 (± 5.6) × 3.7 (± 0.5) µm], 0–3-septate, not constricted at the septa, subhyaline to very pale olivaceous, minutely verruculose (granulate under SEM), walls unthickened or almost so, apex broadly rounded or slightly attenuated towards apex and base, sometimes guttulate, hila protuberant, conspicuous, 0.8–1.5(–2) µm diam, somewhat thickened and darkened-refractive; microcyclic conidiogenesis occurring.

Cultural characteristics: Colonies on PDA reaching 46–49 mm diam after 14 d at 25 ºC, olivaceous to grey-olivaceous due to abundant sporulation, appearing zonate in forming concentric zones, margin entire edge to slightly undulate, white, glabrous, aerial mycelium absent or sparse, growth flat with a somewhat folded and wrinkled colony centre, without prominent exudates, sporulation profuse. Colonies on MEA reaching 48–49 mm diam after 14 d at 25 ºC, grey-olivaceous to olivaceous-grey, velvety, olivaceous-grey to

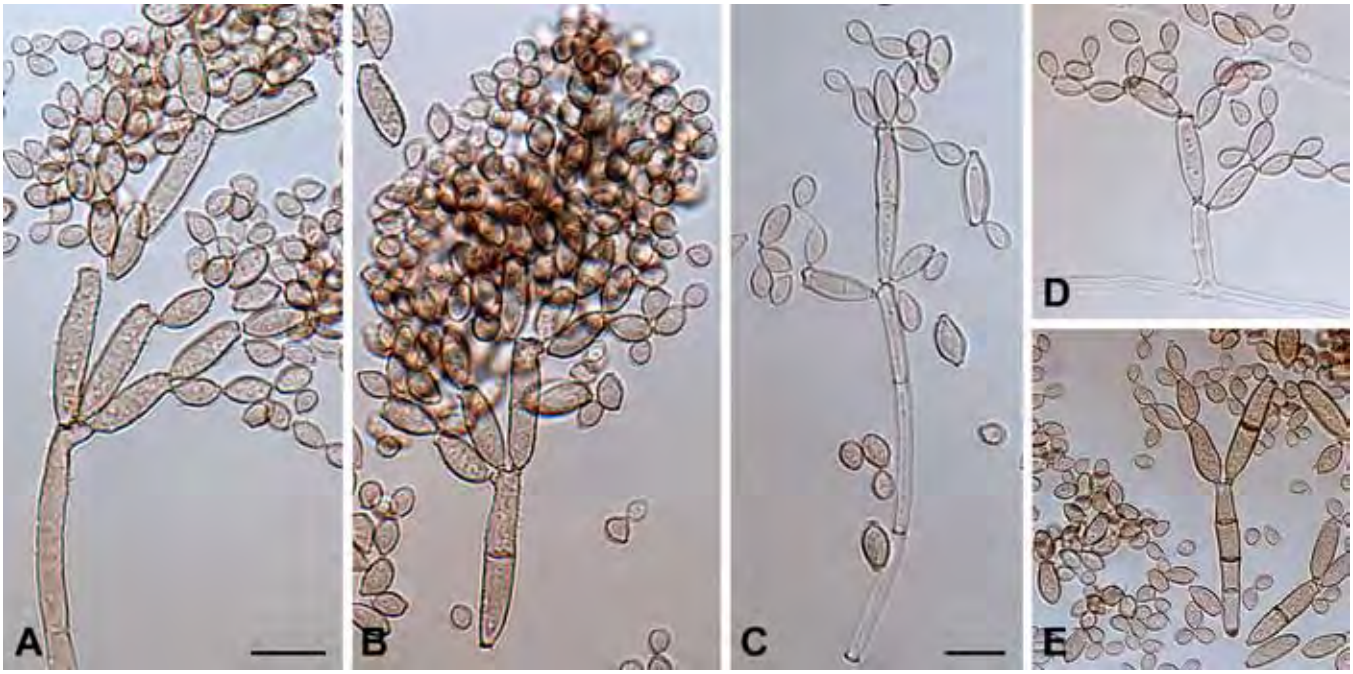

Fig. 32. *Cladosporium ramotenellum* (CPC 12043). A, C. Macronematous conidiophore. B. Conidial chain. D. Micronematous conidiophore. E. Ramoconidia and conidia. Scale bars = 10 µm.

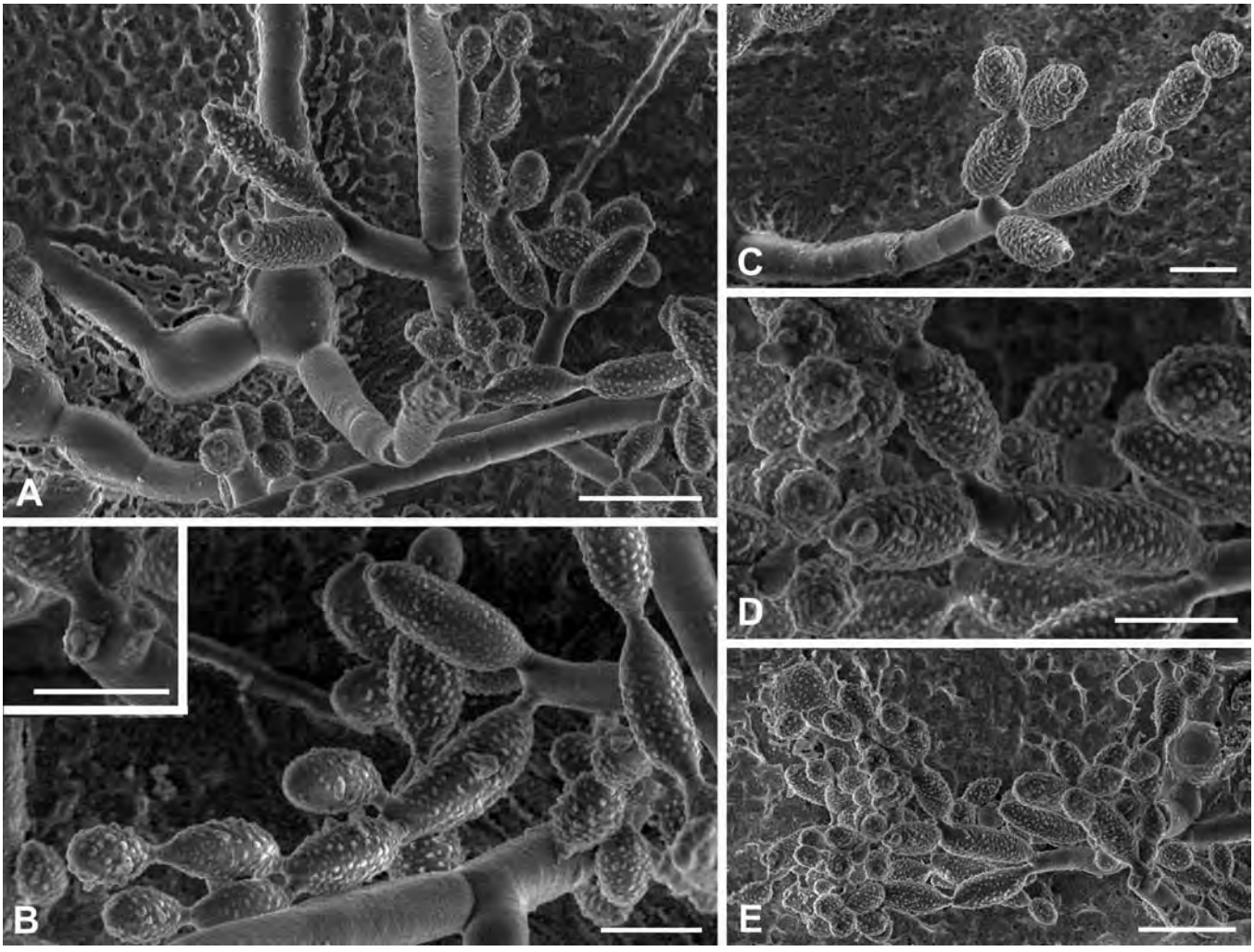

Fig. 33. *Cladosporium ramotenellum* (CPC 12043). A. Survey of colony development showing a large bulbous "foot cell" that gives rise to conidiophores, which can be branched. B. Details of conidiophores showing secondary ramoconidia and conidia. The inset shows scar formation on a conidiophore. C. Conidiophore and several conidia. D. Details of ornamentation on conidia. Note the wide, but relatively low ornamentation units. E. A micrograph illustrating the organisation within a conidiophore. Scale bars A–D = 5 µm, E = 10 µm.

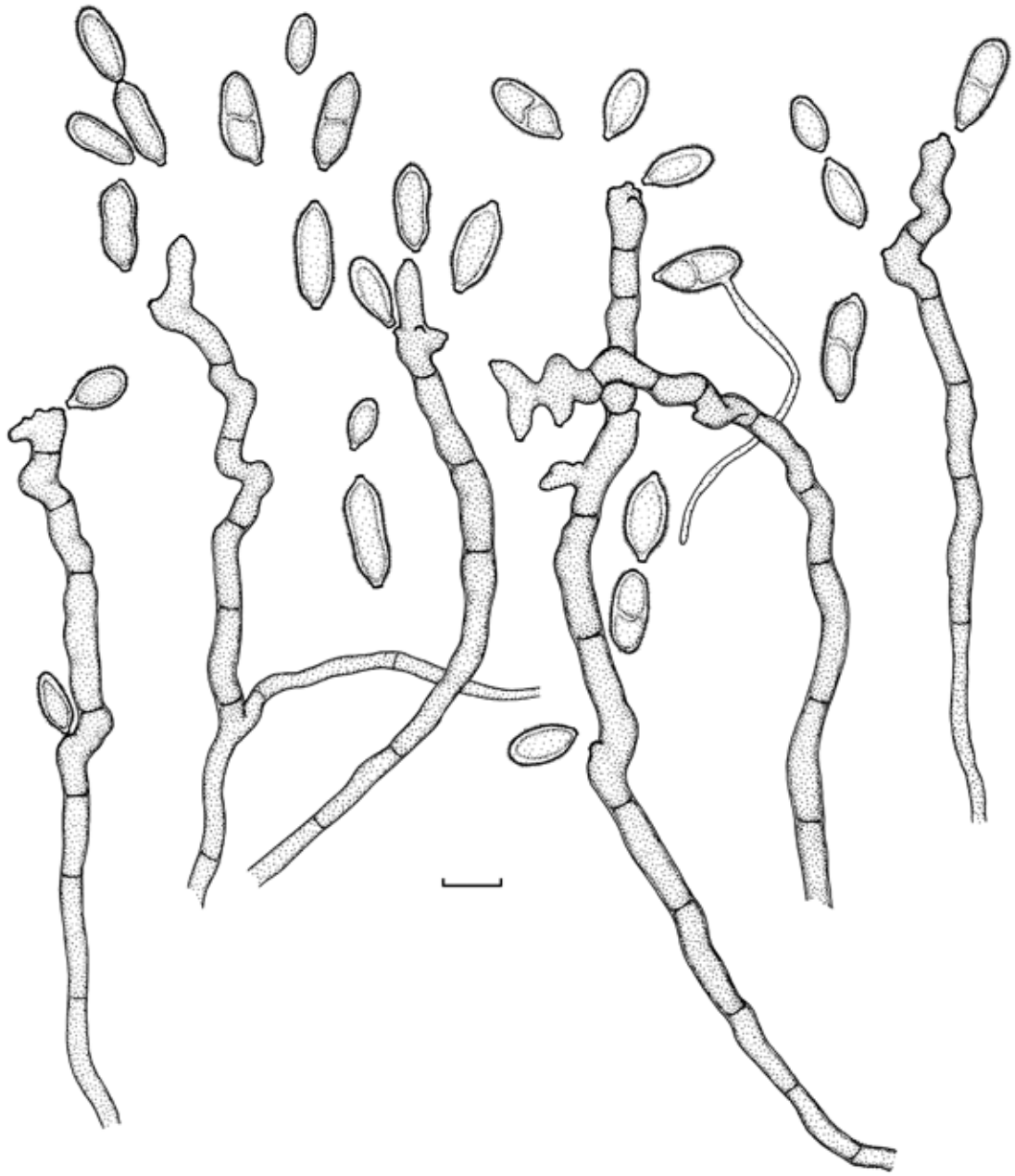

Fig. 34. *Cladosporium sinuosum* (CPC 11839). Conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

iron-grey reverse, margin entire edge to undulate, radially furrowed, colourless, glabrous to feathery, aerial mycelium sparse, diffuse, growth flat with slightly elevated colony centre, distinctly wrinkled, prominent exudates not formed, abundantly sporulating. Colonies on OA attaining 40 mm diam after 14 d at 25 ºC, grey-olivaceous, margin entire edge, colourless or white, glabrous, aerial mycelium absent or sparse, growth flat, without exudates, sporulation profuse.

Specimens examined: **Slovenia**, Ljubljana, isolated from an air conditioning system (bathroom), 2004, M. Butala, CBS 121627 = CPC 12047 = EXF-967; Sečovlje, isolated from hypersaline water from reverse ponds, salterns, 2005, P. Zalar, CBS-H 19862, **holotype**, isotype HAL 2026 F, culture ex-type CBS 121628 = CPC 12043 = EXF-454.

Substrate and distribution: Hypersaline water, air; Slovenia.

Notes: *Cladosporium ramotenellum,* which appears to be a saprobe in air and hypersaline water, morphologically resembles *C. cladosporioides* and *C. tenellum* K. Schub., Zalar, Crous & U. Braun, but is quite distinct from *C. cladosporioides* by having somewhat narrower conidiophores and conidia, 2–4(–5) µm wide, and 0–3-septate, always minutely verruculose conidia. *Cladosporium tenellum*, a newly introduced species (see below) isolated from hypersaline water and plant material, possesses conidiophores with numerous conidiogenous loci, usually crowded towards the apex forming sympodial clusters of pronounced scars, and shorter and somewhat wider, 0–1(–2)-septate conidia, 3–20(–28) × (2.5–)3–5(–6) µm. Besides these morphological differences, *C. ramotenellum* is faster growing in culture than *C. tenellum*.

Cladosporium arthrinioides Thüm. & Beltr. and *C. hypophyllum* Fuckel are also close to *C. ramotenellum*, but *C. arthrinioides*, known from Italy on leaves of *Bougainvillea spectabilis*, deviates in having shorter and wider, 0–1(–2)-septate, mostly smooth conidia (2–18 × 2–6.5 µm) which become larger and more frequently septate with age (up to 32 µm long and with up to four septa); and *C. hypophyllum* occurring in Europe on leaves of *Ulmus minor* differs in having often mildly to distinctly geniculate-sinuous, sometimes subnodulose conidiophores and shorter and somewhat wider, 0–1(–3)-septate conidia, 4–17(–19) × 2–5 µm, becoming distinctly swollen, darker, longer and wider with age, 5–7 µm, with the septa often being constricted (Schubert 2005).

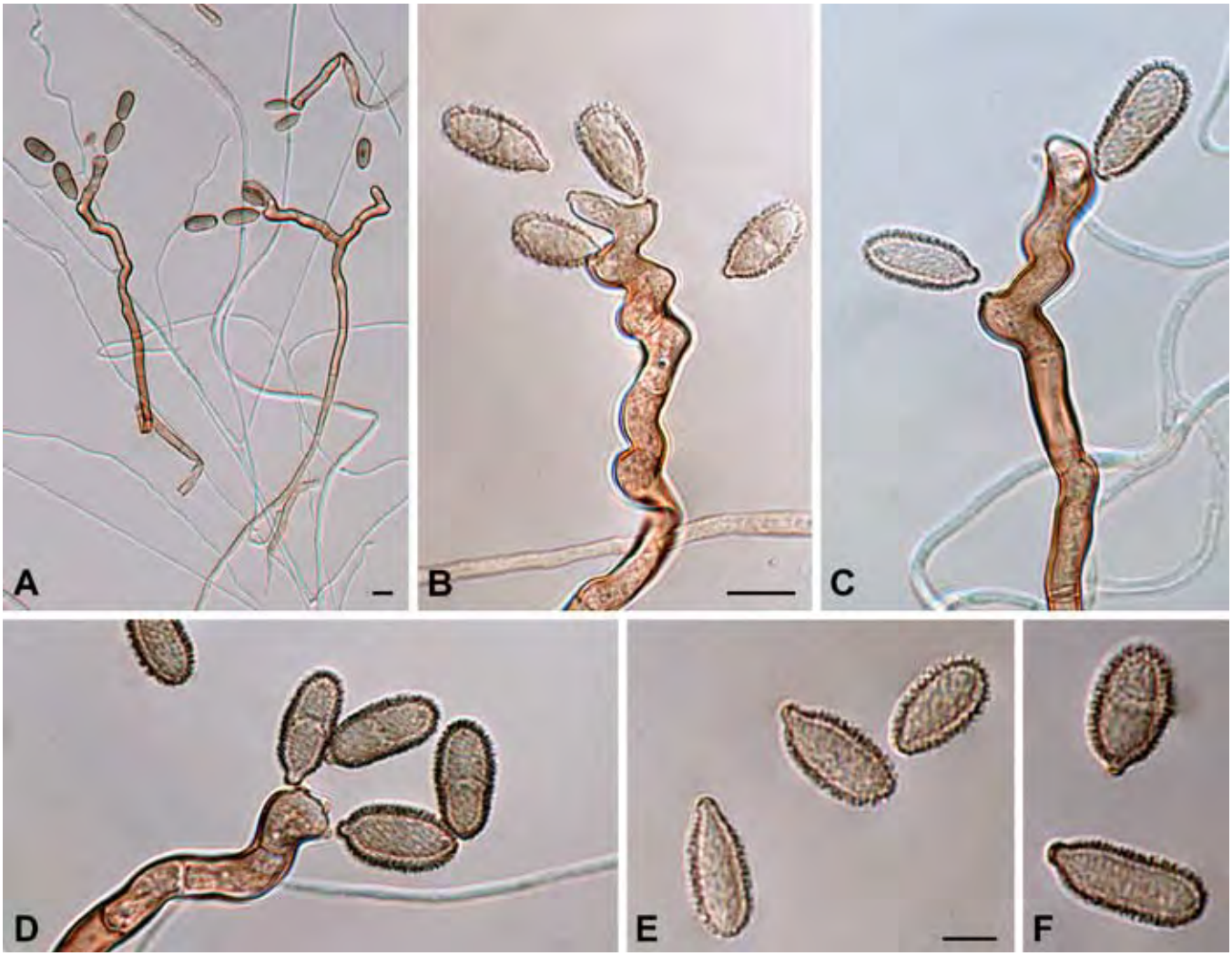

Fig. 35. *Cladosporium sinuosum* (CPC 11839). A–D. Conidiophores. E–F. Conidia. Scale bars = 10 µm.

Cladosporium sinuosum K. Schub., C.F. Hill, Crous & U. Braun, **sp. nov.** MycoBank MB504578. Figs 34–35.

Etymology: Refers to the usually distinctly sinuous conidiophores.

Differt a Cladosporio herbaro conidiophoris distincte sinuosis, conidiis solitariis vel breve catenatis, catenis non ramosis, echinulatis.

Mycelium sparingly branched, 1–7 µm wide, septate, not constricted at the septa, subhyaline to pale brown, smooth to minutely verruculose, walls unthickened or slightly thickened, sometimes with small swellings. *Conidiophores* arising laterally from plagiotropous hyphae or terminally from ascending hyphae, erect, more or less straight to flexuous, often once or several times slightly to distinctly geniculate-sinuous, sometimes even zigzag-like, nodulose with small to large lateral shoulders, shoulders somewhat distant from each other or in close succession giving them a knotty/gnarled appearance, unbranched or once branched, 25–260 × 5–7 µm, shoulders up to 10 µm wide, pluriseptate, septa sometimes in short succession, not constricted at the septa, pale brown to medium brown, smooth to minutely verruculose, walls thickened, often distinctly two-layered, up to 1 µm thick. *Conidiogenous cells* integrated, terminal or intercalary, often slightly to distinctly geniculate-sinuous, nodulose with small to large laterally swollen shoulders, 8–30 µm long, proliferation sympodial, with a single or up to three conidiogenous loci, usually confined to lateral shoulders, protuberant, often denticle-like or on the top of short cylindrical stalk-like prolongations, 1.2–2(–2.2) µm diam, mainly 2 µm, somewhat thickened and darkened-refractive, dome often slightly higher than the surrounding rim. *Conidia* solitary or in short unbranched chains with up to three conidia, straight, obovoid, oval, broadly ellipsoid to subcylindrical or sometimes clavate (broader at the apex), 9–21 × (5–)6–8 µm [av. ± SD, 14.5 (± 2.5) × 6.6 (± 0.7) µm], 0–1-septate, not constricted at the septa, septum more or less median, pale greyish brown, densely echinulate, spines up to 1 µm long, walls thickened, apex mostly broadly rounded or sometimes attenuated, towards the base mostly distinctly attenuated forming a peg-like prolongation, up to 2 µm long, hila protuberant, 1.2–2 µm diam, mainly 2 µm, somewhat thickened and darkened-refractive; microcyclic conidiogenesis not observed.

Cultural characteristics: Colonies on PDA attaining 20 mm diam after 14 d at 25 ºC, pale olivaceous-grey due to abundant aerial mycelium, olivaceous-grey towards margins, iron-grey to olivaceous-black reverse, margin regular, entire edge, aerial mycelium abundant, cottony, dense, high, growth regular, low convex, radially furrowed in the centre, growing deep into the agar, with age numerous small to large prominent exudates, sporulation sparse. Colonies on MEA attaining 16 mm diam after 14 d at 25 ºC, white to pale smoke-grey, fawn reverse, velvety, margin undulate, glabrous, aerial mycelium abundant, dense, high, fluffy, growth raised with elevated colony centre, laterally furrowed, without

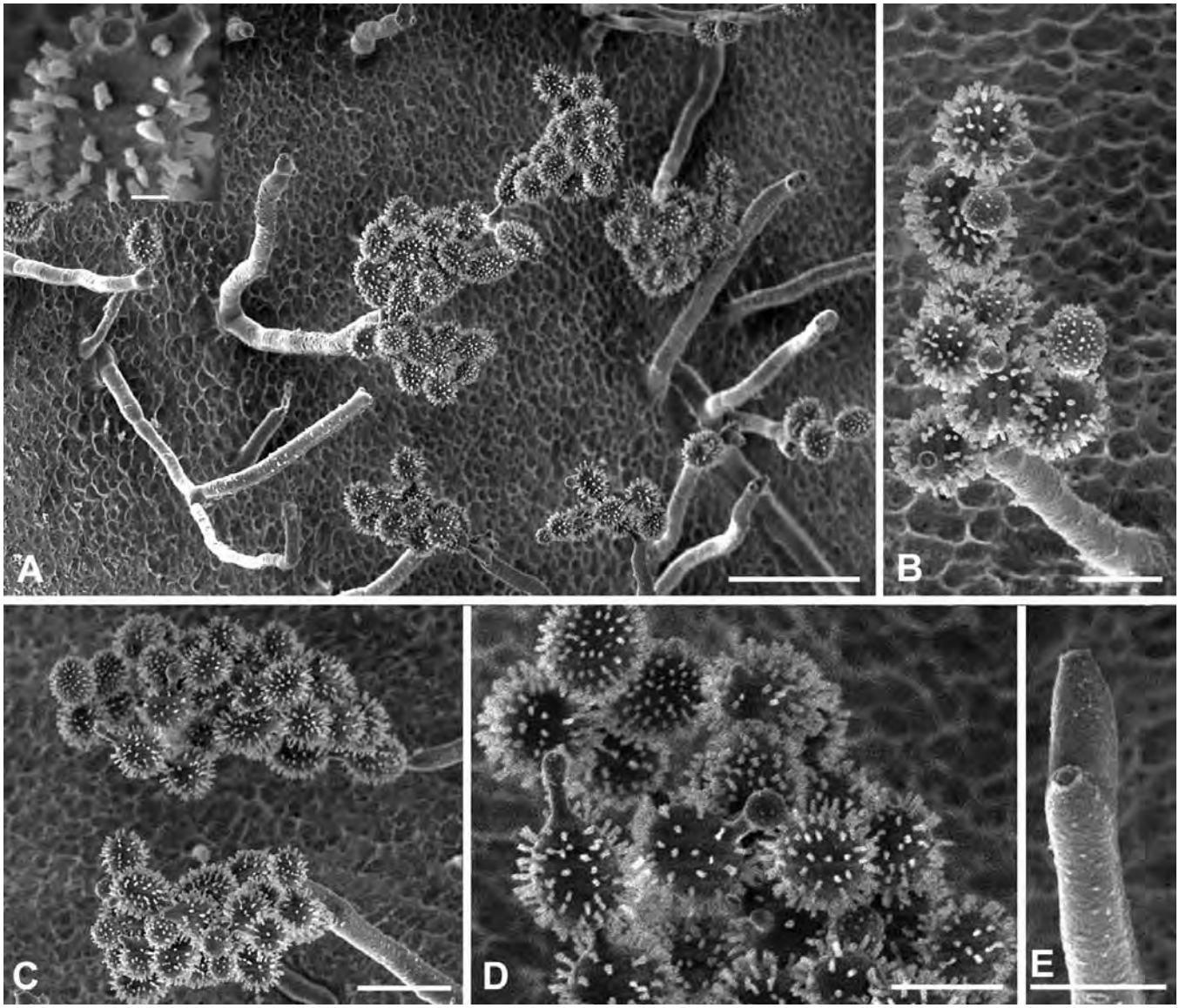

Fig. 36. *Cladosporium spinulosum* (CPC 12040). A. Overview on agar surface with conidiophores arising from the surface. The spore clusters on the conidiophore are very compact. Note several simple, tubular conidiophore ends. The inset shows details of a conidium showing two pronounced hila and a unique, very distinct ornamentation on the cell wall. B. Conidiophore with globose or subsphaerical secondary ramoconidia and conidia. Note the newly forming cells and hila. C. Two conidiophores. D. Details of spores and spore formation. E. The end of a conidiophore and two scars. Scale bars: A = 20 µm, A (inset) = 1 µm, B, D–E = 5 µm, C = 10 µm.

prominent exudates. Colonies on OA attaining 18 mm diam after 14 d at 25 ºC, olivaceous, white to pale olivaceous-grey in the centre due to abundant aerial mycelium, olivaceous-grey reverse, margin white, entire edge, glabrous, aerial mycelium loose to dense, high, fluffy to felty, growth flat to low convex, regular, without prominent exudates, sporulating.

Specimen examined: **New Zealand**, Te Anau, isolated from leaves of *Fuchsia excorticata* (*Onagraceae*), 31 Jan. 2005, A. Blouin, Hill 1134A, CBS-H 19863, **holotype**, culture ex-type CBS 121629 = CPC 11839 = ICMP 15819.

Substrate and distribution: On living leaves of *Fuchsia excorticata*; New Zealand.

Notes: This new species is well characterised by its slightly to distinctly geniculate-sinuous, often zigzag-like conidiophores and its conidia formed solitary or rarely in short unbranched chains and is therefore morphologically not comparable with any of the species described until now. Most *Cladosporium* species with conidia usually formed solitary or in short unbranched chains have previously been treated as species of the genus *Heterosporium* Klotzsch ex Cooke, now considered to be synonymous with *Cladosporium*. All of them, including the newly introduced *C. arthropodii* K. Schub. & C.F. Hill from New Zealand, which also belongs to this species complex (Braun *et al.* 2006), possess very large and wide, often pluriseptate conidia quite distinct from those of *C. sinuosum* (David 1997). *Cladosporium alopecuri* (Ellis & Everh.) U. Braun, known from the U.S.A. on *Alopecurus geniculatus* is also quite different by having larger and wider conidia, 20–40 × 7–13(–15) µm, and wider conidiogenous loci and conidial hila, 3.5–5 µm diam (Braun 2000).

Cladosporium herbarum is superficially similar but the conidiophores of the latter species are sometimes only slightly geniculate-sinuous but never zigzag-like and the verruculose to verrucose conidia are frequently formed in unbranched or branched chains.

Cladosporium spinulosum Zalar, de Hoog & Gunde-Cimerman, Studies in Mycology 58: 180. 2007 – this volume. Fig. 36.

Note: This new species is described and illustrated in Zalar *et al.* (2007 – this volume).

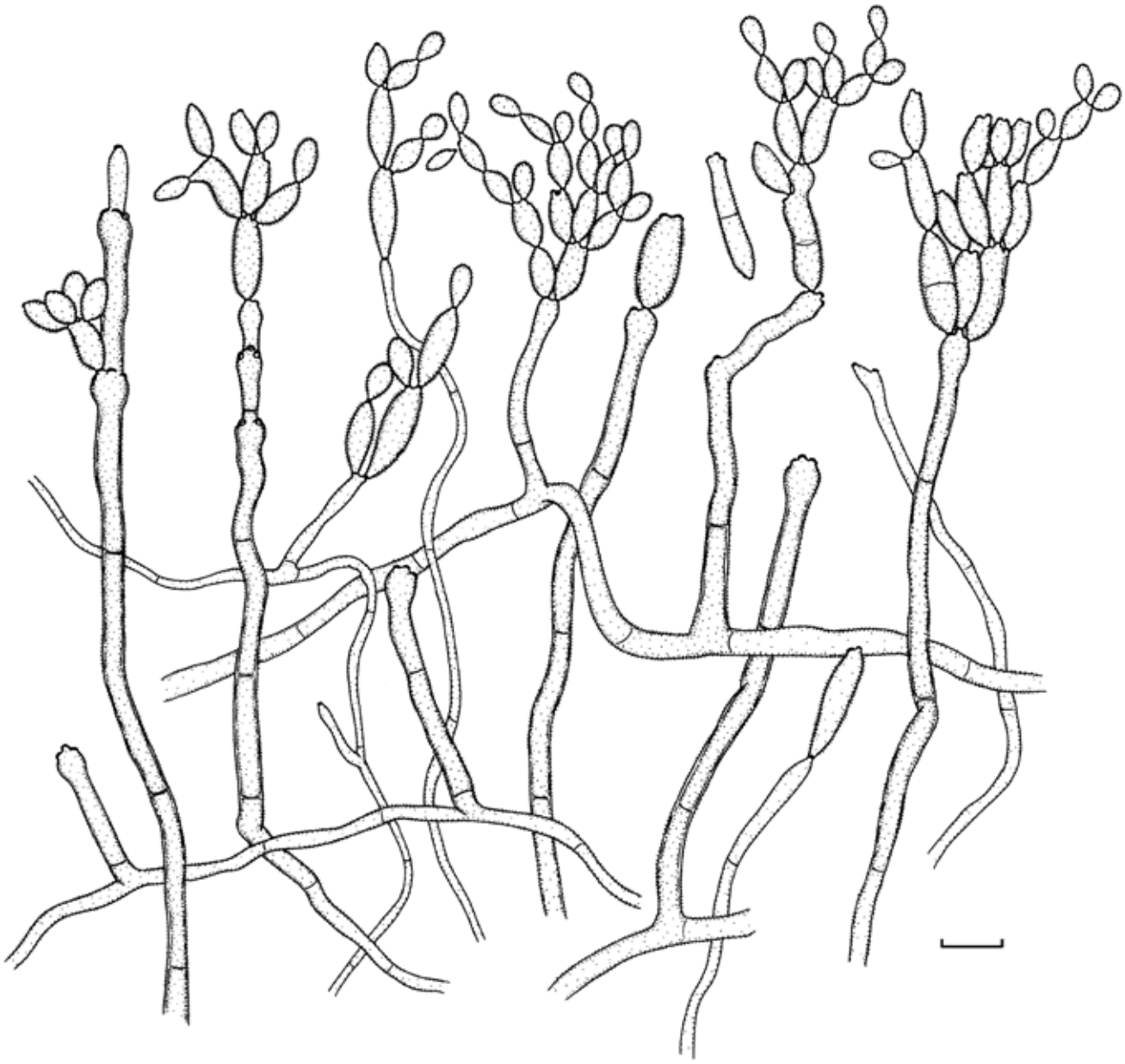

Fig. 37. *Cladosporium subinflatum* (CPC 12041). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

Cladosporium subinflatum K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504579. Figs 37–39.

Etymology: Refers to its nodulose conidiophores.

Differt a Cladosporio bruhnei conidiophoris cum nodulis angustioribus, 3–6.5 µm latis, conidiis brevioribus, 4–17(–22) µm longis, spinulosis, cum spinulis ad 0.8 µm longis; et a Cladosporio spinuloso conidiophoris nodulosis, conidiis spinulosis, cum spinulis brevioribus, ad 0.8 longis, locis conidiogenis et hilis latioribus, (0.5–)1–2 µm latis.

Mycelium unbranched or occasionally branched, 1.5–3 µm wide, later more frequently branched and wider, up to 7 µm wide, septate, not constricted at the septa, hyaline or subhyaline, almost smooth to somewhat verruculose or irregularly rough-walled, walls unthickened. *Conidiophores* mainly macronematous, sometimes also micronematous, arising terminally from ascending hyphae or laterally from plagiotropous hyphae, erect or subdecumbent, straight or flexuous, sometimes bent, cylindrical, nodulose, usually with small head-like swellings, sometimes swellings also on a lower level or intercalary, occasionally geniculate, unbranched, occasionally branched, (5–)10–270 × (1.5–)2.5–4.5(–5.5) µm, swellings 3–6.5 µm wide, aseptate or with few septa, not constricted at the septa, pale brown, pale olivaceous-brown or somewhat reddish brown, smooth, usually verruculose or irregularly rough-walled and paler, subhyaline towards the base, walls thickened, sometimes appearing even two-layered, up to 1 µm thick. *Conidiogenous cells* integrated, usually terminal or conidiophores reduced to conidiogenous cells, cylindrical, nodulose, usually with small head-like swellings with loci confined to swellings, sometimes geniculate, 5–42 µm long, proliferation sympodial, with several loci, up to four situated at nodules or on lateral swellings, protuberant, conspicuous, denticulate, (0.8–)1–2 µm diam, thickened and darkened-refractive. *Conidia* catenate, in branched chains, more or less straight, numerous globose and subglobose conidia, ovoid, obovoid, broadly ellipsoid to cylindrical, 4–17(–22) × (2.5–)3.5–5.5(–7) µm [av. ± SD, 11.7 (± 4.6) × 4.5 (± 0.8) µm], 0–1(–2)-septate, not constricted at septa, pale brown or pale olivaceous-brown, ornamentation variable, mainly densely verruculose to echinulate (loosely muricate under SEM), spines up to 0.8 µm high, sometimes irregularly verrucose with few scattered tubercles

Fig. 38. *Cladosporium subinflatum* (CPC 12041). A–C. Macronematous conidiophores. D–E. Conidia. Scale bar = 10 µm.

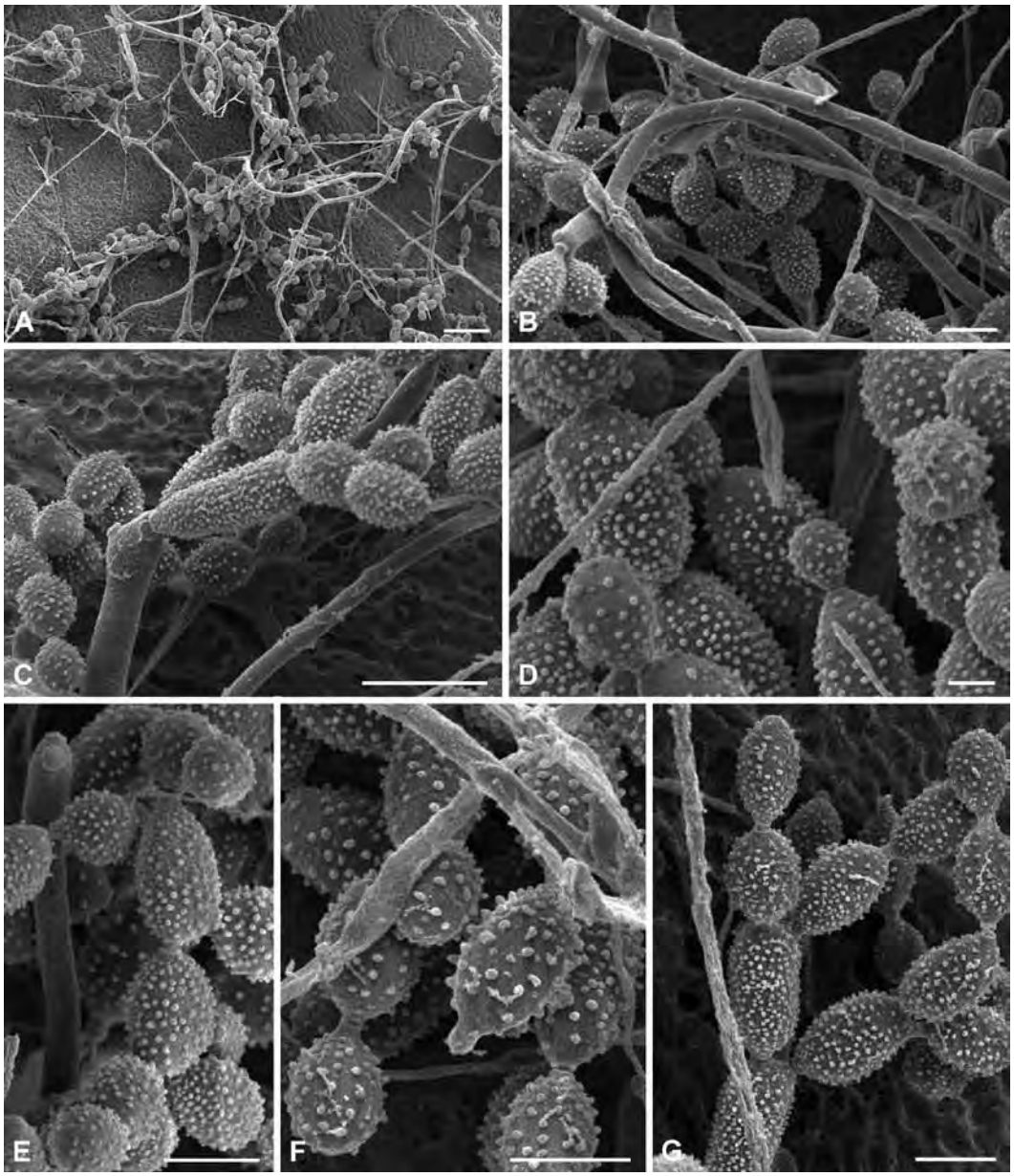

Fig. 39. *Cladosporium subinflatum* (CPC 12041). A–G. Images of an 11-d-old culture on SNA. A. Overview of colony with clusters of conidia and aerial hyphae. Many of the hyphae have a collapsed appearance. B. Detail of colony with conidiophores, conidia and aerial hyphae that are partly collapsed. C. Detail of a conidiophore end and a secondary ramoconidium. Note the scars at the end of the conidiophore. D. Details of conidia and ornamentation. The ornamentation consists out of markedly defined units, which have a relatively large distance from each other. Note the hilum on the right conidium. E. Conidiophore with large scars and conidia. F. Different blastoconidia with very early stages of new spore formation in the middle of the picture. G. Pattern of spore development. Scale bars: A = 20 µm, B, E–G = 5 µm, C = 10 µm, D = 2 µm.

or irregularly echinulate, walls unthickened or slightly thickened, apex rounded or slightly attenuated towards apex and base, hila conspicuous, protuberant, denticulate, 0.5–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis observed.

Cultural characteristics: Colonies on PDA attaining 29 mm diam after 14 d at 25 ºC, olivaceous-black to olivaceous-grey towards margin, margin regular, entire edge, narrow, colourless to white, glabrous to feathery, aerial mycelium formed, fluffy, mainly near margins, growth flat, somewhat folded in the colony centre, deep into the agar, few prominent exudates formed with age, sporulation profuse. Colonies on MEA attaining 25 mm diam after 14 d at 25 ºC, olivaceous-grey to olivaceous due to abundant sporulation in the colony centre, pale greenish grey towards margin, iron-grey reverse, velvety to powdery, margin crenate, narrow, white, glabrous, radially furrowed, aerial mycelium diffuse, growth convex with papillate surface, wrinkled colony centre, without prominent exudates, sporulation profuse. Colonies on OA attaining 26 mm diam after 14 d at 25 ºC, olivaceous, iron-grey to greenish black reverse, growth flat, deep into the agar, with a single exudate, abundantly sporulating.

Specimen examined: **Slovenia**, Sečovlje, isolated from hypersaline water from crystallization ponds, salterns, 2005, S. Sonjak, CBS-H 19864, **holotype**, isotype HAL 2027 F, culture ex-type CBS 121630 = CPC 12041 = EXF-343.

Substrate and distribution: Hypersaline water; Slovenia.

Notes: *Cladosporium subinflatum*, an additional saprobic species isolated from hypersaline water, was at first identified as *C. spinulosum*, but proved to be both morphologically as well as phylogenetically distinct from the latter species in having somewhat wider [(1.5–)2.5–4.5(–5.5) µm], nodulose macronematous conidiophores with conidiogenous loci confined to swellings, wider conidiogenous loci and hila, (0.8–)1–2 µm, and spiny conidia with shorter spines than in *C. spinulosum* (up to 0.8 µm versus 0.5–1.3 µm long) (Zalar *et al.* 2007). With its narrow, nodulose macronematous conidiophores and catenate conidia, *C. bruhnei* is morphologically also similar but differs by having conidiophores with wider swellings, (4–)5–8 µm, and longer conidia 4–24(–31) µm, rarely up to 40 µm long which are minutely verruculose to verrucose but not spiny.

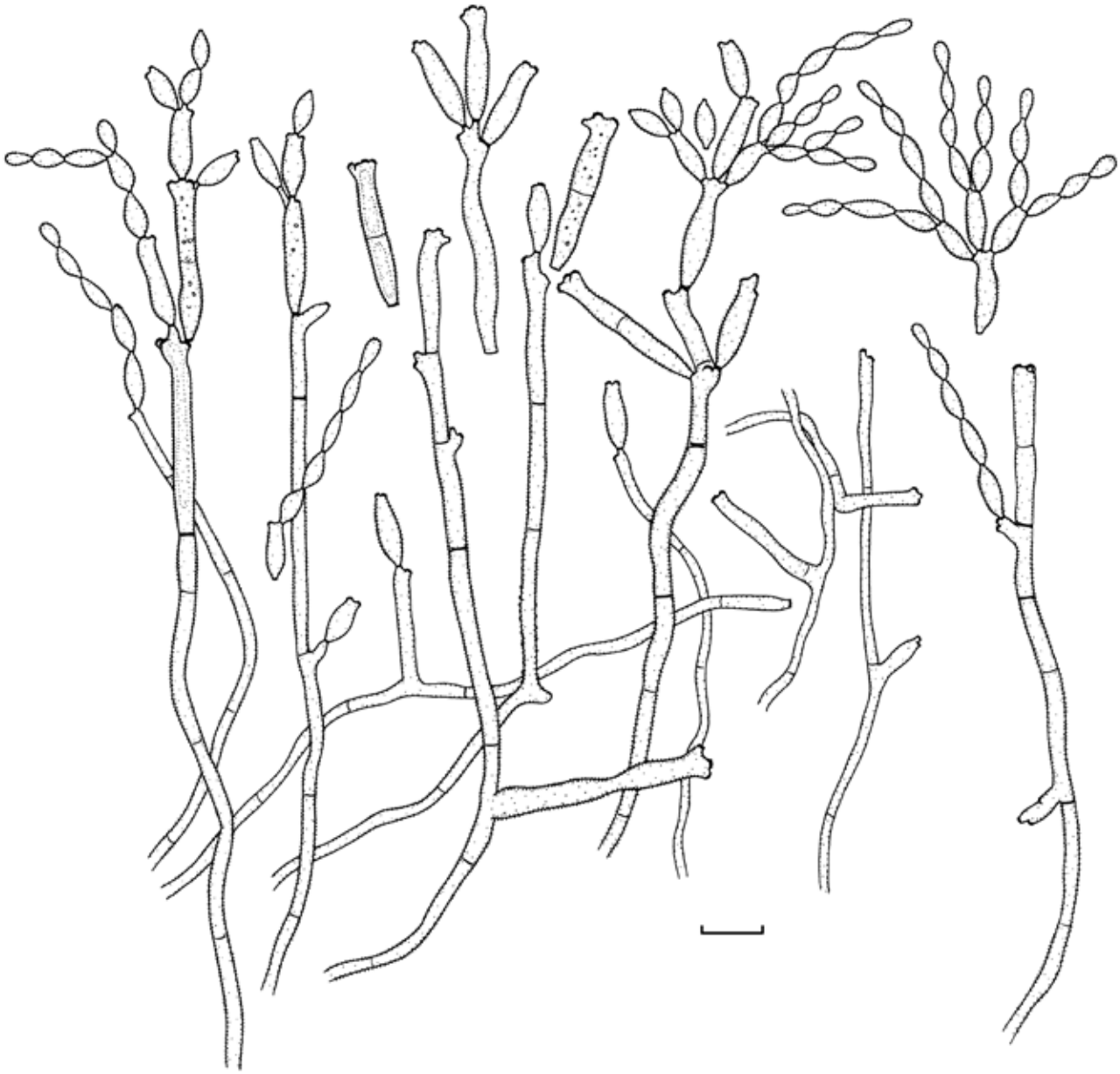

Fig. 40. *Cladosporium subtilissimum* (CBS 113754). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

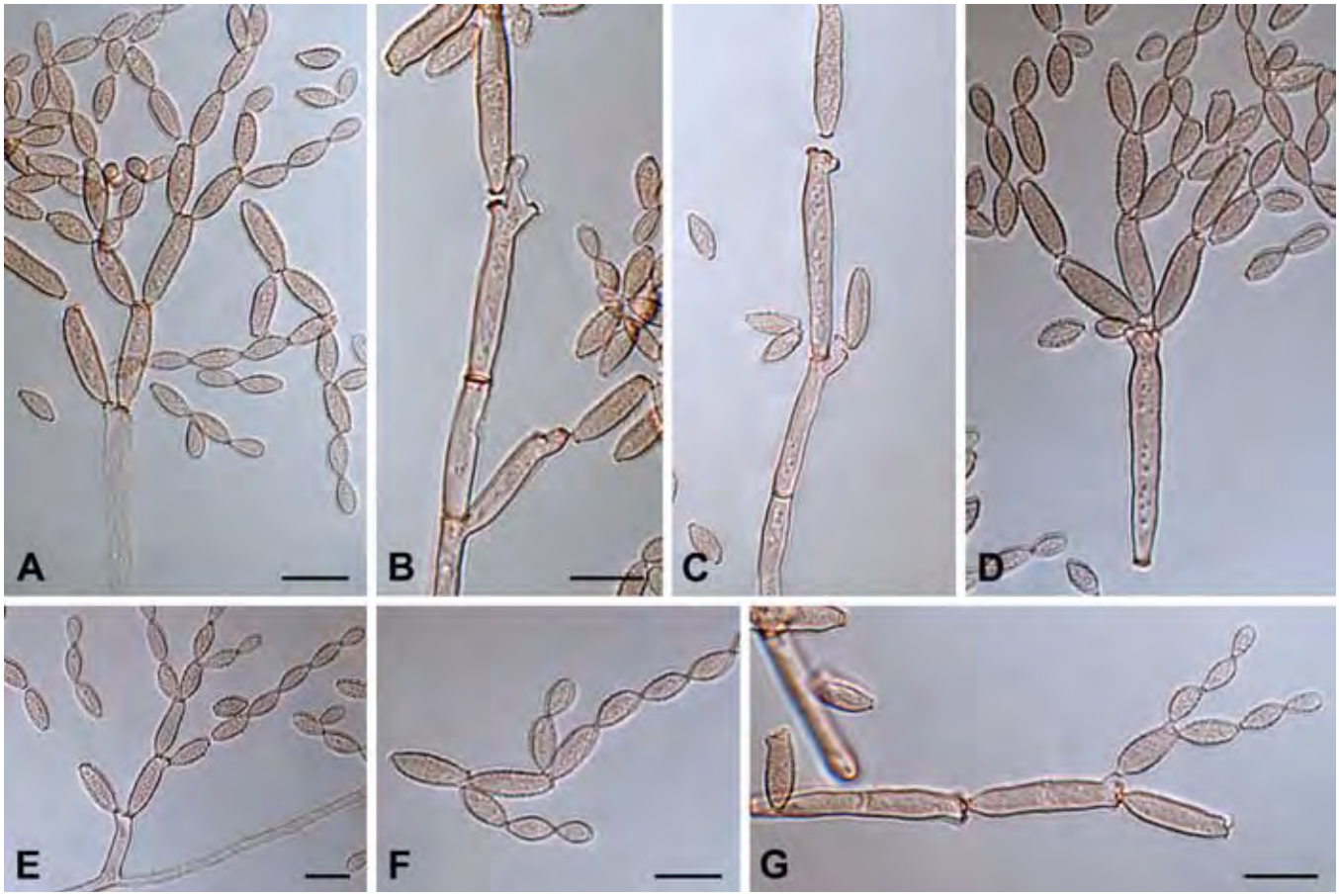

Fig. 41. *Cladosporium subtilissimum* (CBS 113754). A–C. Macronematous conidiophores. D. Conidial chain. E. Micronematous conidiophore. F–G. Conidia. Scale bars = 10 µm.

Cladosporium subtilissimum K. Schub., Dugan, Crous & U. Braun, **sp. nov.** MycoBank MB504580. Figs 40–42.

Etymology: Refers to its narrow conidiophores and conidia.

Differt a Cladosporio cladosporioide conidiophoris et conidiis semper asperulatis ad verruculosis, conidiis 0–1(–2)-septatis.

Mycelium unbranched or sparingly branched, 1–5 µm wide, septate, without swellings and constrictions, hyaline to subhyaline or pale brown, smooth to minutely verruculose, walls unthickened or almost so, protoplasm somewhat guttulate or granular. *Conidiophores* macronematous and micronematous, arising laterally from plagiotropous hyphae or terminally from ascending hyphae, erect, straight to slightly flexuous, filiform to cylindrical-oblong, non-nodulose, sometimes geniculate towards the apex, unbranched or once branched, branches short to somewhat longer, usually formed below a septum, sometimes only short, denticle-like or conical, 25–140 × 2–4 µm, 0–4-septate, not constricted at the septa, subhyaline to pale brown, almost smooth, minutely verruculose to verruculose, sometimes irregularly rough-walled in the lower part, walls unthickened or slightly thickened, protoplasm guttulate or somewhat granular. *Conidiogenous cells* integrated, terminal or pleurogenous, sometimes also intercalary, filiform to narrowly cylindrical, non-nodulose, sometimes geniculate, 14–57 µm long, with usually sympodial clusters of pronounced conidiogenous loci at the apex or on a lower level, denticle-like or situated on short lateral prolongations, up to five loci, intercalary conidiogenous cells usually with a short denticle-like lateral outgrowth below a septum, protuberant, denticulate, somewhat truncate, 1.2–2 µm diam, thickened and darkened-refractive. *Ramoconidia* sometimes occurring, conidiogenous cells seceding at one of the upper septa of the conidiophore and behaving like conidia, filiform or cylindrical, 20–40(–55) µm long, 1.5–4 µm wide, 0–1-septate, concolorous with conidiophores, not attenuated towards apex and base, base broadly truncate, non-cladosporioid, without any dome and raised rim, 2–3.5 µm wide, neither thickened nor darkened, sometimes slightly refractive. *Conidia* catenate, in branched chains, up to 12 or even more in a chain, straight, small terminal conidia numerous, subglobose, narrowly obovoid, limoniform or fusiform, 4–9 × 2–3.5 µm [av. ± SD, 6.4 (± 1.5) × 2.8 (± 0.4) µm], with up to three distal scars, aseptate, hila (0.5–)0.8–1 µm diam, intercalary conidia narrowly ellipsoid, fusiform to subcylindrical, 9–18 × 3–4(–6) µm [av. ± SD, 13.0 (± 2.5) × 3.8 (± 0.3) µm], 0(–1)-septate, hila 1–1.2(–1.8) µm diam, with up to four distal scars, secondary ramoconidia ellipsoid, fusiform or subcylindrical, (13–)17–32(–37) × 3–5(–6) µm [av. ± SD, 21.4 (± 4.4) × 4.1 (± 0.5) µm], 0–1(–2)-septate, septum median or somewhat in the lower half, usually not constricted at the septa, with up to six distal hila crowded at the apex, hila (1.2–)1.5–2(–2.5) µm diam, apex often somewhat laterally enlarged or prolonged with hila crowded there, very pale or pale brown or olivaceous-brown, minutely verruculose to verruculose (granulate under SEM), walls unthickened or only slightly thickened, often slightly attenuated towards apex and base, protoplasm often guttulate or granular, hila protuberant, denticulate, (0.5–)0.8–2(–2.2) µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occasionally observed.

Cultural characteristics: Colonies on PDA attaining 24 mm diam after 14 d at 25 °C, grey-olivaceous to olivaceous, olivaceous-grey, iron-grey or olivaceous-black reverse, velvety, margin regular, entire edge, white or pale greenish olivaceous, glabrous to feathery, aerial mycelium sparse, only few areas with abundant

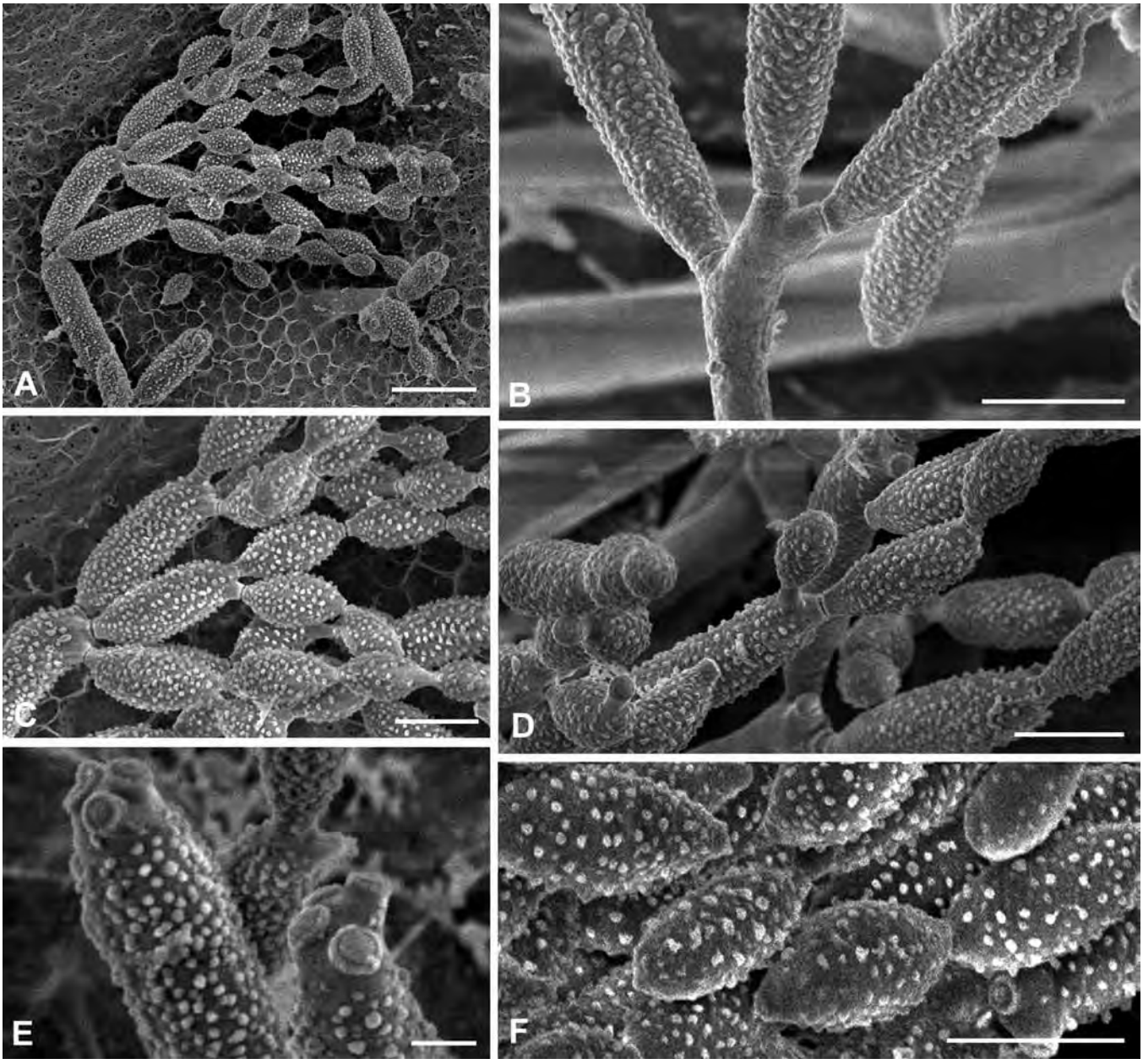

Fig. 42. *Cladosporium subtilissimum* (CBS 113754). A. Overview on the organisation of spore formation. The micrograph shows a large basal secondary ramoconidium which has chains of secondary ramoconidia, intercalary and small terminal conidia. The conidia are formed in rows of often three cells. Note the size difference in the different cells. B. Conidiophore showing very pronounced scars that almost appear as branches. C. Detail of (A), illustrating the scar formation between the cells. D. Conidia during different stages of formation. E. Details of pronounced hila, and prominent ornamentation on secondary ramoconidia with the central dome-formed area. F. Different conidia and hila. Scale bars: A = 10 µm, B–D, F = 5 µm, E = 2 µm.

mycelium, diffuse, growth regular, flat or with a raised and wrinkled colony centre, radially furrowed, effuse, usually without prominent exudates, with age several exudates formed, sporulation profuse, colonies consisting of two kinds of conidiophores, short and a few longer ones. Colonies on MEA reaching 25 mm diam after 14 d at 25 °C, greenish olivaceous to grey-olivaceous in the centre, olivaceous-grey to iron-grey reverse, velvety, margin entire edge, crenate or umbonate, narrow, pale greenish olivaceous, sometimes radially furrowed, aerial mycelium absent or sparse, growth low convex with distinctly wrinkled colony centre, without prominent exudates, abundantly sporulating. Colonies on OA attaining 25 mm diam after 14 d at 25 °C, dark grey-olivaceous to olivaceous due to profuse sporulation, iron-grey reverse, sometimes releasing some olivaceous-buff pigments into the agar, velvety, margin regular, entire edge or crenate, narrow, colourless or white, glabrous or feathery, aerial mycelium sparse, growth flat with slightly raised colony centre, prominent exudates lacking, sporulation profuse.

Specimens examined: **Slovenia**, Sečovlje, isolated from hypersaline water from salterns (reserve pond), 2005, P. Zalar, CPC 12044 = EXF-462. **U.S.A.**, isolated from bing cherry fruits, F. Dugan, CBS 113753; isolated from a grape berry, F. Dugan, wf 99-2-9 sci 1, CBS-H 19865, **holotype**, isotype HAL 2028 F, culture ex-type CBS 113754.

Excluded strains within the subtilissimum complex: **Argentina**, isolated from *Pinus ponderosa* (*Pinaceae*), 2005, A. Greslebin, CPC 12484, CPC 12485. **U.S.A.**, isolated from grape berry, F. Dugan, CBS 113741, CBS 113742; isolated from grape bud, F. Dugan, CBS 113744.

Substrate and distribution: Plant material and hypersaline water; Slovenia, U.S.A.

Notes: *Cladosporium cladosporioides* is morphologically comparable with the new species but deviates in having usually smooth conidiophores and conidia, with the conidia being mainly aseptate. *C. subtilissimum* is represented by three isolates of different origins

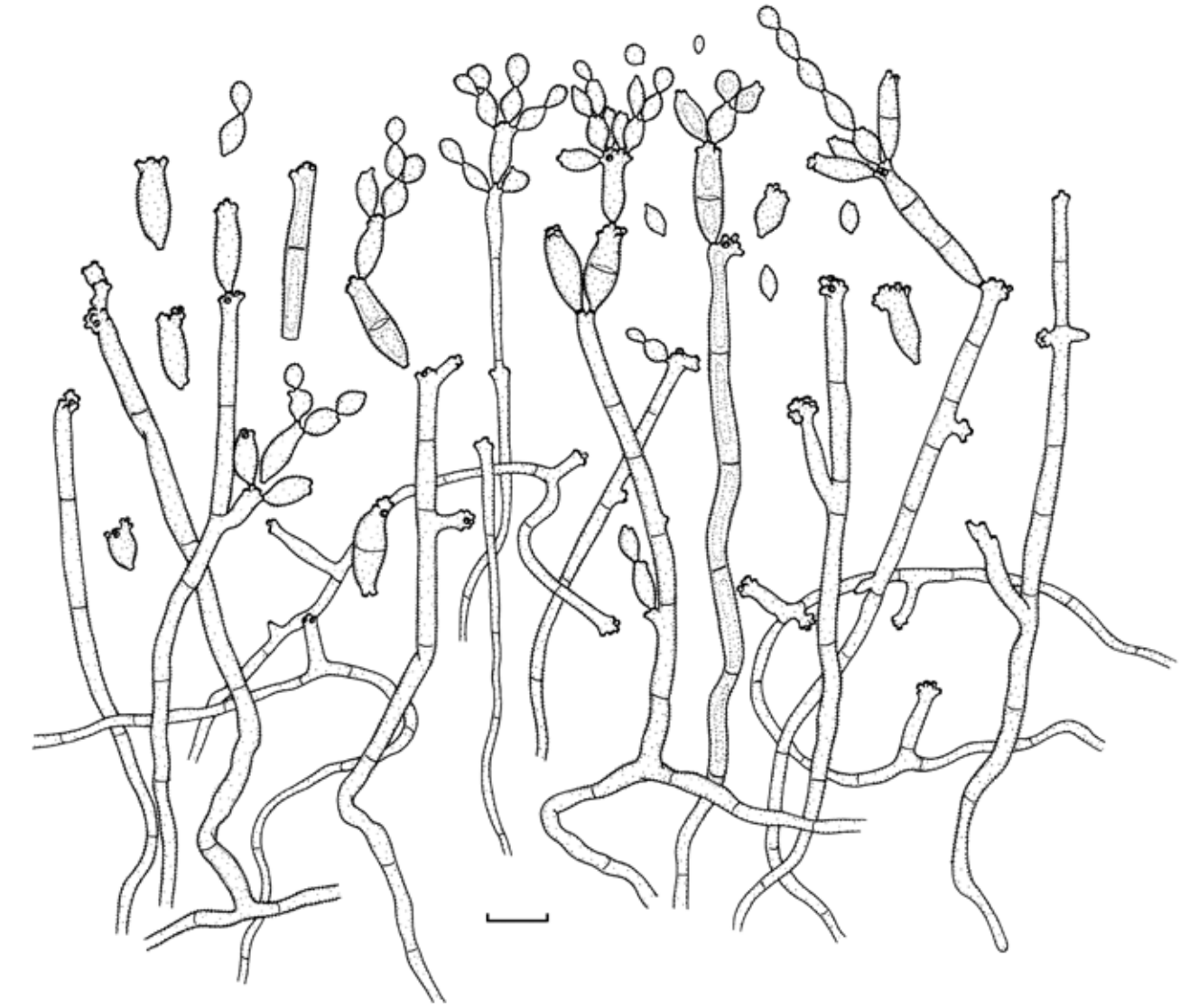

Fig. 43. *Cladosporium tenellum* (CPC 12053). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

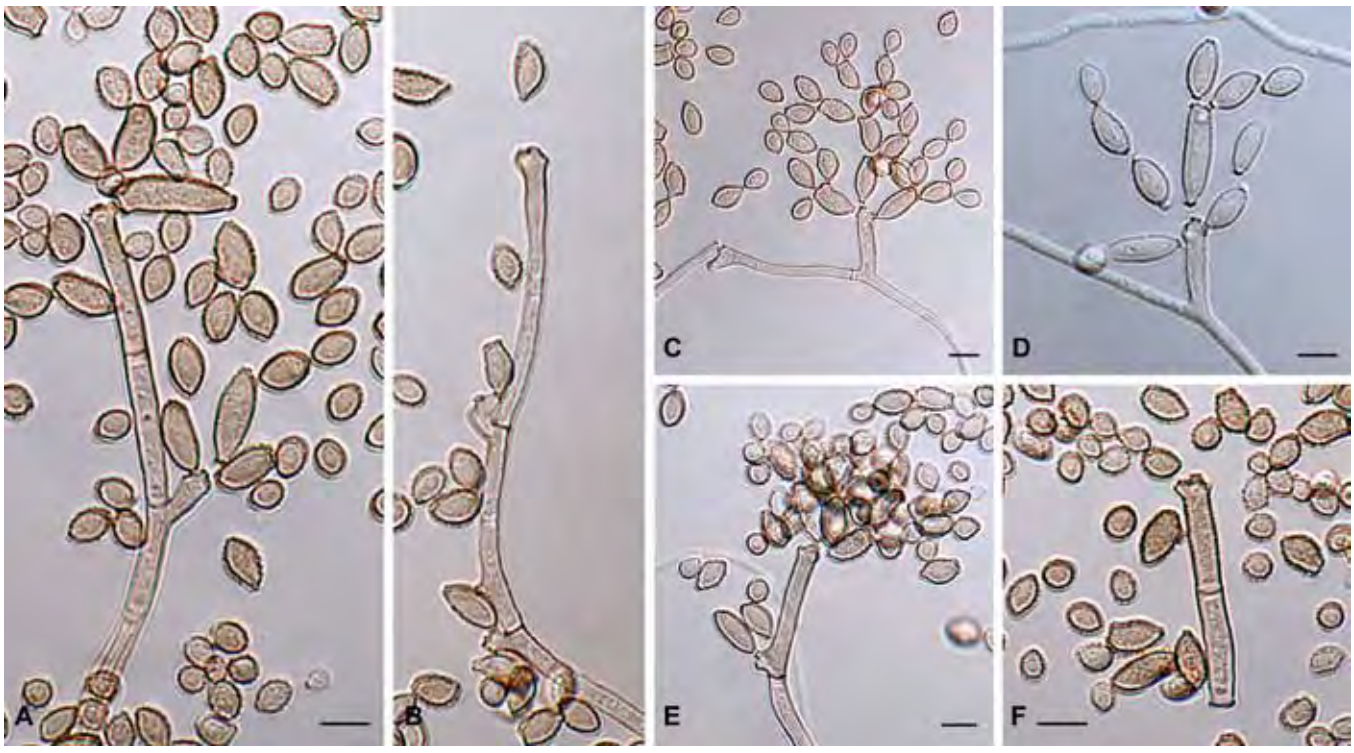

Fig. 44. *Cladosporium tenellum* (CPC 12053). A–C, E. Macronematous conidiophore. D. Micronematous conidiophore. F. Ramoconidium and conidia. Scale bars = 10 µm.

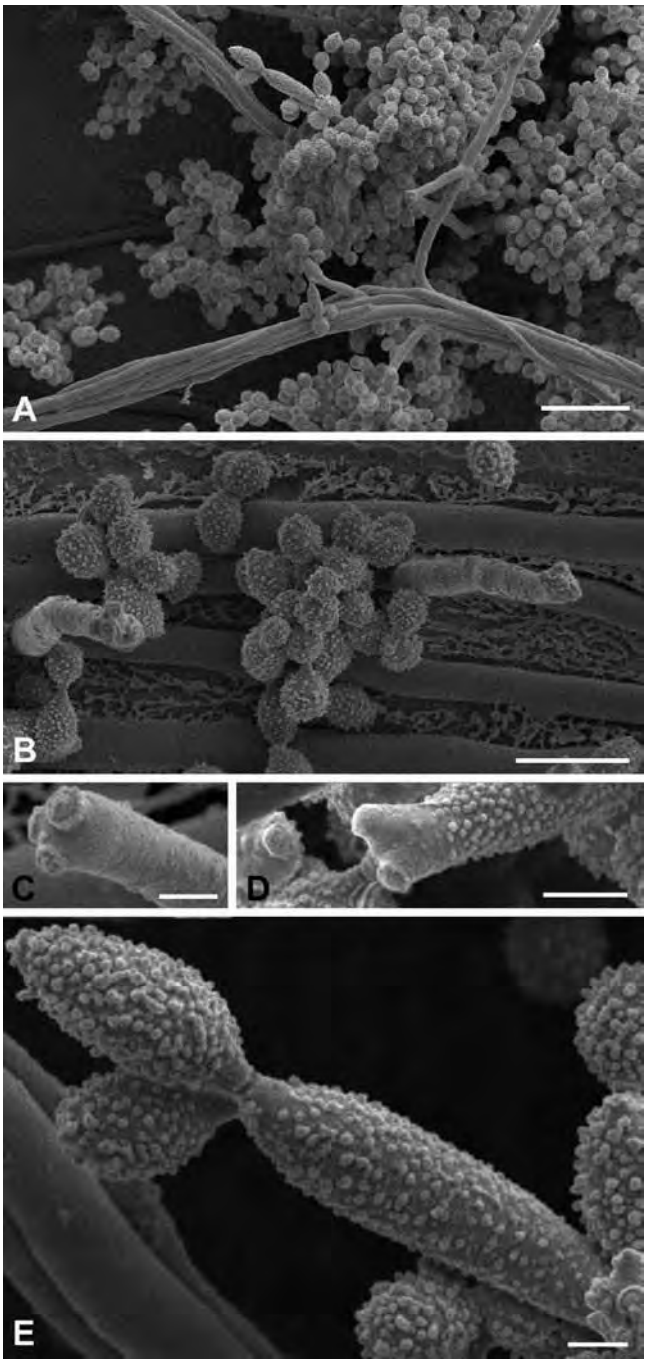

Fig. 45. *Cladosporium tenellum* (CPC 12053). A. A bird's eye view of a colony of *C. tenellum* with its very characteristic bundles of aerial hyphae. Numerous conidia are visible, formed on simple conidiophores. B. Hyphae that run on the agar surface give rise to conidiophores and numerous conidia, that are relatively rounded. C. Conidiophore ends are rather simple and have large scars. D. Hila on a secondary ramoconidium with non-ornamented area. E. Detail of the prominent ornamentation on a secondary ramoconidium. Scale bars: A = 20 µm, B = 10 µm, C, E = 2 µm, D = 5 µm.

and substrates. Besides these strains, several additional isolates listed under excluded strains are morphologically indistinguishable from *C. subtilissimum* in culture, but genetically different, clustering in various subclades. They are indicated as *Cladosporium* sp. in the tree (Fig. 3).

Cladosporium tenellum K. Schub., Zalar, Crous & U. Braun, **sp. nov.** MycoBank MB504581. Figs 43–45.

Etymology: Refers to its narrow conidiophores and conidia.

Differt a Cladosporio cladosporioide conidiophoris et conidiis semper asperulatis, locis conidiogenis apicalibus, numerosis, hilis quoque numerosis, conidiophoris angustioribus, (1–)1.5–3.5(–4) µm latis; et a Cladosporio subtilissimo loci conidiogenis et hilis apicalibus, numerosis, angustioribus, saepe 1–1.5 µm latis, conidiis minutis numerosis, saepe globosis.

Mycelium sparingly branched, 1–3 µm wide, septate, septa often not very conspicuous, not constricted at the septa, sometimes slightly swollen, subhyaline, smooth, walls unthickened. *Conidiophores* macronematous and micronematous, solitary, arising terminally or laterally from plagiotropous or ascending hyphae, erect or subdecumbent, almost straight to more or less flexuous, cylindrical, sometimes geniculate towards the apex, but not nodulose, sometimes with short lateral prolongations at the apex, unbranched to once or twice branched (angle usually 30–45° degree, sometimes up to 90°), branches usually below a septum, 6–200 × (1–)2–4(–5) µm, septate, septa not very conspicuous, not constricted at the septa, subhyaline to pale brown, almost smooth to usually asperulate, walls unthickened or almost so. *Conidiogenous cells* integrated, terminal or intercalary, sometimes conidiophores reduced to conidiogenous cells, cylindrical, sometimes geniculate, non-nodulose, 6–40 µm long, proliferation sympodial, with several conidiogenous loci often crowded at the apex and sometimes also at a lower level, situated on small lateral shoulders, unilateral swellings or prolongations, with up to 6(–10) denticulate loci, forming sympodial clusters of pronounced scars, intercalar conidiogenous cells with short or somewhat long lateral outgrowths, short denticle-like or long branches with several scars at the apex, usually below a septum, loci protuberant, 1–1.5(–2) µm diam, thickened and darkened-refractive. *Ramoconidia* sometimes occurring, cylindrical, up to 32 µm long, 2.5–4 µm wide, with a broadly truncate, unthickened base, about 2 µm wide. *Conidia* catenate, formed in branched chains, straight, small terminal conidia globose, subglobose, ovoid, oval, 3–6 × 2.5–3.5 µm [av. ± SD, 4.5 (± 1.3) × 2.8 (± 0.4) µm], aseptate, asperulate, with 0–2 distal hila, intercalary conidia and secondary ramoconidia ellipsoid-ovoid, ellipsoid to subcylindrical, 3.5–20(–28) × (2.5–)3–5(–6) µm [av. ± SD, 12.4 (± 5.4) × 4.1 (± 0.7) µm], 0–1-septate, rarely with up to three septa, sometimes slightly constricted at the septa, subhyaline, pale brown to medium olivaceous-brown, asperulate or verruculose (muricate, granulate or colliculate under SEM), walls unthickened or slightly thickened, apex rounded or slightly to distinctly attenuated towards apex and base, often forming several apical hila, up to 7(–9), crowded, situated on small lateral outgrowths giving them a somewhat irregular appearance, hila protuberant, 0.5–1.5 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis sometimes occurring.

Cultural characteristics: Colonies on PDA reaching 27–34 mm diam after 14 d at 25 ºC, smoke-grey, grey-olivaceous to olivaceous-grey, olivaceous-grey to iron-grey reverse, velvety to powdery, margin regular, entire edge, narrow, colourless to white, aerial mycelium absent or sparingly formed, felty, whitish, growth regular, flat, radially furrowed, with folded and elevated colony centre, deep into the agar, with age forming few to numerous prominent exudates, sporulation profuse, few high conidiophores formed. Colonies on MEA reaching 25–44 mm diam after 14 d at 25 ºC, olivaceous-grey to olivaceous- or iron-grey due to abundant sporulation in the colony centre, velvety, margin regular, entire edge, narrow, colourless, white to pale olivaceous-grey, aerial mycelium loose, diffuse, growth convex with papillate surface, radially furrowed, wrinkled, without prominent exudates, sporulating. Colonies on OA reaching 23–32 mm diam after 14 d at 25 ºC, grey-olivaceous, olivaceous-

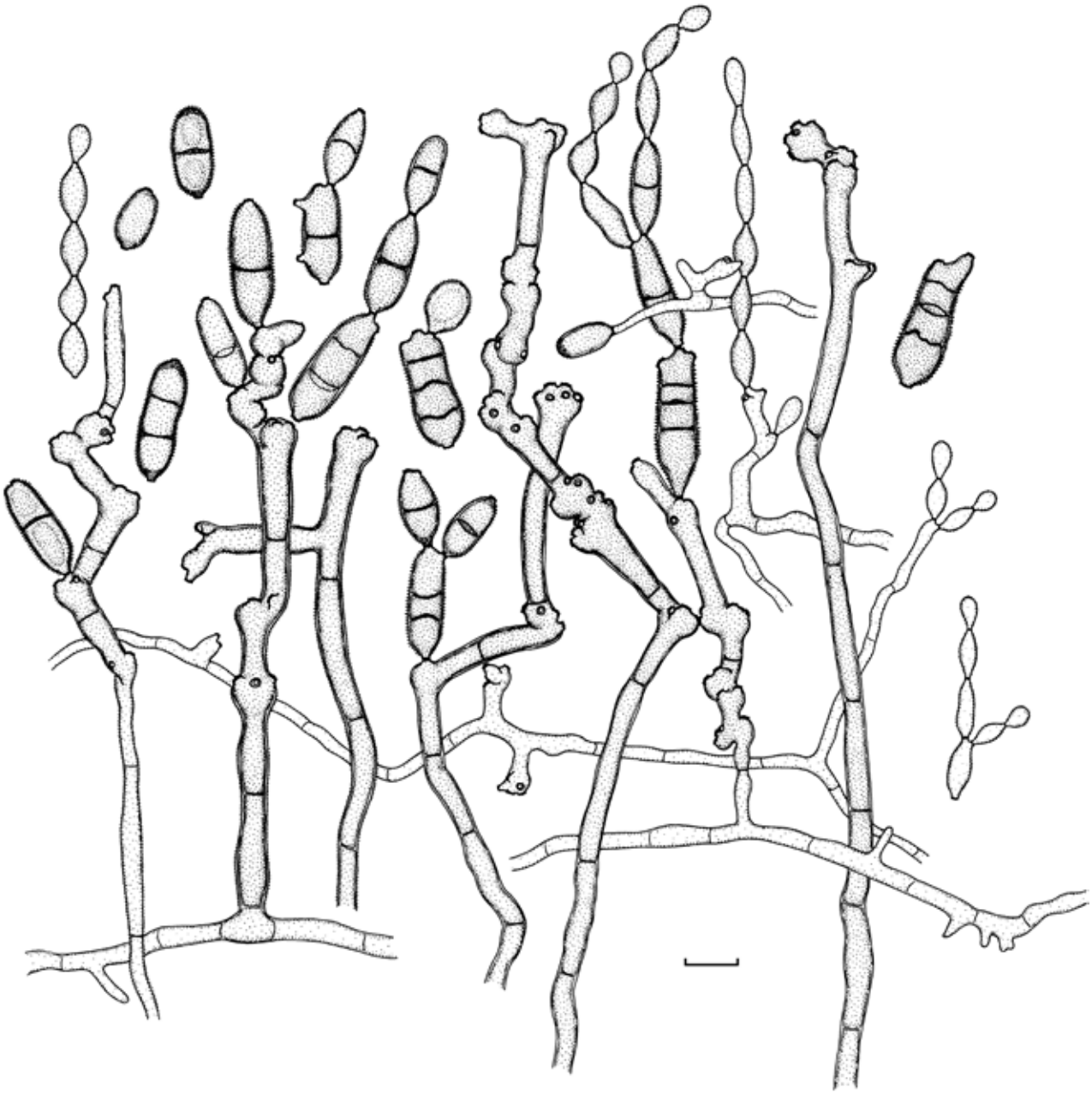

Fig. 46. *Cladosporium variabile* (CPC 12751). Macro- and micronematous conidiophores and conidia. Scale bar = 10 µm. K. Schubert *del*.

grey to olivaceous due to abundant sporulation in the colony centre, olivaceous- or iron-grey reverse, velvety, margin regular, entire edge, narrow, colourless or white, aerial mycelium sparse, diffuse, floccose, growth flat to low convex, radially furrowed, wrinkled, without prominent exudates, sporulation profuse.

Specimens examined: **Israel**, Eilat, isolated from hypersaline water from salterns, 2004, N. Gunde-Cimerman, CBS 121633 = CPC 12051 = EXF-1083; Ein Bokek, isolated from hypersaline water of the Dead Sea, 2004, M. Ota, CBS-H 19866, **holotype**, isotype HAL 2029 F, culture ex-type CBS 121634 = CPC 12053 = EXF-1735. **U.S.A.**, Seattle, University of Washington campus, isolated from *Phyllactinia* sp. (*Erysiphaceae*) on leaves of *Corylus* sp. (*Corylaceae*), 16 Sep. 2004, D. Glawe, CPC 11813.

Substrates and distribution: Hypersaline water and plant material; Israel, U.S.A.

Notes: *Cladosporium subtilissimum* and *C. cladosporioides* are morphologically comparable with the new species *C. tenellum*, but *C. cladosporioides* deviates in having usually smooth conidiophores and conidia with only few conidiogenous loci and conidial hila crowded at the apex and somewhat wider conidiophores, 3–5(–6) µm; and in *C. subtilissimum* the small terminal conidia are not globose but rather narrowly obovoid to limoniform, the conidiogenous loci and conidial hila are somewhat wider, (0.5–)0.8–2(–2.2) µm, and at the apices of conidiophores and conidia only few scars are formed.

Cladosporium ramotenellum, which morphologically also resembles *C. tenellum*, possesses longer and narrower, 0–3-septate conidia, 2.5–35 × 2–4(–5) µm, but forms only few conidiogenous loci and conidial hila at the apices of conidiophores and conidia.

Cladosporium variabile (Cooke) G.A. de Vries, *Contr. Knowl. Genus Cladosporium*: 85. 1952. Figs 46–48.

Basionym: *Heterosporium variabile* Cooke, Grevillea 5(35): 123. 1877.

≡ *Helminthosporium variabile* Cooke, Fungi Brit. Exs. Ser. 1, No. 360. 1870, *nom. inval*.

= *Cladosporium subnodosum* Cooke, Grevillea 17(83): 67. 1889.

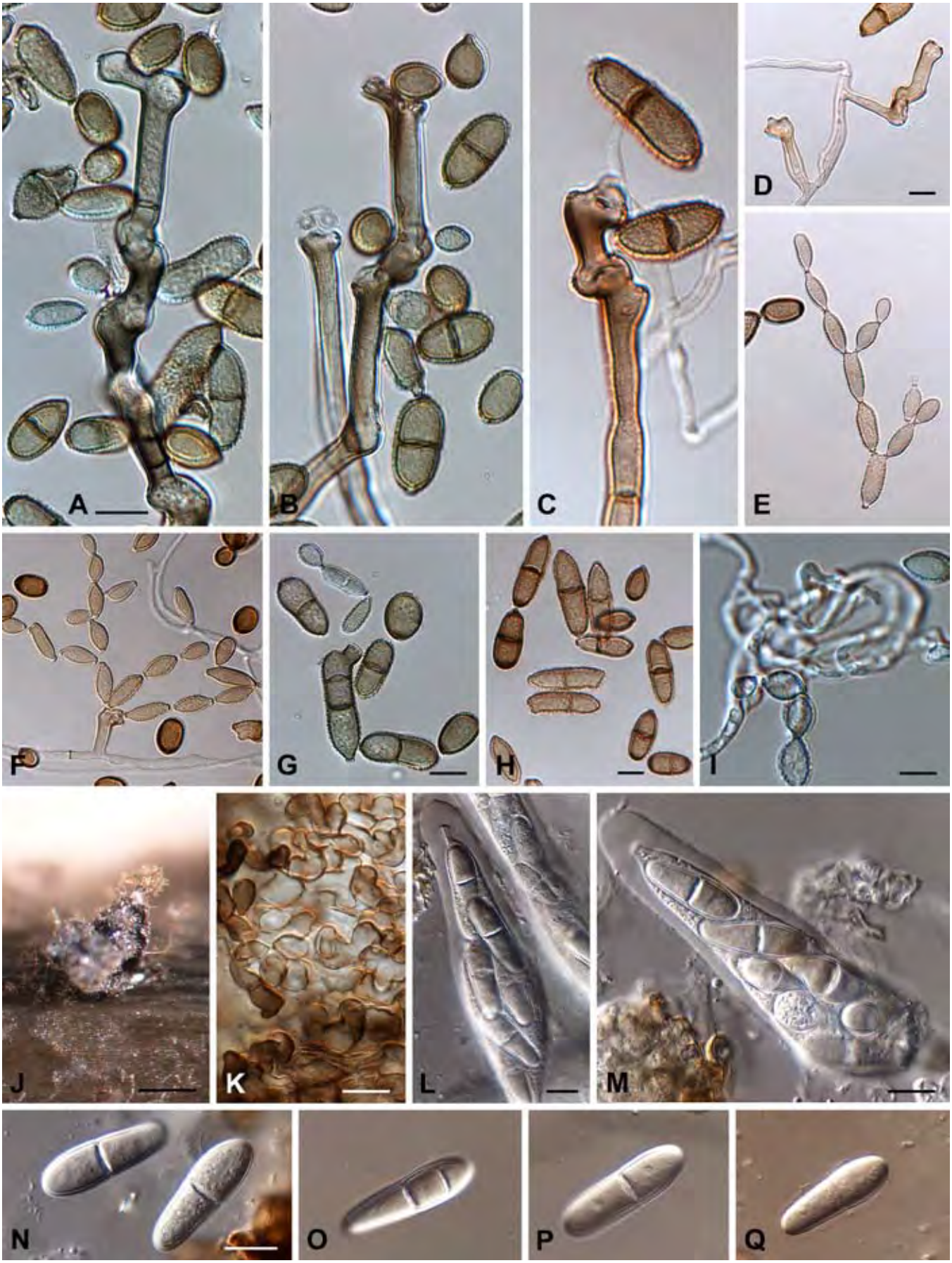

Fig. 47. *Cladosporium variabile* and its teleomorph *Davidiella variabile* (CPC 12751). A–C. Macronematous conidiophores. D, F. Micronematous conidiophores. E, G–H. Conidia. I. Twisted aerial mycelium. J. Ascomata formed on nettle stem in culture. K. Surface view of ascomal wall of *textura epidermoidea*. L–M. Asci. N–P. Ascospores. Q. Ascus with a sheath. Scale bars A, D, G–J, K–N = 10 µm, J = 250 µm.

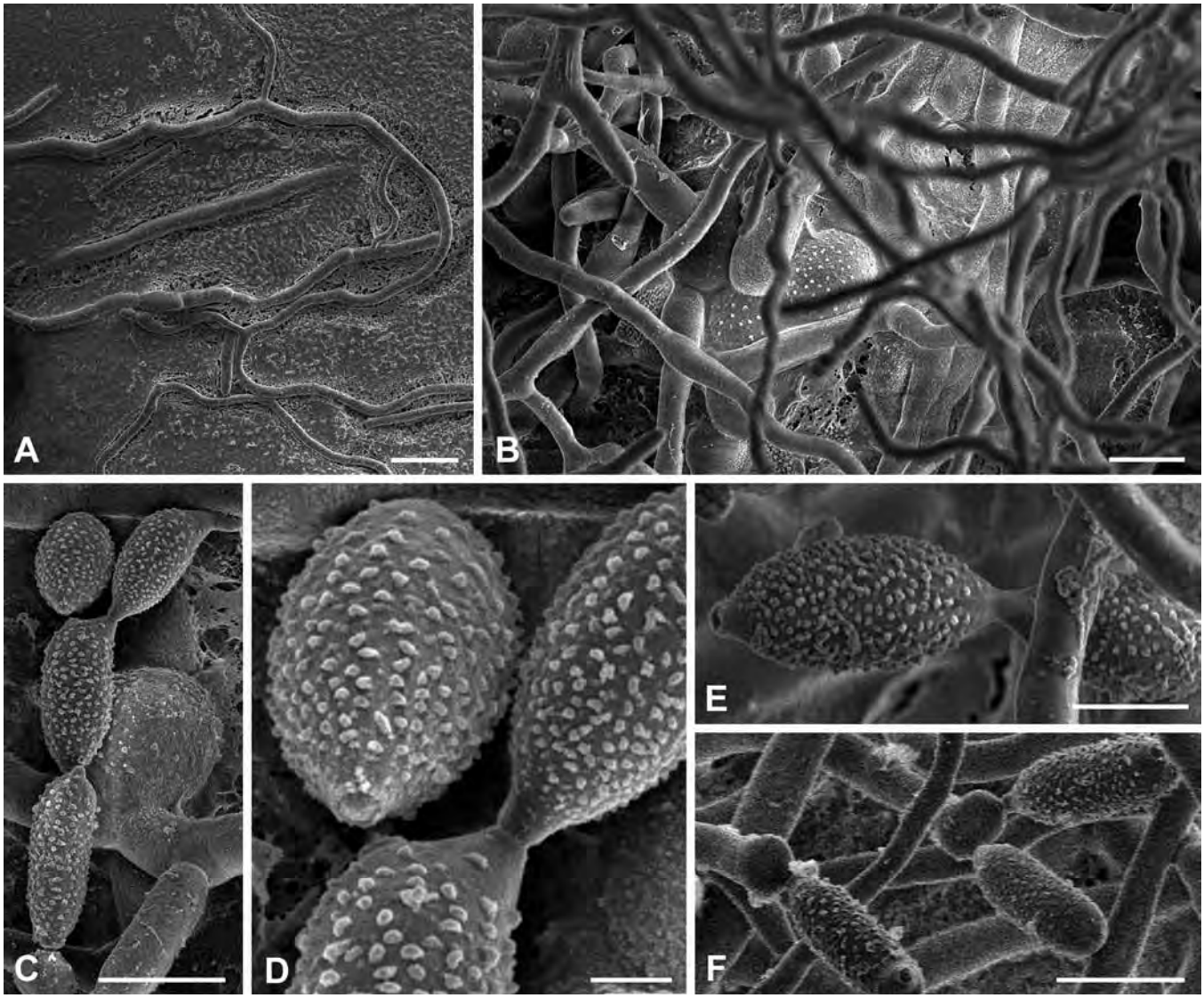

Fig. 48. *Cladosporium variabile* (CPC 12753). A. Survey of hyphae that grow on the agar surface. Some of the fungal cells have a swollen appearance and could develop into a "foot cell" that gives rise to a conidiophore. B. A number of aerial hyphae obstruct the swollen, large structures on the agar surface, which give rise to conidiophores. Some of them appear ornamented. C. A series of conidia formed on a conidiophore (bottom of the micrograph). D. Detail of the ornamented conidia. The ornamentations are isolated and dispersed. Note also the ornamentation-free scar zone and the hilum of the left cell. E. Two conidia behind an aerial hypha. F. Two conidiophores forming secondary ramoconidia. Note the bulbous shape of the spore-forming apparatus. This micrograph is from an uncoated sample. Scale bars: A–C, F = 10 µm, D = 2 µm, E = 5 µm.

Teleomorph: ***Davidiella variabile*** Crous, K. Schub. & U. Braun, **sp. nov.** MycoBank MB504583.

Davidiellae tassianae similis, sed ascosporis maioribus, (22–)26–30(–35) × (7–)7.5–8(–9) µm, et ascis latioribus, plus quam 18 µm.

Ascomata pseudothecial, black, superficial, situated on a small stroma, globose, up to 250 µm diam, with 1–3 ostiolate necks; ostioles periphysate, with apical periphysoids present; wall consisting of 3–6 layers of dark brown *textura angularis*, *textura epidermoidea* in surface view. *Asci* fasciculate, bitunicate, subsessile, obovoid to broadly ellipsoid, straight to slightly curved, 8-spored, 70–95 × 18–28 µm; with pseudoparenchymatal cells of the hamathecium persistent. *Ascospores* tri- to multiseriate, overlapping, hyaline, with irregular lumina, thick-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest near the middle of the apical cell, medianly 1-septate, not to slightly constricted at the septum, at times developing a second septum in each cell, several ascospores with persistent, irregular mucoid sheath, (22–)26–30(–35) × (7–)7.5–8(–9) µm.

Mycelium immersed and superficial, irregularly branched, aerial mycelium twisted and spirally coiled, 1–3 µm wide, septate, sometimes with swellings or small lateral outgrowths, hyaline to subhyaline, smooth, walls unthickened, hyphae which give rise to conidiophores somewhat wider, 3–4.5 µm, subhyaline to pale brown, almost smooth to minutely verruculose, sometimes enveloped by a polysaccharide-like cover. *Conidiophores* usually macronematous, but also micronematous, arising terminally from ascending hyphae or laterally from plagiotropous hyphae. *Macronematous conidiophores* erect, more or less straight to flexuous, often distinctly geniculate-sinuous forming lateral shoulders or unilateral swellings, sometimes zigzag-like or somewhat coralloid, nodulose, swellings at first terminal, then becoming lateral due to sympodial proliferation, often as distinct lateral shoulders, unbranched, sometimes once branched, 6–180 × (2.5–)3–6 µm, swellings (3–)6–11 µm wide, septate, not constricted at the septa, pale to medium olivaceous-brown or brown, usually verruculose, walls somewhat thickened, about 1 µm thick, sometimes appearing to be two-layered. *Conidiogenous cells* integrated, terminal and intercalary, cylindrical, nodulose to nodose,

with a single or two swellings per cell, swellings apart from each other or formed in short succession, loci confined to swellings, up to six per node, protuberant, 1–2 µm diam, thickened and darkened-refractive. *Micronematous conidiophores* erect, straight to slightly flexuous, unbranched, usually without swellings, filiform to narrowly cylindrical, sometimes only as short lateral outgrowths of hyphae, often almost indistinguishable from hyphae, up to 50 µm long, 1.5–2.5(–3) µm wide, longer ones pluriseptate, septa appear to be somewhat more darkened, with very short cells, 4–12 µm long, subhyaline to pale brown, smooth, walls unthickened or almost so. *Conidiogenous cells* integrated, usually terminal, rarely intercalary, cylindrical, non-nodulose, with a single, two or few conidiogenous loci at the distal end, protuberant, up to 2 µm diam, thickened and darkened-refractive. *Conidia* catenate, in branched chains, straight, subglobose, obovoid, oval, broadly ellipsoid to cylindrical, sometimes clavate, 4–26(–30) × (3.5–)5–9(–10) µm [av. ± SD, 16.8 (± 6.9) × 6.5 (± 1.4) µm], 0–3-septate, usually not constricted at the septa, septa becoming sinuous with age, often appearing to be darkened, pale to medium or even dark brown or olivaceous-brown, verruculose to densely verrucose or echinulate (granulate under SEM), walls slightly to distinctly thickened in larger conidia, apex and base broadly rounded, sometimes broadly truncate or somewhat attenuated, apex and base often appear to be darkened or at least refractive, hila protuberant to somewhat sessile (within the outer wall ornamentation), (0.8–)1–2 µm diam, thickened and darkened-refractive; microcyclic conidiogenesis occurring.

Cultural characteristics: Colonies on PDA attaining 29 mm diam after 14 d at 25 ºC, olivaceous to olivaceous-grey or iron-grey, iron-grey or olivaceous-grey reverse, velvety to powdery, margin regular, entire edge to fimbriate, almost colourless, aerial mycelium whitish turning olivaceous-grey, sometimes reddish, greyish rose, woolly-felty, growth flat with elevated colony centre, somewhat folded or radially furrowed, with age forming several very small but prominent exudates, sporulation profuse. Colonies on MEA attaining 27 mm diam after 14 d at 25 ºC, olivaceous-grey to iron-grey, white to pale olivaceous-grey in the centre due to abundant aerial mycelium, velvety, margin very narrow, colourless, more or less entire edge, radially furrowed, aerial mycelium fluffy to floccose, dense, growth low convex with wrinkled and folded centre, without exudates, sporulation profuse. Colonies on OA attaining 25 mm diam after 14 d at 25 ºC, iron-grey or olivaceous, margin regular, entire edge, narrow, white, glabrous, aerial mycelium whitish, at first mainly in the colony centre, high, dense, floccose, growth flat, abundantly sporulating, no exudates.

Specimens examined: **Great Britain**, Wales, Montgomeryshire, Welshpool, Forden Vicarage, on *Spinacia oleracea* (*Chenopodiaceae*), J.E. Vize, Cooke, Fungi Brit. Exs. Ser. I, No. 360, K, **holotype**. **U.S.A.**, Washington, isolated from *Spinacia oleracea*, 1 Jan. 2003, L. DuToit, CBS-H 19867, **epitype designated here** of *C. variabile* and *D. variabile*, cultures ex-epitype CBS 121635 = CPC 12753, CPC 12751.

Substrate and distribution: Leaf-spotting fungus on *Spinacia oleracea*; Asia (China, India, Iraq, Pakistan), Europe (Austria, Belgium, Cyprus, Denmark, France, Germany, Great Britain, Hungary, Italy, Montenegro, Netherlands, Norway, Romania, Spain, Turkey), North America (U.S.A.).

Literature: de Vries (1952: 85–88), Ellis (1971: 315), Ellis & Ellis (1985: 429), David (1995b; 1997: 94, 96–98), Ho *et al.* (1999: 144).

Notes: *In vivo* the conidia are usually longer, somewhat wider and more frequently septate, (6.5–)10–45(–55) × (4.5–)6–14(–17) µm, 0–4(–5)-septate (Schubert 2005). In culture the dimensions tend to be smaller, which was already mentioned by de Vries (1952).

This leaf-spotting fungus superficially resembles *C. macrocarpum*, but besides its pathogenicity to *Spinacia*, *C. variabile* differs from the latter species in having distinctly larger and more frequently septate conidia on the natural host, forming twisted and spirally coiled aerial mycelium in culture and in having lower growth rates in culture (29 mm after 14 d on PDA versus 38 mm on average in *C. macrocarpum*). Furthermore, the conidial septa of *C. variabile* are often distinctly darkened, become sinuous with age and the apex and base of the conidia often appear to be distinctly darkened. A *Davidiella* teleomorph has not previously been reported for this species.

The cladosporioides complex

This species complex will be treated in an additional paper in this series, dealing with the epitypification of this common and widespread species, and with numerous isolates identified and deposited as *C. cladosporioides*.

DISCUSSION

In the present study, a multilocus genealogy supported by light and SEM microscopy, and cultural characteristics was used to redefine species borders within *Cladosporium*, especially within the *C. herbarum* complex. Most of the diagnostic features used for species delimitation on host material (Heuchert *et al.* 2005, Schubert 2005), proved to be applicable in culture. However, morphological features were often more pronounced *in vivo* than *in vitro*. For instance, conidiophore arrangement is not applicable to cultures, conidiophore and conidium widths were often narrower in culture than on the natural host, and macro- as well as microconidiophores were often observed in culture, but not on host material. All species belonging to the *C. herbarum* complex are characterised by possessing conidia which are ornamentated, the ornamentation ranging from minutely verruculose to verrucose, echinulate or spiny whereas in the *C. sphaerospermum* complex species with both smooth-walled as well as ornamented conidia are included (Zalar *et al.* 2007). The surface ornamentation varies based on the length of surface protuberances and in the density of ornamentation. Furthermore, the conidia are mainly catenate, formed in unbranched or branched chains. However, species previously referred to the genus *Heterosporium*, which usually produce solitary conidia or unbranched chains of two or three conidia at the most on the natural host, also belong to this species complex (e.g., *C. iridis*). *In vitro* these chains can become longer and may even be branched. The conidiophores formed in culture are mostly macro- but may also be micronematous, sometimes forming different types of conidia that vary in shape and size from each other. Most of the species possess nodulose conidiophores with the conidiogenesis confined to the usually lateral swellings. However, this phenetic trend is not consistently expressed in all of the species belonging to the *C. herbarum* complex. The various *Cladosporium* species within the *C. herbarum* complex were observed to have subtle differences in their phenotype which were visible via cryo-electron microscopy (cryoSEM), and are discussed below.

Fungal colonies: CryoSEM provides the opportunity to study the organisation of the fungal colony at relatively low magnifications. *Cladosporium tenellum* proved to be the only fungus able to form

aerial hyphal strands under the conditions studied. *Cladosporium variabile* formed abundant aerial hyphae, but in *C. spinulosum* these were sparse, and only conidiophores were observed on the agar surface. Three-day-old colonies of *C. subinflatum* formed numerous, long aerial hyphae, and no conidiophores could be discerned under the binocular. After 11 d the aerial hyphae seemed to have disappeared, giving rise to conidiophores. *Cladosporium antarcticum*, *C. variabile* and *C. ramotenellum* showed very large, swollen (> 10 µm) cells which gave rise to conidiophores. With *C. variabile* possible earlier stages of these cells were visible (Fig. 48), which gave rise to conidiophores. More than one conidiophore could be formed on such a structure (*C. variabile* and *C. ramotenellum*). *Cladosporium herbarum* has very wide hyphae on the agar surface, which gave rise to conidiophores as lateral branches. These wide hyphae were observed to anastomose, which may provide a firm interconnected supporting mycelium for these conidiophores. In *C. herbaroides* these wide hyphae could also be discerned, but conidiophore formation was less obvious. Similarily, *C. tenellum* has wide, parallel hyphae that gave rise to conidiophores.

These observations reveal fungal structures in *Cladosporium* that have not previously been reported on, and that raise intriguing biological questions. For instance, why are hyphal strands observed in some species (*C. tenellum*), and not in others, and what happens to the aerial hyphae during incubation in some species such as *C. subinflatum*? Furthermore, these preliminary results suggest that CryoSEM provide additional features that can be used to distinguish the different species in the *C. herbarum* complex.

Fine details of morphological stuctures: CryoSEM provides the opportunity to study fine details of the conidiophore, (ramo)conidia and scars. Samples can be studied at magnification up to × 8 000, revealing details at a refinement far above what is possible under the light microscope (LM) (Fig. 2). However, the LM micrographs provide information about the different compartments of ramoconidia, as well as the thickness and pigmentation of the cell wall of different structures. With other words, the different techniques are complementary, and both reveal fungal details that build up the picture that defines a fungal species.

Conidiophores can vary with respect to their width and the length. *Cladosporium ramotenellum*, *C. antarcticum* and *C. variabile* have tapered conidiophores formed on large globoid "foot cells". The conidiophore itself can be branched. *Cladosporium spinulosum* has conidiophores that rise from the agar surface, but can have a common point of origin. These conidiophores are not tapered, but parallel and slender. The conidiophores of *C. bruhnei* and *C. herbaroides* are rather long, and can appear as aerial hyphae.

An important feature of the conidiophore is the location were the conidia are formed. Conidiophore ends can be simple and tubular, or rounded to more complex, several times geniculate, with several scars. Conidiophore ends become more elaborate over time. *Cladosporium spinulosum* and *C. tenellum* have nearly tubular conidiophore ends, with often very closely aggregated scars. The conidiophore ends of *C. subinflatum* are also near tubular with a hint of bulbousness. *Cladosporium subtilissimum* is similar, but with somewhat more elevated scars that look denticulate. *Cladosporium variabile* has nodulose, somewhat swollen apices with often sessile, almost inconspicuous scars. In the case of *C. macrocarpum*, these structures are also nodulose to nodose and somewhat bent, with only slightly protuberant loci. *Cladosporium ramotenellum* has tubular conidiophore ends with pronounced scars. *Cladosporium antarcticum* has very characteristic, tapered ends, and widely dispersed (5 µm) scars. More complex conidiophore ends are more irregular in shape, and have scars dispersed over a longer distance, such as observed in *C. bruhnei*, *C. herbaroides*, and *C. herbarum*.

Secondary ramoconidia are usually the first conidia formed on a conidiophore. They are often multicellular, and have one basal cladosporioid hilum, and more at the apex. Few *Cladosporium* species additionally form true ramoconidia representing apical parts of the conidiophore which secede at a septum resulting in an undifferentiated non-coronate base and function as conidia. Ramification of conidial chains is realised through these conidia. They can occur in up to three stages, which results in elaborated spore structures. The basal secondary ramoconidium is invariably the largest, and cell size decreases through a series of additional secondary ramoconidia, intercalary conidia, and small, terminal conidia. The elongation of secondary ramoconidia varies among the different species. *Cladosporium macrocarpum* has broadly ellipsoid to cylindrical secondary ramoconidia usually with broadly rounded ends, like *C. variabile*, while *C. spinulosum* has secondary ramoconidia that can often hardly be discerned from the conidia that are formed at later stages. The conidia of the other species roughly fall between these species. The most notable structures on these conidia are their ornamentation, scar pattern and morphology. *Cladosporium spinulosum* forms numerous globose to subsphaerical spores with digitate, non-tapered surface ornamentation, which is unique for all the species discussed here. In his study on *Cladosporium* wall ornamentation, David (1997) recognised three classes of echinulate surfaces (aculeate, spinulose, digitate), and five classes of verrucose surfaces (muricate, granulate, colliculate, pustulate and pedicellate) (Fig. 2). The ornamentation particles vary in shape, width, height and density. The most strongly ornamented conidia of the species examined by SEM are formed by *C. ossifragi*, with the ornamentation both large (up to 0.5 µm wide) and high, and can be regarded as densely muricately ornamented. Strong ornamentation is also seen in *C. herbaroides*, which is mostly granulate. *Cladosporium tenellum* (with muricate, granulate and colliculate tendencies) and *C. bruhnei* (mostly granulate with some muricate projections) have relatively large ornamentation structures with slightly more space between the units than the other two species. *Cladosporium antarcticum*, *C. ramotenellum*, *C. variabile* and *C. subtilissimum* exhibit rather large granulate ornamentations that have a more irregular and variable shape. *Cladosporium subinflatum* shows the widest dispersed structures of the series, being muricate. In contrast, *C. macrocarpum* has a very neat and regular pattern of muricate ornamentation. The area of formation of new spores on conidia is invariably not ornamented, and hila all have the typical *Cladosporium* morphology with a central dome and a ring-like structure around it.

Branching patterns: Spores usually show a "line of weakness" between them where the coronate scars form. It seems that scars at both sides of the line of weakness have the central dome structure, which appears to play a major role in the effective mechanism *Cladosporium* employs for spore dispersal, with the dome actively pushing the conidia apart. This mechanism is also illustrated in David (1997, fig. 2E). Indeed, conidia of *Cladosporium* are very easily dislodged; even snap freezing or the electrical forces inside the SEM often result in dislodgement of the spores in a powdery "wave". It is no surprise, therefore, that *Cladosporium* conidia are to be found in most air samples. In *Cladosporium*, conidia are mostly formed in chains, with the size invariably decreasing from the base to the apex of the row. Upon formation each conidium is separated from the conidiophore, or previously formed conidium, and hence from its nutrients. The basal ramoconidium or secondary ramoconidia have the nutrients and metabolic power to produce

a number of additional secondary ramoconidia that in turn could produce a chain of intercalary conidia, and finally, some small, single-celled, terminal conidia. Further research is still necessary to determine if specific branching patterns can be linked to different species.

A surprising finding from the present study is the huge diversity in species and genotypes that exist in nature, be it in the indoor environment, on fruit surfaces, or in extreme ecological niches such as salterns, etc. It is clear that detailed studies would be required to find and characterise other species of *Cladosporium* and obtain a better understanding of their host ranges and ecology. A further surprise lay in the fact that several of these species are capable of sexual reproduction, and readily form *Davidiella* teleomorphs in culture. The *Davidiella* states induced here were all from homothallic species. Further attention now needs to be given to elucidating teleomorphs from other species which, as in *Mycosphaerella* (Groenewald *et al*. 2006, Ware *et al*. 2007) could be heterothallic, and experiencing clandestine sex.

Despite the occurrence of many different genotypes in variable genes, the degree of diversity in the entire data set was low. For the majority of the species ITS was almost invariant, with only six genotypes in the entire dataset. This suggests a very recent evolution. The standardised index of association (I^S_A) was high (0.3914), indicating an overabundance of clonality and / or inbreeding, the latter possibly matching with observed homothallism of *Davidiella* teleomorphs. Clonality was visualised with SplitsTree software, where star-shaped representations without any sign of reticulation were obtained for all genes, though at different branch lengths (Fig. 5). With Structure software an optimal subdivision was achieved at six putative groups. Some of them were distinctly separated, yielding a theta (θ) around 0.14, but in most cases there was considerable overlap in representation of motifs, with θ at significantly higher values. Results are difficult to interpret due to the small size of the data set compared to the number of predicted groups, and due to unknown but probably large sampling effects. With optimal subdivision of the 79 strains at a hypothesised value of K = 6 (Fig. 4), still a large degree of inter-group similarity was noted, as was the case at any other level of K. This was particularly obvious when data from the most variable genes (EF and ACT) are superimposed (Fig. 4). The ACT groups are further subdivided by EF data, but in many cases the same EF motif (indicated with arrows) was encountered in different (multilocus) species, for example in *C. antarcticum*, *C. spinulosum*, *Davidiella* sp., and the various clusters comprising *Cladosporium* strains which are phenotypically almost indistinguishable but genetically distinct from *C. subtilissimum*. A similar situation was found with the distribution of EF genotypes (indicated with doughnuts) in *C. herbarum* and *C. macrocarpum*. Nevertheless, the data set showed significant structuring, partly correlating with geography, e.g. the EF-determined cluster of *C. bruhnei* that contained isolates from different sources in The Netherlands. Differences may be over-accentuated by known sampling effects, particularly in *C. herbarum* and *C. macrocarpum*, where single-spore isolates from a single collection are included.

Taken together the data suggest a recent, preponderantly clonal evolution, combined with limited natural selection at a low level of evolutionary pressure. As a result, many genotypes produced by hot spots in the genes analysed have survived, leading to nearly random variation in the data set. Many combinations of motifs that possibly could emerge have maintained in the course of time due to the absence of recombination. This indicates that the observed structure is that of populations within a single species, and consequently a distinction of clonal "species" could be redundant. This conclusion is underlined by the fact that a single source in a single location can be colonised by various genotypes, such as grapes in the U.S.A. containing three different, closely related genotypes. However, the phenomenon of co-inhabitation by different *Mycosphaerella* species on the same lesion of *Eucalyptus* has been described before (Crous 1998, Crous *et al*. 2004) and it is therefore not surprising that different genotypes occurring close together are also observed for the related genus *Cladosporium*. There is no obvious ecological difference between genotypes, and hence isolates seem to have equal fitness.

However, in general we noticed a remarkable concordance of genetic and phenetic characters. The morphological study was done prior to sequencing, and nearly all morphotypes clustered in separate molecular entities. There are some exceptions, such as with *C. antarcticum* with striking morphology that was almost identical on the molecular level to *Cladosporium* spp. that resemble *C. subtilissimum* and would normally have been interpreted to be a mutant. Conversely, nearly all genetically distinguishable groups proved to be morphologically different, with the exception of members of the *C. subtilissimum s. lat.* complex (indicated as *Cladosporium* sp. in Fig. 3 and Table 1). The possibility remains that the found genetic parameters correlate with phenetic markers other than morphology, such as virulence, toxins or antifungal susceptibilities. For this reason we introduce the established entities here as formal species. They can be diagnosed by ACT sequencing or by phenetic characters provided in the key. For simple routine purposes, however, they can be seen and treated as the "*C. herbarum* complex", based on their close phylogenetic relationships.

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Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments

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Abstract: Saprobic *Cladosporium* isolates morphologically similar to *C. sphaerospermum* are phylogenetically analysed on the basis of DNA sequences of the ribosomal RNA gene cluster, including the internal transcribed spacer regions ITS1 and ITS2, the 5.8S rDNA (ITS) and the small subunit (SSU) rDNA as well as β-tubulin and actin gene introns and exons. Most of the *C. sphaerospermum*-like species show halotolerance as a recurrent feature. *Cladosporium sphaerospermum*, which is characterised by almost globose conidia, is redefined on the basis of its ex-neotype culture. *Cladosporium dominicanum*, *C. psychrotolerans*, *C. velox*, *C. spinulosum* and *C. halotolerans*, all with globoid conidia, are newly described on the basis of phylogenetic analyses and cryptic morphological and physiological characters. *Cladosporium halotolerans* was isolated from hypersaline water and bathrooms and detected once on dolphin skin. *Cladosporium dominicanum* and *C. velox* were isolated from plant material and hypersaline water. *Cladosporium psychrotolerans*, which grows well at 4 °C but not at 30 °C, and *C. spinulosum*, having conspicuously ornamented conidia with long digitate projections, are currently only known from hypersaline water. We also newly describe *C. salinae* from hypersaline water and *C. fusiforme* from hypersaline water and animal feed. Both species have ovoid to ellipsoid conidia and are therefore reminiscent of *C. herbarum*. *Cladosporium langeronii* (= *Hormodendrum langeronii*) previously described as a pathogen on human skin, is halotolerant but has not yet been recorded from hypersaline environments.

Taxonomic novelties: *Cladosporium dominicanum* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. fusiforme* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. halotolerans* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. psychrotolerans* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. salinae* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. spinulosum* Zalar, de Hoog & Gunde-Cimerman, sp. nov., *C. velox* Zalar, de Hoog & Gunde-Cimerman, sp. nov.
Key words: Actin, β-tubulin, halotolerance, ITS rDNA, phylogeny, SSU rDNA, taxonomy.

INTRODUCTION

The halophilic and halotolerant mycobiota from hypersaline aqueous habitats worldwide frequently contain *Cladosporium* Link isolates (Gunde-Cimerman *et al*. 2000, Butinar *et al.* 2005). Initially, they were considered as airborne contaminants, but surprisingly, many of these *Cladosporium* isolates were identified as *C. sphaerospermum* Penz. because they formed globoid conidia (data unpublished). *Cladosporium sphaerospermum*, known as one of the most common air-borne, cosmopolitan *Cladosporium* species, was frequently isolated from indoor and outdoor air (Park *et al.* 2004), dwellings (Aihara *et al.* 2001), and occasionally from humans (Badillet *et al.* 1982) and plants (Pereira *et al.* 2002). Strains morphologically identified as *C. sphaerospermum* were able to grow at a very low water activity (a_w 0.816), while other cladosporia clearly preferred a higher, less extreme water activity (Hocking *et al*. 1994). This pronounced osmotolerance suggests a predilection for osmotically stressed environments although *C. sphaerospermum* is reported from a wide range of habitats including osmotically non-stressed niches.

We therefore hypothesised that *C. sphaerospermum* represents a complex of species having either narrow or wide ecological amplitudes. The molecular diversity of strains identified as *C. sphaerospermum* has not yet been determined and isolates from humans have not yet been critically compared with those from environmental samples. Therefore, a taxonomic study was initiated with the aim to define phylogenetically and morphologically distinct entities and to describe their *in vitro* osmotolerance and their natural ecological preferences.

MATERIALS AND METHODS

Sampling

Samples of hypersaline water were collected from salterns located at different sites of the Mediterranean basin (Slovenia, Bosnia and Herzegovina, Spain), different coastal areas along the Atlantic Ocean (Monte Cristy, Dominican Republic; Swakopmund, Namibia), the Red Sea (Eilat, Israel), the Dead Sea (Ein Gedi, Israel), and the salt Lake Enriquillio (Dominican Republic). Samples from the Sečovlje salterns (Slovenia) were collected once per month in 1999. Samples from the Santa Pola salterns and Ebre delta river saltern (Spain) were taken twice (July and November) in 2000. A saltern in Namibia and one in the Dominican Republic were sampled twice (August and October) in 2002. Various salinities, ranging from 15 to 32 % NaCl were encountered in these ponds.

Isolation and maintenance of fungi

Strains were isolated from salterns using filtration of hypersaline water through membrane filters (pore diam 0.45 µm), followed by incubation of the membrane filters on different culture media with lowered water activity (Gunde-Cimerman *et al*. 2000). Only colonies of different morphology on one particular selective medium per sample were analysed further. Strains were carefully selected from different evaporation ponds, collected at different times, in order to avoid sampling of identical clones. Subcultures were maintained at the Culture Collection of Extremophilic Fungi (EXF, Biotechnical Faculty, Ljubljana, Slovenia), while a selection was deposited at the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the Culture Collection of the National Institute of Chemistry (MZKI, Ljubljana, Slovenia). Reference strains were obtained from CBS, and were selected either on the basis of the strain history, name, or on the basis of their ITS rDNA sequence. Strains were maintained on oatmeal agar (OA; diluted OA, Difco: 15 g of Difco 255210 OA medium, 12 g of agar, dissolved in 1 L of distilled water) with or without 5 % additional NaCl. They were preserved in liquid nitrogen or by lyophilisation. Strains studied are listed in Table 1.

Table 1. List of *Cladosporium* strains, with their current and original names, geography, GenBank accession numbers and references to earlier published sequences.

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
Cladosporium bruhnei					
CBS 177.71	Thuja tincture	The Netherlands, Amsterdam	DQ780399 / DQ780938	EF101354	EF101451
CBS 812.71	*Polygonatum odoratum*, leaf	Czech Republic, Lisen	DQ780401 / –	–	–
Cladosporium cladosporioides					
CBS 170.54 **NT**	*Arundo*, leaf	U.K., England, Kew	AY213640 / DQ780940	EF101352	EF101453
EXF-321	Hypersaline water	Slovenia, Sečovlje saltern	DQ780408 / –	–	–
EXF-780			DQ780409 / –	–	–
EXF-946	Hypersaline water	Bosnia and Herzegovina, Ston saltern	DQ780410 / –	–	–
Cladosporium dominicanum					
CPC 11683	*Citrus* fruit (orange)	Iran	DQ780357 / –	EF101369	EF101419
EXF-696	Hypersaline water	Dominican Republic, saltern	DQ780358 / –	EF101367	EF101420
EXF-718	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780356 / –	EF101370	EF101418
EXF-720	Hypersaline water	Dominican Republic, saltern	DQ780355 / –	–	EF101417
EXF-727	Hypersaline water	Dominican Republic, saltern	DQ780354 / –	–	EF101416
EXF-732 **T**; CBS 119415	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780353 / –	EF101368	EF101415
Cladosporium fusiforme					
CBS 452.71	Chicken food	Canada	DQ780390 / –	EF101371	EF101447
EXF-397	Hypersaline water	Slovenia, Sečovlje saltern	DQ780389 / –	EF101373	EF101445
EXF-449 **T**; CBS 119414	Hypersaline water	Slovenia, Sečovlje saltern	DQ780388 / DQ780935	EF101372	EF101446
Cladosporium herbarum					
ATCC 66670, as *Davidiella tassiana*	CCA-treated Douglas-fir pole	U.S.A., New York, Geneva	AY361959[2] & DQ780400 / DQ780939	AY752193[11]	EF101452
Cladosporium halotolerans					
ATCC 26362	Liver and intestine of diseased frog	U.S.A., New Jersey	AY361982[2] / –	–	–
ATCC 64726	Peanut cell suspension tissue culture	U.S.A., Georgia	AY361968[2] / –	–	–
CBS 280.49	Stem of *Hypericum perforatum* identified as *Mycosphaerella hyperici*	Switzerland, Glarus, Mühlehorn	DQ780369 / –	EF101402	EF101432
CBS 191.54	Laboratory air	Great Britain	– / –	–	–
CBS 573.78	*Aureobasidium caulivorum*	Russia, Moscow region	– / –	–	–
CBS 626.82	–	Sweden, Stockholm	– / –	–	–
dH 12862; EXF-2533	Culture contaminant	Brazil	DQ780371 / –	EF101400	EF101422
dH 12941; EXF-2534	Culture contaminant	Turkey	– / –		EF101421
dH 12991; EXF-2535	Brain	Turkey	DQ780372 / –		EF101423
dH 13911; EXF-2422	Ice	Arctics	DQ780370 / –	EF101401	EF101430
EXF-228; MZKI B-840	Hypersaline water	Slovenia, Sečovlje saltern	DQ780365 / DQ780930	EF101393	EF101425
EXF-380	Hypersaline water	Slovenia, Sečovlje saltern	DQ780368 / –	EF101394	EF101427
EXF-564	Hypersaline water	Namibia, saltern	DQ780363 / –	EF101395	EF101433
EXF-565	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-567	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-571	Hypersaline water	Namibia, saltern	– / –	–	–
EXF-572 **T**; CBS 119416	Hypersaline water	Namibia, saltern	DQ780364 / –	EF101397	EF101424
EXF-646	Hypersaline water	Spain, Santa Pola saltern	DQ780366 / –	EF101398	EF101428
EXF-698	Hypersaline water	Dominican Republic, saltern	– / –	–	–
EXF-703	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780367 / –	EF101392	EF101426
EXF-944	Hypersaline water	Bosnia and Herzegovina, Ston saltern	– / –	–	–
EXF-972	Bathroom	Slovenia	– / –	–	–
EXF-977	Bathroom	Slovenia	DQ780362 / –	EF101396	EF101431

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
EXF-1072	Hypersaline water	Israel, Dead Sea	DQ780373 / –	EF101399	EF101428
EXF-2372	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
UAMH 7686	Indoor air ex RCS strip, from *Apis mellifera* overwintering facility	U.S.A., Alta, Clyde Corner	AY625063[5] / –	–	–
–	rDNA from bottlenose dolphin skin infected with *Loboa loboi*	U.S.A., Texas	AF035674[6] / –	–	–
–	Microcolony, on rock	Turkey, Antalya	AJ971409[7] / –	–	–
–	Microcolony, on rock	Turkey, Antalya	AJ971408[7] / –	–	–
–	Tomato leaves	–	L25433[8] / –	–	–
Cladosporium langeronii					
CBS 189.54 **NT**	Mycosis	Brazil	DQ780379 / DQ780932	EF101357	EF101435
CBS 601.84	*Picea abies*, wood	Germany, Göttingen	DQ780382 / –	EF101360	EF101438
CBS 101880	Moist aluminium school window frame	Belgium, Lichtervoorde	DQ780380 / –	EF101359	EF101440
CBS 109868	Mortar of Muro Farnesiano	Italy, Parma	DQ780377 / –	EF101362	EF101434
dH 11736	Biomat in a lake	Antarctics	DQ780381 / –	EF101363	EF101436
dH 12459	Orig. face lesion	Brazil	DQ780378 / –	EF101358	EF101439
dH 13833	Ice	Arctics	DQ780383 / –	EF101361	EF101437
–	Nasal mucus	–	AF455525[4] / –	–	–
–	Nasal mucus	–	AY345352[4] / –	–	–
–	Mycorrhizal roots	–	DQ068982[9] / –	–	–
Cladosporium oxysporum					
ATCC 66669	Creosote-treated southern pine pole	U.S.A., New York, Binghamton	AF393689[10] / DQ780395	AY752192[11]	EF101454
ATCC 76499	Decayed leaf, *Lespedeza bicolor*	–	AF393720	–	–
CBS 125.80	*Cirsium vulgare*, seedcoat	The Netherlands	AJ300332[12] / DQ780941	EF101351	EF101455
EXF-697	Hypersaline water	Dominican Republic, salt lake Enriquilio	DQ780392 / –	–	–
EXF-699	Hypersaline water	Dominican Republic, saltern	DQ780394 / –	–	–
EXF-710	Hypersaline water	Dominican Republic, saltern	DQ780393 / –	–	–
EXF-711	Hypersaline water	Dominican Republic, saltern	DQ780391 / –	–	–
Cladosporium psychrotolerans					
EXF-326	Hypersaline water	Slovenia, Sečovlje saltern	DQ780387 / DQ780934	–	EF101444
EXF-332	Hypersaline water	Slovenia, Sečovlje saltern	DQ780385 / DQ780933	EF101364	EF101441
EXF-391 **T**; CBS 119412	Hypersaline water	Slovenia, Sečovlje saltern	DQ780386 / –	EF101365	EF101442
EXF-714	Hypersaline water	Dominican Republic	DQ780384 / –	EF101366	EF101443
Cladosporium ramotenellum					
EXF-454 **T**; CPC 12043	Hypersaline water	Slovenia, Sečovlje saltern	DQ780403 / –	–	–
Cladosporium salinae					
EXF-322	Hypersaline water	Slovenia, Sečovlje	DQ780375 / –	EF101391	EF101403
EXF-335 **T**; CBS 119413	Hypersaline water	Slovenia, Sečovlje	DQ780374 / DQ780931	EF101390	EF101405
EXF-604	Hypersaline water	Spain, Santa Pola	DQ780376 / –	EF101389	EF101404
***Cladosporium* sp.**					
CBS 300.96	Soil along coral reef coast	Papua New Guinea, Madang, Jais Aben	DQ780352 / –	EF101385	–
EXF-595	Hypersaline water	Spain, Santa Pola saltern	DQ780402 / –	–	–
Cladosporium sphaerospermum					
ATCC 12092	Soil	Canada	AY361988[2] / –	–	–
ATCC 200384	Compost biofilter	The Netherlands	AY361991[2] / –	–	–
CBS 109.14; ATCC 36950	*Carya illinoensis* leaf scale	U.S.A.	DQ780350 / –	EF101384	EF101410
CBS 122.47; IFO 6377; IMI 49640; VKM F-772; ATCC 11292	Decaying stem of *Begonia* sp., with *Thielaviopsis basicola*	The Netherlands, Aalsmeer	AJ244228[1] / –	–	–
CBS 188.54; ATCC 11290; IMI 049638	de Vries (Engelhardt strain)	–	AY361990[2] & AY251077[3] / –	–	–
CBS 190.54; ATCC 11293; IFO 6380; IMI 49641	–	–	AY361992[2] / –	–	–
CBS 192.54; ATCC 11288; IMI 49636	Nail of man	–	AY361989[2] / –	–	–

Table 1. (Continued).

Strain Nr.[a]	Source	Geography	GenBank accession Nr.[b]		
			ITS rDNA / 18S rDNA	actin	β-tubulin
CBS 193.54 **NT**; ATCC 11289; IMI 49637	Human nails	–	DQ780343 & AY361958[2] / DQ780925	EF101380	EF101406
CBS 122.63	Plywood of *Betula* sp.	Finland, Helsinki	– / –	–	–
CBS 102045; EXF-2524; MZKI B-1066	Hypersaline water	Spain, Barcelona, Salines de la Trinitat	DQ780351 / –	EF101378	EF101411
CBS 114065	Outdoor air	Germany, Stuttgart	– / –	–	–
CPC 10944	Gardening peat substrate	Russia, Kaliningrad	DQ780350 / –	–	–
EXF-131; MZKI B-1005	Hypersaline water	Slovenia, Sečovlje saltern	AJ238670[1] / –	–	–
EXF-328	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-385	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-446	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-455	Hypersaline water	Slovenia, Sečovlje saltern	DQ780349 / –	EF101375	EF101412
EXF-458	Hypersaline water	Slovenia, Sečovlje saltern	DQ780345 / –	EF101374	EF101409
EXF-461	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-464	Hypersaline water	Slovenia, Sečovlje saltern	– / DQ780927	–	–
EXF-465	Hypersaline water	Slovenia, Sečovlje saltern	– / –	–	–
EXF-598	Hypersaline water	Spain, Santa Pola	– / –	EF101377	–
EXF-644	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-645	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-649	Hypersaline water	Spain, Santa Pola	– / –	–	–
EXF-715	Hypersaline water	Dominican Republic, saltern	– / –	–	–
EXF-738	Bathroom	Slovenia	DQ780348 / –	EF101383	EF101414
EXF-739	Bathroom	Slovenia	DQ780344 / –	EF101381	EF101407
EXF-781; MZKI B-899	Hypersaline water	Slovenia, Sečovlje	– / –	–	–
EXF-788	Hypersaline water	Slovenia, Sečovlje	– / –	–	–
EXF-962	Bathroom	Slovenia	DQ780347 / –	EF101382	EF101413
EXF-965	Bathroom	Slovenia	– / –	–	–
EXF-1069	Hypersaline water	Israel, Eilat saltern	– / –	EF101376	–
EXF-1061	Hypersaline water	Israel, Dead Sea	DQ780346 / –	EF101379	EF101408
EXF-1726	Hypersaline water	Israel, Dead Sea	– / –	–	–
EXF-1732	Hypersaline water	Israel, Eilat saltern	– / DQ780928	–	–
–	*Bryozoa* sp.	–	AJ557744 / –	–	–
–	Nasal mucus	–	AF455481[4] / –	–	–
Cladosporium spinulosum					
EXF-333	Hypersaline water	Slovenia, Sečovlje saltern	DQ780404 / –	–	–
EXF-334 **T**	Hypersaline water	Slovenia, Sečovlje saltern	DQ780406 / –	EF101355	EF101450
EXF-382	Hypersaline water	Slovenia, Sečovlje saltern	DQ780407 / DQ780936	EF101356	EF101449
Cladosporium subinflatum					
EXF-343 **T**; CPC 12041	Hypersaline water	Slovenia, Sečovlje saltern	DQ780405 / –	EF101353	EF101448
Cladosporium tenuissimum					
ATCC 38027	Soil	New Caledonia	AF393724 / –	–	–
EXF-324	Hypersaline water	Slovenia, Sečovlje saltern	– / DQ780926	–	–
EXF-371	Hypersaline water	Slovenia, Sečovlje saltern	DQ780396 / –	–	–
EXF-452	Hypersaline water	Slovenia, Sečovlje saltern	DQ780397 / –	–	–
EXF-563	Hypersaline water	Namibia, saltern	DQ780398 / –	–	–
Cladosporium velox					
CBS 119417 **T**; CPC 11224	*Bamboo* sp.	India, Charidij	DQ780361 / DQ780937	EF101388	EF101456
EXF-466	Hypersaline water	Slovenia, Sečovlje saltern	DQ780359 / –	EF101386	–
EXF-471	Hypersaline water	Slovenia, Sečovlje saltern	DQ780360 / –	EF101387	–

Cultivation and microscopy

For growth rate determination and phenetic description of colonies, strains were point inoculated on potato-dextrose agar (PDA, Difco), OA and Blakeslee malt extract agar (MEA, Samson *et al*. 2002) and incubated at 25 °C for 14 d in darkness. Surface colours were rated using the colour charts of Kornerup & Wanscher (1967). For studies of microscopic morphology, strains were grown on synthetic nutrient agar (SNA, Gams *et al*. 2007) in slide cultures. SNA blocks of approximately 1 × 1 cm were cut out aseptically, placed upon sterile microscope slides, and inoculated at the upper four edges by means of a conidial suspension (Pitt 1979). Inoculated agar blocks were covered with sterile cover slips and incubated in moist chambers for 7 d at 25 °C in darkness. The structure and branching pattern of conidiophores were observed at magnifications × 100, × 200 and × 400 in intact slide cultures under the microscope without removing the cover slips from the agar blocks. For higher magnifications (× 400, × 1 000) cover slips were carefully removed and mounted in lactic acid with aniline blue.

Morphological parameters

Morphological terms follow David (1997), Kirk *et al.* (2001) and Schubert *et al.* (2007 – this volume). Conidiophores in *Cladosporium* are usually ascending and sometimes poorly differentiated. Though the initiation point of conidiophore stipes could sometimes be determined only approximately, their lengths were in some cases useful for distinguishing morphologically similar species when observed in slide cultures. The branching patterns can be rotationally symmetric or unilateral. Characters of conidial scars were studied by light and scanning electron microscopy (SEM). Conidial chains show different branching patterns, determined by the numbers of conidia in unbranched parts, the nature of ramoconidia as well as their distribution in conidial chains. Measurements are given as (i) n_1–n_2 or (ii) (n_1–)n_3–n_4(–n_2), with n_1 = minimum value observed; n_2 = maximum value observed; n_3/n_4 = first/third quartile. For conidia and ramoconidia also average values and standard deviations are listed. The values provided are based on at least 25 measurements for the conidiophores of each strain, and at least 50 measurements for conidia.

Ecophysiology

To determine the degree of halotolerance, strains were point-inoculated on MEA without and with additional NaCl at concentrations of 5, 10, 17 and 20 % NaCl (w/v) and incubated at 25 °C for 14 d. To determine cardinal temperature requirements for growth, plates were incubated at 4, 10, 25, 30 and 37 °C, and colony diameters measured after 14 d of incubation.

DNA extraction, sequencing and analysis

For DNA isolation strains were grown on MEA for 7 d. DNA was extracted according to Gerrits van den Ende & de Hoog (1999) by mechanical lysis of approx. 1 cm^2 of mycelium. A fragment of the rDNA including the Internal Transcribed Spacer region 1, 5.8S rDNA and the ITS 2 (ITS) was amplified using the primers V9G (de Hoog & Gerrits van den Ende 1998) and LS266 (Masclaux *et al.* 1995). Sequence reactions were done using primers ITS1 and ITS4 (White *et al*. 1990). For amplification and sequencing of the partial actin gene, primers ACT-512F and ACT-783R were applied according to Carbone & Kohn (1999). For amplification and sequencing of the β-tubulin gene primers T1 and T22 were used according to O'Donnell & Cigelnik (1997). A BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) was used in sequence reactions. Sequences were obtained with an ABI Prism 3700 DNA Analyzer (Applied Biosystems). They were assembled and edited using SeqMan v. 3.61 (DNAStar, Inc., Madison, U.S.A.). Sequences downloaded from GenBank are indicated in the trees by their GenBank accession numbers; newly generated sequences are indicated by strain numbers (see also Table 1). Sequences were automatically aligned using ClustalX v. 1.81 (Jeanmougin *et al*. 1998). The alignments were adjusted manually using MEGA3 (Kumar *et al*. 2004). Phylogenetic relationships of the taxa were estimated from aligned sequences by the maximum parsimony criterion as implemented in PAUP v. 4.0b10 (Swofford 2003). Data sets of the SSU rDNA, ITS rDNA and the β-tubulin and actin genes are analysed separately. Species of *Cladosporium s. str.* were compared with various taxa of the *Mycosphaerellaceae* using SSU rDNA sequences and *Fusicladium effusum* G. Winter (*Venturiaceae*) as outgroup. The other data sets focus on *Cladosporium s. str.*, using *Cladosporium salinae* Zalar, de Hoog & Gunde-Cimerman as an outgroup, because this species was most deviant within *Cladosporium* in the SSU rDNA analysis (see below). Heuristic searches were performed on all characters, which were unordered and equally weighted. Gaps were treated as missing characters. Starting tree(s) were obtained via stepwise, random, 100 times repeated sequence addition. Other parameters included a "MaxTrees" setting to 9 000, the tree-bisection-reconnection as branch-swapping algorithm, and the "MulTrees" option set to active. Branch robustness was tested in the parsimony analysis by 10 000 search replications, each on bootstrapped data sets using a fast step-wise addition bootstrap analysis. Bootstrap values larger than 60 are noted near their respective branches. Newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov); their accession numbers are listed in Table 1. Alignments and trees were deposited in TreeBASE (www.treebase.org).

Table 1. (Page 158–160).

[a] Abbreviations used: ATCC = American Type Culture Collection, Virginia, U.S.A.; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC = Culture Collection of Pedro Crous, housed at CBS, Utrecht, The Netherlands; dH = de Hoog Culture Collection, housed at CBS, Utrecht, The Netherlands; EXF = Culture Collection of Extremophilic Fungi, Ljubljana, Slovenia; IFO = Institute for Fermentation, Culture Collection of Microorganizms, Osaka, Japan; IMI = The International Mycological Institute, Egham, Surrey, U.K.; MZKI = Microbiological Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia; UAMH = University of Alberta Microfungus Collection, Alberta, Canada; VKM = All-Russian Collection of Microorganisms, Russian Academy of Sciences, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia; NT = ex-neotype strain; T = ex-type strain.

[b] Reference: [1]de Hoog *et al.* 1999; [2]Park *et al.* 2004; [3]Braun *et al.* 2003; [4]Buzina *et al.* 2003; [5]Meklin *et al.* 2004; [6]Haubold *et al.* 1998; [7]Sert & Sterflinger, unpubl.; [8]Curtis *et al.* 1994; [9]Menkis *et al.* 2005; [10]Managbanag *et al.* unpubl.; [11]Crous *et al.* 2004; [12]Wirsel *et al.* 2002. All others are newly reported here.

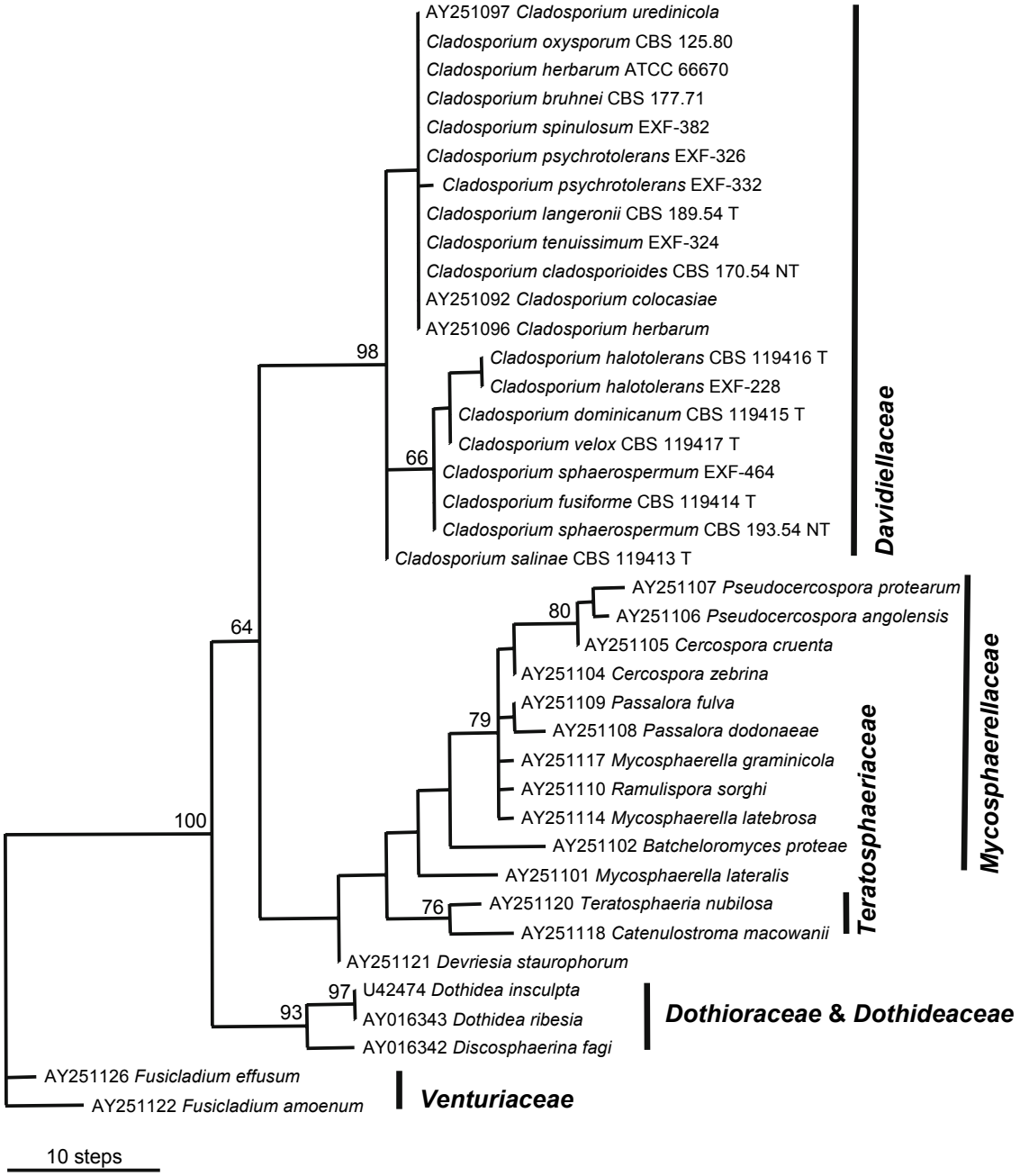

Fig. 1. One of 30 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned small subunit ribosomal DNA sequences. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Species of *Cladosporium s. str.*, including the seven newly described species, form a strongly supported monophyletic group among other taxa of the *Mycosphaerellaceae* (*Dothideomycetes*) (CI = 0.631, RI = 0.895, PIC = 50).

Table 2. Statistical parameters describing phylogenetic analyses performed on sequence alignments of four different loci.

Parameter	SSU rDNA	ITS rDNA[1]	β-tubulin[2]	Actin[3]
Number of alignment positions	1031	498	654	210
Number of parsimony informative characters (PIC)	50	68	220	103
Length of tree / number of steps	103	102	714	338
Consistency Index (CI)	0.631	0.804	0.538	0.586
Retention Index (RI)	0.895	0.975	0.883	0.885
Rescaled Consistency Index (RC)	0.565	0.784	0.475	0.518
Homoplasy index (HI)	0.369	0.196	0.462	0.414
Number of equally parsimonious trees retained	30	600	90	32

[1]Including the internal transcribed spacer region 1 and 2 and the 5.8S rDNA.

[2]Including partial sequences of 4 exons and complete sequences of 3 introns.

[3]Including partial sequences of 3 exons and 2 introns.

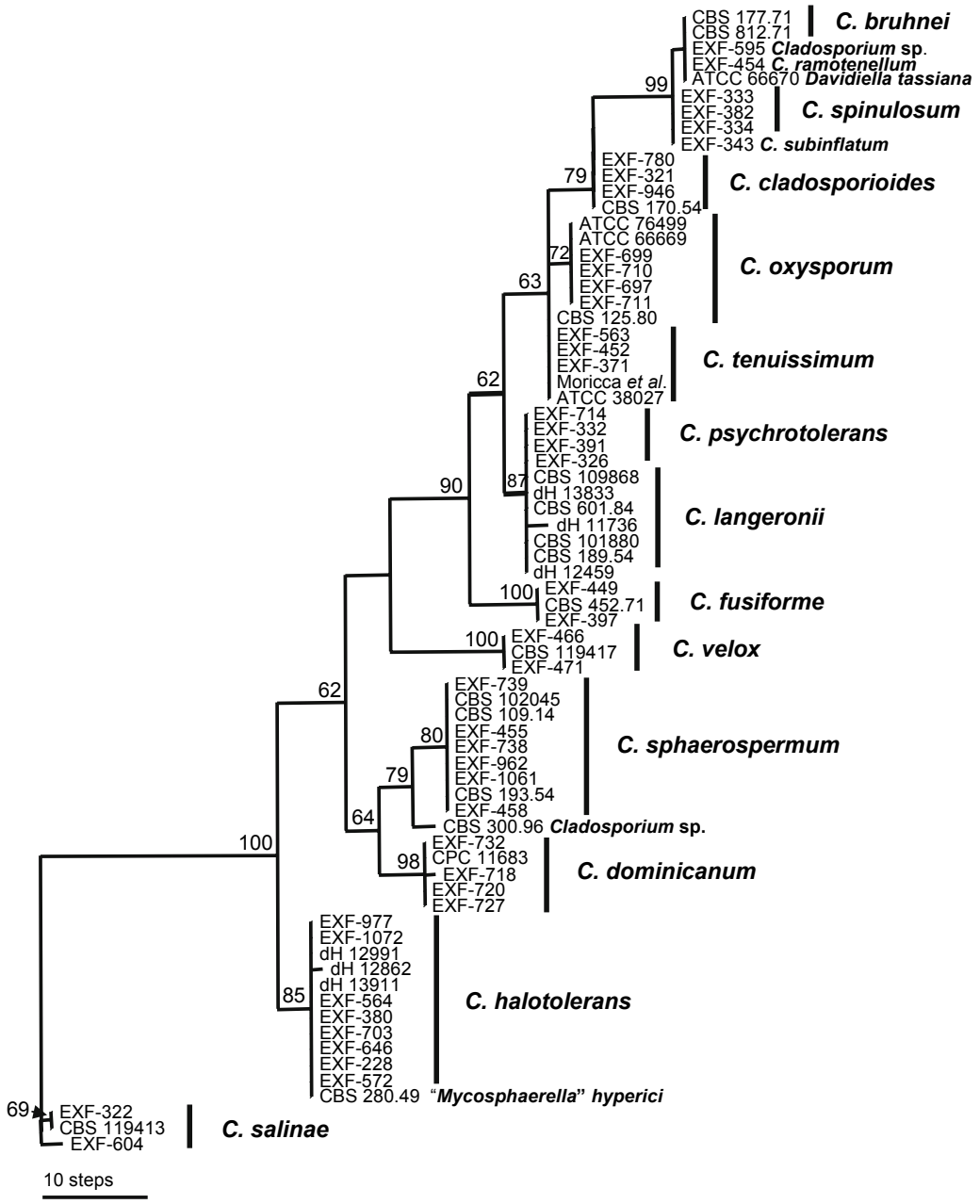

Fig. 2. One of 600 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned sequences of the internal transcribed spacer regions 1 and 2 and the 5.8S rDNA. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but some deeper branches moderate, bootstrap support (CI = 0.804, RI = 0.975, PIC = 68).

RESULTS

Descriptive statistical parameters of phylogenetic analyses and calculated tree scores for each analysed sequence locus are summarised in Table 2. Mainly reference material such as ex-type or ex-neotype strains was analysed on the level of SSU rDNA sequences. Downloaded and newly generated SSU rDNA sequences of members of *Cladosporium s. str.* were compared with related taxa of the *Mycosphaerellaceae*, *Dothioraceae* and *Dothideaceae*. The somewhat more distantly related *Fusicladium effusum* (*Venturiaceae*) (Braun *et al.* 2003: Fig. 2) was selected as outgroup. *Anungitopsis amoena* R.F. Castañeda & Dugan (now placed in *Fusicladium* Bonord., see Crous *et al*. 2007b), also a member of the *Venturiaceae*, was included in the analyses. All taxa included in the SSU rDNA analysis belong to the *Dothideomycetes* (Schoch *et al*. 2006), within which the ingroup is represented by the orders *Capnodiales* (*Davidiellaceae*, *Mycosphaerellaceae*, *Teratosphaeriaceae*) and *Dothideales* (*Dothioraceae*, *Dothideaceae*) (see also Schoch *et al.* 2006). The genus *Cladosporium*, of which some species are linked to *Davidiella* Crous & U. Braun teleomorphs (Braun *et al.* 2003), forms a statistically strongly supported monophyletic group (*Davidiellaceae*). It also accommodates species newly described in this paper, namely, *C. halotolerans* Zalar, de Hoog & Gunde-Cimerman, *C. fusiforme* Zalar, de Hoog & Gunde-Cimerman, *C. dominicanum* Zalar, de Hoog & Gunde-Cimerman, *C. salinae*, *C. psychrotolerans* Zalar, de Hoog & Gunde-Cimerman, *C. velox* Zalar, de Hoog & Gunde-Cimerman and *C. spinulosum* Zalar, de Hoog & Gunde-Cimerman (Fig. 1). A sister group relationship of *Cladosporium s. str.* with a clade of taxa characterised, among others, by *Mycosphaerella* Johanson teleomorphs, containing various anamorphic genera such as *Septoria* Sacc.,

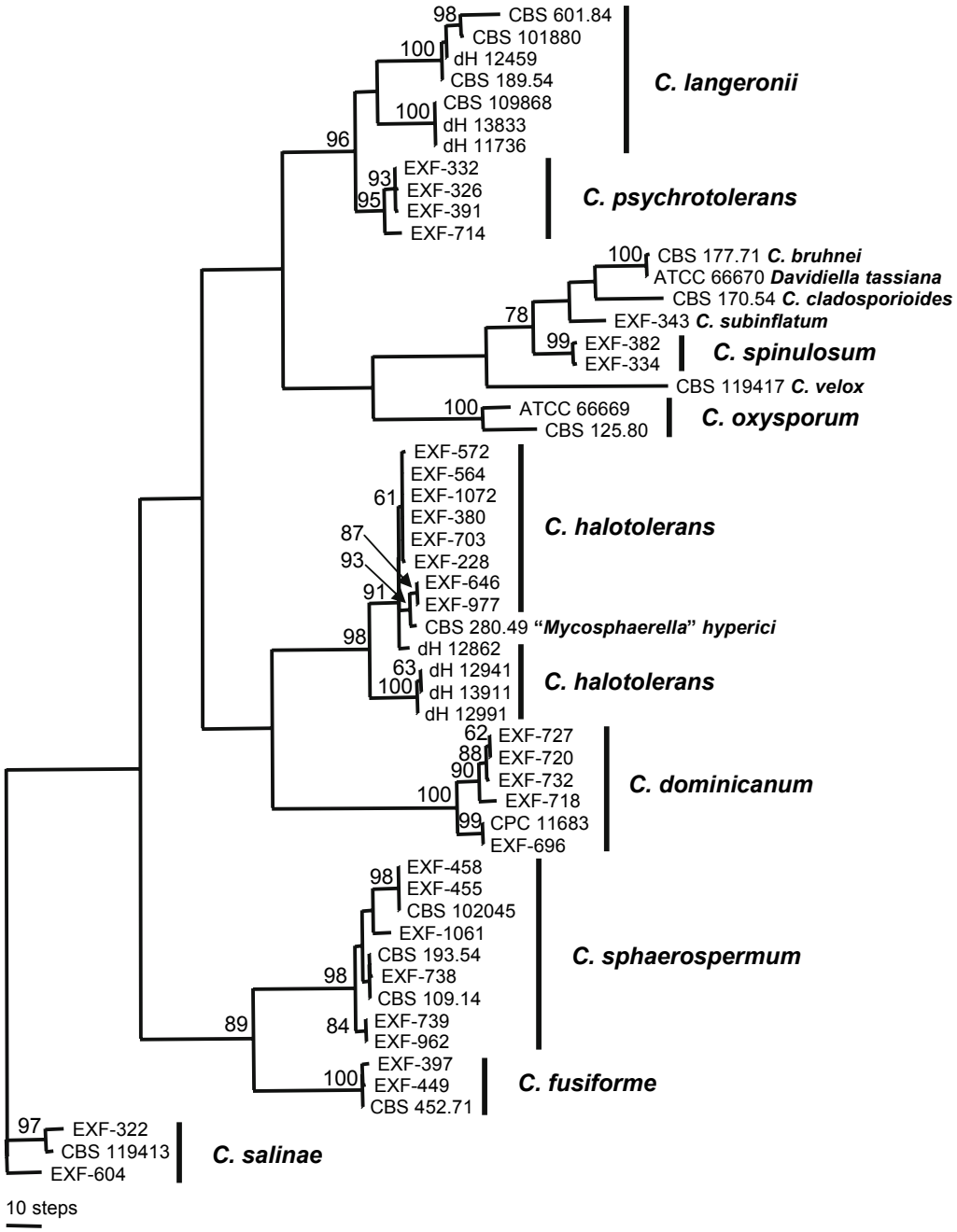

Fig. 3. One of 90 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned exons and introns of a part of the β-tubulin gene. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but deeper branches weak or no, bootstrap support (CI = 0.538, RI = 0.883, PIC = 220).

Ramularia Unger, *Cercospora* Fresen., *Pseudocercospora* Speg., "*Trimmatostroma*" Corda (now *Catenulostroma* Crous & U. Braun) (see Crous *et al*. 2004, 2007a – this volume) and the somewhat cladosporium-like genus *Devriesia* Seifert & N.L. Nick. (Seifert *et al.* 2004), was statistically only moderately supported (Fig. 1), whereas in an analogous analysis by Braun *et al.* (2003: Fig. 2) it was highly supported. These data also support the conclusion by Braun *et al.* (2003) and Crous *et al.* (2006) that *Cladosporium* is not a member of the distantly related *Herpotrichiellaceae* (*Chaetothyriomycetes*), which is also rich in cladosporium-like taxa (Crous *et al.* 2006). None of the fungi isolated from hypersaline environments belonged to the *Herpotrichiellaceae*. The SSU rDNA sequences do not resolve a phylogenetic structure within *Cladosporium s. str*. Only a moderately supported clade comprising *C. halotolerans*, *C. dominicanum*, *C. velox*, *C. sphaerospermum* and *C. fusiforme* is somewhat distinguished from a statistically unsupported clade with *C. herbarum* (Pers. : Fr.) Link, *C. cladosporioides* (Fresen.) G.A. de Vries, *C. oxysporum* Berk. & Broome, *C. spinulosum*, and *C. psychrotolerans*, etc. Because *C. salinae* appeared most distinct within the genus *Cladosporium* in analyses of the SSU rDNA (Fig. 1), it was used as outgroup in analyses of the ITS rDNA and the β-tubulin and actin genes.

Analyses of the more variable ITS rDNA and partial β-tubulin and actin gene introns and exons supported the species clades of *C. halotolerans*, *C. dominicanum*, *C. sphaerospermum*, *C. fusiforme* and *C. velox* (Figs 2–4), of which *C. velox* was distinguished in the β-tubulin tree by a particular long terminal branch of the only sequenced strain (Fig. 3). *Cladosporium salinae* also clustered as a well-supported species clade in preliminary analyses using various *Mycosphaerella* species as outgroup (not shown). All strains of *C. langeronii* (Fonseca, Leão & Nogueira) Vuill. are particularly well distinguishable from other *Cladosporium* species by strikingly slow-

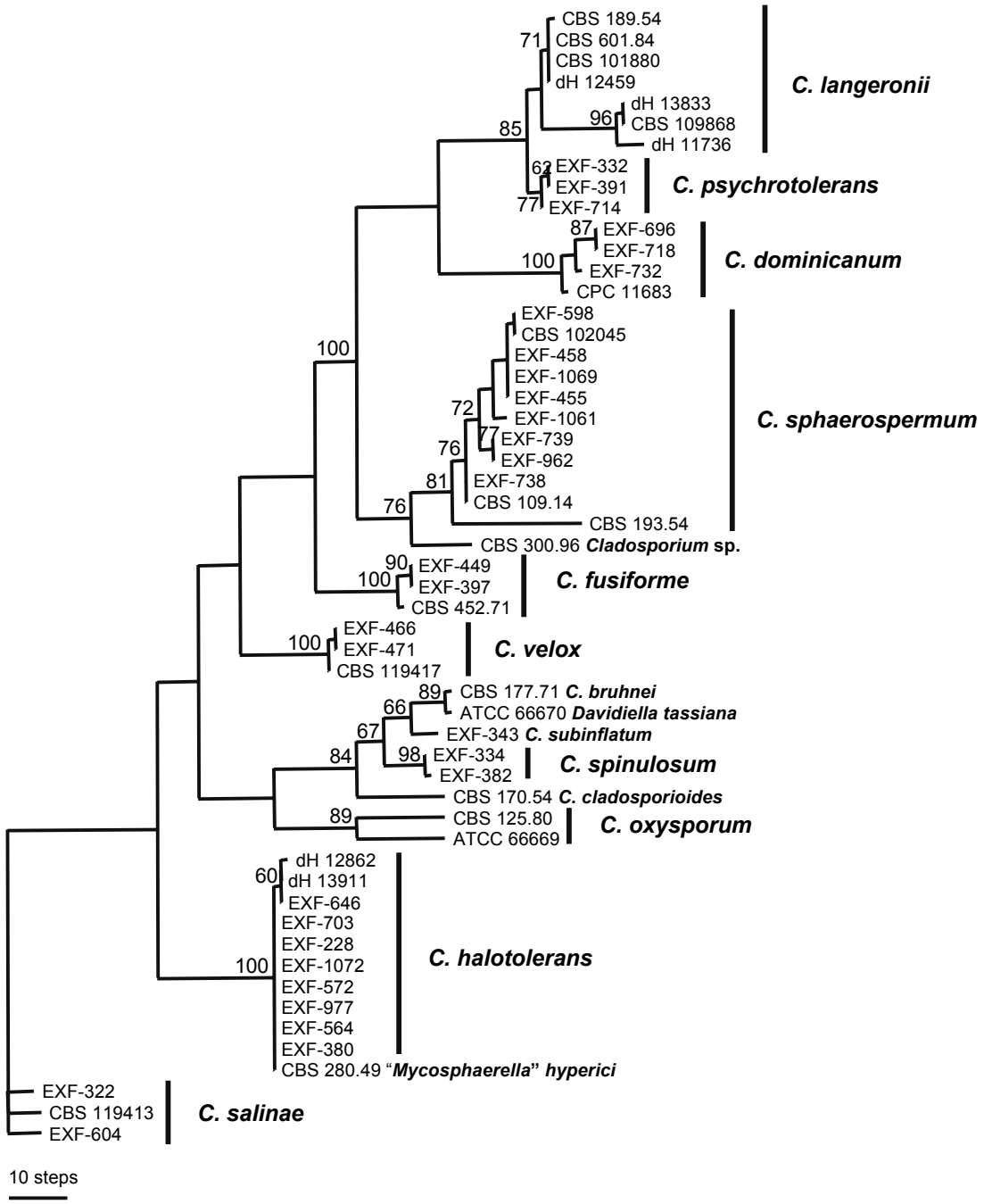

Fig. 4. One of 32 equally most parsimonious and equally looking phylogenetic trees based on a heuristic tree search using aligned exons and introns of the partial actin gene. The tree was randomly selected. Support based on 10 000 replicates of a fast step-wise addition bootstrap analysis is indicated near the branches. Trees were rooted with the strains of *Cladosporium salinae*. Most monophyletic species clades received high, but deeper branches weak or no, bootstrap support (CI = 0.586, RI = 0.885, PIC = 103).

growing colonies at all tested temperatures and relatively large, oblong conidia. However, phylogenetic analyses of the β-tubulin and actin gene indicate that *C. langeronii* presents two cryptic species (Figs 3–4). The species clade of *C. psychrotolerans* is moderately supported in analyses of the actin gene but highly by means of the β-tubulin gene. It is evident from all three analyses (Figs 2–4) that *C. langeronii* and *C. psychrotolerans* are closely related species. The species node of *Cladosporium spinulosum*, which is morphologically clearly distinguished from all other species by its conspicuous ornamentation consisting of digitate projections (Fig. 5), is supported by β-tubulin (Fig. 3) and actin (Fig. 4) sequence data but not by those of the ITS rDNA (Fig. 2). Analyses of all loci, however, indicate that it is a member of the *C. herbarum* complex.

The analyses of sequences of the ITS and the β-tubulin and actin gene introns and exons (Figs 2–4) do not allow the full elucidation of phylogenetic relationships among these *Cladosporium* species. Statistical support of the interior tree branches resulting from analyses of the β-tubulin and actin genes is low (bootstrap values mostly < 50 %). While the sister group relationship of *C. sphaerospermum* and *C. fusiforme* is highly supported in the analysis based on the β-tubulin gene, analysis of the ITS rDNA indicate that these two species are unrelated, and that *C. sphaerospermum* is closely related to *C. dominicanum*. It is clear from the data that the species morphologically resembling *C. sphaerospermum* are not phylogenetically closely related and that the data we present here do not allow their classification in natural subgroups of the genus *Cladosporium*. Only *C. spinulosum* was placed in all analyses among species of the *C. herbarum* complex

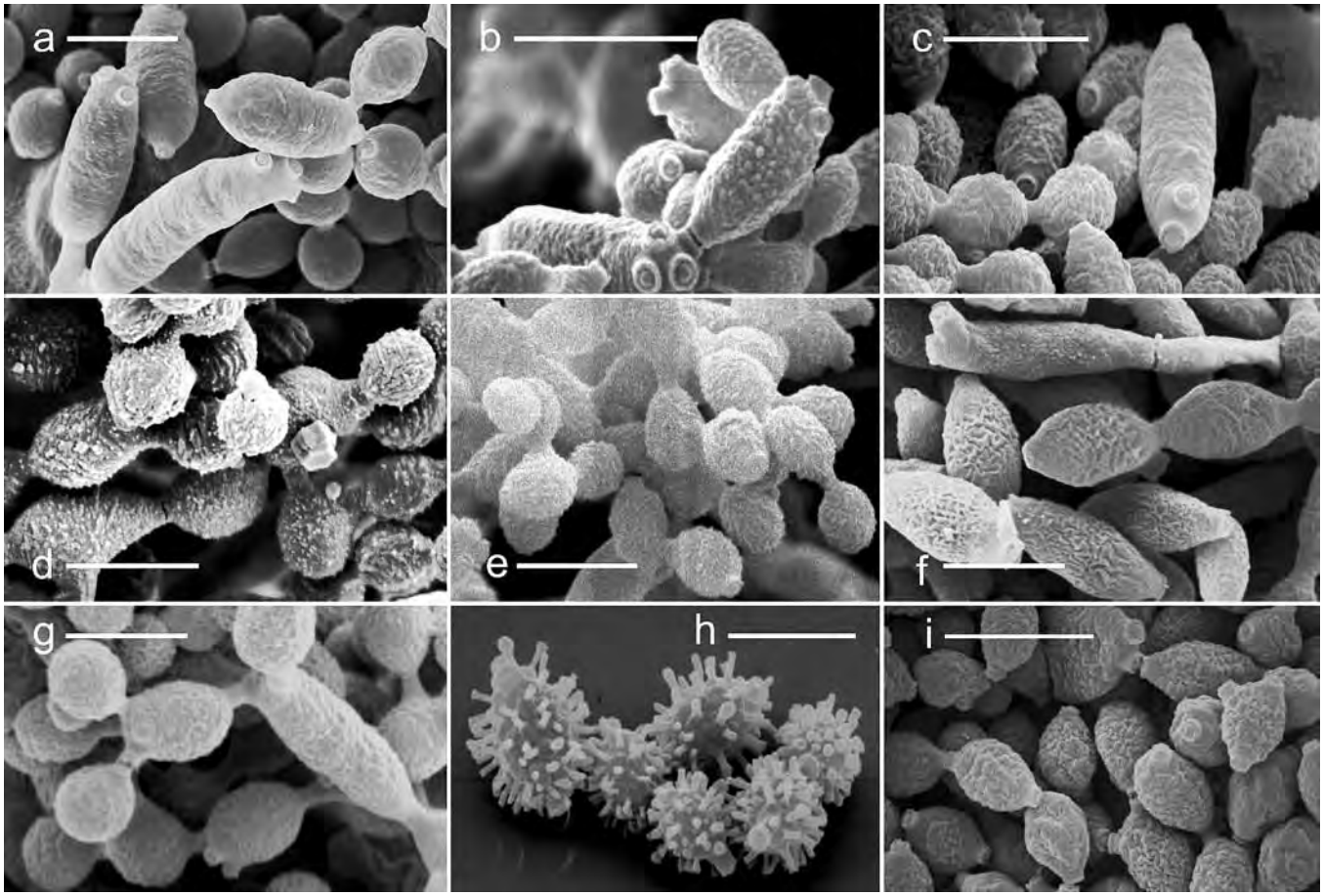

Fig. 5. Conidial scars and surface ornamentation of ramoconidia and conidia (SEM). A. *C. dominicanum* (strain EXF-732). B. *C. fusiforme* (strain EXF-449). C. *C. halotolerans* (strain EXF-572). D. *C. langeronii* (strain CBS 189.54). E. *C. psychrotolerans* (strain EXF-391). F. *C. salinae* (strain EXF-335 = CBS 119413). G. *C. sphaerospermum* (strain CBS 193.54). H. *C. spinulosum* (strain EXF-334). I. *C. velox* (strain CBS 119417). Scale bars = 5 µm. (Photos: K. Drašlar).

and all analyses supported close relatedness of *C. langeronii* and *C. psychrotolerans*.

The majority of species described here have slightly ornamented conidia ranging from minutely verruculose (*C. fusiforme*, *C. langeronii, C. psychrotolerans*, *C. sphaerospermum*, *C. velox*) to verrucose (*C. halotolerans*) (Fig. 5). The verrucose conidia of *C. halotolerans* can be recognised also under the light microscope and used as a distinguishing character. Almost smooth to minutely verruculose conidia are encountered in *C. dominicanum* and *C. salinae* (Fig. 5). *Cladosporium spinulosum*, a member of the *C. herbarum* species complex, has conidia with a digitate ornamentation that can appear spinulose under the light microscope; however, when using the SEM it became clear that its projections have parallel sides and a blunt end (Fig. 5).

DISCUSSION

The genus *Cladosporium* was established by Link (1816) who originally included four species, of which *C. herbarum* is the type species of the genus (Clements & Shear 1931). In 1950, von Arx reported a teleomorph connection for this species with *Mycosphaerella tassiana* (De Not.) Johanson. Based on SSU rDNA data the majority of *Mycosphaerella* species, including the type species of the genus, *M. punctiformis* (Pers.) Starbäck, clustered within the *Mycosphaerellaceae*, a family separated from *M. tassiana* (Braun *et al*. 2003). Therefore, *Mycosphaerella tassiana* was reclassified as *Davidiella tassiana* (De Not.) Crous & U. Braun, the type of the new genus *Davidiella.* All anamorphs with a cladosporium- and heterosporium-like appearance and with a supposed *Dothideomycetes* relationship were maintained under the anamorph name *Cladosporium*, morphologically characterised by scars with a protuberant hilum consisting of a central dome surrounded by a raised rim (David 1997).

The concept of distinguishing ramoconidia from secondary ramoconidia has been adopted from Schubert *et al.* (2007). In the species described here, ramoconidia have been observed often in *C. sphaerospermum*, sometimes in *C. psychrotolerans*, *C. langeronii* and *C. spinulosum*, and only sporadically in all other species. Therefore, ramoconidia can be seen as important for distinguishing species although sometimes, they can be observed only with difficulty. When using ramoconidia as a diagnostic criterion, colonies only from SNA and not older than 7 d should be taken into account.

Cladosporium sphaerospermum was described by Penzig (1882) from decaying *Citrus* leaves and branches in Italy. He described *C. sphaerospermum* as a species with (i) branched, septate and dark conidiophores having a length of 150–300 µm and a width of the main conidiophore stipe of 3.5–4 µm, (ii) spherical to ellipsoid, acrogenously formed conidia of 3.4–4 µm diam, and (iii) ramoconidia of 6–14 × 3.5–4 µm. Penzig's original material is not known to be preserved. Later, a culture derived from CBS 193.54, originating from a human nail, was accepted as typical of *C. sphaerospermum.* However, de Vries (1952), incorrectly cited it as "lectotype", and thus the same specimen is designated as neotype in this study (see below), with the derived culture (CBS 193.54)

used as ex-neotype strain. Numerous strains with identical or very similar ITS rDNA sequences as CBS 193.54 were isolated from hypersaline water or organic substrata including plants or walls of bathrooms. It is not clear yet whether surfaces in bathrooms and of plants, colonised by *C. sphaerospermum*, can have a similar low water activity as salterns. In our experiments, the strains of this species, however, grew under *in vitro* conditions at a water activity of up to 0.860, while Hocking *et al.* (1994) and Aihara *et al.* (2002) reported that it can grow even at 0.815. Therefore, we consider *C. sphaerospermum* as halo- or osmotolerant. Hardly any reports are available unambiguously proving that *C. sphaerospermum* is a human pathogen. It is therefore possible that CBS 193.54 was not involved in any disease process but rather occurred as a contaminant on dry nail material. *Cladosporium sphaerospermum* is a phylogenetically well-delineated species (Figs 2–4).

Strains of *C. halotolerans* were isolated sporadically from substrata such as peanut cell suspension, tissue culture, bathroom walls and as culture contaminants. This surprising heterogeneity of substrata suggests that *C. halotolerans* is distributed by air and that it can colonise whatever substrata available, although it may have its natural niche elsewhere. We have recurrently isolated it from hypersaline water of salterns and other saline environments and it was also detected with molecular methods (but not isolated) from skin of a salt water dolphin. There are only few reports of this species from plants (Table 1). It is therefore possible that *C. halotolerans* is a species closely linked to salty or hypersaline environments although additional sampling is necessary to prove that. *Cladosporium halotolerans* is morphologically recognisable by relatively oblong to spherical, coarsely rough-walled conidia. The ITS rDNA sequence of a fungus in the skin of a bottlenose dolphin, suffering from lobomycosis, is identical to the sequences of *C. halotolerans*. This sequence was deposited as *Lacazia loboi* Taborda, V.A. Taborda & McGinnis (GenBank AF035674) by Haubold *et al*. (1998), who apparently concluded wrongly that a fungus with a cladosporium-like ITS rDNA sequence similar to that of *C. halotolerans* can be the agent of lobomycosis. Later, Herr *et al*. (2001) showed that *Lacazia loboi* phylogenetically belongs to the *Onygenales* on the basis of amplified SSU rDNA and chitin synthase-2 gene sequences generated from tissue lesions. By this, they confirmed an earlier supposition by Lacaz (1996) who reclassified the organism as *Paracoccidioides loboi* O.M. Fonseca & Silva Lacaz (*Onygenales*). It is therefore possible that *C. halotolerans* was not the main etiologic agent for the lobomycosis and it was colonising the affected dolphin skin secondarily while inhabiting other seawater habitats.

Cladosporium langeronii and *C. psychrotolerans* are closely related but *C. langeronii* is particularly well distinguishable from all other *Cladosporium* species by its slow growing colonies (1–7 mm diam / 14 d) and relatively large conidia (4–5.5 × 3–4 µm). *Cladosporium psychrotolerans* has smaller conidia (3–4 × 2.5–3 µm) but a similar length : width ratio and faster expanding colonies (8–18 mm diam / 14 d). *Cladosporium langeronii* is most likely a complex of at least two species. Strains isolated from the Arctic and the Antarctic may need to be distinguished from *C. langeronii s. str.* on species level. This inference is particularly supported by analyses of the β-tubulin and actin genes (Figs 3–4). *Cladosporium langeronii s. str.*, represented by an authentic strain of *Hormodendrum langeronii* Fonseca, Leão & Nogueira, CBS 189.54 (Trejos 1954), has been isolated from a variety of substrata but is tolerating only up to 10 % NaCl. It was originally described by da Fonseca *et al*. (1927a, b) and subsequently reclassified as *Cladosporium langeronii* by Vuillemin (1931). The authentic strain derived from an ulcerating nodular lesion on the arm of a human patient. Because other strains of this species are ubiquitous saprobes originating from various substrata, we suspect that *C. langeronii* is not an important human pathogen. *Cladosporium psychrotolerans* has been isolated from hypersaline environments only, and tolerates up to 20 % NaCl in culture media.

In general, the human- or animal-pathogenic role of the *C. sphaerospermum*-like species described here seems to be limited. It is possible that pathogenic species of *Cladophialophora* Sacc. have been misidentified as *C. sphaerospermum* or as other species of *Cladosporium* (de Hoog *et al*. 2000). Alternatively, true *Cladosporium* species isolated as clinical strains could have been secondary colonisers since they are able to dwell on surfaces poor in nutrients, possibly in an inconspicuous dormant phase and may then be practically invisible. More likely, they could be air-borne contaminations of lesions, affected nails etc. (Summerbell *et al*. 2005) or are perhaps disseminated by insufficiently sterilised medical devices, as melanised fungi can be quite resistant to disinfectants (Phillips *et al.* 1992). They can easily be isolated and rapidly become preponderant at isolation and thus difficult to exclude as etiologic agents of a disease. For example, in 2002, a case report on an intrabronchial lesion by *C. sphaerospermum* in a healthy, non-asthmatic woman was described (Yano *et al*. 2002), but we judge the identification of the causal agent to remain uncertain, as it was based on morphology alone and no culture is available. The present authors have the opinion that all clinical cases ascribed to *Cladosporium* species need careful re-examination.

General characteristics and description of *Cladosporium sphaerospermum*-like species

The present paper focuses on *Cladosporium* strains isolated from hypersaline environments. Comparison of data from deliberate sampling and analysis of reference strains from culture collections inevitably leads to statistical bias, and therefore a balanced interpretation of ecological preferences of the species presented is impossible. Nevertheless, some species appeared to be consistent in their choice of habitat, and for this reason we summarise isolation data for all species described. Strains belonging to a single molecular clade proved to have similar cultural characteristics and microscopic morphology. Although within most of the species there was some molecular variation noted (particularly when intron-rich genes were analysed), some consistent phenetic trends could be observed.

Conidiophores of all *C. sphaerospermum*-like species lack nodose inflations (McKemy & Morgan-Jones 1991). They are usually ascending and can sometimes be poorly differentiated from their supporting hyphae. Though the initiation point of conidiophore stipes could sometimes be determined only approximately, their lengths were in some cases useful for distinguishing morphologically similar species when observed in slide cultures. Generally, the branched part of a conidiophore forms a complex tree-like structure. The number and orientation of early formed secondary ramoconidia, however, determines whether it is rotationally symmetric or unilateral.

The variability in ITS rDNA sequences observed in all *C. sphaerospermum*-like species (about 10 %) spans the variation observed in all members of the genus *Cladosporium* sequenced to date. Thus, the *C. sphaerospermum*-like species described here may not present a single monophyletic group but may belong to various species complexes within *Cladosporium*. Verifying existing literature with sequence data of these species (Wirsel *et al.* 2002, Park *et al*. 2004), we noticed that names of the common saprobes seem to be distributed nearly at random over phylogenetic trees.

For most commonly used names, no type material is available for sequencing. Also verification of published reports is difficult without available voucher strains.

Cladosporium cladosporioides was incorrectly lectotypified based on CBS 170.54 (de Vries 1952), which Bisby considered a standard culture of *C. herbarum*. The *C. cladosporioides* species complex requires revision, and will form the basis of a future study. *Cladosporium herbarum* is maintained as a dried specimen in the Leiden herbarium; Prasil & de Hoog (1988) selected CBS 177.71 as a representative living strain. Strains, earlier accepted as living representatives of *C. herbarum*, CBS 177.71 and CBS 812.71 (Prasil & de Hoog 1988, Wirsel *et al.* 2002) and ATCC 66670 (Braun *et al.* 2003, as *Davidiella tassiana*) have been re-identified as *C. bruhnei* Linder by Schubert *et al.* (2007 – this volume). Ho *et al*. (1999) used strain ATCC 38027 as a representative of *C. tenuissimum* Cooke and this strain has identical ITS sequences as the non-deposited *C. tenuissimum* material used by Moricca *et al.* (1999). We tentatively accept this concept although we could not include ATCC 38027 in our analyses. The ITS sequence of strain CBS 125.80, identified by Wirsel *et al.* (2002) as *C. oxysporum*, is identical to the sequence of ATCC 38027. Strain ATCC 76499, published by Ho *et al.* (1999) as *C. oxysporum*, appears to be identical to a number of currently unidentified *Cladosporium* strains from Slovenian salterns that compose a cluster separate from all remaining species. Strains of this cluster, represented in Fig. 2 by strain ATCC 76499, morphologically resemble *C. oxysporum.*

Strain CBS 300.96 has not been identified to species level in the present study. It clusters outside the species clade of *C. sphaerospermum*, with the latter being its nearest relative. CBS 300.96 differs from *C. sphaerospermum* by having smaller structures: conidiophore stipes [(5–)20–80(–150) × (2–)2.5–3(–4) µm], 0–1 septate ramoconidia [(13–)19–27(–32) × 2–2.5 µm], conidia [(2.5–)3–3.5(–4) × (2–)2–2.5(–3) µm] and secondary ramoconidia [(5–)9–18(–30) × (2–)2.5–2.5(–3) µm]. However, based on a single isolate, we currently refrain from describing it as a new species.

Key to species treated in this study

Macro-morphological characters used in the key are from colonies grown on PDA and MEA 14 d at 25 °C, if not stated otherwise; microscopical characters are from SNA slide cultures grown for 7 d at 25 °C.

1. Conidial ornamentation conspicuously echinulate / digitate because of up to 1.3 µm long projections that have more or less parallel sides ..... ***C. spinulosum***
1. Conidial ornamentation verruculose to verrucose or smooth, not conspicuously echinulate or digitate 2

2. Conidiophores micronematous, poorly differentiated, once or several times geniculate-sinuous, short, up to 60 µm long; terminal conidia obovoid ..... ***C. salinae***
2. Conidiophores micro- or macronematous, not geniculate or only slightly so, usually up to 100 µm or 220 µm long or even longer; terminal conidia globose, subglobose to ovoid or fusiform 3

3. Secondary ramoconidia 0–3(–4)-septate; septa of conidiophores and conidia darkened and thickened 4
3. Secondary ramoconidia 0–1(–2)-septate; septa neither darkened nor thickened 5

4. Conidiophores (5–)10–50(–300) × (2–)2.5–3(–5.5) µm; terminal conidia (2–)3–4(–6) × (2–)2.5–3(–5) µm; secondary ramoconidia (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm; ramoconidia sporadically formed ..... ***C. halotolerans***
4. Conidiophores mostly longer and somewhat wider, (10–)45–130(–300) × (2.5–)3–4(–6) µm; terminal conidia mostly wider, (2.5–)3–4(–7) × (2–)3–3.5(–4.5) µm; secondary ramoconidia (4–)8.5–16(–37.5) × (2–)3–3.5(–5) µm; ramoconidia often formed, up to 40 µm long, with up to 5 septa ..... ***C. sphaerospermum***

5. Terminal conidia usually fusiform ..... ***C. fusiforme***
5. Terminal conidia globose, subglobose or ovoid 6

6. Conidia and secondary ramoconidia irregularly verruculose to sometimes loosely verrucose; radial growth on PDA at 25 °C after 14 d typically less than 5 mm ..... ***C. langeronii***
6. Conidia and secondary ramoconidia smooth to minutely verruculose; radial growth on PDA at 25 °C after 14 d typically more than 10 mm 7

7. Conidiophores (3–)3.5–4(–7.5) µm wide, thick-walled; conidiogenous loci and conidial hila 0.5–2 µm diam; ramoconidia sometimes formed with a broadly truncate, up to 2 µm wide non-cladosporioid base; no growth observed after 14 d at 30 °C on MEA ..... ***C. psychrotolerans***
7. Conidiophores mostly narrower, 2–4 µm wide, only with slightly thickened walls; conidiogenous loci and conidial hila narrower, 0.5–1.5 µm diam; ramoconidia rarely formed; colony showing at least weak growth after 14 d at 30 °C on MEA 8

8. Secondary ramoconidia (4–)6.5–13(–24.5) µm long; no visible colony growth after 14 d at 10 °C on MEA..... ***C. dominicanum***
8. Secondary ramoconidia mostly longer, (3.5–)5.5–19(–42) µm; radial growth of colonies after 14 d at 10 °C on MEA more than 5 mm ..... ***C. velox***

Description of *Cladosporium* species

Cladosporium dominicanum Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB510995. Fig. 6.

Etymology: Refers to the the Dominican Republic, where most strains were encountered.

Conidiophora lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (5–)10–100(–200) × (1.5–)2–2.5(–3.5) µm, olivaceo-brunneus, levis vel leniter verruculosus, tenuitunicatus, plerumque unicellularis, simplex vel ramosus. Conidiorum catenae undique divergentes, ad 8 conidia in parte continua continentes. Cellulae conidiogenae indistinctae. Conidia levia vel leniter verruculosa, dilute brunnea, unicellularia, plerumque breviter ovoidea, utrinque angustata, (2.5–)3–3.5(–5.5) × (2–)2–2.5(–2.5) µm, long.: lat. 1.4–1.6; ramoconidia secundaria cylindrica vel quasi globosa, 0–1-septata, (4–)6.5–13(–24.5) × (2–)2.5–3(–4.5) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, protuberantes, 0.5–1.2 µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium without extracellular polysaccharide-like material. *Conidiophores* arising laterally and terminally on erect hyphae, micronematous and semimacronematous, stipes of variable length, (5–)10–100(–200) × (1.5–)2–2.5(–3.5) µm, olivaceous-brown, smooth to minutely verruculose, thin-walled, almost non-septate, unbranched or branched. *Conidial chains* branching in all directions, up to eight conidia in the unbranched parts. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* smooth to minutely verruculose, subhyaline to light brown, non-septate, usually short-ovoid, narrower at both ends, length : width ratio = 1.4–1.6; (2.5–)3–3.5(–5.5) × (2–)2–2.5(–2.5) µm [av. (± SD) 3.4 (± 0.6) × 2.2 (± 0.2)]; *secondary ramoconidia* cylindrical to almost spherical, 0–1-septate, (4–)6.5–13(–24.5) × (2–)2.5–3(–4.5) µm [av. (± SD) 10.3 (± 5.2) × 2.7 (± 0.6)], with up to four distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–1.2 µm diam.

Cultural characteristics: Colonies on PDA reaching 18–36 mm diam, olive-yellow (2D6), hairy granular, flat or slightly furrowed, with flat margin. Droplets of light reseda-green (2E6) exudate sometimes present. Reverse dark green to black. Colonies on OA reaching 19–34 mm diam, olive (2F5), loosely powdery with raised central part due to fasciculate bundles of conidiophores. Reverse dark green. Colonies on MEA reaching 30–32 mm diam, reseda green (2E6), velvety, furrowed, with undulate margin. Reverse dark green-brown. Colonies on MEA + 5 % NaCl reaching 37–41 mm diam, reseda-green (2E6), radially furrowed, velvety, sporulating in the central part or all over the colony, margin white and regular. Reverse brownish green.

Maximum tolerated salt concentration: 75 % of tested strains develop colonies at 20 % NaCl after 7 d, while after 14 d all strains grow and sporulate.

Cardinal temperatures: No growth at 4 and 10 °C, optimum 25 °C (30–32 mm diam), maximum 30 °C (2–15 mm diam), no growth at 37 °C.

Specimen examined: **Dominican Republic**, from hypersaline water of salt lake Enriquillo, coll. Nina Gunde-Cimerman, Jan. 2001, isol. P. Zalar 25 Feb. 2001, CBS H-19733, **holotype**, culture ex-type EXF-732 = CBS 119415.

Habitats and distribution: Fruit surfaces; hypersaline waters in (sub)tropical climates.

Differential parameters: No growth at 10 °C, ovoid conidia, large amounts of sterile mycelium.

Strains examined: CPC 11683, EXF-696, EXF-718, EXF-720, EXF-727, EXF-732 (= CBS 119415; ex-type strain).

Note: Cultures of *C. dominicanum* sporulate less abundantly than *C. sphaerospermum* and *C. halotolerans* and tend to lose their ability to sporulate with subculturing.

Cladosporium fusiforme Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB510997. Fig. 7.

Etymology: Refers to its usually fusiform conidia.

Conidiophora erecta, lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (10–)25–50(–100) × (2–)2–3.5(–4) µm, olivaceo-brunneus, levis, crassitunicatus, compluries septatus (cellulis 9–23 µm longis), plerumque simplex. Conidiorum catenae undique divergentes, in parte continua ad 5 conidia continentes. Cellulae conidiogenae indistinctae. Conidia leniter verruculosus, dilute brunnea, unicellularia, plerumque fusiformia, utrinque angustata, (2.5–)3.5–5(–6.5) × (2–)2–2.5(–3) µm, long. : lat. 1.8–2.0; ramoconidia secundaria cylindrica, 0(–1)-septata, (5–)6–11(–22) × (2.5–)2.5–3(–3) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, 0.7–1.0 µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium without extracellular polysaccharide-like material. *Conidiophores* erect, arising laterally and terminally from straight hyphae, stipes of variable length, (10–)25–50(–100) × (2–)2–3.5(–4) µm, olivaceous-brown, smooth- and thick-walled, regularly-septate (cell length 9–23 µm), mostly unbranched. *Conidial chains* branching in all directions, up to 5 conidia in the unbranched parts. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* minutely verruculose, light brown, aseptate, usually fusiform and narrower at both ends, length : width ratio = 1.8–2.0; (2.5–)3.5–5(–6.5) × (2–)2–2.5(–3) µm [av. (± SD) 4.4 (± 0.8) × 2.2 (± 0.2)]; *secondary ramoconidia* cylindrical, 0(–1)-septate, (5–)6–11(–22) × (2.5–)2.5–3(–3) µm [av. (± SD) 9.0 (± 4.7) × 2.6 (± 0.3)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.0 µm diam.

Cultural characteristics: Colonies on PDA reaching 20–26 mm diam, dull green (30E3), granular due to profuse sporulation, flat, with flat margin. Sterile mycelium absent. Reverse blackish green. Colonies on OA reaching 24–28 mm diam, olive (3F3), granular in concentric circles, consisting of two kinds of conidiophores (low and high), flat, with flat margin. Reverse black. Colonies on MEA reaching 23–28 mm diam, olive (3E5), deeply furrowed, velvety (sporulating all over) with undulate, white margin. Reverse brownish green. Colonies on MEA + 5 % NaCl reaching 28–43 mm diam, olive (3E6), granular due to profuse sporulation, slightly furrowed with flat, olive-grey (3F2) margin. Reverse dark green.

Maximum tolerated salt concentration: Only one of three strains tested (CBS 452.71) developed colonies at 17 % NaCl after 14 d, the other two strains grew until 10 % NaCl.

Cardinal temperatures: For one of three strains (CBS 452.71) the minimum temperature of growth was 4 °C (6 mm diam), for the other two 10 °C (8–9 mm diam); optimum 25 °C (23–28 mm diam), maximum 30 °C (only strain CBS 452.71 grew 5 mm diam), no growth at 37 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. L. Butinar, Dec. 1999, CBS H-19732, **holotype**, culture ex-type EXF-449 = CBS 119414.

Habitats and distribution: Osmotic environments worldwide.

Differential parameters: Oblong conidia, relatively low degree of halotolerance.

Strains examined: CBS 452.71, EXF-397, EXF-449 (= CBS 119414; ex-type strain).

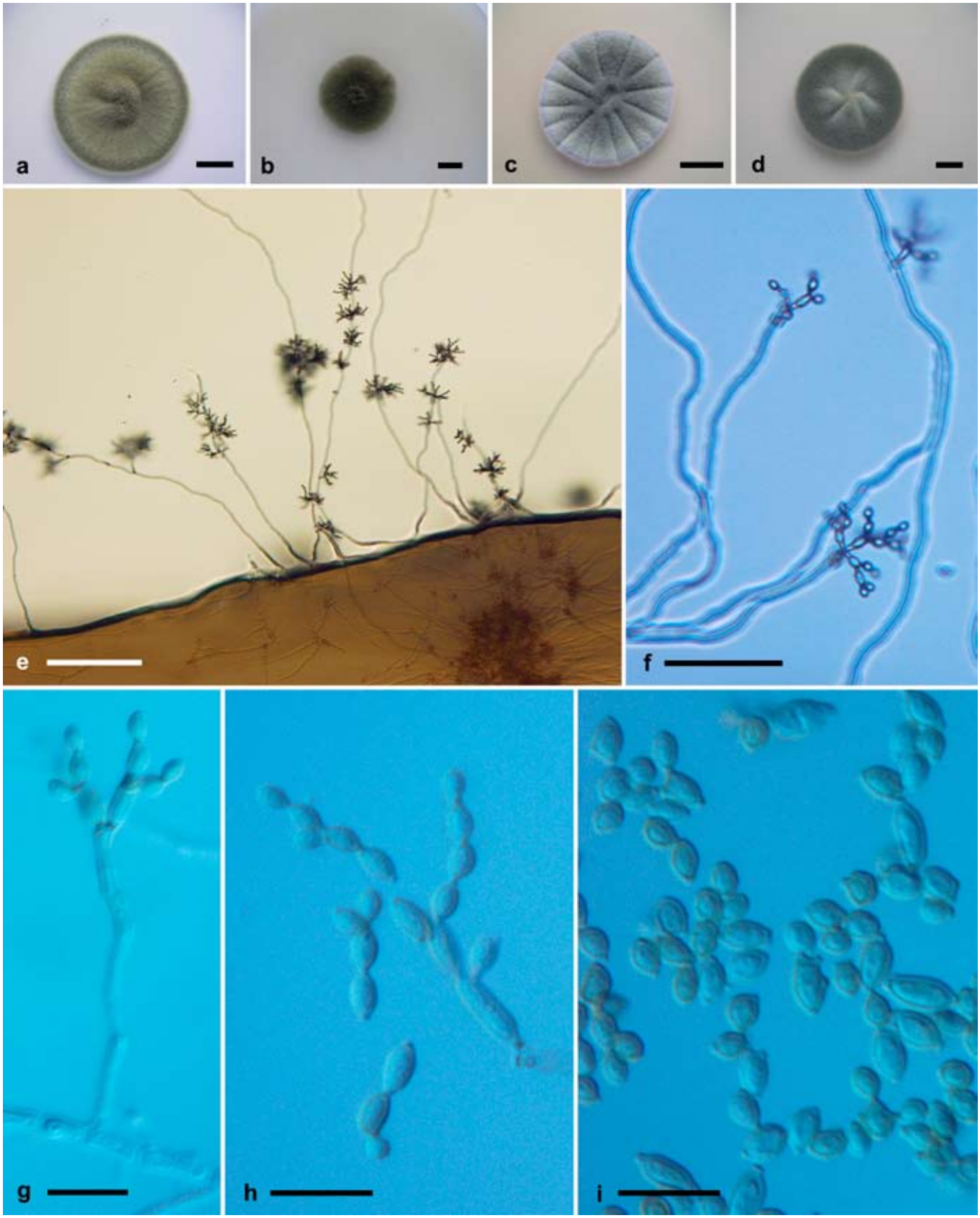

Fig. 6. *Cladosporium dominicanum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A, D, F–H, from EXF-2519; B, C, E from EXF-727; I, EXF-732 (ex-type strain). Scale bars A–D = 10 mm, E = 100 µm, F = 30 µm, G–I = 10 µm.

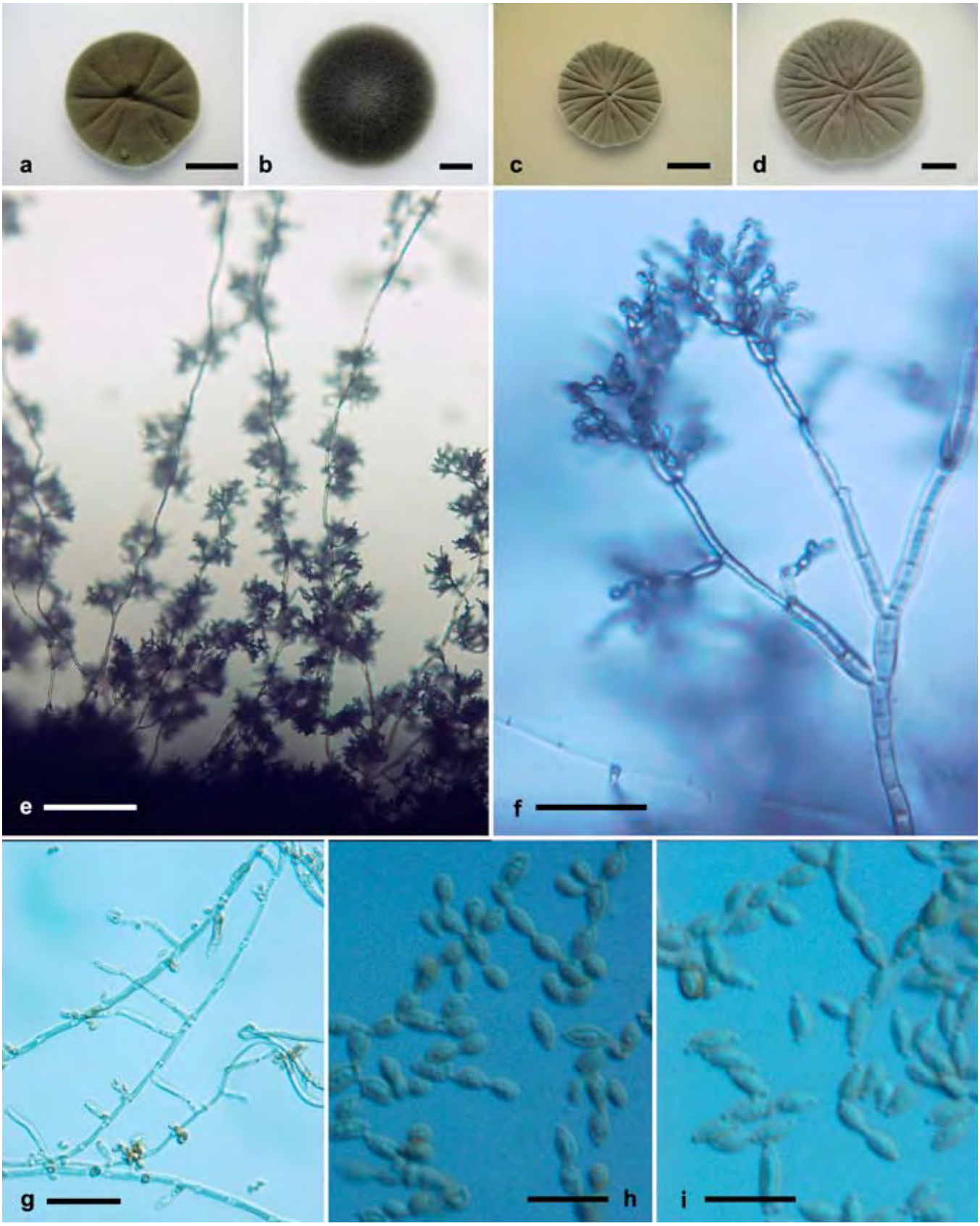

Fig. 7. *Cladosporium fusiforme*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–G. Habit of conidiophores. H–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–H, from EXF-449 (ex-type strain); I, from CBS 452.71. Scale bars A–D = 10 mm, E = 100 µm, F–G = 30 µm, H–I = 10 µm.

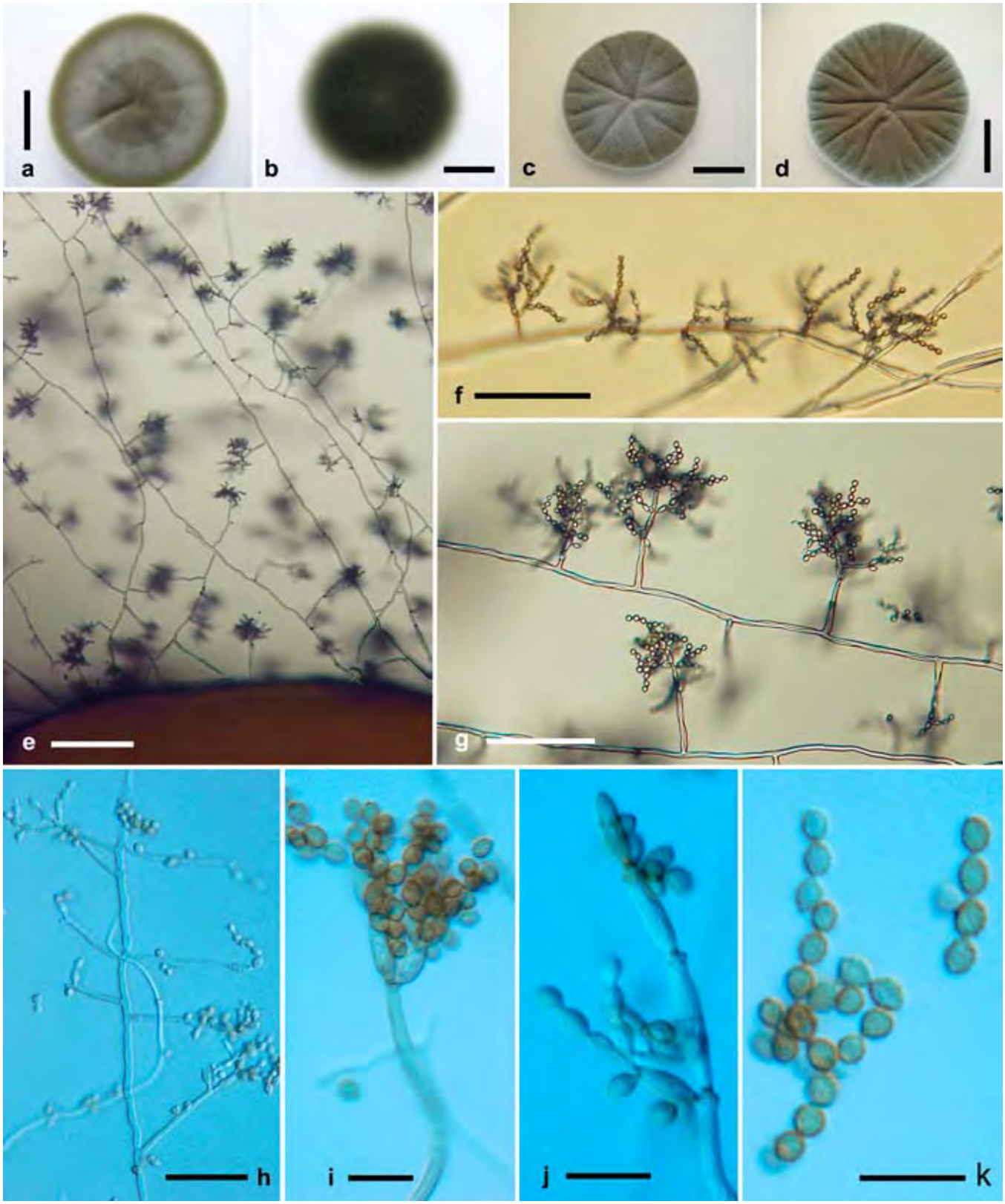

Fig. 8. *Cladosporium halotolerans.* Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–H. Habit of conidiophores. I. Conidiophore. J. Succession of secondary ramoconidia. K. Conidia. E–K. All from 7-d-old SNA slide cultures. A–B, from EXF-572 (ex-type strain); C–D, from EXF-977; E, G, from EXF-972; F, from EXF-564; H, I, K, from EXF-1072; J, from dH 12862. Scale bars A–D = 10 mm, E = 100 µm, F–G = 50 µm, H = 30 µm, I–K = 10 µm.

Cladosporium halotolerans Zalar, de Hoog & Gunde-Cimerman **sp. nov.** MycoBank MB492439. Fig. 8.

Etymology: Refers to its halotolerant habit.

Conidiophora erecta, lateralia vel terminalia ex hyphis rectis oriunda; stipes longitudine variabili, (5–)10–50(–300) × (2–)2.5–3(–5.5) µm, pallide olivaceo-brunneus, levis vel leniter verruculosus, tenuitunicatus, 0–3-septatus, interdum pluriseptatus, simplex, denticulatus. Conidiorum catenae undique divergentes, terminales ad 9 conidia continentes. Cellulae conidiogenae indistinctae. Conidia verrucosa, brunnea vel fusca, unicellularia, plerumque subglobosa vel globosa, raro breviter ovoidea, utrinque angustata, (2–)3–4(–6) × (2–)2.5–3(–5) µm, long. : lat. 1.2–1.5; ramoconidia secundaria cylindrica vel quasi globosa, 0(–1)-septata, (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm , ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, protuberantes, 0.7–1.0(–1.5) µm diam. Hyphae vagina polysaccharidica carentes.

Mycelium partly submerged, partly superficial; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect, arising laterally and terminally from straight hyphae, stipes of variable length, (5–)10–50(–300) × (2–)2.5–3(–5.5) µm, pale olivaceous-brown, smooth to minutely verruculose, thin-walled, 0–3-septate, unbranched, with pronounced denticles. *Conidial chains* branching in all directions, terminal chains with up to 9 conidia. *Conidiogenous cells* undifferentiated. *Ramoconidia* rarely formed. *Conidia* verrucose, brown to dark brown, non-septate, usually subglobose to globose, less often short-ovoid, narrower at both ends, length : width ratio = 1.2–1.5; (2–)3–4(–6) × (2–)2.5–3(–5) µm [av. (± SD) 3.5 (± 0.7) × 2.7 (± 0.5)]; *secondary ramoconidia* cylindrical to almost spherical, 0–1-septate, (5–)7–12(–37.5) × (2–)2.5–3(–6.5) µm [av. (± SD) 10.3 (± 4.8) × 2.9 (± 0.6)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.0(–1.5) µm diam.

Cultural characteristics: Colonies on PDA reaching 27–43 mm diam, olive (2F5), slightly furrowed, often covered with grey secondary mycelium, except at the marginal area where only sporulating structures can be observed. Margin white and regular, with submerged hyphae. Reverse pale green to black. Colonies on OA reaching 29–40 mm diam, olive (2F6), flat, uniform, granular due to profuse sporulation and fasciculate bundles of conidiophores, without sterile mycelium. Reverse dark green to black. Colonies on MEA reaching 18–44 mm diam, highly variable in colour, but mainly olive (2E5), and from flat with regular margin to deeply furrowed with undulate margin. Colony centre wrinkled with crater-shaped appearance. Reverse pale to dark green. Colonies on MEA + 5 % NaCl reaching 24–48 mm diam, olive (3E8), furrowed, velvety, with more pale, undulate margins. Reverse dark green to black.

Maximum tolerated salt concentration: Only 15 % of tested strains develop colonies at 20 % NaCl after 7 d, whereas after 14 d all cultures grow and sporulate.

Cardinal temperatures: No growth at 4 °C, optimum 25 °C (18–44 mm diam), maximum 30 °C (6–23 mm diam). No growth at 37 °C.

Specimen examined: **Namibia**, from hypersaline water of salterns, coll. Nina Gunde-Cimerman, 1 Sep. 2000, isol. P. Zalar, 1 Oct. 2000, CBS H-19734, **holotype**, culture ex-type EXF-572 = CBS 119416.

Habitats and distribution: Hypersaline water in subtropical climates; indoor environments; Arctic ice; contaminant in lesions of humans and animals; plant phyllosphere; rock.

Literature: Haubold *et al.* (1998), Meklin *et al.* (2004).

Differential parameters: Verrucose conidia, short unbranched and non-septate conidiophores which arise laterally alongside erect hyphae.

Strains examined: CBS 191.54, CBS 573.78, CBS 626.82, dH 12862, dH 12991, dH 13911, EXF-228, EXF-380, EXF-565, EXF-567, EXF-571, EXF-572 (= CBS 119416; ex-type strain), EXF-646, EXF-698, EXF-703, EXF-944, EXF-972, EXF-977, EXF-1072, EXF-2372.

Notes: *Cladosporium halotolerans* strongly resembles *C. sphaerospermum*. Several strains of this species such as dH 12862, dH 12941, CBS 191.54 and UAMH 7686 have been isolated sporadically from various indoor habitats in Europe, Brazil and the U.S.A. and repeatedly from bathrooms in Slovenia (Table 1). Probably sometimes as uncertain culture contaminations, it has been isolated from plants (GenBank accession no. L25433), inner organs of a diseased frog (AY361982) and human brain (Kantarcioglu *et al*. 2002). The presence of *C. halotolerans* species in gypsum sediments entrapped in Arctic ice, the fact that it was repeatedly isolated from hypersaline water and possibly its presence in dolphin skin (see Discussion) suggest that it has a clear preference for (hyper)osmotic habitats. This is supported by its ability to grow at 20 % NaCl.

The teleomorph of *C. halotolerans* is predicted to be a *Davidiella* species. Strain CBS 280.49 was isolated by J.A. von Arx from teleomorphic material of a fungus labelled as *Mycosphaerella hyperici* (Auersw.) Starbäck on *Hypericum perforatum* in Switzerland. According to Aptroot (2006) this species may belong in *Davidiella* and produces a *Septoria* anamorph. In the original herbarium specimen, CBS H-4867, a *Mycosphaerella* teleomorph was present, but no sign of a *Cladosporium* anamorph. We assume that CBS 280.49 was a culture contaminant.

Cladosporium langeronii (Fonseca, Leão & Nogueira) Vuill., Champ. Paras.: 78. 1931. Fig. 9.

Basionym: *Hormodendrum langeronii* Fonseca, Leão & Nogueira, Sci. Med. 5: 563. 1927.

≡ *Cladosporium langeronii* (Fonseca, Leão & Nogueira) Cif., Manuale di Micologia Medica, ed. 2: 488 (1960), comb. superfl.

Mycelium partly submerged, partly superficial; hyphae sometimes enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, micronematous and macronematous, stipes of variable length, (20–)50–130(–200) × (3–)3.5–4.5(–6.5) µm, dark brown, rough- and thick-walled, regularly septate (cell length 9–22 µm), arising laterally and terminally from submerged or aerial hyphae, branched. *Conidial chains* dichotomously branched, up to 6 conidia in the unbranched parts. *Conidiogenous cells* undifferentiated, sometimes seceding and forming ramoconidia. *Ramoconidia* cylindrical, 0–1 septate, (10–)11–22(–42) × (3–)3.5–4.5(–5) µm, base broadly truncate, 2–3.5 µm wide, slightly thickened and somewhat darkened. *Conidia* irregularly verruculose to sometimes loosely verrucose, dark brown, non-septate, usually ovoid, length : width ratio = 1.3–1.5; conidial size (3–)4–5.5(–8) × (2–)3–4(–5) µm [av. (± SD) 4.8 (± 1.0) × 3.5 (± 0.6)]; *secondary ramoconidia* cylindrical to almost spherical, mostly 0–1(–2)-septate, (5.5–)7.5–12.5(–35.5) × (2.5–)3–4.5(–5.5) µm [av. (± SD) 10.7 (± 4.7) × 3.6 (± 0.8)], with 2, rarely 3 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.9–1.5(–2.3) µm diam.

Cultural characteristics: Colonies on PDA, OA and MEA with restricted growth, attaining 2.5–4.5, 1.5–7.0 and 1.0–5.5 mm diam, respectively. Colonies flat or heaped (up to 3 mm), dark green (30F4), with black reverse and slightly undulate margin with immersed mycelium. Sporulating on all media. On MEA + 5 % NaCl growth is faster, colonies attaining 8.5–12.0 mm diam, sporulating and growing deeply into the agar.

Maximum tolerated salt concentration: All strains develop colonies at 17 % NaCl after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum / maximum 25 °C (1.0–5.5 mm diam), no growth at 30 °C.

Specimen examined: **Brazil**, from man ulcero-nodular mycosis of hand and arm, 1927, coll. and isol. da Fonseca, CBS H-19737, **holotype**, culture ex-type CBS 189.54.

Habitats and distribution: Polar ice and biomats; conifer wood and window frame in Europe; humans; strains originating from nasal mucus (Buzina *et al*. 2003) have 100 % sequence homology with

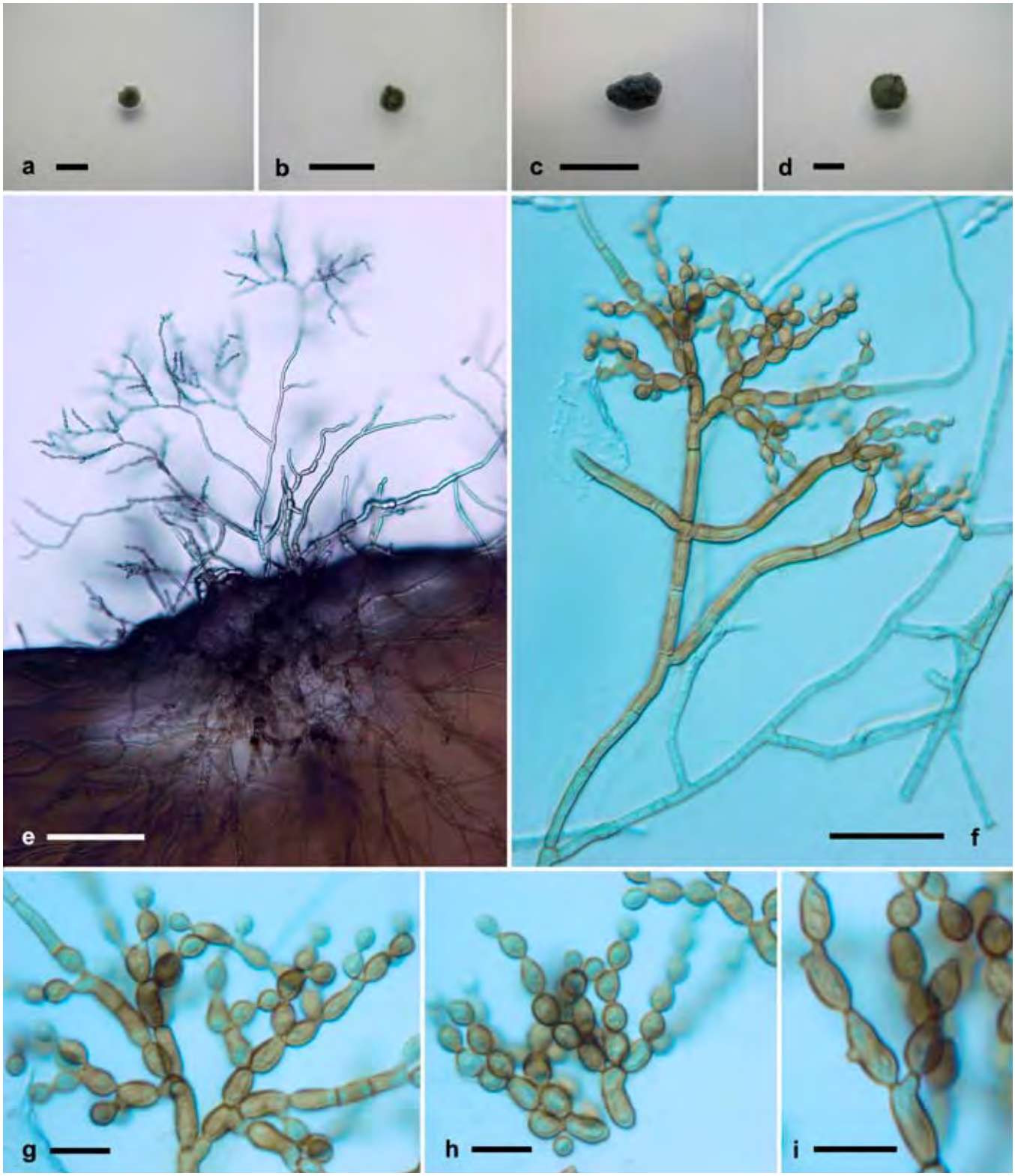

Fig. 9. *Cladosporium langeronii*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–D, from CBS 189.54 (ex-type strain); E, from CBS 109868; F–I, from EXF-999. Scale bars A, C–D = 10 mm, B = 5 mm, E = 100 µm, F = 30 µm, G–I = 10 µm.

the strains studied, as well as with a clone from mycorrhizal roots (Menkis *et al*. 2005). The species is distributed worldwide, without any apparent predilection for a particular habitat. The strains from clinical cases probably were culture contaminants.

Literature: da Fonseca *et al.* (1927a, b).

Differential parameters: Restricted growth; lowest salt halotolerance taxon of all *C. sphaerospermum*-like species.

Strains examined: CBS 189.54 (ex-type strain), CBS 601.84, CBS 101880, CBS 109868, dH 11736, dH 12459 = EXF-999, dH 13833 = EXF-1933.

Notes: De Vries (1952) synonymised the isolate identified as *Hormodendrum langeronii* with *C. sphaerospermum*. Strains of this species have often been identified as *C. cladosporioides* (Buzina *et al.* 2003, Menkis *et al.* 2005) although it has slightly longer conidia.

Cladosporium psychrotolerans Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492428. Fig. 10.

Etymology: Refers to its ability to grow at low temperatures.

Mycelium partim submersum; hyphae vagina polysaccharidica carentes. Conidiophora erecta vel adscendentia; stipes (10–)50–100(–150) × (3–)3.5–4(–7.5) µm, olivaceo-brunneus, levis, crassitunicatus, compluries regulariter septatus (cellulis 10–40 µm longis), identidem dichotome ramosus. Conidiorum catenae undique divergentes, terminales partes simplices ad 4 conidia continentes. Cellulae conidiogenae indistinctae. Ramoconidia primaria cylindrica, (18–)19–22(–43) × (2.5)3–3.5(–4.5) µm, 0(–1)-septata. Conidia leves vel leniter verruculosa, dilute brunnea, unicellularia, globosa vel ovoidea, (2.5–)3–4(–4.5) × (2–)2.5–3(–3) µm, long.: lat. 1.3–1.4; ramoconidia secundaria cylindrica, 0–1(–2)-septata, (5–)8–16(–36) × (2–)2.5–3(–5) µm, ad 4 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, 0.5–2 µm diam.

Mycelium partly superficial partly submerged; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect or ascending, macronematous, stipes (10–)50–100(–150) × (3–)3.5–4(–7.5) µm, olivaceous-brown, smooth or almost so, thick-walled, regularly septate (cell length 10–40 µm), arising laterally from aerial hyphae, repeatedly dichotomously branched. *Conidial chains* branching in all directions, up to 4 conidia in the unbranched parts. *Ramoconidia* sometimes formed, cylindrical, (18–)19–22(–43) × (2.5)3–3.5(–4.5) µm, aseptate, rarely 1-septate, with a broadly truncate base, up to 2 µm wide, unthickened or slightly thickened, somewhat darkened-refractive. *Conidia* smooth to minutely verruculose, light brown, non-septate, spherical to ovoid, length : width ratio = 1.3–1.4; conidial size (2.5–)3–4(–4.5) × (2–)2.5–3(–3) µm [av. (± SD) 3.4 (± 0.5) × 2.5 (± 0.2)]; *secondary ramoconidia* cylindrical, 0–1(–2)-septate, (5–)8–16(–36) × (2–)2.5–3(–5) µm [av. (± SD) 12.7 (± 6.5) × 3.0 (± 0.5)], with up to 4 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–2 µm diam.

Cultural characteristics: Colonies on PDA reaching 13–18 mm diam, velvety, olive (3F4) due to profuse sporulation, flat with straight margin. Reverse dark green. Colonies on OA reaching 13–15 mm diam, olive (2F8), of granular appearance due to profuse sporulation; aerial mycelium sparse. Margin regular. Reverse black. Colonies on MEA reaching 8–15 mm diam, olive (2F4), velvety, radially furrowed with undulate white margin. Colonies on MEA with 5 % NaCl growing faster than on other media, reaching 25–27 mm diam, olive (3E6) and granular due to profuse sporulation, either slightly furrowed or heavily wrinkled with regular or undulate margin. Reverse dark green.

Maximum tolerated salt concentration: 17 % NaCl after 14 d.

Cardinal temperatures: Minimum at 4 °C (5 mm diam), optimum and maximum at 25 °C (8–15 mm diam).

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, May 1999, CBS H-19730, **holotype**, culture ex-type EXF-391 = CBS 119412.

Habitats and distribution: Hypersaline water in the Mediterranean basin.

Differential parameters: Growth at 4 °C; maximal NaCl concentration 17 % NaCl, which differentiates it from other species with similar conidia, like *C. sphaerospermum*, *C. halotolerans* and *C. dominicanum*.

Strains examined: EXF-326, EXF-332, EXF-391 (= CBS 119412; ex-type strain), EXF-714.

Cladosporium salinae Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492438. Fig. 11.

Etymology: Refers to salterns (= Latin *salinae*) as the habitat of this species.

Mycelium partim submersum; hyphae multa rostra lateralia ferentes, hyphae vagina polysaccharidica involutae. Conidiophora vix distincta, lateralia vel terminalia ex hyphis aeriis oriunda; stipes longitudine variabili, (5–)25–50(–60) × (2–)2.5–3(–4) µm, olivaceo-brunneus, levis vel leniter verruculosus, crassitunicatus, irregulariter dense septatus (cellulis 6–29 µm longis), simplex, interdum ramosus. Conidiorum catenae undique divergentes, terminales ad 6 conidia continentes. Cellulae conidiogenae nonnumquam integratae, in summo sequentiam sympodialem denticulorum formantes. Conidia levia, interdum leniter verruculosa, dilute brunnea, unicellularia, plerumque fusiformia, (4.5–)5.5–7.5(–10) × (2–)2.5–3(–3.5) µm, long. : lat. 1.9–2.4; ramoconidia secundaria cylindrica, 0–1(–2)-septata, (7.5–)9.5–13.5(–19) × (2.5–)2.5–3.5(–4.5) µm, ad 5 cicatrices terminales ferentia; cicatrices inspissatae, conspicuae, protuberantes, 0.7–1.8 µm diam.

Mycelium partly superficial partly submerged, with numerous lateral pegs, consistently enveloped in polysaccharide-like material. *Conidiophores* poorly differentiated, micronematous, stipes (5–)25–50(–60) × (2–)2.5–3(–4) µm, olivaceous-brown, smooth to often minutely verruculose or irregularly rough-walled, thick-walled, irregularly densely septate (length of cells 6–29 µm), arising laterally and terminally from aerial hyphae, unbranched, occasionally branched. *Conidial chains* branching in all directions, terminal chains with up to 6 conidia. *Conidiogenous cells* sometimes integrated, producing sympodial clusters of pronounced denticles at their distal ends. *Conidia* usually smooth, occasionally minutely verruculose, light brown, aseptate, usually oblong ellipsoidal to fusiform, length : width ratio = 1.9–2.4; (4.5–)5.5–7.5(–10) × (2–)2.5–3(–3.5) µm [av. (± SD) 6.7 (± 1.3) × 2.9 (± 0.4)]; *secondary ramoconidia* cylindrical, 0–1(–2)-septate, (7.5–)9.5–13.5(–19) × (2.5–)2.5–3.5(–4.5) µm [av. (± SD) 12.1 (± 3.3) × 3.2 (± 0.6)], with up to 5 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.7–1.8 µm diam.

Cultural characteristics: Colonies on PDA reaching 10–27 mm diam, granular, olive (2E4) due to profuse sporulation, with white undulate margin. Aerial mycelium absent. Colonies either heaped or radially furrowed, in the marginal area growing deeply into the agar. Reverse dark brown to dark green. Colonies on OA reaching 7–20 mm diam, olive (3E6), of granular appearance due to profuse sporulation, aerial mycelium present. Margin either undulate or arachnoid, deeply furrowed. Reverse pale brown to dark green. Colonies on MEA reaching 8–19 mm diam, velvety, reseda-green (2E6), heaped. Margin furrowed, growing deeply into the agar. Colonies on MEA with 5 % NaCl growing much faster than on other media, reaching 25–38 mm diam, of different colours, mostly reseda-green (2E6) and granulate due to profuse sporulation, margin olive-yellow (2D6). Reverse yellow to dark green.

Maximum tolerated salt concentration: MEA + 17 % NaCl after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum and maximum temperature at 25 °C (8–19 mm diam), no growth at 30 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, Feb. 1999, CBS H-19731, **holotype**, culture ex-type EXF-335 = CBS 119413.

Habitats and distribution: Hypersaline water in the Mediterranean basin.

Differential parameters: Sympodial conidiogenous cells with pronounced denticles, narrow temperature amplitude.

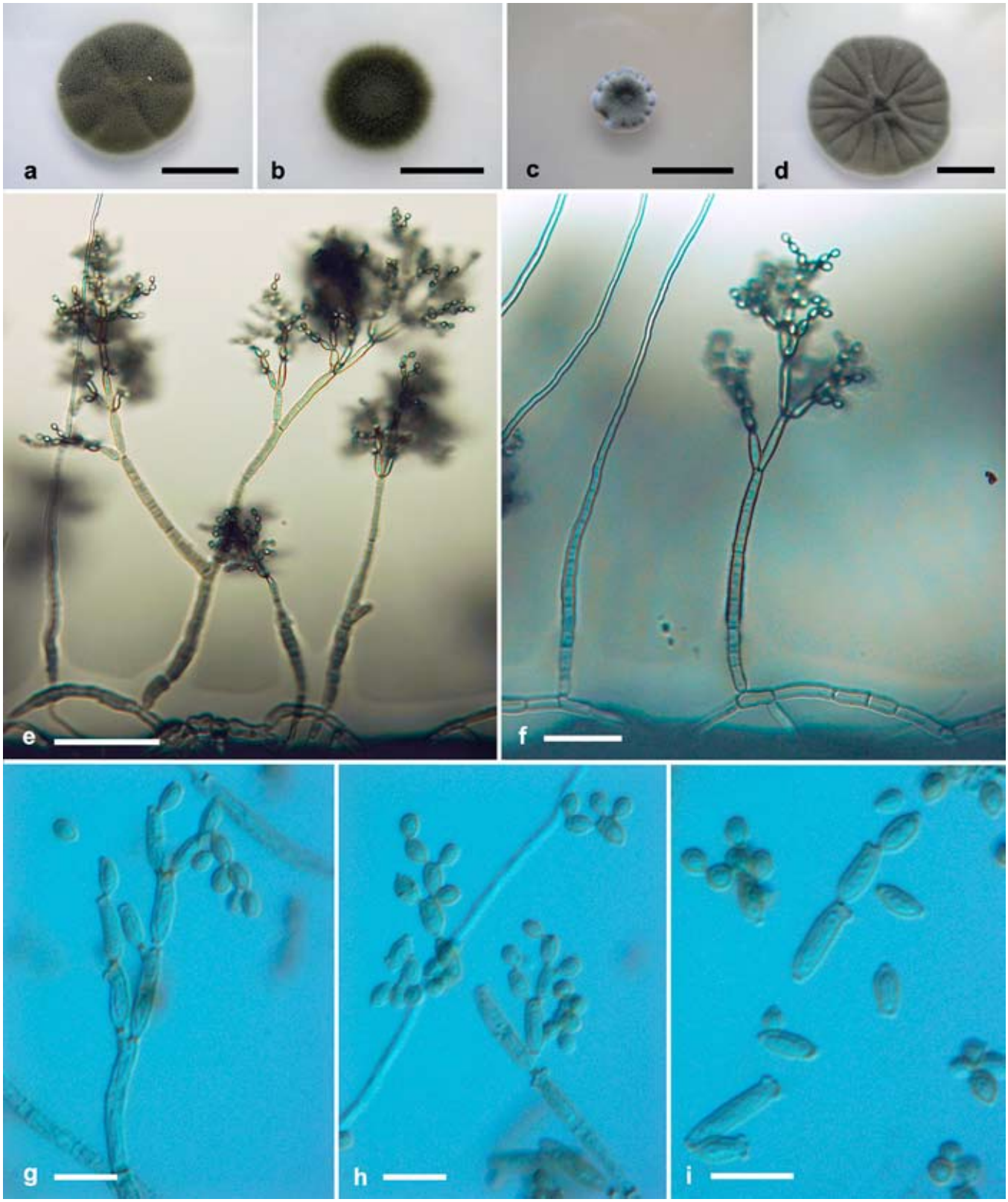

Fig. 10. *Cladosporium psychrotolerans*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Conidiophores. G. Apical part of a conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. All but C, from EXF-391 (ex-type strain); C, from EXF-714. Scale bars A–D = 10 mm, E = 100 µm, F = 50 µm, G–I = 10 µm.

Strains examined: EXF-322, EXF-335 (= CBS 119413; ex-type strain), EXF-604.

Notes: *Cladosporium salinae* morphologically resembles species of the genus *Fusicladium* because its conidia are oblong ellipsoidal to fusiform and conidiogenous loci of ramoconidia are placed closely together. As any other *Cladosporium* species, its conidia show typical cladosporioid scar structures, however. *Cladosporium salinae* seems to have a separate position within the genus *Cladosporium* since it seems to be distantly related to any other described *Cladosporium* species or currently known species complex within *Cladosporium*.

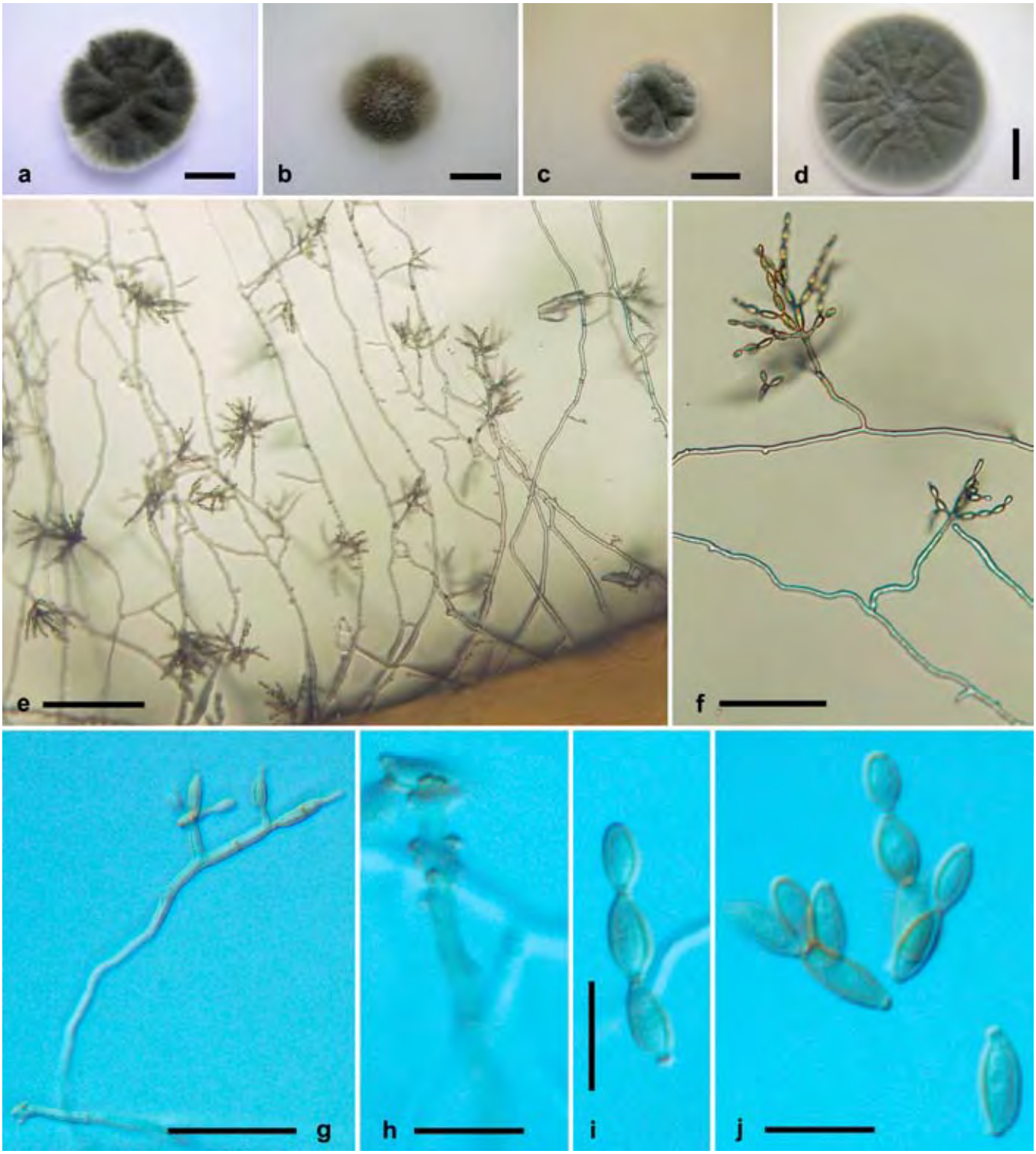

Fig. 11. *Cladosporium salinae*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H. Detail of apical part of conidiophore. I. Conidia. J. Secondary ramoconidia and conidia. E–J. All from 7-d-old SNA slide cultures. A–D, from EXF-604; E–J, from EXF-335 (ex-type strain). Scale bars A–C = 5 mm, D = 10 mm, E = 100 µm, F = 50 µm, G = 30 µm, H–J = 10 µm.

Cladosporium sphaerospermum Penzig, Michelia 2(8): 473. 1882. Fig. 12.

Mycelium partly submerged, partly superficial; hyphae thick, darkly pigmented and densely septate in submerged mycelium, not enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, micronematous and macronematous, stipes of variable length, (10–)45–130(–300) × (2.5–)3–4(–6) µm, olivaceous-brown, smooth to minutely verruculose, thick-walled, with relatively dense septation (cells mostly 4.5–23 long), septa darkened and somewhat thickened, arising laterally and terminally from immersed or aerial hyphae, either unbranched or branched. *Conidial chains* branching in all directions, up to 6 conidia in the unbranched parts. *Conidiogenous cells* not differentiated. *Ramoconidia* often formed, cylindrical, (11.5–)20.5–40(–48) × (2.5–)3(–3.5) µm, with up to 5 septa, base broadly truncate, 2 µm wide, slightly thickened and somewhat darkened-refractive. *Conidia* verruculose, brown to dark brown, non-septate, usually subspherical to spherical, less often short-ovoid, narrower at both ends, with length : width ratio = 1.1–1.5; conidial size (2.5–)3–4(–7) × (2–)3–3.5(–4.5) µm [av. (±

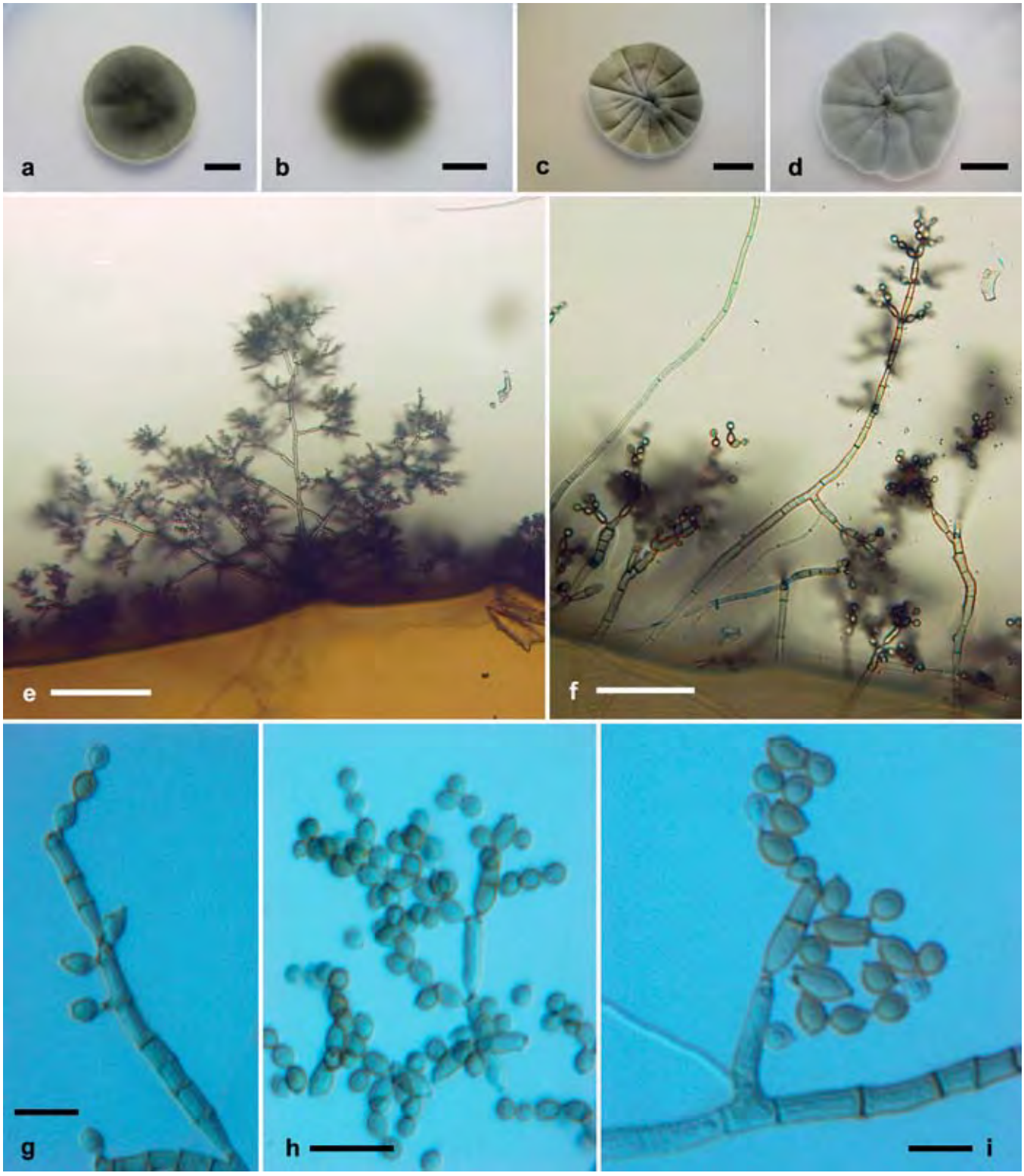

Fig. 12. *Cladosporium sphaerospermum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G–I. Ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A, C–D, F–H, from CBS 193.54 (ex-neotype strain); B, from EXF-738; E, EXF-455; I, EXF-458. Scale bars A–D = 10 mm; E = 100 µm; F = 50 µm; G–I = 10 µm.

SD) 3.8 (± 0.8) × 3.1 (± 0.4)]; *secondary ramoconidia* cylindrical to almost spherical, 0–3(–4) septate, (4–)8.5–16(–37.5) × (2–)3–3.5(–5) µm [av. (± SD) 13.1 (± 6.3) × 3.2 (± 0.5)], with up to 4, rarely up to 6 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.9–1.1(–1.4) µm diam.

Cultural characteristics: Colonies on PDA reaching 21–44 mm diam, velvety, olive (2F5) due to profuse sporulation, either with white and regular, or exceptionally undulate margin. Aerial mycelium sparse. Colonies flat or rarely radially furrowed with elevated colony centre. Exudates not prominent, some strains release green soluble pigments into the agar. Reverse blackish blue to pale green. Growth deep into the agar. Colonies on OA reaching 21–38 mm diam, olive (2F8), of granular appearance due to profuse and uniform sporulation, almost no aerial mycelium. Margin either regular or arachnoid, deeply radially furrowed. Reverse black. Colonies on MEA reaching 15–35 mm diam, velvety, linden-green (2C5), radially furrowed. Colony centre wrinkled, forming a crater-like structure; margin furrowed, lighter in colour, consisting of

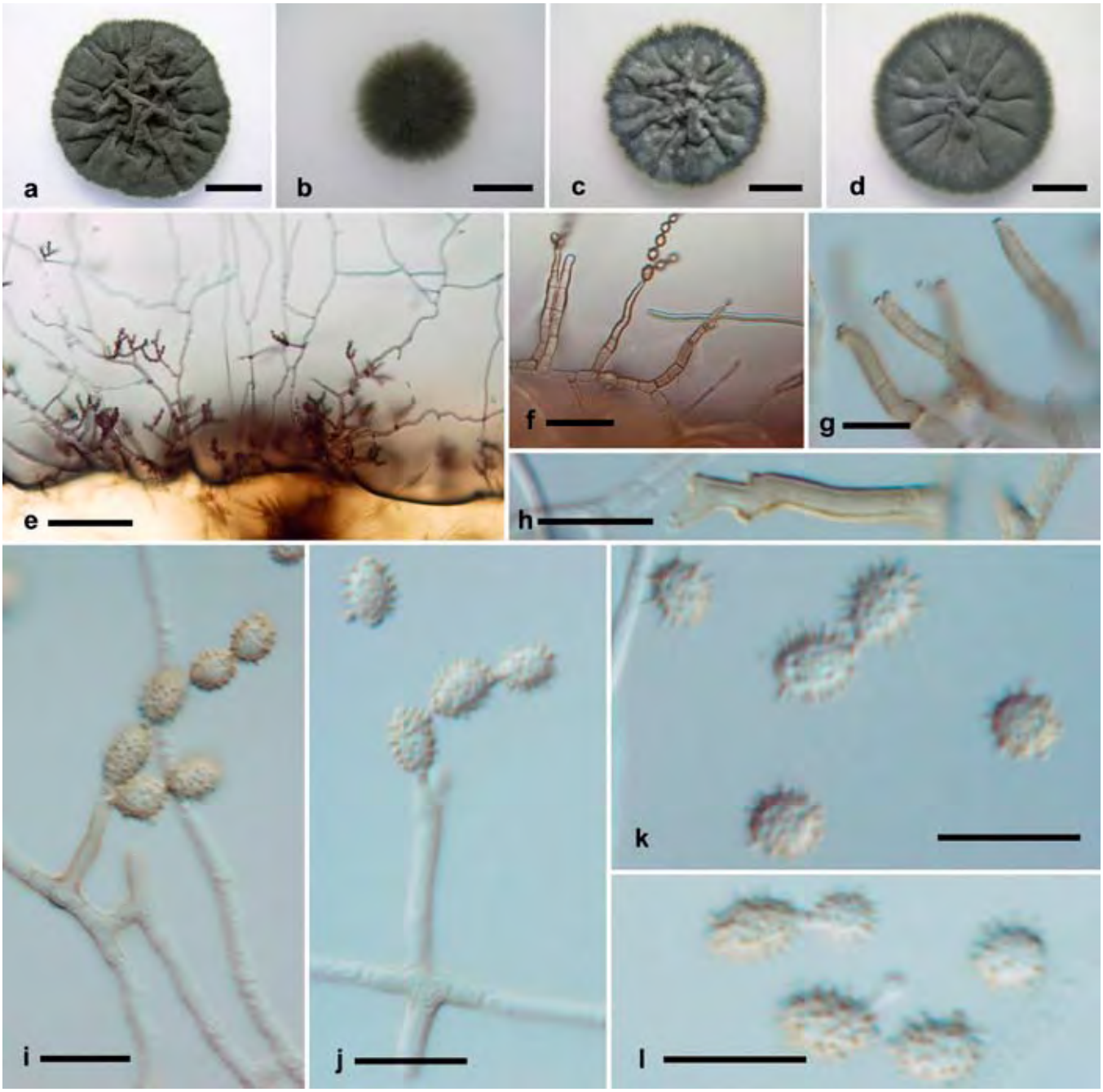

Fig. 13. *Cladosporium spinulosum*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E. Habit of conidiophores. F–J. Conidiophores. K–L. Conidia (also visible in I–J). E–L. All from 7-d-old SNA slide cultures. A–L, from EXF-334 (ex-type strain). Scale bars A–D = 10 mm, E = 100 µm, F = 30 µm, G–L = 10 µm.

submerged mycelium. Reverse pale to dark brown. Colonies on MEA with 5 % NaCl growing faster than on other media, reaching 31–60 mm diam, mainly olive (2D4), either being almost flat or radially furrowed, with margin of superficial mycelium; sporulation dense. Reverse ochraceous or dark green.

Maximum tolerated salt concentration: On MEA + 20 % NaCl 89 % of all strains tested develops colonies after 7 d, 96 % after 14 d.

Cardinal temperatures: No growth at 4 °C, optimum 25 °C (15–35 mm diam), maximum 30 °C (2–15 mm diam). No growth at 37 °C.

Specimen examined: **Netherlands**, from nail of man, 1949, coll. and isol. R.W. Zappey, CBS H-19738, **neotype designated here,** incorrectly selected by de Vries (1952) as "lectotype", culture ex-neotype CBS 193.54 = ATCC 11289 = IMI 049637.

Habitats and distribution: Hypersaline water in mediterranean and tropics; soil and plants in temperate climates; indoor wet cells; humans. The species does not seem to have any particular preference. Human isolates were probably culture contaminants.

Literature: Penzig (1882), de Vries (1952), Ellis (1971), de Hoog *et al.* (2000), Samson *et al.* (2002).

Diagnostic parameters: Thick-walled, melanised, densely septate mycelium, almost spherical, verruculose to verrucose terminal conidia, growth on 20 % NaCl after 7 d.

Strains examined: CBS 109.14, CBS 122.63, CBS 190.54, CBS 192.54, CBS 193.54 (ex-neotype strain), CBS 102045, CPC 10944, EXF-131, EXF-328, EXF-385, EXF-446, EXF-455, EXF-458, EXF-461, EXF-464, EXF-465, EXF-598, EXF-644, EXF-645, EXF-649, EXF-715, EXF-738, EXF-739, EXF-781, EXF-962, EXF-965, EXF-1061, EXF-1726, EXF-1732.

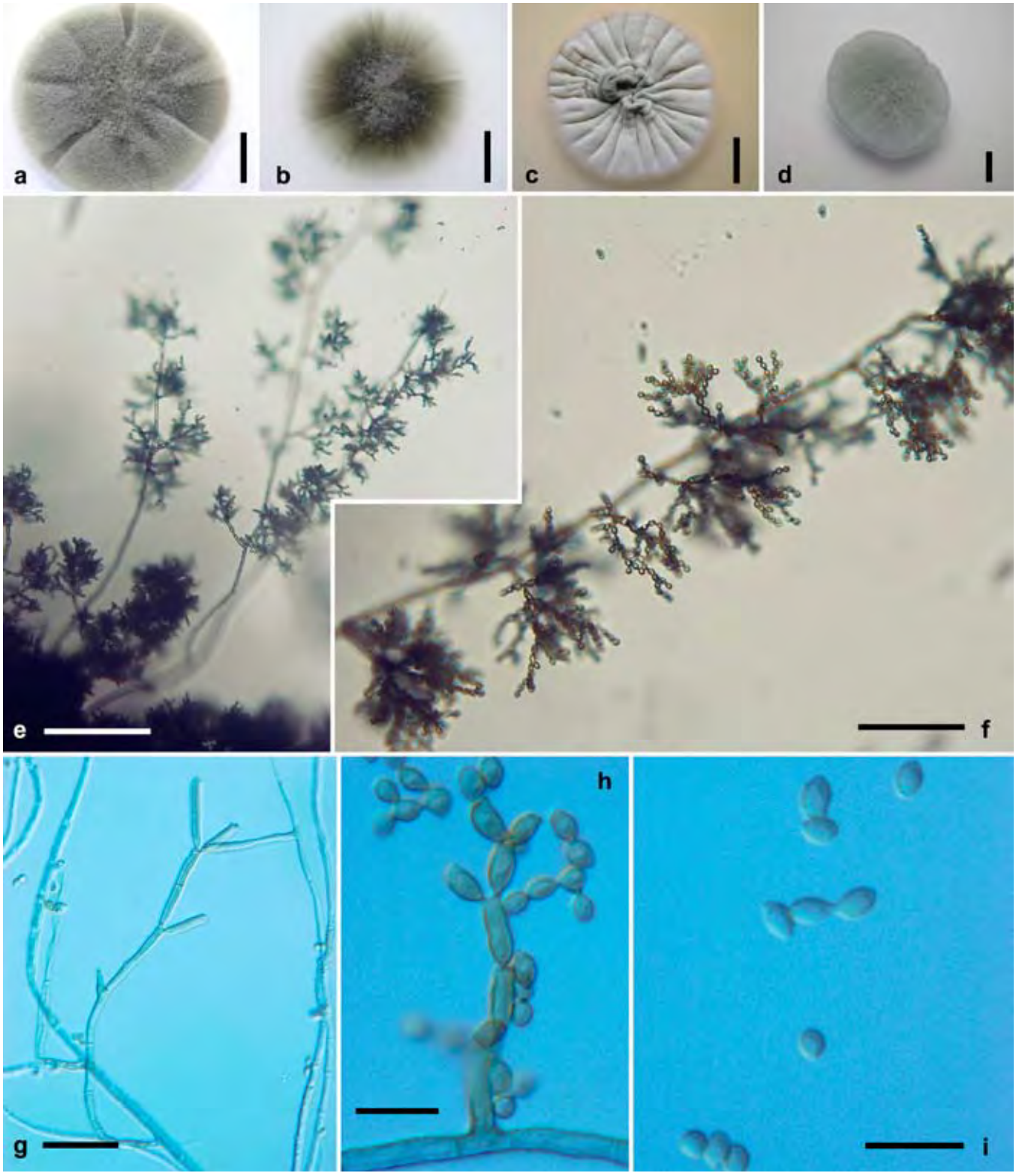

Fig. 14. *Cladosporium velox*. Macro- and micromorphological characters. A–D. Colony surface grown on PDA (A), OA (B), MEA (C) and MEA plus 5 % NaCl (D) of strains incubated for 14 d at 25 ºC in darkness. E–F. Habit of conidiophores. G. Conidiophore. H–I. Secondary ramoconidia and conidia. E–I. All from 7-d-old SNA slide cultures. A–D, G, from CBS 119417 (ex-type strain); E–F, H–I, from EXF-466. Scale bars A–D = 10 mm, E = 100 µm, F = 50 µm, G = 30 µm, H–I = 10 µm.

Cladosporium spinulosum Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB501099. Fig. 13.

Etymology: Refers to its conspicuously digitate conidia.

Conidiophora erecta vel adscendentia; stipites longitudine variabili, (15–)25–50(–155) × (2.5–)3–4(–5) µm, olivaceo-brunneus, levis, crassitunicatus, 0–6(–9)-septatus (cellulis 6–20 µm longis), ex hyphis submersis vel aeriis lateraliter vel terminaliter oriundus, simplex vel ramosus. Conidiorum catenae undique ramosae, ad 4 conidiis in partibus linearibus continuis cohaerentibus. Cellulae conidiogenae integratae vel discretae, acervos distales denticulorum conspicuorum sympodialium proferentes. Conidia echinulata vel digitata, brunnea vel fusca, continua, vulgo subglobosa vel globosa, (4.5–)5.5–7(–8) × (3–)4–4.5(–5) µm, long.: lat. = 1.1–1.6, digiti 0.6–1.3 µm longi; ramoconidia secundaria etiam digitata, cylindrica vel subglobosa, 0(–1)-septata, (6–)6.5–8(–18) × (4–)4.5–5(–5.5) µm, 1–3 cicatrices distales ferentia. Cicatrices inspissatae, conspicuae, protuberantes, 0.8–1.2 µm diam. Hyphae nonnumquam polysaccharido circumdatae.

Hyphae sometimes enveloped in polysaccharide-like material. *Conidiophores* erect or ascending, stipes of variable length, (15–)

25–50(–155) × (2.5–)3–4(–5) µm, olivaceous-brown, smooth, sometimes irregularly rough-walled to verrucose near the base, thick-walled, 0–6(–9)-septate (cells mostly 6–20 µm long), arising laterally and terminally from immersed or aerial hyphae, either unbranched or branched, somewhat tapering towards the apex. *Conidial chains* branching in all directions, up to 4 conidia in the unbranched parts. *Conidiogenous cells* sometimes integrated, producing sympodial clusters of pronounced denticles at their distal ends. *Ramoconidia* rarely formed. *Conidial wall ornamentation* conspicuously digitate, with up to 1.3 µm long projections having parallel sides and blunt ends. *Conidia* brown to dark brown, aseptate, usually subspherical to spherical, length : width ratio = 1.1–1.6; conidial size (4.5–)5.5–7(–8) × (3–)4–4.5(–5) µm [av. (± SD) 6.2 (± 1.0) × 4.2 (± 0.5)]; *secondary ramoconidia* ornamented as conidia, cylindrical to almost spherical, 0(–1)-septate, (6–)6.5–8(–18) × (4–)4.5–5(–5.5) µm [av. (± SD) 8.6 (± 4.0) × 4.8 (± 0.4)], with up to 3 distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.8–1.2 µm diam.

Cultural characteristics: Colonies on PDA reaching 20–30 mm diam, velvety, dull green (29E4) to dark green (29F6) due to profuse sporulation, either with white and regular, or undulate margin. Aerial mycelium sparse. Colonies flat or radially furrowed with elevated colony centre. Growth deep into the agar. Exudates not prominent. Colonies on OA reaching 20–25 mm diam, dull green (29E4) to dark green (29F6), sometimes olive (3D4), of granular appearance due to profuse and uniform sporulation; almost without aerial mycelium. Margin arachnoid. Reverse pale brown to black. Colonies on MEA reaching 17–28 mm diam, velvety, dull green (29E4) to dark green (29F6), either flat or radially furrowed. Colony centre wrinkled, forming a crater-like structure; margin furrowed, paler in colour, consisting of submerged mycelium only. Reverse pale to dark green. Colonies on MEA with 5 % NaCl reaching 12–18 mm diam, of different colours, greenish grey (29D2), greyish green (29D5) to dark green (29F6); colony appearance variable, mostly either being almost flat with immersed colony centre or radially furrowed, with white to dark green margin consisting of superficial mycelium; sporulation dense. Reverse pale to dark green.

Maximum tolerated salt concentration: On MEA + 17 % NaCl, two of three strains tested developed colonies after 14 d.

Cardinal temperatures: Growth at 4 °C, optimum and maximum at 25 °C (17–28 mm). No growth at 30 °C.

Specimen examined: **Slovenia**, from hypersaline water of Sečovlje salterns, coll. and isol. S. Sonjak, Feb. 1999, CBS H-19796, **holotype**, culture ex-type EXF-334 = CBS 119907.

Habitats and distribution: Hypersaline water in temperate climate.

Diagnostic parameters: Conidia and ramoconidia with a digitate ornamentation.

Strains examined: EXF-334 (= CBS 119907; ex-type strain), EXF-382.

Notes: *Cladosporium spinulosum* is a member of the *C. herbarum* species complex (Figs 2–4) although its globoid conidia are reminiscent of *C. sphaerospermum*. Within *Cladosporium*, the species is unique in having conspicuously digitate conidia and ramoconidia. The two strains are differing in the size of conidia. The average size of conidia in EXF-334 is 6.2 (± 0.9) × 4.2 (± 0.5) µm, and in EXF-382 it is 3.9 (± 0.6) × 3.3 (± 0.4) µm.

Cladosporium velox Zalar, de Hoog & Gunde-Cimerman, **sp. nov.** MycoBank MB492435. Fig. 14.

Etymology: Refers to the quick growth of strains of this species.

Mycelium partim submersum; hyphae vagina polysaccharidica carentes. Conidiophora erecta, lateralia vel terminalia ex hyphis aeriis oriunda; stipes (10–)25–150(–250) × (2.5–)3–4(–4.5) µm, olivaceo-brunneus, levis, crassitunicatus, ad 7–septatus (cellulis 10–60 µm longis), identidem dichotome ramosus. Conidiorum catenae undique divergentes, terminales partes simplices ad 5 conidia continentes. Cellulae conidiogenae indistinctae. Conidia levia vel leniter verruculosa, dilute brunnea, unicellularia, ovoidea, (2–)3–4(–5.5) × (1.5–)2–2.5(–3) µm, long. : lat. 1.4–1.7; ramoconidia secundaria cylindrica, 0–1-septata, (3.5–)5.5–19(–42) × (2–)2.5–3(–4.5) µm, ad 4(–5) cicatrices terminales ferentia; cicatrices inspissatae, protuberantes, conspicuae, 0.5–1.5 µm diam.

Mycelium partly superficial partly submerged; hyphae without extracellular polysaccharide-like material. *Conidiophores* erect, stipes (10–)25–150(–250) × (2.5–)3–4(–4.5) µm, slightly attenuated towards the apex, olivaceous-brown, smooth- and thick-walled, arising terminally and laterally from aerial hyphae, dichotomously branched [up to 5(–7)-septate, cell length 10–60 µm]. *Ramoconidia* rarely formed. *Conidial chains* branching in all directions, terminal chains with up to 5 conidia. *Conidia* smooth to very finely verruculose, pale brown, non-septate, ovoid, length : width ratio = 1.4–1.7; (2–)3–4(–5.5) × (1.5–)2–2.5(–3) µm [av. (± SD) 3.6 (± 0.6) × 2.3 (± 0.2)]; *secondary ramoconidia* cylindrical, 0–1-septate, (3.5–)5.5–19(–42) × (2–)2.5–3(–4.5) µm [av. (± SD) 13.4 (± 10.2) × 2.8 (± 0.5)], with up to 4(–5) distal scars. *Conidiogenous scars* thickened and conspicuous, protuberant, 0.5–1.5 µm diam.

Cultural characteristics: Colonies on PDA reaching 35–45 mm diam, velvety, dark green due to profuse sporulation, on some parts covered with white sterile mycelium, flat with straight white margin. Reverse dark green to black. Colonies on OA reaching 30–43 mm diam, dark green, mycelium submerged, aerial mycelium sparse. Margin regular. Reverse black. Colonies on MEA reaching 30–42 mm diam, pale green, radially furrowed, with raised, crater-shaped central part, with white, undulate, submerged margin. Sporulation poor. Colonies on MEA with 5 % NaCl reaching 35–45 mm diam, pale green, velvety, flat with regular margin. Reverse pale green. Sporulation poor.

Maximum tolerated salt concentration: 20 % NaCl after 14 d.

Cardinal temperatures: Minimum at 10 °C (9 mm diam), optimum at 25 °C (30–42 mm diam) and maximum at 30 °C (5–18 mm diam).

Specimen examined: **India**, Charidij, isolated from *Bambusa* sp., W. Gams, CBS H-19735, **holotype**, culture ex-type CBS 119417.

Habitats and distribution: Hypersaline water in Slovenia; bamboo, India.

Strains examined: CBS 119417 (ex-type strain), EXF-466, EXF-471.

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Opportunistic, human-pathogenic species in the *Herpotrichiellaceae* are phenotypically similar to saprobic or phytopathogenic species in the *Venturiaceae*

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Abstract: Although morphologically similar, species of *Cladophialophora* (*Herpotrichiellaceae*) were shown to be phylogenetically distinct from *Pseudocladosporium* (*Venturiaceae*), which was revealed to be synonymous with the older genus, *Fusicladium*. Other than being associated with human disorders, species of *Cladophialophora* were found to also be phytopathogenic, or to occur as saprobes on organic material, or in water, fruit juices, or sports drinks, along with species of *Exophiala*. *Caproventuria* and *Metacoleroa* were confirmed to be synonyms of *Venturia*, which has *Fusicladium* (= *Pseudocladosporium*) anamorphs. *Apiosporina*, based on *A. collinsii*, clustered basal to the *Venturia* clade, and appears to represent a further synonym. Several species with a pseudocladosporium-like morphology *in vitro* represent a sister clade to the *Venturia* clade, and are unrelated to *Polyscytalum*. These taxa are newly described in *Fusicladium*, which is morphologically close to *Anungitea*, a heterogeneous genus with unknown phylogenetic affinity. In contrast to the *Herpotrichiellaceae*, which were shown to produce numerous synanamorphs in culture, species of the *Venturiaceae* were morphologically and phylogenetically more uniform. Several new species and new combinations were introduced in *Cladophialophora, Cyphellophora* (*Herpotrichiellaceae*), *Exophiala*, *Fusicladium, Venturia* (*Venturiaceae*), and *Cylindrosympodium* (*incertae sedis*).

Taxonomic novelties: *Cladophialophora australiensis* Crous & A.D. Hocking, sp. nov., *Cladophialophora chaetospira* (Grove) Crous & Arzanlou, comb. nov., *Cladophialophora hostae* Crous, U. Braun & H.D. Shin, sp. nov., *Cladophialophora humicola* Crous & U. Braun, sp. nov., *Cladophialophora potulentorum* Crous & A.D. Hocking, sp. nov., *Cladophialophora scillae* (Deighton) Crous, U. Braun & K. Schub., comb. nov., *Cladophialophora sylvestris* Crous & de Hoog, sp. nov., *Cylindrosympodium lauri* Crous & R.F. Castañeda, sp. nov., *Cyphellophora hylomeconis* Crous, de Hoog & H.D. Shin, sp. nov., *Exophiala eucalyptorum* Crous, sp. nov., *Fusicladium africanum* Crous, sp. nov., *Fusicladium amoenum* (R.F. Castañeda & Dugan) Crous, K. Schub. & U. Braun, comb. nov., *Fusicladium brevicatenatum* (U. Braun & Feiler) Crous, U. Braun & K. Schub., comb. nov., *Fusicladium fagi* Crous & de Hoog, sp. nov., *Fusicladium intermedium* (Crous & W.B. Kendr.) Crous, comb. nov., *Fusicladium matsushimae* (U. Braun & C.F. Hill) Crous, U. Braun & K. Schub., comb. nov., *Fusicladium pini* Crous & de Hoog, sp. nov., *Fusicladium ramoconidii* Crous & de Hoog, sp. nov., *Fusicladium rhodense* Crous & M.J. Wingf., sp. nov., *Venturia hystrioides* (Dugan, R.G. Roberts & Hanlin) Crous & U. Braun, comb. nov.
Key words: *Anungitea*, *Anungitopsis*, *Cladophialophora*, *Exophiala*, *Fusicladium*, phylogeny, *Pseudocladosporium*, systematics, *Venturia*.

INTRODUCTION

Species of *Cladophialophora* Borelli are relatively simple hyphomycetes with brown hyphae that give rise to branched chains of pale brown conidia. Phylogenetically they are defined to belong to the *Chaetothyriales* (Haase *et al*. 1999, Untereiner 2000), an order containing numerous opportunists (de Hoog *et al*. 2000); teleomorph relationships are with *Capronia* Sacc. in the *Herpotrichiellaceae*. In several cases cladophialophora-like synanamorphs are found accompanying black yeasts of the genus *Exophiala* J.W. Carmich. (de Hoog *et al*. 1995). Braun & Feiler (1995) placed several saprobic hyphomycetes in *Cladophialophora*, and described *Capronia hanliniana* U. Braun & Feiler as teleomorph of *Cladophialophora brevicatenata* U. Braun & Feiler. This work was continued by Dugan *et al.* (1995), who described an additional teleomorph, *Capronia hystrioides* Dugan, R.G. Roberts & Hanlin for *Cladophialophora hachijoensis* (Matsush.) U. Braun & Feiler. Untereiner (1997) reduced *Capronia hystrioides* to synonymy with *C. hanliniana*, and placed them in *Venturia* Sacc. (*Venturiaceae, Pleosporales*). The concept of *Cladophialophora hachijoensis*, which is based on *Phaeoramularia hachijoensis* Matsush. (Matsushima 1975) is confused, however, and phylogenetic studies have revealed that isolates attributed to this name in recent studies, were in fact representatives of three different species in phylogenetically distinct genera (Braun *et al*. 2003). The separation of *Cladophialophora* with *Capronia* teleomorphs (*Herpotrichiellaceae, Chaetothyriales*; commonly isolated as human pathogens), from predominantly saprobic or phytopathogenic isolates in the *Dothideomycetes* was recognised by Braun (1998). Recently the cactus endophyte *Cladophialophora yegresii* de Hoog was reported to be the nearest neighbour of *C. carrionii* (Trejos) de Hoog *et al*., a major agent of human chromoblastomycosis (de Hoog *et al*. 2007), so that the main distinction between the two anamorph genera remains in their phylogenetic positions. *Capronia hanliniana* and *C. hystrioides* were again recognised as distinct species, and placed in a new genus, *Caproventuria* U. Braun (*Venturiaceae*), while their anamorphs were accommodated in *Pseudocladosporium* U. Braun. *Caproventuria* was primarily distinguished from *Venturia* based on its distinct *Pseudocladosporium* anamorphs. Recently, Crous *et al*. (2007b) introduced a third genus, namely *Sympoventuria* Crous & Seifert, which produces a sympodiella-like anamorph in culture. To complicate matters further, Beck *et al*. (2005) concluded, based on an ITS DNA phylogeny, that the morphology attributed to the form genera *Spilocaea* Fr., *Pollaccia* E. Bald. & Cif., and *Fusicladium* Bonord. has evolved several times within *Venturia*, and that a single anamorph genus should be used for *Venturia*, namely *Fusicladium* (see Schubert *et al*. 2003 for additional generic synonyms).

In their treatment of *Venturia* anamorphs, Schubert *et al*. (2003) excluded *Pseudocladosporium*, and stated that its status needs to be confirmed along with that of other genera such as *Anungitea* B. Sutton, *Fusicladium* and *Polyscytalum* Riess. In the study by Beck *et al*. (2005) an isolate of *Caproventuria hystrioides* (*Pseudocladosporium* sp.) was included to confirm the link to the *Venturiaceae*, though this was not well resolved, nor was the status of the older generic names mentioned above addressed.

The aim of the present study, therefore, was to use DNA sequence comparisons in conjunction with morphology in an attempt to clarify these generic issues, as well as to determine which morphological characters could be used to distinguish *Pseudocladosporium* from *Cladophialophora*.

MATERIALS AND METHODS

Isolates

Cultures were obtained from the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands, or isolated from plant material incubated in moist chambers to promote sporulation. Isolates were cultured on 2 % malt extract plates (MEA; Gams *et al*. 2007), by obtaining single conidial colonies as explained in Crous (2002). Colonies were subcultured onto fresh MEA, oatmeal agar (OA), potato-dextrose agar (PDA) and synthetic nutrient-poor agar (SNA) (Gams *et al*. 2007), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation.

DNA extraction, amplification and phylogeny

Fungal colonies were established on agar plates, and genomic DNA was isolated following the CTAB-based protocol described in Gams *et al*. (2007). The primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene (LSU). Four internal primers, namely ITS4 (White *et al*. 1990), LR0R (Rehner & Samuels 1994), LR3R (www.biology.duke.edu/fungi/mycolab/primers.htm), and LR16 (Moncalvo *et al*. 1993), were used for sequencing to ensure good quality overlapping sequences were obtained. The PCR conditions, sequence alignment and subsequent phylogenetic analysis followed the methods of Crous *et al*. (2006a). The ITS1, ITS2 and 5.8S rRNA gene were only sequenced for isolates of which these data were not available. The ITS data were not included in the analyses but deposited in GenBank where applicable. Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as missing data. Sequence data were deposited in GenBank (Table 1) and alignments in TreeBASE (www.treebase.org).

Taxonomy

Structures were mounted in lactic acid, and 30 measurements (× 1 000 magnification) determined wherever possible, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 2–4 wk on OA and PDA at 25 °C in the dark, using the colour charts of Rayner (1970). All cultures obtained in this study are maintained in the CBS collection (Table 1). Nomenclatural novelties and descriptions were deposited in MycoBank (www.MycoBank.org).

RESULTS

DNA phylogeny

Amplicons of approximately 1 700 bases were obtained for the isolates listed in Table 1. These sequences were used to obtain additional sequences from GenBank which were added to the alignment. The manually adjusted LSU alignment contained 116 sequences (including the two outgroup sequences) and 1 157 characters including alignment gaps (available in TreeBASE). Of the 830 characters used in the phylogenetic analysis, 326 were parsimony-informative, 79 were variable and parsimony-uninformative, and 425 were constant. Neighbour-joining analyses using three substitution models on the sequence data yielded trees with identical topologies to one another. The neighbour-joining trees support the same clades as obtained from the parsimony analysis, but with a different arrangement at the deep nodes, for example, the clade containing *Protoventuria alpina* (Sacc.) M.E. Barr (CBS 140.83) is placed as sister to the *Venturiaceae* using parsimony but basal to the *Herpotrichiellaceae* using neighbour-joining. Because of the large number of different strain associations in the *Venturia* clade (see the small number of strict consensus branches for this clade in Fig. 1), only the first 5 000 equally most parsimonious trees (TL = 1 752 steps; CI = 0.392; RI = 0.849; RC = 0.333) were saved, one of which is shown in Fig. 1.

Bayesian analysis was conducted on the same aligned LSU data set using a general time-reversible (GTR) substitution model with inverse gamma rates and dirichlet base frequencies. The Markov Chain Monte Carlo (MCMC) analysis of 4 chains started from a random tree topology and lasted 2 000 000 generations. Trees were saved each 1 000 generations, resulting in 2 000 saved trees. Burn-in was set at 500 000 generations after which the likelihood values were stationary, leaving 1 500 trees from which the consensus tree (Fig. 2) and posterior probabilities (PP's) were calculated. The average standard deviation of split frequencies was 0.06683 at the end of the run. The same overall topology as that observed using parsimony was obtained, with the exception of the position of *Anungitopsis speciosa* R.F. Castañeda & W.B. Kendr., which is placed between the *Leotiomycetes* and the *Sordariomycetes* based on the Bayesian analysis. Also, similar to the results obtained using neighbour-joining, the clade containing *Protoventuria alpina* (CBS 140.83) is placed as sister to the *Herpotrichiellaceae* and not to the *Venturiaceae*. The phylogenetic affinity of specific genera or species are discussed below.

Taxonomy

Several collections represented novel members of the *Herpotrichiellaceae* and *Venturiaceae*, and these are described below. Taxa that were cladophialophora- or pseudocladosporium-like, but that clustered elsewhere, are treated under excluded species.

Members of *Chaetothyriales, Herpotrichiellaceae*

Cladophialophora australiensis Crous & A.D. Hocking, **sp. nov.** MycoBank MB504525. Fig. 3.

Etymology: Named after its country of origin, Australia.

Cladophialophorae carrionii similis, sed conidiis secundis majoribus, (7–)8–12(–15) × 3–4 µm.

In vitro: *Mycelium* consisting of branched, septate, smooth, pale brown, guttulate, 2–3 µm wide hyphae; hyphal coils not seen. *Conidiophores* dimorphic; macroconidiophores mononematous, subcylindrical, multi-septate, straight to curved, up to 150 µm long (including conidiogenous cells), and 4 µm wide, pale to medium brown, smooth, guttulate; microconidiophores integrated with hyphae, which terminate in subcylindrical conidiogenous cells that give rise to branched chains of conidia; conidiophores (including

conidiogenous cells) up to 5-septate, 50 µm long, with terminal and lateral conidiogenous cells. *Conidiogenous cells* pale to medium brown, smooth, guttulate, terminal and lateral, subcylindrical, 20–35 × 2–3.5 µm, or reduced to indistinct subtruncate to truncate loci, scars up to 2 µm wide, mono- to polyblastic, proliferating sympodially, scars neither darkened, thickened, nor refractive. *Conidia* pale to medium brown, guttulate, smooth; ramoconidia subcylindrical, 0–1-septate, 20–35 × 2–3 µm, hila subtruncate, inconspicuous, up to 2 µm wide, giving rise to branched chains of conidia; conidia ellipsoid, pale brown, but becoming dark brown and thick-walled in older cultures, guttulate, tapering towards subtruncate terminal loci, 0–1-septate, occurring in chains of up to 20 conidia, (7–)8–12(–15) × 3–4 µm (older, dark brown conidia are ellipsoid, up to 5 µm wide).

Cultural characteristics: Colonies erumpent, somewhat spreading, margins crenate, feathery, aerial mycelium sparse; colonies on PDA olivaceous-grey to iron-grey (surface); reverse iron-grey; on OA and SNA olivaceous-grey. Colonies reaching 5 mm diam after 2 wk at 25 °C in the dark; colonies fertile. Not able to grow at 37 °C.

Specimen examined: **Australia**, isolated from apple juice, Dec. 1986, A.D. Hocking, **holotype** CBS H-19899, culture ex-type CBS 112793 = CPC 1377.

Notes: *Cladophialophora australiensis* is one of two novel species of *Cladophialophora* originally isolated from sports drinks in Australia. *Cladophialophora* spp. are commonly associated with human disorders (Honbo *et al*. 1984, de Hoog *et al*. 2000, Levin *et al*. 2004), and thus their occurrence in sports drinks is cause for concern. However, none of the new species described here had the ability to grow at 37 °C, and therefore it is not expected that they could pose a danger to humans. Comparing ITS diversity, the species shows more than 12 % difference to established pathogens such as *C. carrionii* and *C. bantiana* (Sacc.) de Hoog *et al.*

Cladophialophora chaetospira (Grove) Crous & Arzanlou, **comb. nov.** MycoBank MB504526. Fig. 4.

Basionym: *Septocylindrium chaetospira* Grove, J. Bot. Lond. 24: 199. 1886.

≡ *Septonema chaetospira* (Grove) S. Hughes, Naturalist, London 840: 9. 1952.

≡ *Heteroconium chaetospira* (Grove) M.B. Ellis, in Ellis, *More Dematiaceous Hyphomycetes*: 64. 1976.

In vitro: *Mycelium* consisting of branched, septate, smooth, medium brown hyphae, 2–3.5 µm wide. *Conidiophores* reduced to conidiogenous cells, or a single supporting cell, 20–40 × 3–4 µm. *Conidiogenous cells* subcylindrical, erect, straight to irregularly curved, medium brown, smooth, 15–30 × 3–4 µm. *Conidia* in branched, acropetal chains with up to 30 conidia; subcylindrical to fusiform, medium brown, smooth, tapering slightly at subtruncate ends, 1(–3)-septate, thin-walled, becoming slightly constricted at septa of older conidia, (20–)25–30(–45) × 3–4(–5) µm; conidia remaining attached in long chains; hila neither thickened, nor darkened-refractive.

Cultural characteristics: Colonies erumpent, convex, spreading, with sparse to dense aerial mycelium; margins smooth, undulate; on PDA iron-grey (surface), margins olivaceous-black; reverse olivaceous-black; on OA olivaceous-grey in the middle due to fluffy aerial mycelium, iron-grey in wide outer margin; on SNA olivaceous-grey. Colonies reaching 12 mm diam after 2 wk on PDA at 25 °C in the dark. Not able to grow at 37 °C.

Specimens examined: **China**, Yunnan, Yiliang, isolated from *Phyllostachys bambusoides* (*Gramineae*), decaying bamboo, freshwater, 6 Jul. 2003, L. Cai, CBS 114747; China, Yunnan, stream in Kunming, isolated from bamboo wood, 15 Jun. 2003, C. Lei, CBS 115468. **Denmark**, isolated from roots of *Picea abies* (*Pinaceae*), isol. by D.S. Malla, CBS 491.70. **Germany**, Schleswig-Holstein, Kiel-Kitzeberg, isolated from wheat field soil, isol. by W. Gams, CBS 514.63 = ATCC 16274 = MUCL 8310.

Notes: Two cultures of *Heteroconium chaetospira* were originally deposited as *Spadicoides minuta* L. Cai, McKenzie & K.D. Hyde (Cai *et al*. 2004), but later found to represent *Heteroconium chaetospira*, a species commonly found on rotting wood in Europe (Ellis 1976). The genus *Heteroconium* Petr. has in recent years been used to name leaf spotting fungi with chains of brown, disarticulating conidia (Crous *et al*. 2006b), which have phylogenetic affinities to several orders, obviously being polyphyletic. The type species of *Heteroconium*, *H. citharexyli* Petr., is a plant pathogen on *Cytharexylum* (Petrak 1949) with hitherto unknown phylogenetic position. The fact that *H. chaetospira* is linked to the *Chaetothyriales*, was rather unexpected. The species appears to be similar to others placed in *Cladophialophora* by having short, lateral conidiogenous cells, and long chains of branched subcylindrical conidia that largely remain attached. It is, however, quite distinct from other members of *Cladophialophora* in having medium brown conidia, and in lacking the ellipsoid conidia observed in several species.

Cladophialophora hostae Crous, U. Braun & H.D. Shin, **sp. nov.** MycoBank MB504527. Figs 5–6.

Etymology: Epithet derived from the host genus, *Hosta*.

Cladophialophorae scillae similis, sed conidiophoris in vitro brevioribus et leniter angustioribus, 10–15 × 1.5–2 µm, conidiis brevioribus, (7–)10–15(–20) µm.

In vivo: *Leaf spots* amphigenous, subcircular to somewhat angular-irregular, 1–5 mm wide, scattered to aggregated, sometimes confluent, pale to medium brown or with a reddish brown tinge, later greyish brown, margin indefinite or on the upper leaf surface with a narrow slightly raised marginal line or very narrow lighter halo, yellowish, ochraceous to brownish. *Caespituli* epiphyllous, punctiform to confluent, dingy greyish brown. *Mycelium* immersed, forming fusicladium-like hyphal strands or plates; hyphae septate, sometimes with constrictions at the septa, thin-walled, pale olivaceous, 1.5–7 µm wide. *Stromata* immersed, small, 10–40 µm diam, composed of swollen hyphal cells, subcircular to somewhat angular-irregular in outline, 2–8 µm diam, wall somewhat thickened, brown. *Conidiophores* in small to moderately large fascicles, loose, divergent to moderately dense, rarely solitary, arising from stromatic hyphal aggregations, erumpent, erect, usually unbranched, rarely branched, straight, subcylindrical to distinctly geniculate-sinuous, 5–40 × 2–5 µm, 0–6-septate, pale to medium olivaceous to olivaceous-brown, thin-walled, up to 0.5 µm, smooth. *Conidiogenous cells* integrated, terminal, 5–15(–20) µm long, sympodial, conidiogenous loci rather inconspicuous to subdenticulate, flat-tipped, 1–1.5 µm diam, unthickened or almost so, not to slightly darkened-refractive. *Conidia* in simple or branched chains, narrowly ellipsoid-subcylindrical, 10–15 × 1.5–3.5 µm, 0–1-septate, subhyaline to pale olivaceous, thin-walled, smooth, ends truncate or with two denticle-like hila in ramoconidia, (0.75–)1–1.5(–2) µm diam, unthickened or almost so, at most slightly darkened-refractive.

In vitro: *Mycelium* composed of branched, smooth, pale olivaceous to medium brown hyphae, frequently forming hyphal coils, guttulate, septa inconspicuous, not constricted, hyphae somewhat irregular in width, 1–2 µm wide. *Conidiophores* reduced to conidiogenous cells, integrated in hyphae, terminal, subcylindrical, pale olivaceous to pale brown, smooth, 0–1-septate, proliferating sympodially at

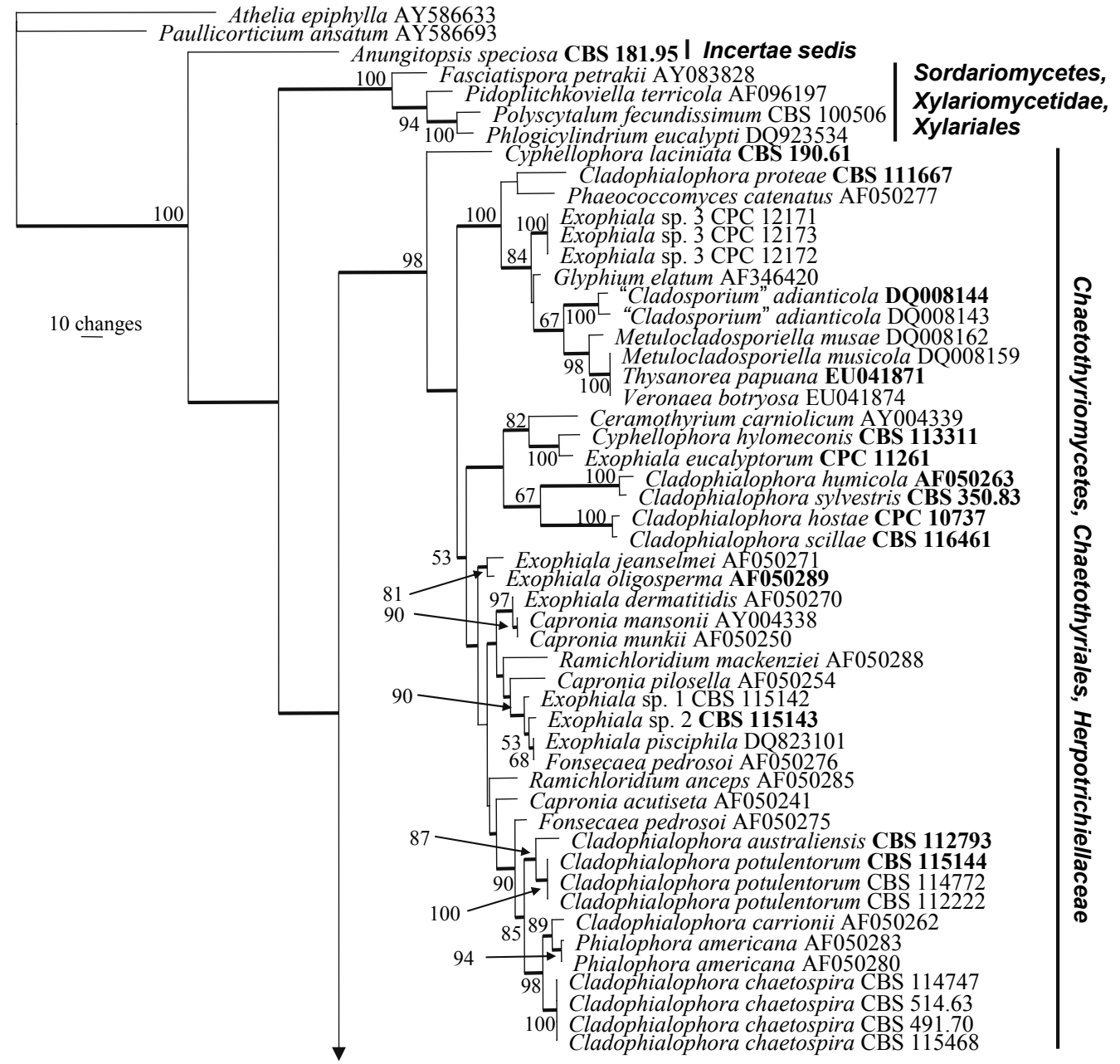

Fig. 1. (Page 188–189). One of 5 000 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

apex via 1–2(–3) flat-tipped, minute, denticle-like loci, 1–1.5 µm wide, 10–15 × 1.5–2 µm; scars minutely darkened and thickened, but not refractive. *Conidia* in extremely long chains (–60), simple or branched, subcylindrical, or narrowly ellipsoid, smooth, pale olivaceous, 0–1-septate, (7–)10–15(–20) × (1.5–)2(–2.5) µm, hila truncate, 1–1.5 µm wide, minutely thickened and darkened-refractive.

Cultural characteristics: Colonies on PDA erumpent, spreading, with smooth, undulate margins and dense aerial mycelium; surface hazel (middle), outer zone isabelline; reverse fuscous-black in middle, isabelline in outer zone. Colonies reaching 25 mm diam on SNA, and 40 mm diam on PDA after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Korea**, Pyongchang, *Hosta plantaginea* (*Hostaceae*), 20 Sep. 2003, H.D. Shin, HAL 2030 F, **holotype**, culture ex-type SMK 19664, CPC 10737 = CBS 121637, CPC 10738–10739.

Notes: Although this species is morphologically similar to *Cladophialophora scillae* (Deighton) Crous, U. Braun & K. Schub. described below in this paper, *C. hostae* is treated as a separate taxon due to the differences in the length and width of its conidiophores and conidia *in vitro*, as well as 17 bp differences in the ITS DNA sequence data and a distinct ecology causing leaf-spots on a different, unrelated host. Based on disease symptoms caused on the living host leaves, *C. hostae* is a very unusual, unexpected member of the genus *Cladophialophora*. *In vivo*, the mycelium forms obvious hyphal strands and plates which are characteristic for *Fusicladium* species. The conidiophores and conidia are also fusicladium-like. Nevertheless, this species clusters within the *Herpotrichiellaceae*, i.e., it has to be placed in the genus *Cladophialophora*. Biotrophic species like *C. hostae* and *C. scillae* without phialidic synanamorphs render the differentiation between *Cladophialophora* and *Fusicladium* (incl. *Pseudocladosporium*) almost impossible without sequence data. Furthermore, the

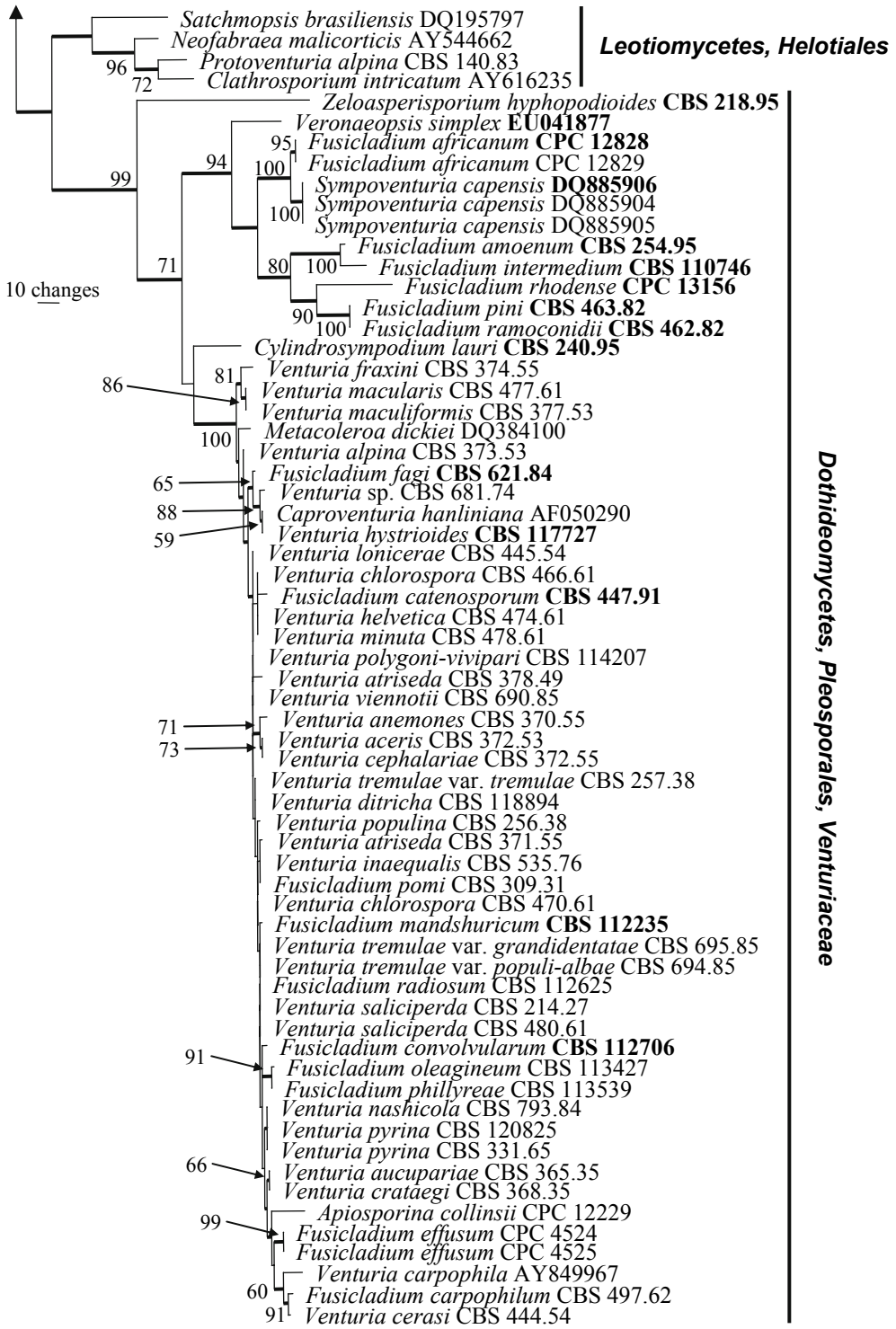

Fig 1. (Continued).

morphology of *C. hostae in vivo* and *in vitro* shows remarkable differences in conidiophore morphology, i.e., the growth *in vivo* is characteristically fusicladium-like (conidiophores macronematous, long, septate), whereas habit *in vitro* is rather pseudocladosporium-like (conidiophores less developed, usually reduced to conidiogenous cells, short). However, several *Fusicladium* species have also been observed to exibit a *Pseudocladosporium* growth habit in culture, suggesting this growth plasticity to be rather common, and strongly influenced by growth conditions.

Cladophialophora humicola Crous & U. Braun, **sp. nov.** MycoBank MB504528. Figs 7–8.

Etymology: Named after its ecology, namely occurring in soil.

Cladophialophorae bantianae similis, sed conidiis majoribus, (8–)11–14(–17) × (1.5–)2(–2.5) µm, locis conidiogenis et hilis angustioribus, 1–1.5 µm latis.

In vitro: *Mycelium* composed of branched, smooth, pale olivaceous to pale brown hyphae, frequently forming hyphal coils, prominently guttulate, not to slightly constricted at the septa, 1–2 µm wide, cells somewhat uneven in width. *Conidiophores* solitary, mostly inconspicuous and integrated in hyphae, varying from inconspicuously truncate lateral loci on hyphal cells, 1–1.5 µm wide, to occasionally terminal conidiophores, 0–3-septate, subcylindrical, proliferating sympodially, 10–30 × 1.5–3 µm, pale brown, smooth.

Table 1. Isolates used for sequence analysis.

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank numbers[2] (ITS, LSU)
Anungitopsis speciosa		CBS 181.95*; INIFAT C94/135	Leaf litter of *Buchenavia capitata*	Cuba	R.F. Castañeda	EU035401, EU035401
Cladophialophora australiensis		CBS 112793*; CPC 1377	Sports drink	Australia	–	EU035402, EU035402
Cladophialophora chaetospira		CBS 114747	*Phyllostachys bambusoides*	China	L. Cai	EU035403, EU035403
		CBS 115468; HKUCC 10147	Bamboo	China	–	EU035404, EU035404
		CBS 491.70	Roots of *Picea abies*	Denmark	–	EU035405, EU035405
		CBS 514.63; ATCC 16274; MUCL 8310	Soil, wheat field	Germany	–	EU035406, EU035406
Cladophialophora hostae		CPC 10737*	*Hosta plantaginea*	Korea	H.D. Shin	EU035407, EU035407
Cladophialophora humicola		CBS 117536*; BBA 65570	Soil, arable	Germany	Z. Zaspel & H. Nirenberg	EU035408, AF050263
Cladophialophora potulentorum		CBS 112222; CPC 1376; FRR 4946	Sports drink	Australia	N.J. Charley	EU035409, EU035409
		CBS 114772; CPC 1375; FRR 4947	Sports drink	Australia	N.J. Charley	EU035410, EU035410
		CBS 115144*; CPC 11048; FRR 3318	Apple juice	–	–	DQ008141, DQ008141
Cladophialophora proteae		CBS 111667*; CPC 1514	*Protea cynaroides*	South Africa	L. Viljoen	EU035411, EU035411
Cladophialophora scillae		CBS 116461*	*Scilla peruviana*	New Zealand	C.F. Hill	EU035412, EU035412
Cladophialophora sylvestris		CBS 350.83	*Pinus sylvestris*	Netherlands	–	EU035413, EU035413
"Cladosporium" adianticola		CBS 735.87*; ATCC 200931; INIFAT C87/40	*Adiantum* sp.	Cuba	R.F. Castañeda & G. Arnold	DQ008125, DQ008144
Cylindrosympodium lauri		CBS 240.95*; INIFAT C95/3-2	*Laurus* sp.	Spain, Canary Islands	R.F. Castañeda	EU035414, EU035414
Cyphellophora hylomeconis		CBS 113311*	*Helomeco velane*	Korea	H.D. Shin	EU035415, EU035415
Cyphellophora laciniata		CBS 190.61*; ATCC 14166; MUCL 9569	Man, skin	Switzerland	K.M. Wissel	EU035416, EU035416
Exophiala eucalyptorum		CPC 11261*	*Eucalyptus* sp.	New Zealand	J. Stalpers	EU035417, EU035417
Exophiala sp. 1		CBS 115142; CPC 11044; FRR 5582	Fruit-based drink	–	–	DQ008139, EU035418
Exophiala sp. 2		CBS 115143*; CPC 11047; FRR 5599	Bottled spring water	–	–	DQ008140, EU035419
Exophiala sp. 3		CPC 12171	*Prunus* sp.	Canada	K.A. Seifert	EU035420, EU035420
		CPC 12172	*Prunus* sp.	Canada	K.A. Seifert	EU035421, EU035421
		CPC 12173	*Prunus* sp.	Canada	K.A. Seifert	EU035422, EU035422
Fusicladium africanum		CPC 12828*	*Eucalyptus* sp.	South Africa	P.W. Crous	EU035423, EU035423
		CPC 12829	*Eucalyptus* sp.	South Africa	P.W. Crous	EU035424, EU035424
Fusicladium amoenum		CBS 254.95*; ATCC 200947; CPC 3681; IMI 367525; INIFAT C94/155; MUCL 39143	Leaf litter of *Eucalyptus grandis*	Cuba	R.F. Castañeda	EU035425, EU035425
Fusicladium carpophilum	*Venturia carpophila*	CBS 497.62; ETH 4568	*Prunus* sp.	Switzerland	–	EU035426, EU035426

Fusicladium catenosporum		CBS 447.91*	*Salix triandra*	Germany	H. Butin	EU035427, EU035427
Fusicladium convolvularum		CBS 112706*; CPC 3884; IMI 383037	*Convolvulus arvensis*	New Zealand	C.F. Hill	AY251082, EU035428
Fusicladium effusum		CPC 4524	*Carya illinoinensis*	U.S.A.	K. Stevenson	AY251084, EU035429
		CPC 4525	*Carya illinoinensis*	U.S.A.	K. Stevenson	AY251085, EU035430
Fusicladium fagi		CBS 621.84*; ATCC 200937	*Fagus sylvatica*	Netherlands	G.S. de Hoog	EU035431, EU035431
Fusicladium intermedium		CBS 110746*; CPC 778; IMI 362702	*Eucalyptus* sp.	Madagascar	P.W. Crous	EU035432, EU035432
Fusicladium mandshuricum	*Venturia mandshurica*	CBS 112235*; CPC 3639	*Populus simonii*	China	–	EU035433, EU035433
Fusicladium oleagineum		CBS 113427	*Olea europaea*	New Zealand	C.F. Hill	EU035434, EU035434
Fusicladium phillyreae		CBS 113539; UPSC 1329	–	Portugal	B. d'Oliveira	EU035435, EU035435
Fusicladium pini		CBS 463.82*	*Pinus sylvestris*	Netherlands	G.S. de Hoog	EU035436, EU035436
Fusicladium pomi	*Venturia inaequalis*	CBS 309.31	–	–	–	EU035437, EU035437
		CBS 535.76	*Sorbus aria*	Switzerland	–	EU035460, EU035460
Fusicladium radiosum	*Venturia tremulae*	CBS 112625; CPC 3638	*Populus tremula*	France	–	EU035438, EU035438
Fusicladium ramoconidii		CBS 462.82*; CPC 3679	*Pinus* sp.	Netherlands	G.S. de Hoog	AY251086, EU035439
Fusicladium rhodense		CPC 13156*	*Ceratonia siliqua*	Greece	P.W. Crous	EU035440, EU035440
Polyscytalum fecundissimum		CBS 100506	*Fagus sylvatica*	Netherlands	W. Gams	EU035441, EU035441
Zeloasperisporium hyphopodioides		CBS 218.95*; IMI 367520; INIFAT C94/114; MUCL 39155	Air	Cuba	R. F. Castañeda	EU035442, EU035442
	Apiosporina collinsii	CPC 12229	*Amelanchier alnifolia*	Canada	L.J. Hutchinson	EU035443, EU035443
	Protoventuria alpina	CBS 140.83	*Arctostaphylos uva-ursi*	Switzerland	–	EU035444, EU035444
	Sympoventuria capensis	CBS 120136; CPC 12838	*Eucalyptus* sp.	South Africa	P.W. Crous	DQ885906, DQ885906
		CPC 12839	*Eucalyptus* sp.	South Africa	P.W. Crous	DQ885905, DQ885905
		CPC 12840	*Eucalyptus* sp.	South Africa	P.W. Crous	DQ885904, DQ885904
	Venturia aceris	CBS 372.53	*Acer pseudoplatanus*	Switzerland	–	EU035445, EU035445
	Venturia alpina	CBS 373.53	*Arctostaphylos alpina*	Switzerland	–	EU035446, EU035446
	Venturia anemones	CBS 370.55; IMI 163998	*Anemone alpina*	France	–	EU035447, EU035447
	Venturia atriseda	CBS 371.55	*Gentiana punctata*	Switzerland	–	EU035448, EU035448
		CBS 378.49	*Gentiana lutea*	Switzerland	J.A. von Arx	EU035449, EU035449
	Venturia aucupariae	CBS 365.35; IMI 163987	*Sorbus aucuparia*	Germany	–	EU035450, EU035450
	Venturia cephalariae	CBS 372.55	*Cephalaria alpina*	Switzerland	–	EU035451, EU035451
	Venturia cerasi	CBS 444.54; ATCC 12119; IMI 163988	*Prunus cerasus*	Germany	–	EU035452, EU035452
	Venturia chlorospora	CBS 466.61; ETH 543	*Salix caesia*	Switzerland	J. Nüesch	EU035453, EU035453
		CBS 470.61	*Salix daphnoides*	France	J. Nüesch	EU035454, EU035454
	Venturia crataegi	CBS 368.35	*Crataegus* sp.	Germany	–	EU035455, EU035455

Table 1. (Continued).

Anamorph	Teleomorph	Accession number[1]	Host	Country	Collector	GenBank numbers[2] (ITS, LSU)
	Venturia ditricha	CBS 118894	*Betula pubescens* var. *tortuosa*	Finland	–	EU035456, EU035456
	Venturia fraxini	CBS 374.55	*Fraxinus excelsior*	Switzerland	–	EU035457, EU035457
	Venturia helvetica	CBS 474.61; ETH 2571; IMI 163990	*Salix helvetica*	Switzerland	J. Nüesch	EU035458, EU035458
	Venturia hystrioides	CBS 117727*; ATCC 96019; CPC 5391	*Prunus avium* cv. Bing	U.S.A.	R.G. Roberts	EU035459, EU035459
	Venturia lonicerae	CBS 445.54; IMI 163997	*Lonicera coerulea*	Switzerland	–	EU035461, EU035461
	Venturia macularis	CBS 477.61; ETH 2831	*Populus tremula*	France	–	EU035462, EU035462
	Venturia maculiformis	CBS 377.53	*Epilobium montanum*	France	–	EU035463, EU035463
	Venturia minuta	CBS 478.61; ETH 523; IMI 163991	*Salix nigricans*	Switzerland	J. Nüesch	EU035464, EU035464
	Venturia nashicola	CBS 793.84	*Pyrus serotina*	Japan	–	EU035465, EU035465
	Venturia polygoni-vivipari	CBS 114207; UPSC 2754	*Polygonum viviparum*	Norway	K. & L. Holm	EU035466, EU035466
	Venturia populina	CBS 256.38; IMI 163996	*Populus canadensis*	Italy	–	EU035467, EU035467
	Venturia pyrina	CBS 120825	*Pyrus communis*	Brazil	–	EU035468, EU035468
		CBS 331.65	*Pyrus* sp.	–	–	EU035469, EU035469
	Venturia saliciperda	CBS 214.27; IMI 163993	–	–	–	EU035470, EU035470
		CBS 480.61; ETH 2836	*Salix cordata*	Switzerland	–	EU035471, EU035471
	Venturia sp.	CBS 681.74	*Cedrus atlantica*	France	W. Gams	EU035472, EU035472
	Venturia tremulae var. *grandidentatae*	CBS 695.85	*Populus tremuloides*	Canada	–	EU035473, EU035473
	Venturia tremulae var. *populi-albae*	CBS 694.85	*Populus alba*	France	–	EU035474, EU035474
	Venturia tremulae var. *tremulae*	CBS 257.38	*Populus tremula*	Italy	–	EU035475, EU035475
	Venturia viennotii	CBS 690.85	*Populus tremula*	France	–	EU035476, EU035476

[1]ATCC: American Type Culture Collection, Virginia, U.S.A.; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, Germany; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; ETH: Eidgenössische Technische Hochschule, Institute for Special Botany, Zürich, Switzerland; FRR: Division of Food Research, CSIRO, North Ryde, N.S.W., Australia; HKUCC: The University of Hong Kong Culture Collection, Dept. of Ecology and Biodiversity, University of Hong Kong, Pokfulam Road, China; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; INIFAT: Alexander Humboldt Institute for Basic Research in Tropical Agriculture, Ciudad de La Habana, Cuba; MUCL: Mycotheque de l' Université Catholique de Louvain, Louvain-la-Neuve, Belgium; UPSC: Uppsala University Culture Collection of Fungi, Museum of Evolution, Botany Section, Evolutionary Biology Centre, Uppsala, Sweden.

[2]ITS: internal transcribed spacer regions, LSU: partial 28S rDNA sequence.

*Ex-type cultures.

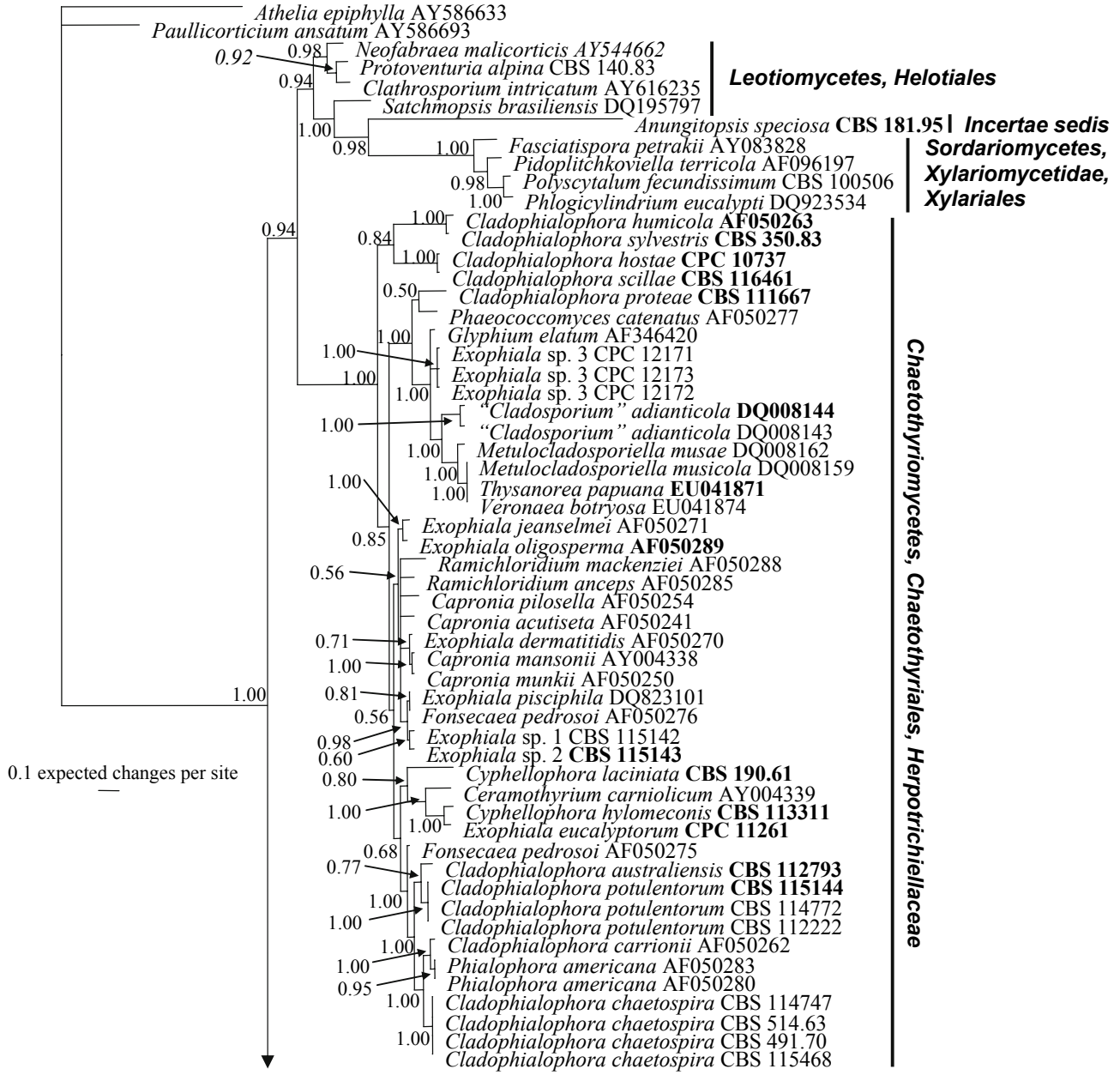

Fig. 2. (Page 193–194). Consensus phylogram (50 % majority rule) of 1 500 trees resulting from a Bayesian analysis of the LSU sequence alignment using MRBAYES v. 3.1.2. Bayesian posterior probabilities are indicated at the nodes. Ex-type sequences are printed in bold face. The tree was rooted to two sequences obtained from GenBank (*Athelia epiphylla* AY586633 and *Paullicorticium ansatum* AY586693).

Conidiogenous cells integrated, inconspicuous, truncate, lateral loci 1–1.5 µm wide, or conidiogenous cells subcylindrical with 1–3 sympodial loci (which appear as minute lateral denticles), 7–17 × 1.5–2 µm; scars inconspicuous, neither darkened, refractive nor thickened. *Conidia* in short chains of up to 10, simple or branched, subcylindrical to narrowly ellipsoid, 0–1-septate, (8–)11–14(–17) × (1.5–)2(–2.5) µm, pale olivaceous to olivaceous-brown or pale brown, smooth, hila truncate, 1–1.5 µm wide, unthickened, neither darkened, nor refractive.

Cultural characteristics: Colonies erumpent, spreading, with uneven, feathery margins and dense aerial mycelium on PDA; pale olivaceous-grey in the middle, becoming olivaceous-grey in the outer zone (surface); reverse olivaceous-black, with grey-olivaceous margins. Colonies reaching 7 mm diam after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **Germany**, Brandenburg, Müncheberg, from soil, Zaspel, Zalf & H. Nirenberg, **holotype** CBS H-19902, culture ex-type BBA 65570 = CBS 117536.

Notes: Phylogenetically *Cladophialophora humicola* is closely related to *C. sylvestris* Crous & de Hoog (see below). Morphologically the two species can be distinguished in that *C. humicola* lacks ramoconidia, and has 1-septate conidia, while those of *C. sylvestris* are 0–3-septate.

Cladophialophora potulentorum Crous & A.D. Hocking, **sp. nov.** MycoBank MB504529. Figs 9–10.

Etymology: Refers to its presence in fruit juices and sports drinks.

Cladophialophorae carrionii similis, sed conidiis secundis majoribus, (6–)8–10(–13) × 2–3 µm.

In vitro: *Mycelium* consisting of branched, septate, smooth, pale brown, guttulate, 1.5–2.5 µm wide hyphae. *Conidiophores* solitary, macronematous, well distinguishable under the dissecting microscope from aerial mycelium, pale to medium brown, subcylindrical, straight to somewhat curved, erect, with apical apparatus appearing as a tuft due to extremely long conidial

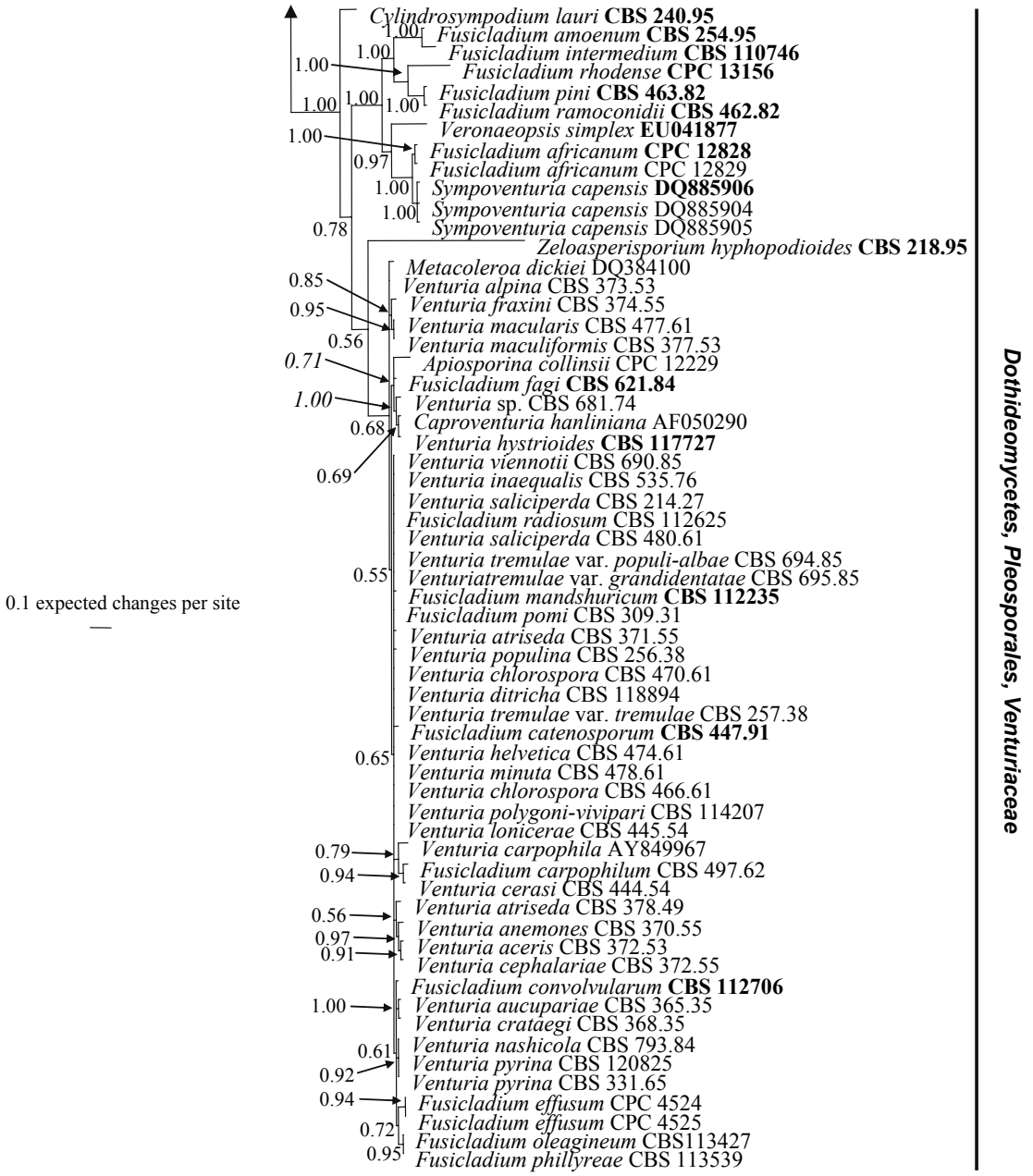

Fig. 2. (Continued).

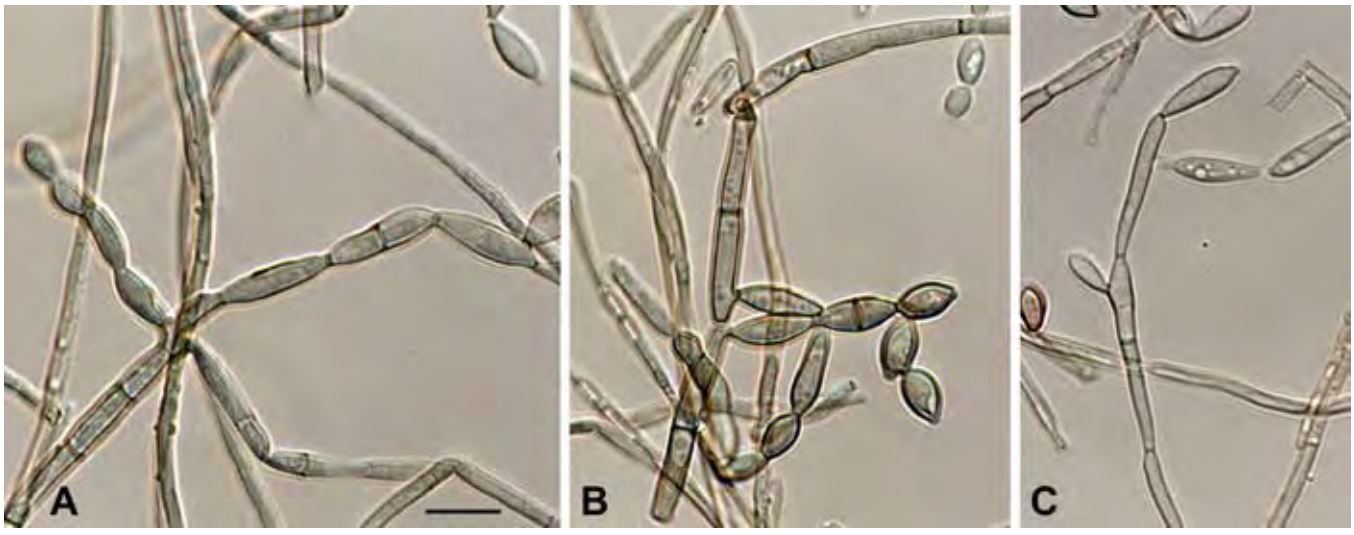

Fig. 3. *Cladophialophora australiensis* (CBS 112793). A. Conidiophore. B–C. Subcylindrical ramoconidia, and ellipsoid conidia. Scale bar = 10 µm.

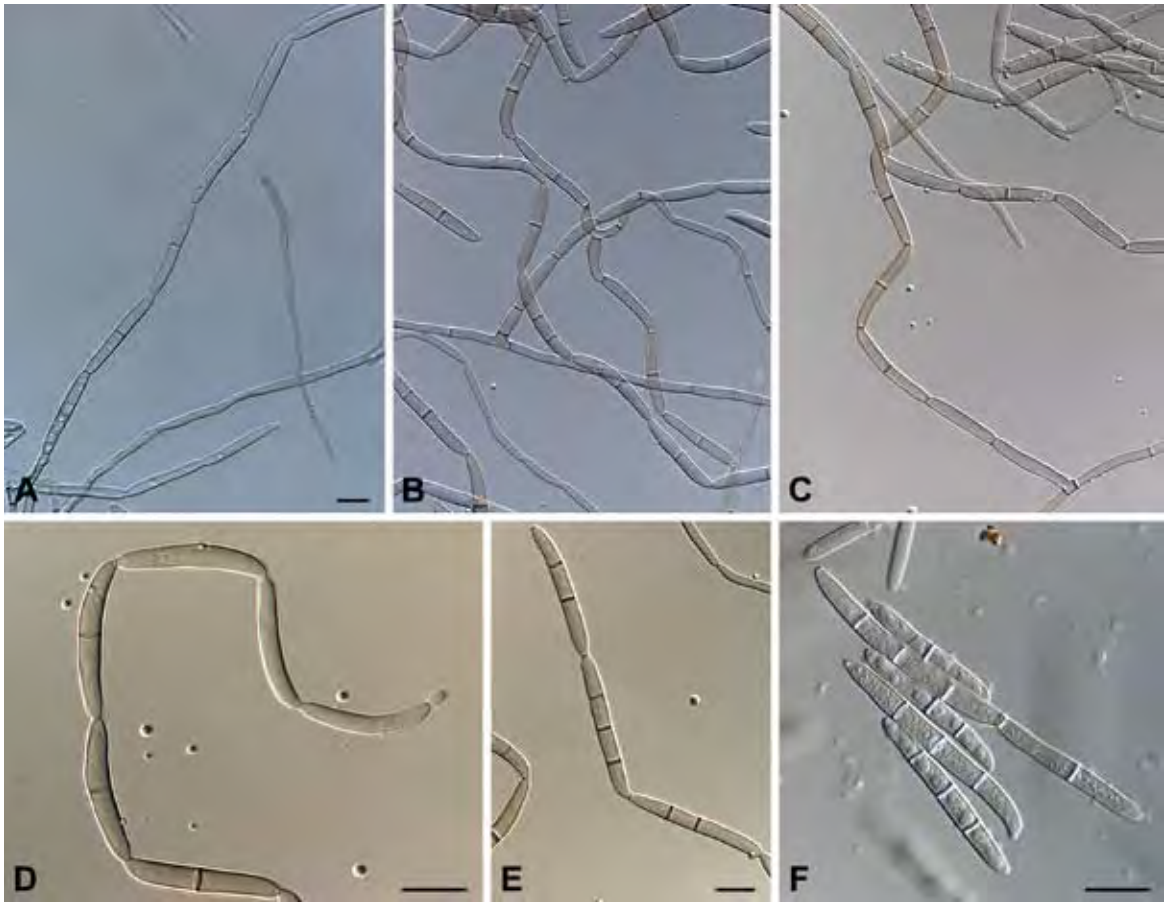

Fig. 4. *Cladophialophora chaetospira* (CBS 114747). A–C. Hyphae giving rise to conidiophores with catenulate conidia. D–F. Conidia become up to 3-septate, frequently remaining attached in chains. Scale bars = 10 µm.

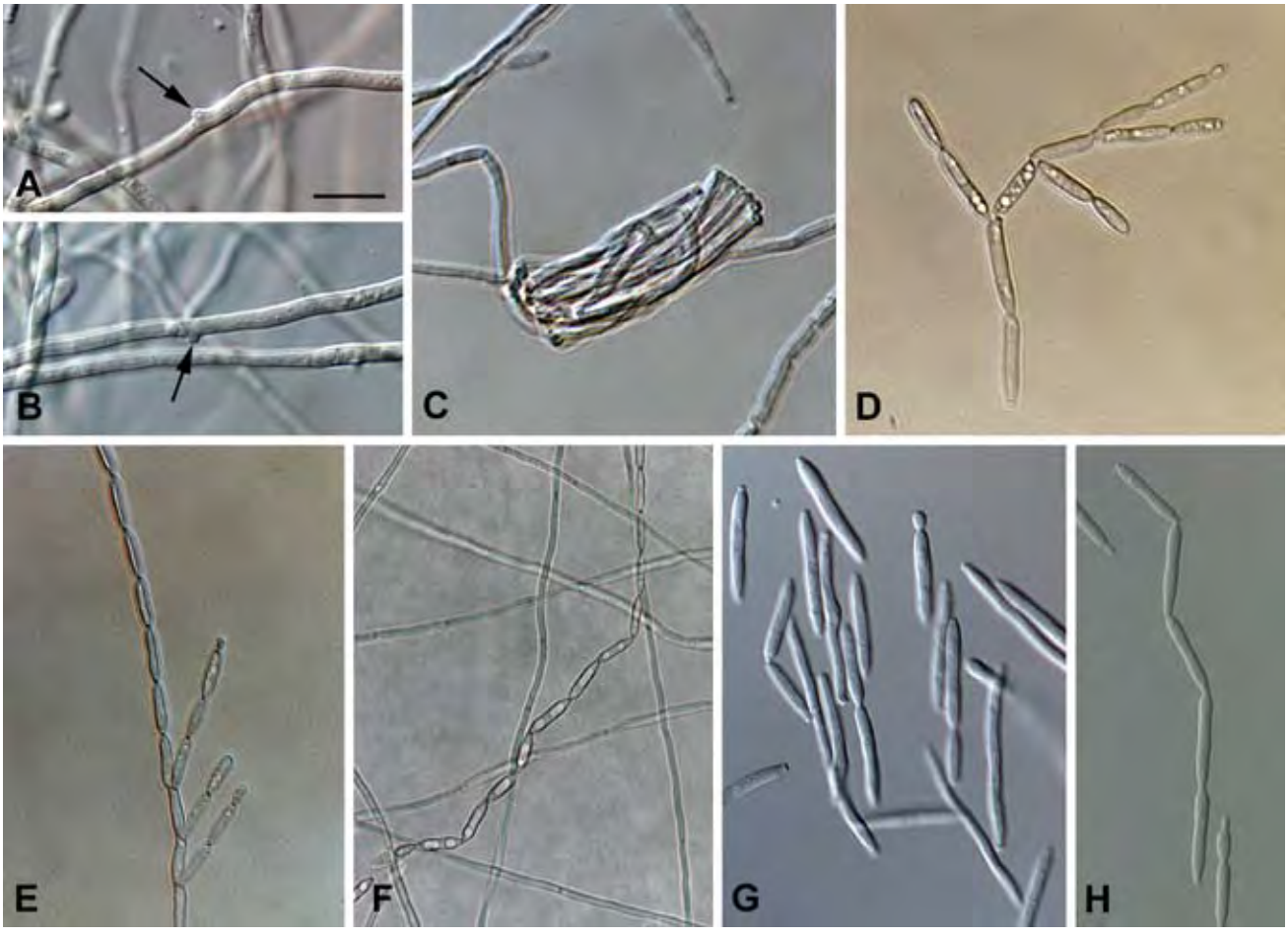

Fig. 5. *Cladophialophora hostae* (CPC 10737). A–B. Conidiogenous loci (arrows). C. Hyphal coil. D–F. Branched conidial chains. G–H. Conidia. Scale bar = 10 µm.

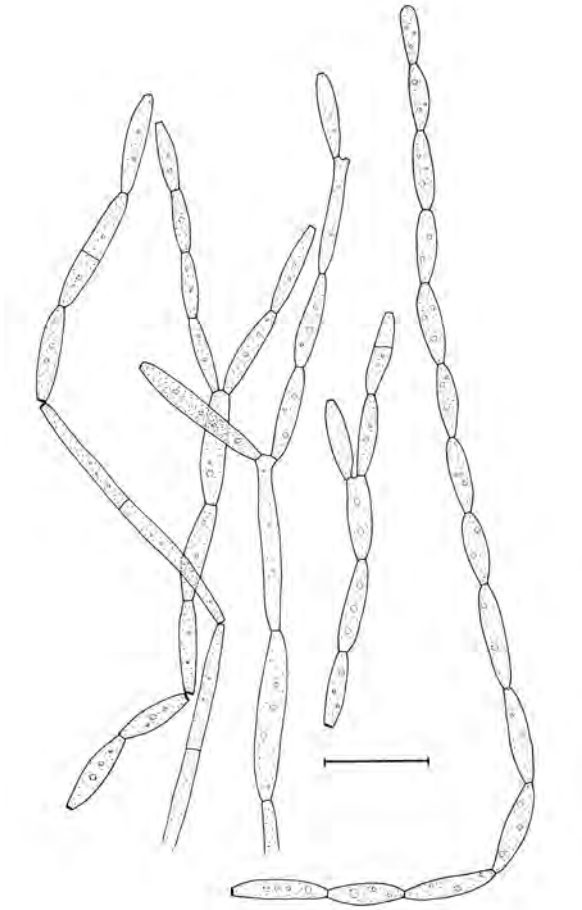

Fig. 6. *Cladophialophora hostae* (CPC 10737). Branched conidial chains with ramoconidia and conidia. Scale bar = 10 µm.

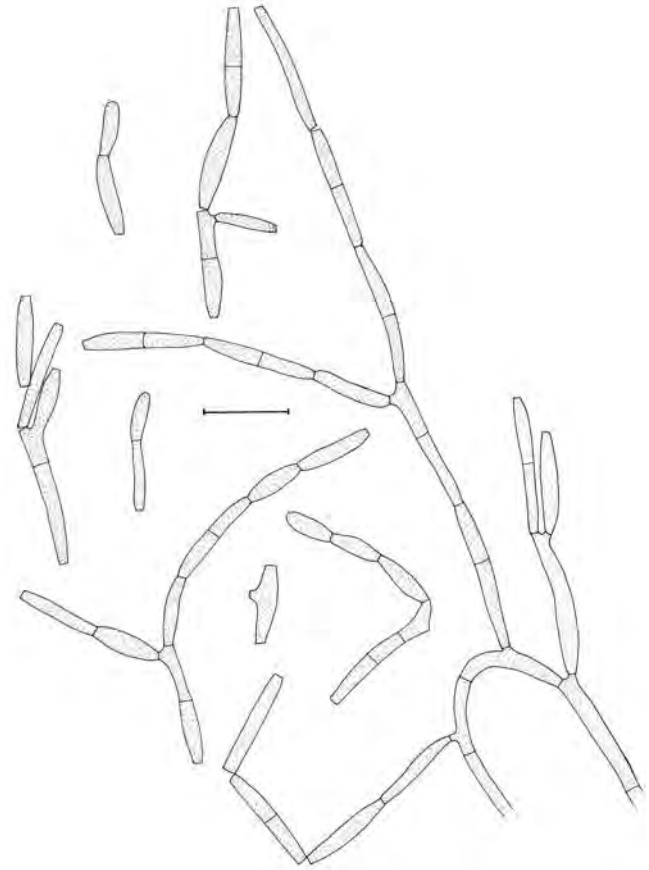

Fig. 7. *Cladophialophora humicola* (CBS 117536). Conidiophore with branched conidial chains. Scale bar = 10 µm.

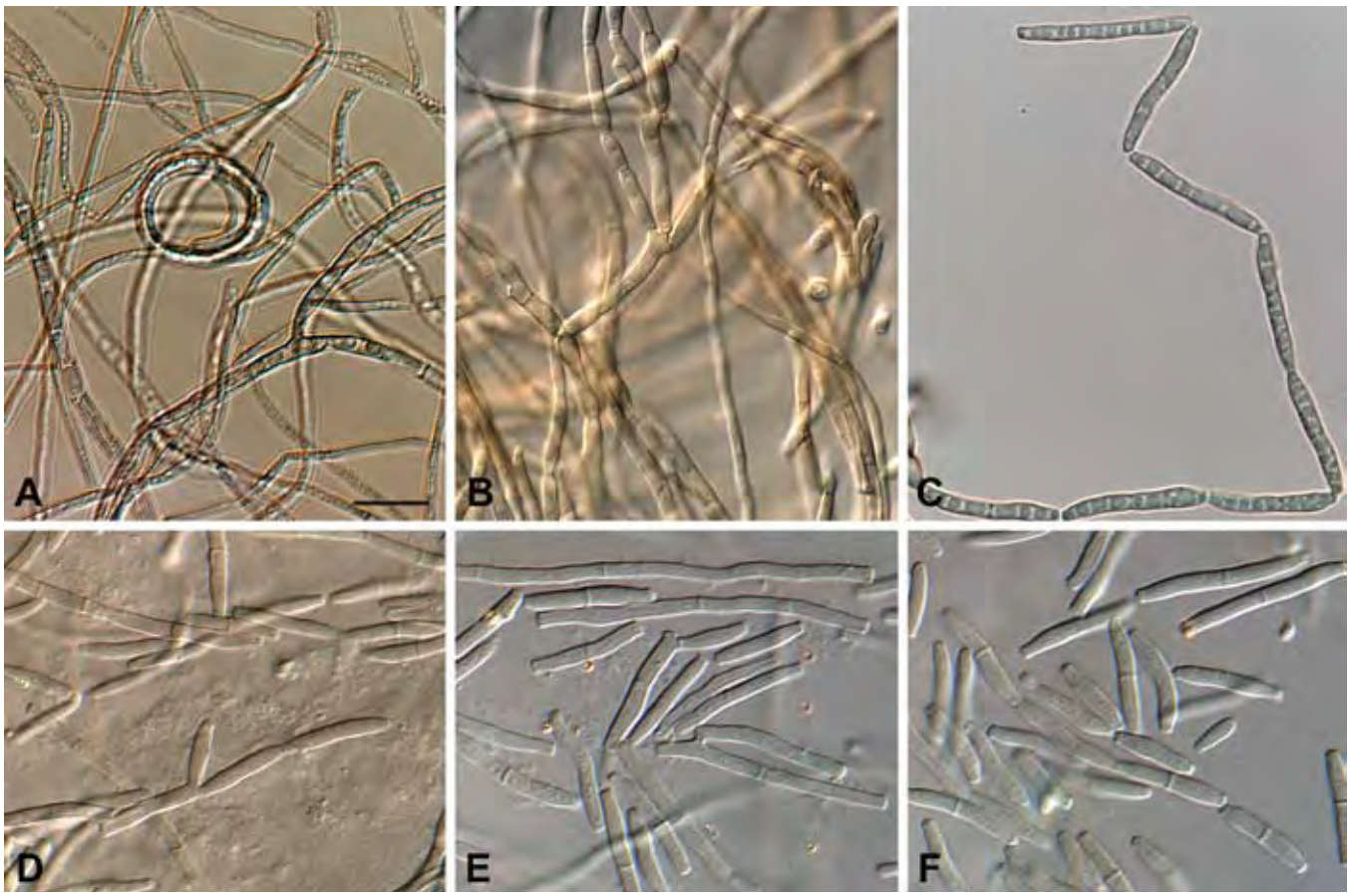

Fig. 8. *Cladophialophora humicola* (CBS 117536). A. Hyphal coil. B. Conidiophore. C–F. Conidial chains with ramoconidia and conidia. Scale bar = 10 µm.

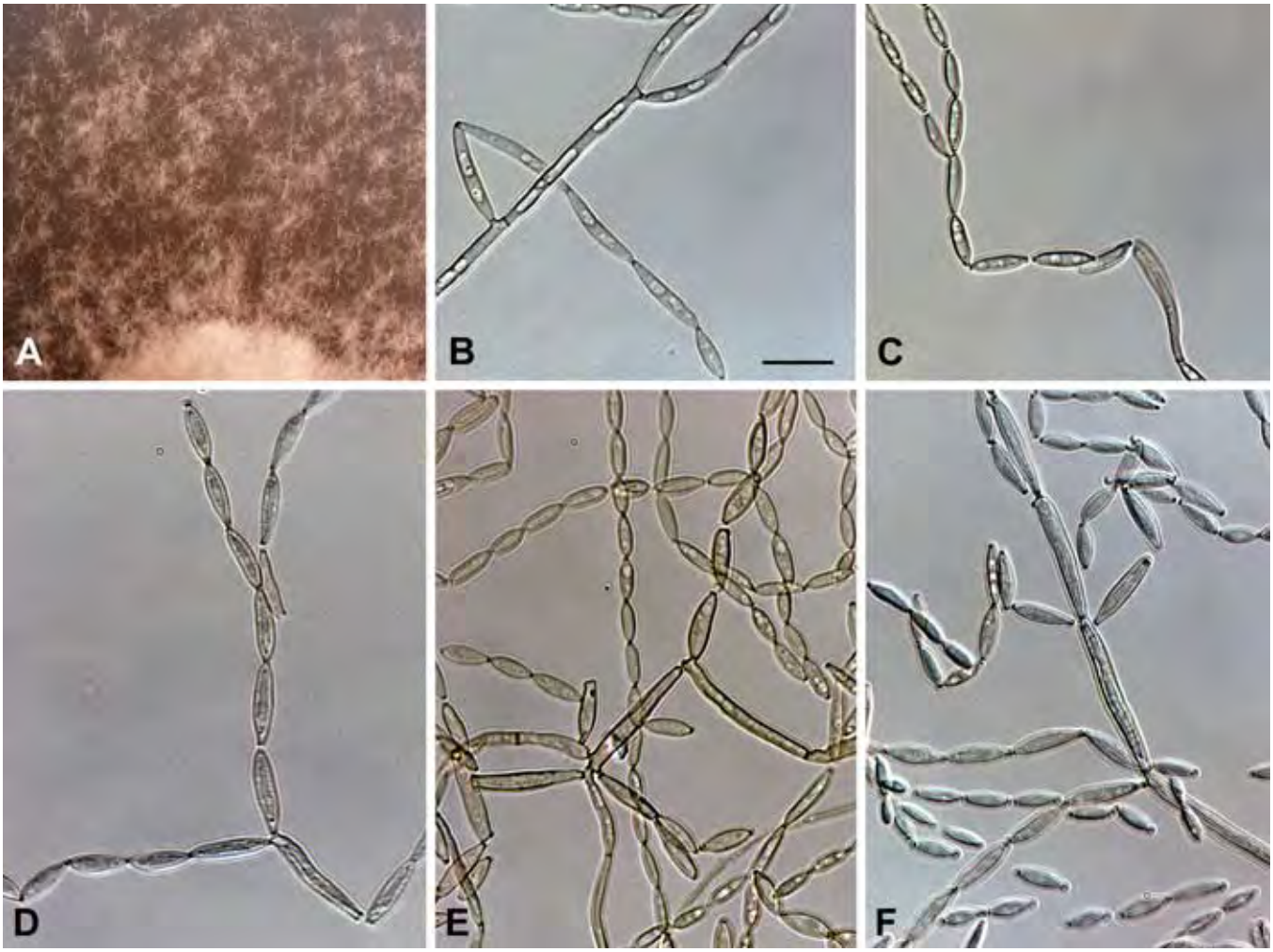

Fig. 9. *Cladophialophora potulentorum* (CBS 115144). A. Colony on PDA. B. Conidiophore. C–D. Conidial chains. E–F. Ramoconidia and conidia. Scale bar = 10 µm.

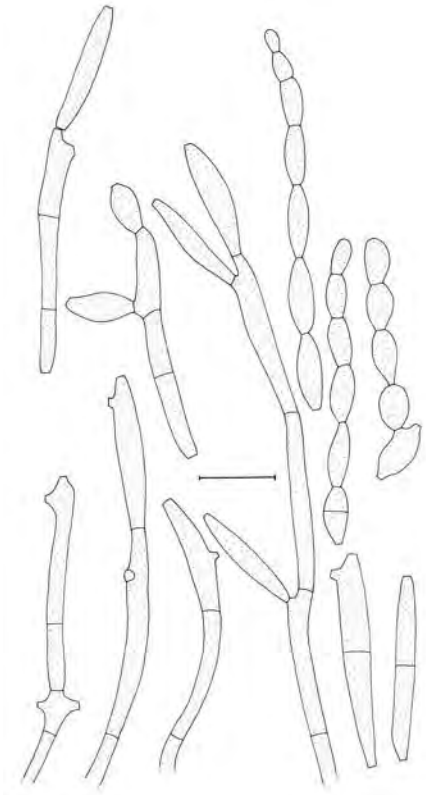

Fig. 10. *Cladophialophora potulentorum* (CBS 115144). Conidiophores with chains of ramoconidia and conidia. Scale bar = 10 µm.

chains; conidiophores up to 5-septate, and 100 µm tall (excluding conidiogenous cells). *Conidiogenous cells* pale brown, smooth, terminal and lateral, subcylindrical, tapering towards subtruncate to truncate loci, 1 µm wide, somewhat darkened, thickened, but not refractive, loci appearing subdenticulate on lateral conidiogenous cells, mono- to polyblastic, proliferating sympodially, 10–35 × 1.5–2 µm. *Conidia* pale brown, smooth, guttulate, occurring in branched chains of up to 60; hila somewhat darkened and thickened, but not refractive, 0.5 µm wide; ramoconidia subcylindrical, 0–1-septate, 15–17(–20) × 2.5–3 µm; conidia ellipsoid, (6–)8–10(–13) × 2–3 µm.

Cultural characteristics: Colonies erumpent, spreading, with smooth margins and dense aerial mycelium on PDA, olivaceous-grey (surface), with a thin, olivaceous-black margin; reverse olivaceous-black; on OA olivaceous-grey (surface) with a wide olivaceous-black margin. Colonies reaching 25–30 mm diam after 1 mo at 25 °C in the dark; colonies fertile, also sporulating in the agar. Not able to grow at 37 °C.

Specimens examined: **Australia**, isolated from apple juice drink, Dec. 1986, A.D. Hocking, **holotype** CBS H-19901, culture ex-type CBS 115144 = CPC 11048; **Australia**, isolated from sports drink, Feb. 1996, A.D. Hocking, CBS 114772 = CPC 1375 = FRR 4947; **Australia**, isolated from sports drink, Feb. 1996, A.D. Hocking, CBS 112222 = FRR 4946.

Notes: Originally this taxon, isolated from fruit and sports drinks, was thought to be an undescribed species of *Pseudocladosporium* (= *Fusicladium*, see below). However, upon closer examination, this

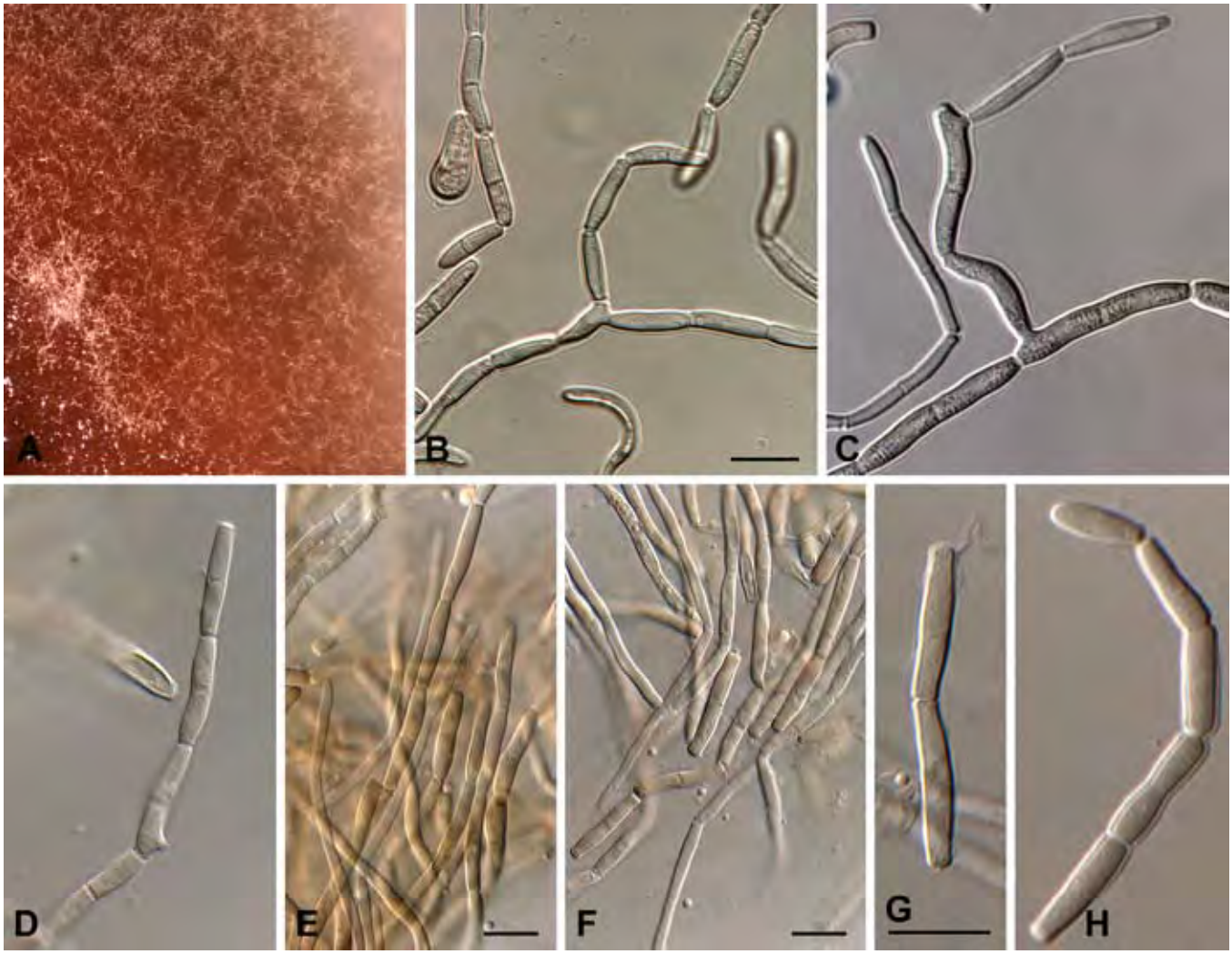

Fig. 11. *Cladophialophora proteae* (CBS 111667). A. Colony on OA. B–C. Conidiophores. D–H. Catenulate conidia. Scale bars = 10 µm.

proved not to be the case. Conidiophores appear as distinct tufts under the dissecting microscope, and are readily distinguishable from the superficial mycelium, as is normally observed in species of *Fusicladium*, but the conidial chains are extremely long, and the conidia tend to be more ellipsoid than the predominantly fusiform or subcylindrical conidia observed in species of *Fusicladium.* Hyphal coils were also not observed in cultures of *C. potulentorum*, but are rather common in species of *Fusicladium*. The phylogenetic position of this taxon within the *Herpotrichiellaceae* clade also supports inclusion in the genus *Cladophialophora*.

Cladophialophora proteae Viljoen & Crous, S. African J. Bot. 64: 137. 1998. Fig. 11.

≡ *Pseudocladosporium proteae* (Viljoen & Crous) Crous, in Crous *et al*., *Cultivation and Diseases of Proteaceae: Leucadendron, Leucospermum and Protea*: 101. 2004.

In vitro: *Mycelium* consisting of branched, septate hyphae, often forming strands, anastomosing, smooth to finely verruculose, frequently constricted at septa, olivaceous, 3–4 µm wide; hyphal cells in older cultures becoming swollen, up to 6 µm wide. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, integrated, forming short, truncate protuberances, 2–3 × 1.5–2 µm, concolorous with mycelium, subcylindrical. *Conidia in vitro* arranged in long acropetal chains (up to 20), simple or branched, subcylindrical to oblong-doliiform, (9–)13–17(–22) × 2.5–3(–4) µm *in vitro* on MEA, (9–)16–22(–25) × (2.5–)3–4(–6) µm on SNA; 0–1(–4)-septate, pale brown to pale olivaceous, smooth, hila subtruncate to truncate, not thickened, but somewhat refractive.

Cultural characteristics: Colonies erumpent, with sparse aerial mycelium on PDA; margins irregular, feathery; greyish rose, with patches of pale olivaceous-grey (surface); reverse olivaceous-grey. Colonies reaching 10 mm diam after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **South Africa**, Western Cape Province, Stellenbosch, J.S. Marais Nature Reserve, leaves of *Protea cynaroides* (*Proteaceae*), 26 Aug. 1996, L. Viljoen, **holotype** PREM 55345, culture ex-type CBS 111667.

Notes: *Cladophialophora proteae* differs from species of *Fusicladium* (= *Pseudocladosporium*) based on its colony colour, the slimy nature of colonies, as well as its conidia that have inconspicuous, unthickened hila (Fig. 11) (Crous *et al*. 2004), unlike those observed in species of *Fusicladium*. Sequence data show that this species is not allied to the *Venturiaceae*, but to the *Herpotrichiellaceae*.

Cladophialophora scillae (Deighton) Crous, U. Braun & K. Schub., **comb. nov.** MycoBank MB504530. Fig. 12.

Basionym: *Cladosporium scillae* Deighton, N. Zealand J. Bot. 8: 55. 1970.

≡ *Fusicladium scillae* (Deighton) U. Braun & K. Schub., *IMI Descriptions of Fungi and Bacteria* 152: 1518. 2002.

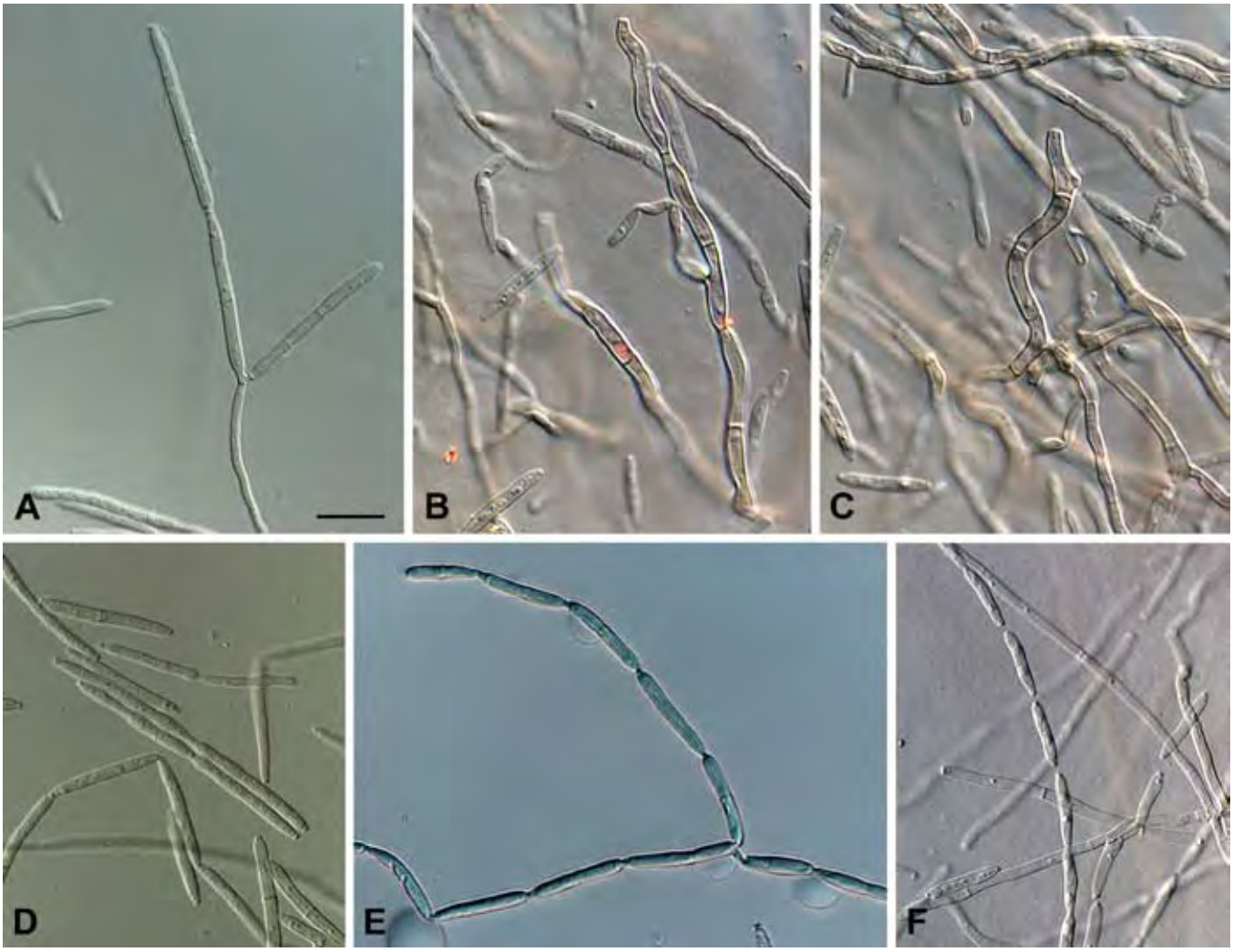

Fig. 12. *Cladophialophora scillae* (CBS 116461). A–C. Conidiophores. D–F. Catenulate conidia. Scale bar = 10 µm.

In vivo: see Schubert & Braun (2002a) and Schubert *et al*. (2003).

In vitro: *Mycelium* consisting of branched, septate, smooth, green-brown to medium brown, guttulate hyphae, variable in width, 1.5–3 µm diam. *Conidiophores* lateral or terminal on hyphae, erect, straight to slightly flexuous, solitary, in some cases aggregated, subcylindrical, curved to geniculate-sinuous, unbranched, up to 55 µm long, 2–3 µm wide, 0–7-septate, septa in short succession, pale to medium brown, somewhat paler towards apices, smooth. *Conidiogenous cells* integrated, terminal or lateral as individual loci on hyphal cells, straight to curved, subcylindrical, up to 14(–18) µm long and 2 µm wide, pale to medium brown, smooth, with a single or few subdenticulate to denticulate loci at the apex due to sympodial proliferation, or reduced to individual loci, 0.8–1.5(–2) µm wide; scars minutely thickened and darkened, but not refractive. *Conidia* occurring in long, unbranched or loosely branched chains (–30), straight to slightly curved, ellipsoid to mostly narrowly subcylindrical, obclavate in some larger, septate conidia, (5–)10–20(–35) × 1.5–3 µm, 0–1(–3)-septate, sometimes slightly constricted at the septa, subhyaline to pale brown, smooth, guttulate, tapering at ends to subtruncate hila, 0.8–1.5 µm wide, minutely thickened and darkened, but not refractive; microcyclic conidiogenesis occurring.

Cultural characteristics: Colonies erumpent, spreading, with smooth, even margins and dense, abundant aerial mycelium on PDA; grey-olivaceous (surface); reverse dark olivaceous. Colonies on OA olivaceous-grey, smoke-grey due to profuse sporulation, reverse olivaceous-grey to iron-grey, velvety, aerial mycelium sparse, diffuse. Colonies reaching 20 mm diam on SNA, and 40 mm on PDA after 1 mo at 25 °C in the dark; colonies fertile.

Specimens examined: **New Zealand**, Levin, on *Scilla peruviana* (*Hyacinthaceae*), 21 Dec. 1965, G.F. Laudon, IMI 116997 **holotype**; Auckland, Manurewa, Auckland Botanic Gardens, on leaf spots of *Scilla peruviana*, 25 Apr. 2004, C.F. Hill, 1044, CBS H-19903, **epitype designated here**, culture ex-type CBS 116461.

Notes: In culture *Cladophialophora scillae* forms a pseudocladosporium-like state, though the scars are somewhat darkened and thickened, but not refractive. Conidiophores are reduced to conidiogenous cells that are integrated in the mycelium, terminal or lateral, frequently also as an inconspicuous lateral denticle, with a flat-tipped scar. Conidia occur in long, branched chains, which are subcylindrical to narrowly ellipsoid, and are up to 35 µm long, 1.5–3 µm wide, thus longer and thinner than reported on the host, which were 0–3-septate, subcylindrical to ellipsoid-ovoid, 7–22 × 2.5–4 µm. Due to the fusicladioid habit of this species *in vivo*, Schubert & Braun (2002a) reallocated it to *Fusicladium*. Based on ITS sequence data, morphology and cultural characteristics, *Cladophialophora scillae* was almost identical to an isolate obtained from leaf spots of *Hosta plantaginea* in Korea. These isolates appeared to resemble species of *Fusicladium*, but phylogenetically they clustered in the *Herpotrichiellaceae*. Therefore, "*Fusicladium*" *scillae* was placed in the genus *Cladophialophora*. As far as we are aware, this species and *C. hostae* are first reports of phytopathogenic species within the genus *Cladophialophora*.

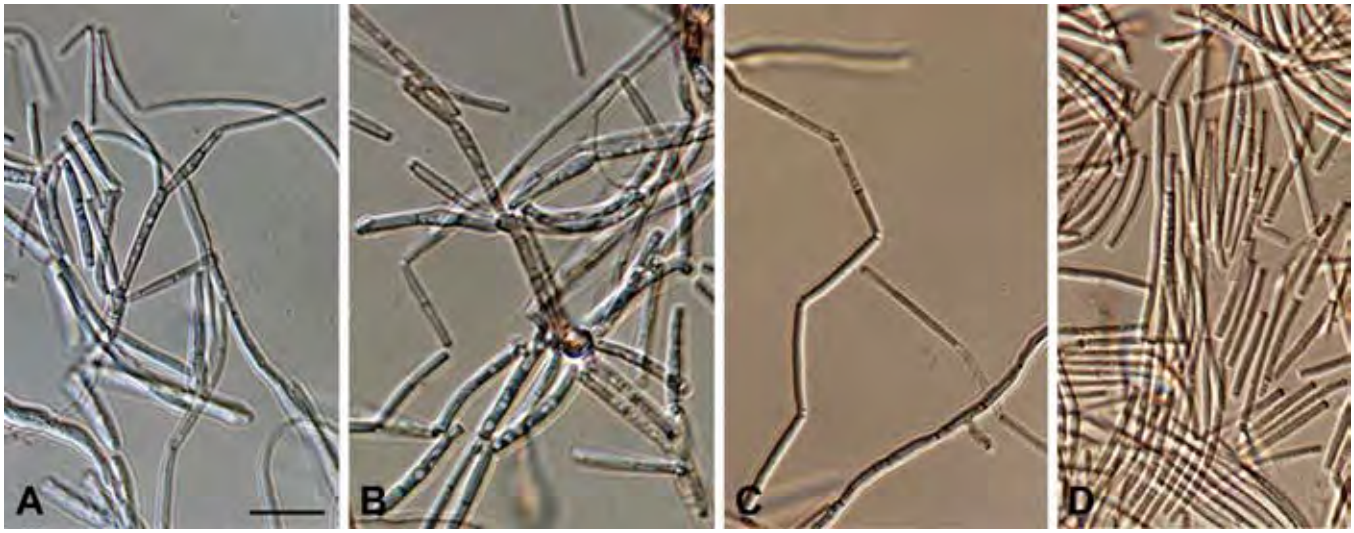

Fig. 13. *Cladophialophora sylvestris* (CBS 350.83). A–B. Conidiophores. C. Catenulate conidia. D. Conidial mass. Scale bar = 10 µm.

Fig. 14. *Cyphellophora hylomeconis* (CBS 113311). A. Colony on PDA. B–C. Hyphae with truncate conidiogenous loci. D–F. Conidia. Scale bars = 10 µm.

Cladophialophora sylvestris Crous & de Hoog, **sp. nov.** MycoBank MB504531. Fig. 13.

Etymology: Refers to its host, *Pinus sylvestris*.

Cladophialophorae humicolae similis, sed conidiis 0–3-septatis, (7–)10–16(–20) × 1.5–2 µm.

Mycelium composed of branched, smooth, pale olivaceous to pale brown hyphae, frequently forming hyphal coils, not to slightly constricted at the septa, 1–2 µm wide. *Conidiophores* medium brown, subcylindrical, flexuous, mononematous, multiseptate, up to 50 µm long, and 2–3 µm wide. *Conidiogenous cells* apical, sympodial, pale brown, 5–12 × 2–3 µm; scars somewhat darkened and thickened, not refractive. *Conidia* occurring in branched chains; ramoconidia up to 2 µm wide, giving rise apically to disarticulating chains of conidia; smooth, 0–3-septate, pale olivaceous, subcylindrical, (7–)10–16(–20) × 1.5–2 µm, with truncate ends; hila somewhat darkened and thickened, not refractive.

Cultural characteristics: Colonies erumpent on PDA, with smooth, catenulate margins; iron-grey (surface); reverse greenish black. Colonies reaching 15 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Netherlands**, Kootwijk, needle litter of *Pinus sylvestris* (*Pinaceae*), 8 Nov. 1982, G.S. de Hoog, **holotype** CBS H-19917, culture ex-type CBS 350.83.

Notes: Morphologically CBS 350.83 was originally identified as *Polyscytalum griseum* Sacc., but the latter is reported to have conidia that are 5–5.5 × 1 µm (Saccardo 1877), which is much smaller than that observed for the present isolate. Furthermore, the type species of *Polyscytalum*, *P. fecundissimum* Riess (CBS 100506), does not cluster within the *Herpotrichiellaceae*, thus suggesting that CBS 350.83 is best treated as a new species of *Cladophialophora.*

Cyphellophora hylomeconis Crous, de Hoog & H.D. Shin, **sp. nov.** MycoBank MB504532. Fig. 14.

Etymology: Named after its host genus, *Hylomecon*.

Cyphellophorae lacinatae similis, sed conidiis longioribus et leniter angustioribus, (15–)25–35(–55) × (2.5–)3(–4) µm.

Mycelium consisting of branched, greenish brown, septate, branched, smooth, 3–5 µm wide hyphae, constricted at septa. *Conidiogenous cells* phialidic, intercalary, appearing denticulate, 1 µm tall, 1.5–2 µm wide, with minute collarettes (at times

proliferating percurrently). *Conidia* sickle-shaped, smooth, medium brown, guttulate, (1–)3(–5)-septate, constricted at septa, widest in middle, or lower third of the conidium; apex subacutely rounded, base subtruncate, or having a slight constriction, giving rise to a foot cell, 1 µm long, 0.5–1 µm wide, subacutely rounded, (15–)25–35(–55) × (2.5–)3(–4) µm; a marginal frill is visible above the foot cell, suggesting this foot cell may be the onset of basal germination; conidia also anastomose and undergo microcyclic conidiation in culture.

Cultural characteristics: Colonies slow-growing, slimy, aerial mycelium absent, margins smooth, catenate; surface crumpled, olivaceous-black to iron-grey. Colonies reaching 20 mm diam after 1 mo at 25 °C in the dark on PDA, 12 mm on SNA; colonies fertile.

Specimen examined: **Korea**, Yangpyeong, on leaves of *Hylomecon verlance* (*Papaveraceae*), 4 Jun. 2003, H.D. Shin, **holotype** CBS H-19907, **isotype** SMK 19550, culture ex-type CBS 113311.

Notes: *Cyphellophora hylomeconis* is related to the type species of the genus, *Cyphellophora laciniata* G.A. de Vries, which also resides in the *Herpotrichiellaceae*. The genus *Cyphellophora* G.A. de Vries is phenetically distinguished from *Pseudomicrodochium* B.C. Sutton, typified by *P. aciculare* B.C. Sutton (1975) by melanized *versus* hyaline thalli. Phylogenetic confirmation is pending due to unavailability of sequence data. Decock *et al*. (2003) synonymised the hyaline genus *Kumbhamaya* M. Jacob & D.J. Bhat (Jacob & Bhat 2000) with *Cyphellophora*, but as no cultures of this fungus are available this decision seems premature. Nearly all *Cyphellophora* species accepted by Decock *et al.* (2003) have been found to be involved in cutaneous infections in humans. This also holds true for the species originally described as being environmental, *C. vermispora* Walz & de Hoog, which is closely related to *C. suttonii* (Ajello *et al*.) Decock and *C. fusarioides* (C.K. Campbell & B.C. Sutton) Decock known from proven human and animal infections. Decock *et al*. (2003) added the melanized species *C. guyanensis* Decock & Delgado, isolated as a saprobe from tropical leaf litter. *Cyphellophora hylomeconis* is the first species of the genus infecting a living plant host. ITS sequences are remote from those of the remaining *Cyphellophora* species, the nearest neighbour being *C. pluriseptata* G.A. de Vries, Elders & Luykx at 19.1 % distance (data not shown). *Cyphellophora hylomeconis* can be distinguished based on its conidial dimensions and septation. Conidia are larger than those of *C. fusarioides* (11–20 × 2–2.5 µm, 1–2-septate), and those of *C. laciniata* (11–25 × 2–5 µm, 1–3-septate) (for a key to the species see Decock *et al*. 2003).

***Exophiala* sp. 1.** Fig. 15.

Mycelium consisting of smooth, branched, septate, medium brown, 2–3 µm wide hyphae, regular in width, forming hyphal strands and hyphal coils, with free yeast-like cells present in culture; chlamydospores terminal on hyphae, frequently forming clusters or chains, medium brown, ellipsoid, 0–1-septate, up to 10 µm long and 5 µm wide. *Conidiophores* reduced to conidiogenous cells, or consisting of one supporting cell, giving rise to a single conidiogenous cell, subcylindrical to ellipsoid, medium brown, smooth, 5–12 × 3.5–4 µm, with 1(–3) phialidic loci, somewhat protruding, appearing subdenticulate at first glance under the light microscope. *Conidiogenous cells* integrated as lateral loci on hyphal cells, inconspicuous, 1–1.5 µm wide, with a slightly flaring collarette, (1–)1.5(–2) µm long. *Conidia* ellipsoid, smooth, guttulate, becoming brown, swollen and elongated, and at times 1-septate, 4–5(–7) × (2.5–)3(–4) µm (description based on CBS 115142).

Cultural characteristics: Colonies erumpent, spreading, with sparse to dense aerial mycelium on PDA, olivaceous-grey (surface), with a thin to wide, smooth, olivaceous-black margins; reverse olivaceous-black; on OA olivaceous-grey (surface) with wide, olivaceous-black margins. Colonies reaching 40–50 mm diam after 1 mo at 25 °C in the dark; colonies fertile, but sporulation sparse. Not able to grow at 37 °C.

Specimen examined: **Australia**, from a fruit drink, May 2002, N.J. Charley, CBS 115142 = CPC 11044 = FRR 5582.

Notes: Species of *Exophiala* are frequently observed as agents of human mycoses in immunocompromised patients (de Hoog *et al*. 2000). They are found in the environment as slow-growing, oligotrophic colonisers of moist substrates. For example the thermotolerant species *E. dermatitidis* (Kano) de Hoog and *E. phaeomuriformis* (Matsumoto *et al.*) Matos *et al.* are common in public steam baths (Matos *et al*. 2003), while *E. mesophila* Listemann & Freiesleben can be found in showers and swimming pools (unpubl. data). Both species are able to cause infections in humans (Zeng *et al*. 2007). Several other species have been associated primarily with infections in fish and cold-blooded animals (Richards *et al.* 1978) and are occasionally found on humans (Madan *et al.* 2006). The occurrence of the present species in fruit drinks, therefore, is cause of concern, although it was unable to grow at 37 °C. This species forms part of a larger study, and will be treated elsewhere.

***Exophiala* sp. 2.** Fig. 16.

Mycelium consisting of smooth, branched, septate, pale brown, 1.5–3 µm wide hyphae, forming hyphal strands and hyphal coils; hyphae at times terminating in chains of ellipsoid chlamydospores that are medium brown, smooth, up to 10 µm long and 5 µm wide. *Conidiophores* subcylindrical, medium brown, smooth, consisting of a supporting cell and a single conidiogenous cell, or reduced to a conidiogenous cell, straight to curved, up to 30 µm long and 2–3 µm wide. *Conidiogenous cells* pale to medium brown, subcylindrical to narrowly ellipsoid or subclavate, with 1–3 apical, phialidic loci, 1 µm wide, 1–2 µm tall, collarette somewhat flaring, but mostly cylindrical, 7–20 × 2–2.5 µm; at times proliferating percurrently. *Conidia* ellipsoid, smooth, guttulate, hyaline, becoming pale olivaceous, apex obtuse, base subtruncate, (4–)5–7(–10) × 2–2.5(–3) µm.

Cultural characteristics: Colonies spreading with smooth, submerged margins, moderate aerial mycelium on PDA, sparse on OA, on PDA and OA olivaceous-grey (surface), with a wide, iron-grey margin; reverse iron-grey. Colonies reaching 40–50 mm diam after 1 mo at 25 °C in the dark; colonies fertile. Not able to grow at 37 °C.

Specimen examined: **Australia**, from bottled spring water, May 2003, N.J. Charley, CBS 115143 = CPC 11047 = FRR 5599.

Notes: This strain represents another taxon occurring in bottled drinks destined for human consumption. As it is unable to grow at 37 °C, it does not appear to pose any serious threat to human health. This species forms part of a larger study, and will be treated elsewhere.

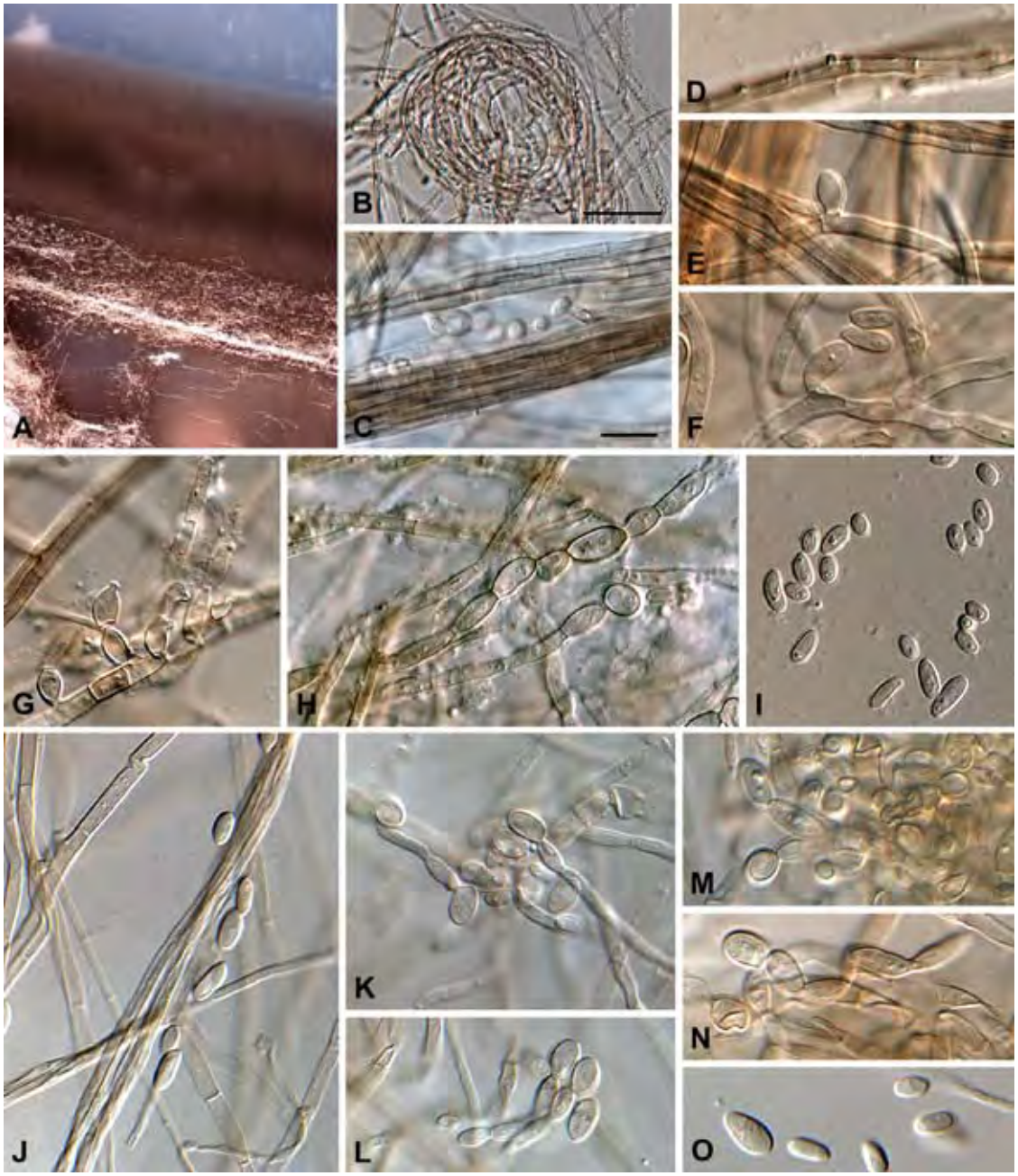

Fig. 15. *Exophiala* sp. 1 (CBS 115142). A. Colony on PDA. B. Hyphal coil. C. Hyphal strand. D–H. Conidiogenous cells and loci. I–O. Conidiogenous cells and conidia. Scale bars = 10 µm.

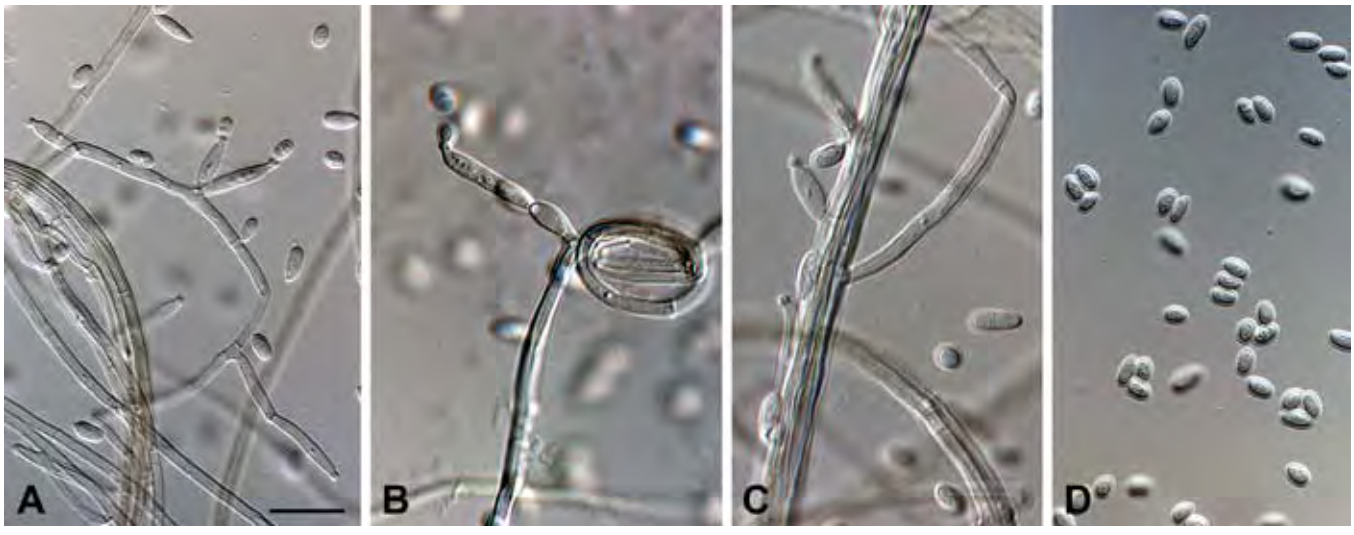

Fig. 16. *Exophiala* sp. 2 (CBS 115143). A. Conidiogenous cells. B. Conidiophore with hyphal coil. C. Conidiogenous cell with hyphal strand. D. Conidia. Scale bar = 10 µm.

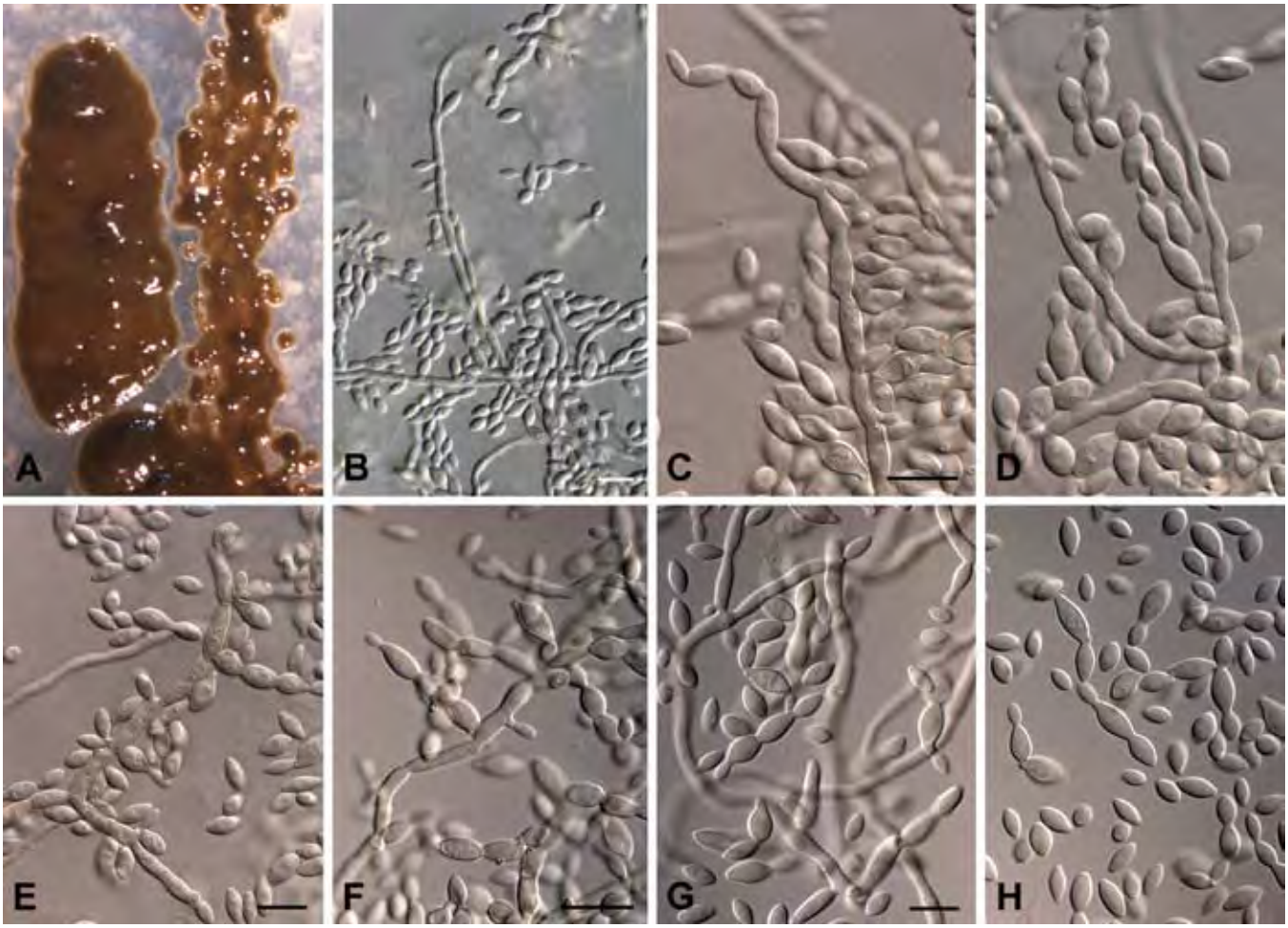

Fig. 17. *Exophiala eucalyptorum* (CPC 11261). A. Colony on PDA. B–H. Hyphae, conidiogenous cells and conidia. Scale bars = 10 µm.

Exophiala eucalyptorum Crous, **sp. nov.** MycoBank MB504533. Fig. 17.

Etymology: Named after its occurrence on *Eucalyptus* leaves.

Exophialae spiniferae similis, sed conidiis fusoidibus-ellipsoideis, (5–)6–8(–10) × (3–)4–5(–7) µm, et cellulis conidiogenis saepe catenatis, in catenis brevibus, dividentibus.

Mycelium consisting of smooth to finely verruculose, branched, septate, 2–4 µm wide hyphae, at times giving rise to chains of dark brown, fusoid-ellipsoid chlamydospores, which can still have phialides, suggesting they were conidiogenous cells; hyphae becoming constricted at septa when fertile. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* numerous, terminal and lateral, mono- to polyphialidic, 5–15 × 3–5 µm; loci 1–1.5 µm wide and tall, with inconspicuous collarettes, at time proliferating percurrently; conidiogenous cells fusoid-ellipsoid, and frequently breaking off, appearing as short chains of conidia, but distinct in having conidiogenous loci. *Conidia* fusoid-ellipsoid, apex acutely rounded, base subtruncate, (5–)6–8(–10) × (3–)4–5(–7) µm; frequently becoming fertile, septate and brown with age.

Cultural characteristics: Colonies erumpent, convex, smooth, slimy, margins feathery to crenate and smooth; aerial mycelium absent, growth yeast-like. Colonies on PDA, OA and SNA chestnut on surface and reverse. Colonies reaching 4 mm diam after 2 wk on PDA at 25 °C in the dark.

Specimen examined: **New Zealand**, Wellington Botanical Garden, on leaf litter of *Eucalyptus* sp. (*Myrtaceae*), Mar. 2004, J.A. Stalpers, **holotype** CBS H-19905, culture ex-type CBS 121638 = CPC 11261.

Notes: *Exophiala eucalyptorum* is rather characteristic in that, in culture, chains of conidiogenous cells frequently detach from hyphae, appearing as short, intact chains of fertile conidia. Its phylogenetic position is somewhat outside the core of the *Herpotrichiellaceae* containing most *Capronia* teleomorphs and the remaining opportunistic *Exophiala* species, but still within the *Chaetothyriales* (Figs 1–2).

Members of *Venturiaceae*

Anungitea B. Sutton and *Anungitopsis* R.F. Castañeda & W.B. Kendr.

Sutton (1973) erected the genus *Anungitea* to accommodate species with brown, mononematous conidiophores bearing apically aggregated, flat-tipped, subdenticulate conidiogenous loci that give rise to chains of pale brown subcylindrical conidia with thickened, darkened hila. He compared the type species, *A. fragilis* B. Sutton with anamorph genera of the *Mycosphaerellaceae*, but did not compare it to *Fusicladium*, to which it is remarkably similar. Castañeda & Kendrick (1990b) introduced the genus *Anungitopsis* based on *A. speciosa* R.F. Castañeda & W.B. Kendr. This genus was distinguished from *Anungitea* by its formation of subdenticulate conidiogenous loci distributed along the apical region of the conidiophore, and by the relatively poorly defined appearance of these loci. No cultures are available of the ex-type species of *Anungitea*, but we studied strains of *Anungitopsis*

Fig. 18. *Cylindrosympodium lauri* (CBS 240.95). A–C. Conidiophores with conidiogenous loci. D. Conidia. Scale bar = 10 µm.

amoena R.F. Castañeda & Dugan (CBS 254.95, ex-type), and *Anungitopsis intermedia* Crous & W.B. Kendr. (CBS 110746, ex-epitype), and found them to cluster adjacent to *Fusicladium* (*Venturiaceae*). However, the ex-type strain of *Anungitopsis speciosa* (CBS 181.95), type species of *Anungitopsis*, clustered distantly from all other species, confirming that the genus name *Anungitopsis* is not available for any of the taxa treated here. In any case, *A. speciosa* has unusual subdenticulate conidiogenous loci with indistinct marginal frills, and these are obviously different from those of anungitea- and fusicladium-like anamorphs, including *A. amoena* and *A. intermedia*. The latter two species previously referred to as *Anungitopsis* belong to a sister clade of the *Venturia* (*Fusicladium*, incl. *Pseudocladosporium*) clade. *Sympoventuria* (Crous *et al.* 2007b), which produces a sympodiella-like anamorph in culture, is the only teleomorph of this clade hitherto known. The venturia-like habit of *Sympoventuria*, connected with fusicladium- / pseudocladosporium-like anamorphs distributed in both clades, indicates a close relation between these clades, suggesting a placement in the *Venturiaceae*. Schubert *et al*. (2003) referred to the difficulty to distinguish between *Anungitea* and *Fusicladium*. *Anungitea* is undoubtedly heterogeneous. *Anungitea rhabdospora* P.M. Kirk (Kirk 1983) is, for instance, intermediate between *Anungitea* (conidiophores with a terminal denticulate conidiogenous cell, but conidia disarticulating in an arthroconidium-like manner) and *Sympodiella* B. Kendr. (conidiophores distinctly sympodial, forming arthroconidia). Other species assigned to *Anungitea* possess a distinctly swollen, lobed conidiophore base, e.g. *A. heterospora* P.M. Kirk (Kirk 1983), which is comparable with other morphologically similar genera, e.g., *Parapleurotheciopsis* P.M. Kirk (Kirk 1982), *Rhizocladosporium* Crous & U. Braun (see Crous *et al*. 2007a – this volume), and *Subramaniomyces* Varghese & V.G. Rao (Varghese & Rao 1979, Kirk 1982). The application of *Anungitea* depends, however, on the affinity of *A. fragilis*, the type species, of which sequence data are not yet available. The best solution for this problem is the widened application of *Fusicladium* (incl. *Pseudocladosporium*) to both sister clades, i.e., to the whole *Venturiaceae*. Morphologically a distinction between fusicladioid anamorphs of both clades is impossible. The more "fusicladium-like" growth is mainly characteristic for the fruiting *in vivo*, above all in biotrophic taxa, whereas the more "pseudocladosporium-like" habit is typical for the growth *in vitro* and in saprobic taxa, a phenomenon which is also evident in species of the morphologically similar genus *Cladophialophora* (see *C. hostae* and *C. scillae*). A potential placement of *Anungitea fragilis* within the *Venturiaceae*, which has still to be proven, would render the genus *Anungitea* a synonym of *Fusicladium*, but in the case of a quite distinct phylogenetic position a new circumscription of this genus, excluding the *Venturiaceae* anamorphs, would be necessary. Thus, a final conclusion about *Anungitea* has to be postponed, awaiting cultures and sequence analyses of its type species.

The taxonomic placement of a fungus from the Canary Islands, isolated from leaf litter of *Laurus* sp. (CBS 240.95), is somewhat problematic. It clusters within the *Venturiaceae*, but not within *Venturia s. str.* itself, and it does not fit into the current morphological concept of *Fusicladium* (incl. *Pseudocladosporium*). Based on its solitary, cylindrical, hyaline conidia and pale brown conidiogenous structures, it resembles species accommodated in *Cylindrosympodium* W.B. Kendr. & R.F. Castañeda (Castañeda & Kendrick 1990a, Marvanová & Laichmanová 2007).

Cylindrosympodium lauri Crous & R.F. Castañeda, **sp. nov.** MycoBank MB504534. Fig. 18.

Etymology: Named after the host genus it was collected from, *Laurus*.

Cylindrosympodii variabilis similis, sed conidiophoris longioribus, ad 70 µm, conidiis subhyalinis vel dilute olivaceis.

Mycelium consisting of brown, smooth, septate, branched hyphae, 1.5–2.5 µm wide. *Conidiophores* macronematous, mononematous, solitary, erect, subcylindrical, straight to geniculate-sinuous, medium brown, smooth, 35–70 × 2.5–4 µm, 1–5-septate. *Conidiogenous cells* terminal, integrated, pale to medium brown, smooth, 10–35 × 2–3 µm, proliferating sympodially, with one to several flat-tipped loci, 1.5–2 µm wide; scars somewhat darkened, minutely thickened, but not refractive. *Conidia* solitary, subacicular to narrowly subcylindrical, apex subobtuse, base truncate, or somewhat swollen, straight or curved, smooth, subhyaline to very pale olivaceous, guttulate, (45–)60–70(–80) × 2.5–3(–3.5) µm, (4–)6–8-septate; scars are somewhat darkened, minutely thickened, but not refractive, 2.5–3 µm wide.

Cultural characteristics: Colonies erumpent, convex, with smooth, lobed margins, and moderate, dense aerial mycelium on PDA; mouse-grey in the central part, and dark mouse-grey in the outer zone (surface); reverse dark mouse-grey. Colonies reaching 5 mm diam after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **Spain**, Canary Islands, leaf litter of *Laurus* sp. (*Lauraceae*), 4 Jan. 1995, R.F. Castañeda, **holotype** CBS H-19909, culture ex-type CBS 240.95.

Note: The present fungus differs from *Cylindrosympodium variabile* (de Hoog) W.B. Kendr. & R.F. Castañeda (de Hoog 1985) in that the conidiophores are much longer, the conidia are subhyaline to very pale olivaceous, and the scars and hila are thin, slightly darkened, but not refractive.

Venturia Sacc. and its anamorph *Fusicladium*

Venturia Sacc., Syll. fung. (Abellini) 1: 586. 1882.
= *Apiosporina* Höhn., Sitzungsber. Kaiserl. Akad. Wiss., Math.-Naturw. Cl., Abt. 1, 119: 439. 1910, **syn. nov.**
= *Metacoleroa* Petr., Ann. Mycol. 25: 332. 1927, **syn. nov.**
= *Caproventuria* U. Braun, *A Monograph of Cercosporella, Ramularia and Allied Genera (Phytopathogenic Hyphomycetes)* 2: 396. 1998, **syn. nov.**
For additional synonyms see Sivanesan, *The bitunicate Ascomycetes and their anamorphs*: 604. 1984.
Anamorph: ***Fusicladium*** Bonord., *Handb. Mykol.*: 80. 1851.
= *Pseudocladosporium* U. Braun, *A Monograph of Cercosporella, Ramularia and Allied Genera (Phytopathogenic Hyphomycetes)* 2: 392. 1998, **syn. nov.**
For additional synonyms, see Schubert *et al*. (2003).

Notes: The genus *Caproventuria*, based on *C. hanliniana* (U. Braun & Feiler) U. Braun, was erected to accommodate saprobic, soil-borne venturia-like ascomycetes with numerous ascomatal setae, and an anamorph quite distinct from *Fusicladium* (Braun 1998). The genus *Metacoleroa* is based on *M. dickiei* (Berk. & Broome) Petr., which clusters in the *Venturiaceae*, adjacent to *Caproventuria*, which has *Pseudocladosporium* anamorphs. *Metacoleroa* was retained by Barr (1987) as separate from *Venturia* based on its superficial ascomata with a thin, stromatic layer beneath the ascomata. Whether these criteria still justify the separation of *Caproventuria* and *Metacoleroa* from *Venturia* is debatable, and the names *Venturia dickiei* (Berk. & Broome) Ces. & de Not. and *Venturia hanliniana* (U. Braun & Feiler) Unter. are available for these organisms. The genus *Apiosporina*, which is based on *Apiosporina collinsii* (Schwein.) Höhn., clusters in the *Venturiaceae*, as was to be expected based on its *Fusicladium* anamorph (Schubert *et al*. 2003). It was distinguished from *Venturia* species by having ascospores strictly septate near the lower end (Sivanesan 1984).

The anamorph genus *Fusicladium* has been monographed by Schubert *et al*. (2003). Morphological as well as molecular studies (Beck *et al*. 2005) demonstrated that the genus *Venturia* with its *Fusicladium* anamorphs is monophyletic. A separation of *Venturia* into various uniform subclades based on the previous anamorph genera *Fusicladium, Pollaccia* and *Spilocaea* was not evident and could be rejected. As in cercosporoid anamorphs of *Mycosphaerella*, features such as the arrangement of the conidiophores (solitary, fasciculate, sporodochial), the proliferation of conidiogenous cells (sympodial, percurrent) and shape, size as well as formation of conidia (solitary, catenate) proved to be of little taxonomic value at generic level. Hence, Schubert *et al*. (2003) proposed to maintain *Fusicladium emend.* as sole anamorph genus for *Venturia*. The genus *Fusicladosporium* Partridge & Morgan-Jones (type species: *Cladosporium carpophilum* Thüm.) (Partridge & Morgan-Jones 2003), recently erected to accommodate fusicladium-like species with catenate conidia, represents a further synonym of *Fusicladium*.

Similar to their occurrence *in vivo* the conidiophores *in vitro* of species previously referred to the genera *Spilocaea* and *Pollaccia* are usually micronematous, conidia often appear to be directly formed on the mycelium, unilocal, determinate, mostly reduced to conidiogenous cells, sometimes forming a few percurrent proliferations, whereas the conidiophores of species of *Fusicladium s. str.* are mostly macronematous, but sometimes also micronematous. They are often initiated as short lateral, peg-like outgrowths of hyphae which proliferate sympodially, becoming slightly geniculate, forming a single, several or numerous subdenticulate to denticulate, truncate, unthickened or only slightly thickened, somewhat darkened-refractive conidiogenous loci.

The genus *Pseudocladosporium* was described to be quite distinct from *Fusicladium* by being saprobic and connected with a different teleomorph, viz. *Caproventuria* (Braun 1998). However, since the type species of *Caproventuria*, *C. hanliniana*, with its anamorph *Pseudocladosporium brevicatenatum* (U. Braun & Feiler) U. Braun clusters together with numerous *Venturia* species, the genus *Pseudocladosporium* should be reduced to synonymy with *Fusicladium*. Morphologically there is no clear delimitation between *Fusicladium* and *Pseudocladosporium*. The typically pseudocladosporium-like habit, characterised by forming solitary conidiophores, often reduced to conidiogenous cells or even micronematous, and conidia formed in long chains, is mainly found in culture, above all in saprobic taxa. The fusicladium-like growth with well-developed macronematous conidiophores is usually more evident *in vivo*, above all in biotrophic taxa. There are, however, all kinds of transitions between these two genera.

Fusicladium africanum Crous, **sp. nov.** MycoBank MB504535. Fig. 19.

Etymology: Named after the continent from which it was collected, Africa.

Fusicladio brevicatenato similis, sed conidiophoris brevioribus, 5–10 µm longis, conidiis minoribus, ad 20 × 3.5 µm, 0(–1)-septatis, locis conidiogenis et hilis angustioribus, 1–1.5 µm latis.

Mycelium composed of smooth, medium brown, branched, septate, 1.5–2 µm wide hyphae, frequently forming hyphal coils. *Conidiophores* reduced to conidiogenous cells, solitary, pale to medium brown, smooth, inconspicuous, integrated in hyphae, varying from small, truncate lateral loci on hyphal cells, 1–1.5 µm wide, to micronematous conidiogenous cells, 5–10 × 2–3 µm; mono- to polyblastic, sympodial, scars inconspicuous, 1 µm wide. *Conidia* in long, branched chains of up to 40, subcylindrical, 0(–1)-septate, pale brown, smooth; hila truncate, 1 µm wide, unthickened, neither darkened nor refractive; ramoconidia (11–)15–17(–20) × 2–3(–3.5) µm; conidia (8–)11–17 × 2–2.5 µm.

Cultural characteristics: Colonies somewhat erumpent, with moderate aerial mycelium and smooth, lobate margins on PDA, ochreous to umber (surface); reverse dark umber; on OA umber; on SNA ochreous. Colonies reaching 9 mm diam on PDA after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **South Africa**, Western Cape Province, Malmesbury, *Eucalyptus* leaf litter, Jan. 2006, P.W. Crous, **holotype** CBS H-19904, cultures ex-type CPC 12828 = CBS 121639, CPC 12829 = CBS 121640.

Notes: *Fusicladium africanum* is a somewhat atypical member of the genus, as its conidial hila are quite unthickened and inconspicuous. Among biotrophic, leaf-spotting *Fusicladium* species a wider morphological variation was found pertaining to the structure of the conidiogenous loci and conidial hila, ranging from being indistinct, unthickened and not darkened-refractive to unthickened or almost so, but somewhat darkened-refractive (Schubert *et al*. 2003). *Fusicladium africanum* was found occurring with *Sympoventuria capensis* Crous & Seifert on *Eucalyptus* leaf litter in South Africa (Crous *et al*. 2007b).

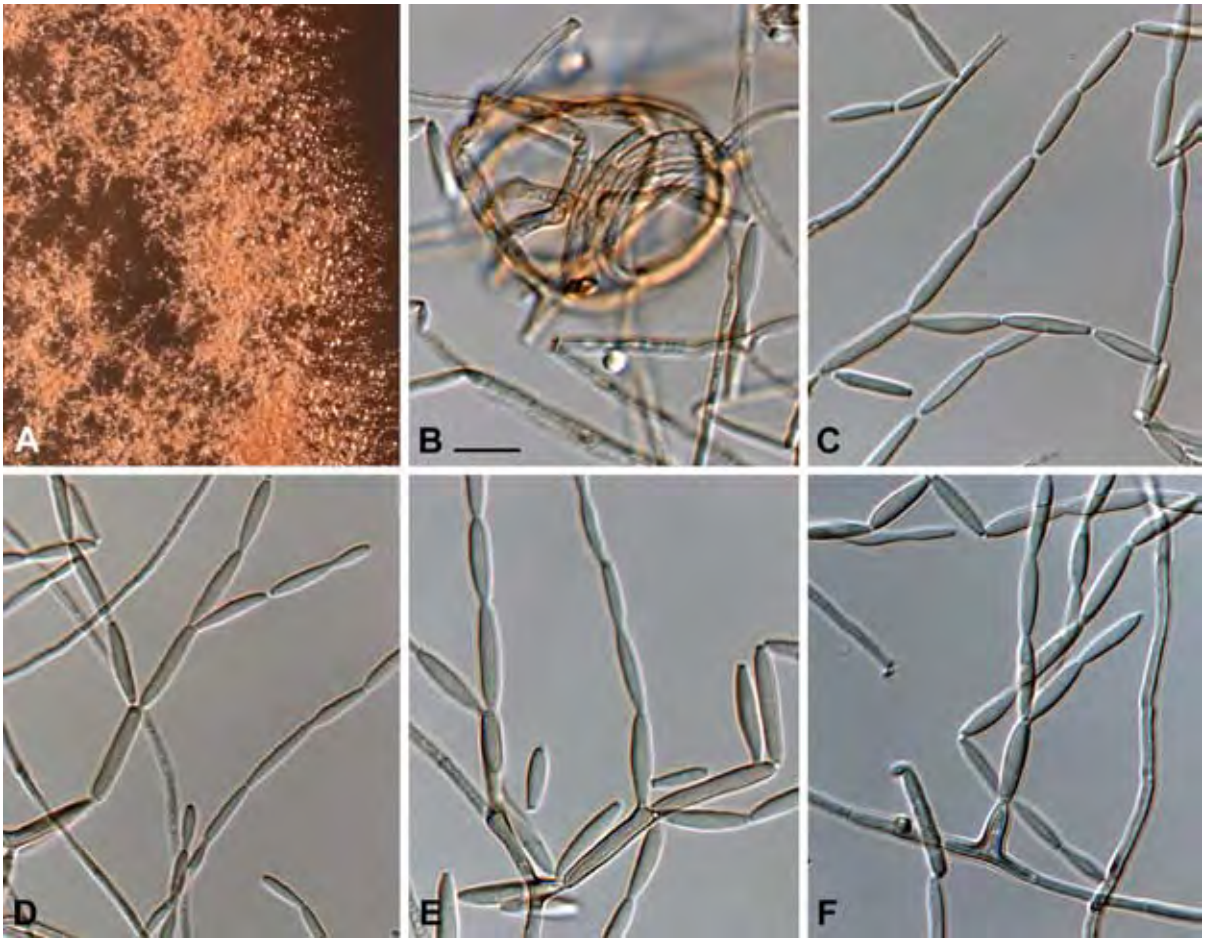

Fig. 19. *Fusicladium africanum* (CPC 12828). A. Colony on MEA. B. Hyphal coil. C. Branched conidial chain. D–F. Conidiophores with catenulate conidia. Scale bar = 10 µm.

Fig. 20. *Fusicladium amoenum* (CBS 254.95). A–E. Conidiophores with conidiogenous loci. F. Conidia. Scale bar = 10 µm.

Fig. 21. *Fusicladium convolvularum* (CBS 112706). A–B, D–I. Conidiophores with conidiogenous loci. C, J–K. Ramoconidia and conidia. Scale bars = 10 µm.

Fusicladium amoenum (R.F. Castañeda & Dugan) Crous, K. Schub. & U. Braun, **comb. nov.** MycoBank MB504536. Fig. 20.

Basionym: *Anungitopsis amoena* R.F. Castañeda & Dugan, Mycotaxon 72: 118. 1999.

≡ *Cladosporium amoenum* R.F. Castañeda, in Untereiner *et al.*, 1998, *nom. nud.*

Specimen examined: **Cuba**, Santiago de Cuba, La Gran Piedra, fallen leaves of *Eucalyptus* sp. (*Myrtaceae*), 2 Nov. 1994, R.F. Castañeda, (Ho *et al.* 1999: 117, figs 2–3) **iconotype**, culture ex-type CBS 254.95 = ATCC 200947 = IMI 367525 = INIFAT C94/155 = MUCL 39143.

Note: In culture *F. amoenum* has a typical pseudocladosporium-like morphology, though the scars are neither prominently thickened, nor refractive.

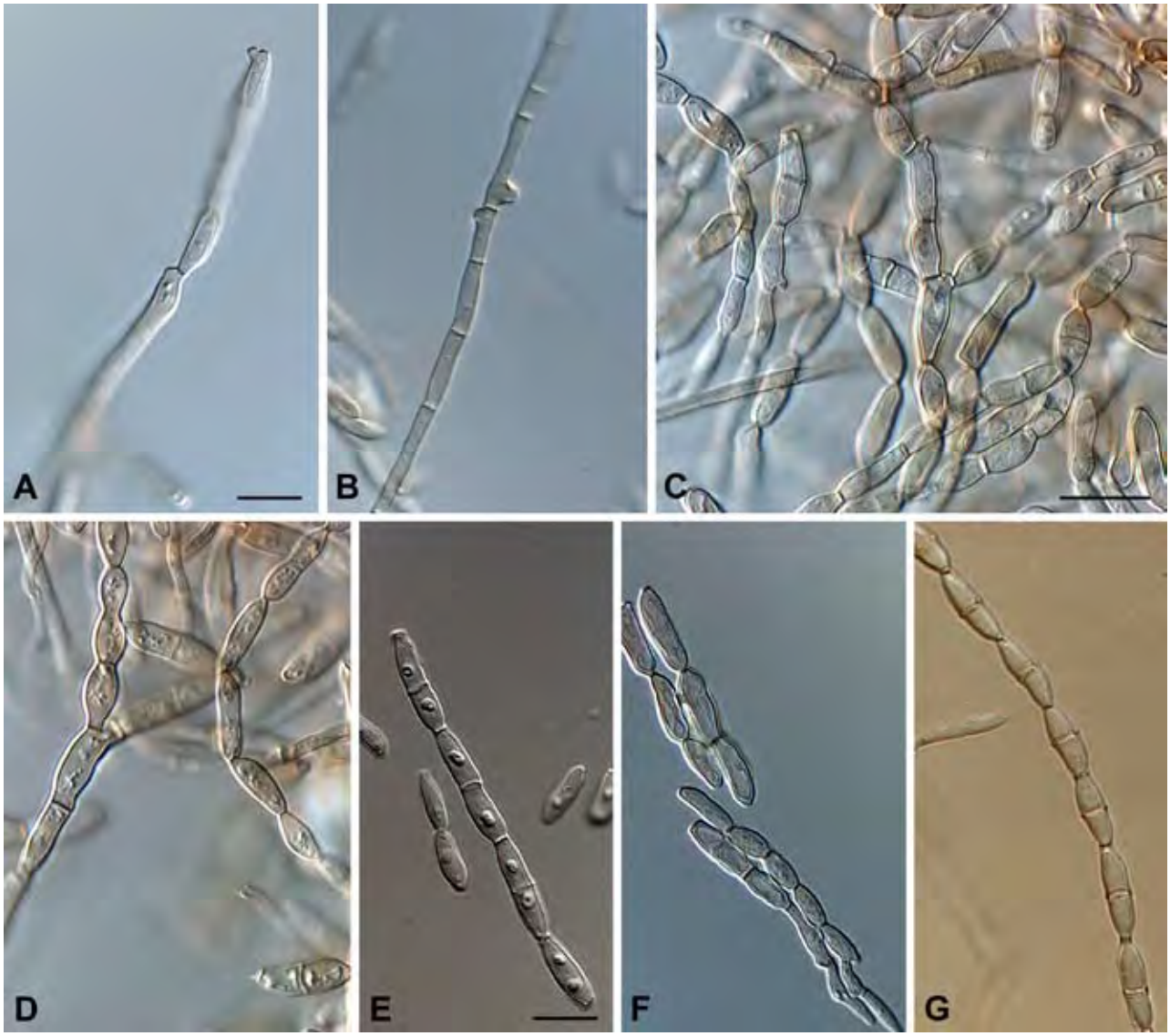

Fig. 22. *Fusicladium fagi* (CBS 621.84). A. Conidiophore with truncate conidiogenous loci. B. Hypha with conidiogenous loci. C–G. Conidial chains. Scale bars = 10 µm.

Fusicladium caruanianum Sacc., Ann. Mycol. 11: 20. 1913.
≡ *Pseudocladosporium caruanianum* (Sacc.) U. Braun, Schlechtendalia 9: 114. 2003.

Fusicladium convolvularum Ondřej, Česká Mycol. 25: 171. 1971. Fig. 21.

In vivo: Schubert *et al.* (2003: 37).

In vitro on SNA: *Mycelium* unbranched or only sparingly branched, 2–3 µm wide, septate, not constricted at septa, subhyaline to pale brown, smooth, walls unthickened or almost so. *Conidiophores* laterally arising from hyphae, erect, straight to somewhat flexuous, sometimes geniculate, unbranched, (6–)12–75 × (2.5–)3–4.5 µm, aseptate or septate, pale brown or pale medium brown, smooth, walls somewhat thickened, sometimes only as short lateral conical prolongations of hyphae, occasionally irregular in shape. *Conidiogenous cells* integrated, terminal or conidiophores reduced to conidiogenous cells, sometimes geniculate, 6–29 µm long, proliferation sympodial, with several denticle-like loci, broadly truncate, 1.5–2(–2.5) µm wide, unthickened, somewhat refractive or darkened. *Ramoconidia* occurring, 20–28 × 5 µm, 0–1-septate, somewhat darker, pale medium brown, with a broadly truncate base, 3–4 µm wide, usually with several denticle-like apical loci. *Conidia* catenate, formed in unbranched or loosely branched chains, straight to sometimes curved, cells sometimes irregularly swollen, fusiform, subcylindrical, sometimes obpyriform, 13–35 × 3.5–5.5(–6) µm, 0–3-septate, occasionally slightly constricted at the median septum, few very large conidia with up to five septa, up to 75 µm long, 4.5–6 µm wide, subhyaline to pale brown, smooth, walls slightly thickened, slightly attenuated towards apex and base, hila broadly truncate, 1–2 µm wide, unthickened or only slightly thickened, somewhat darkened-refractive; microcyclic conidiogenesis occurring, conidia often germinating.

Cultural characteristics: Colonies on PDA spreading, somewhat erumpent, with moderate aerial mycelium and regular, but feathery margins; surface fuscous black, and reverse dark fuscous black. Colonies reaching 15 mm diam after 1 mo on PDA at 25 °C in the dark.

Specimens examined: **Czech Republic**, Libina, okraj pole pod nadrazim (okr. Sumperk), on *Convolvulus arvensis* (*Convolvulaceae*), 7 Sep. 1970, Ondřej, **holotype** BRA. **New Zealand**, on leaves of *Convolvulus arvensis*, 7 Nov. 2000, C.F. Hill, **epitype designated here** CBS H-19911, culture ex-epitype CBS 112706 = CPC 3884 = IMI 383037.

Note: Conidiophores are somewhat longer and narrower *in vitro* than *in vivo*, and ramoconidia occur (Schubert & Braun 2002b, Schubert *et al.* 2003).

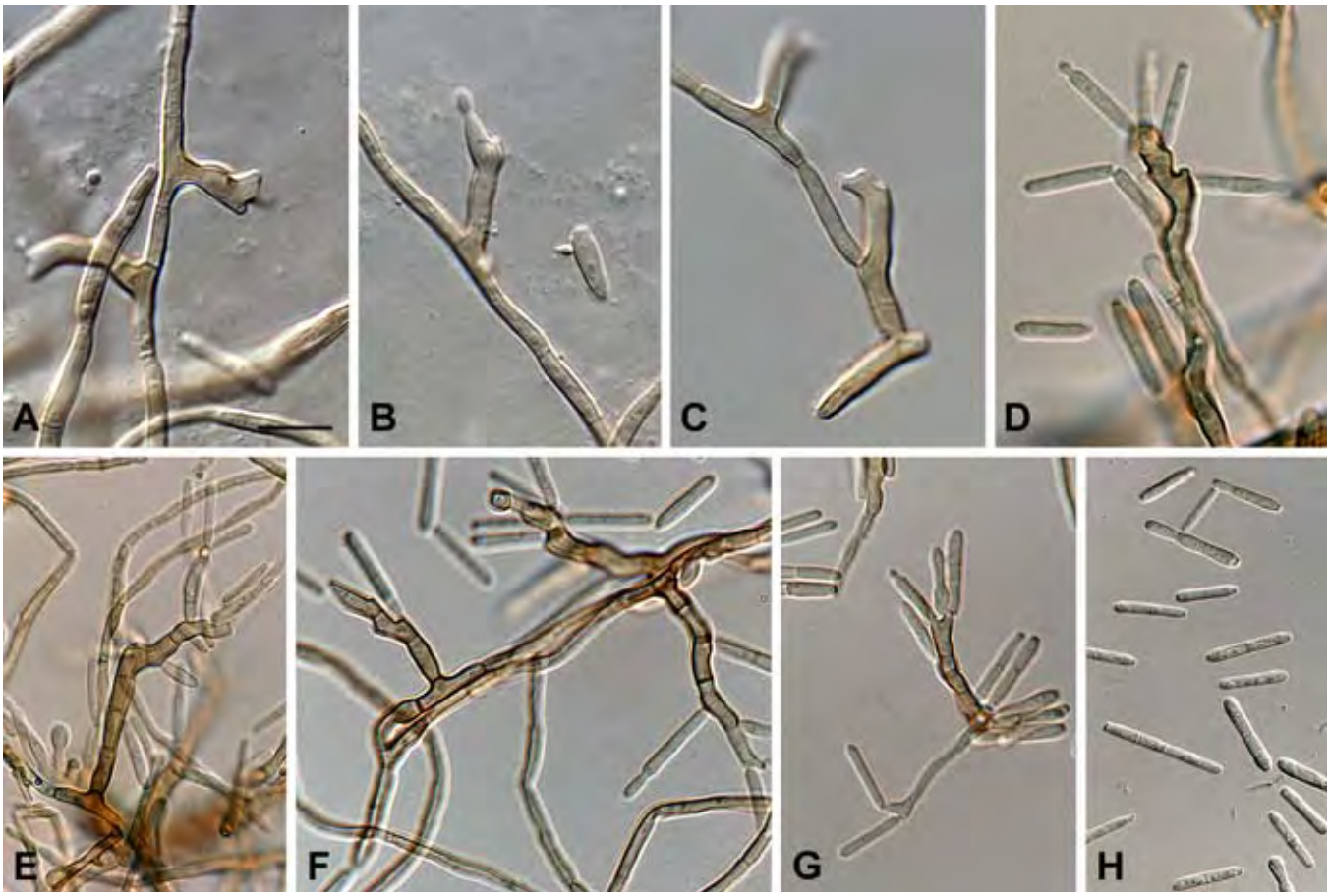

Fig. 23. *Fusicladium intermedium* (CBS 110746). A–G. Conidiophores with sympodial conidiogenous loci. H. Conidia. Scale bar = 10 µm.

Fusicladium fagi Crous & de Hoog, **sp. nov.** MycoBank MB504537. Fig. 22.

Etymology: Named after its host, *Fagus sylvatica*.

Fusicladio brevicatenato similis, sed conidiis secundis minoribus, (8–)11–17(–20) × 3–3.5 µm, locis conidiogenis et hilis angustioribus, 1–1.5 µm latis.

Mycelium consisting of pale to medium brown, smooth to finely verruculose, branched, 2–3 µm wide hyphae. *Conidiophores* integrated, terminal on hyphae, 0–1-septate, mostly reduced to conidiogenous cells, also lateral, visible as small, protruding, denticle-like loci, 10–15 × 2–3.5 µm. *Conidiogenous cells* subcylindrical, 5–15 × 2–3.5 µm, pale to medium brown, smooth to finely verruculose, tapering to 1–3 apical loci, 1–1.5 µm wide; scars inconspicuous. *Conidia* pale brown, smooth, guttulate, subcylindrical to narrowly ellipsoid, occurring in simple or branched chains, 0–1(–2)-septate, tapering towards subtruncate ends, 1.5–2.5 µm wide, aseptate conidia (8–)11–17(–20) × 3–3.5 µm, septate conidia up to 40 µm long and 4 µm wide; hila inconspicuous, i.e. neither thickened nor darkened-refractive; microcyclic conidiation common in older cultures.

Cultural characteristics: Colonies erumpent, spreading, with abundant aerial mycelium on PDA, and feathery to smooth margins; isabelline to patches of fuscous-black due to the absence of aerial mycelium, which collapses with age (surface); reverse fuscous-black. Colonies reaching 50 mm diam after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Netherlands**, Baarn, Maarschalksbosch, decaying leaves of *Fagus sylvatica* (*Fagaceae*), 1 Oct. 1984, G.S. de Hoog, **holotype** CBS H-10366, culture ex-type CBS 621.84 = ATCC 200937.

Notes: Isolate CBS 621.84 was until recently preserved at the CBS as representative of *Cladosporium nigrellum* Ellis & Everh., a species known from bark of *Robinia* sp. in the U.S.A. Morphologically it is, however, quite distinct in having somewhat larger, and more subcylindrical to ellipsoid conidia. Conidia of *C. nigrellum* are fusiform to limoniform, 0–3-septate, 5–15 × 4–7 µm (Ellis 1976), possessing the typical cladosporioid scars with a central convex dome and a periclinal rim which characterise it as a true member of the genus *Cladosporium* Link, which has been confirmed by a re-examination of type material of *C. nigrellum* (on inner bark of railroad ties, U.S.A., West Virginia, Fayette Co., Nuttallburg, 20 Oct. 1893, L.A. Nuttall, Flora of Fayette County No. 172, NY; also Ellis & Everh., N. Amer. Fungi 3086 and Fungi Columb. 382, BPI, NY, PH).

Fusicladium intermedium (Crous & W.B. Kendr.) Crous, **comb. nov.** Mycobank MB504538. Fig. 23.
Basionym: *Anungitopsis intermedia* Crous & W.B. Kendr. S. Afr. J. Bot. 63: 286. 1997.

Specimens examined: **South Africa**, Mpumalanga, from leaf litter of *Eucalyptus* sp. (*Myrtaceae*), Oct. 1992, M.J. Wingfield, PREM 51438 **holotype**. **Madagascar**, Tamatave, *Eucalyptus* leaf litter, Apr. 1994, P.W. Crous, CBS H-19918, **epitype designated here**, culture ex-epitype CPC 778 = IMI 362702 = CBS 110746.

Note: Conidiophores are dimorphic in culture, being macronematous, anungitopsis-like, and micronematous, more pseudocladosporium-like.

Fusicladium matsushimae (U. Braun & C.F. Hill) Crous, U. Braun & K. Schub., **comb. nov.** Mycobank MB504539.
Basionym: *Pseudocladosporium matsushimae* U. Braun & C.F. Hill, Australas. Pl. Pathol. 33: 492. 2004.

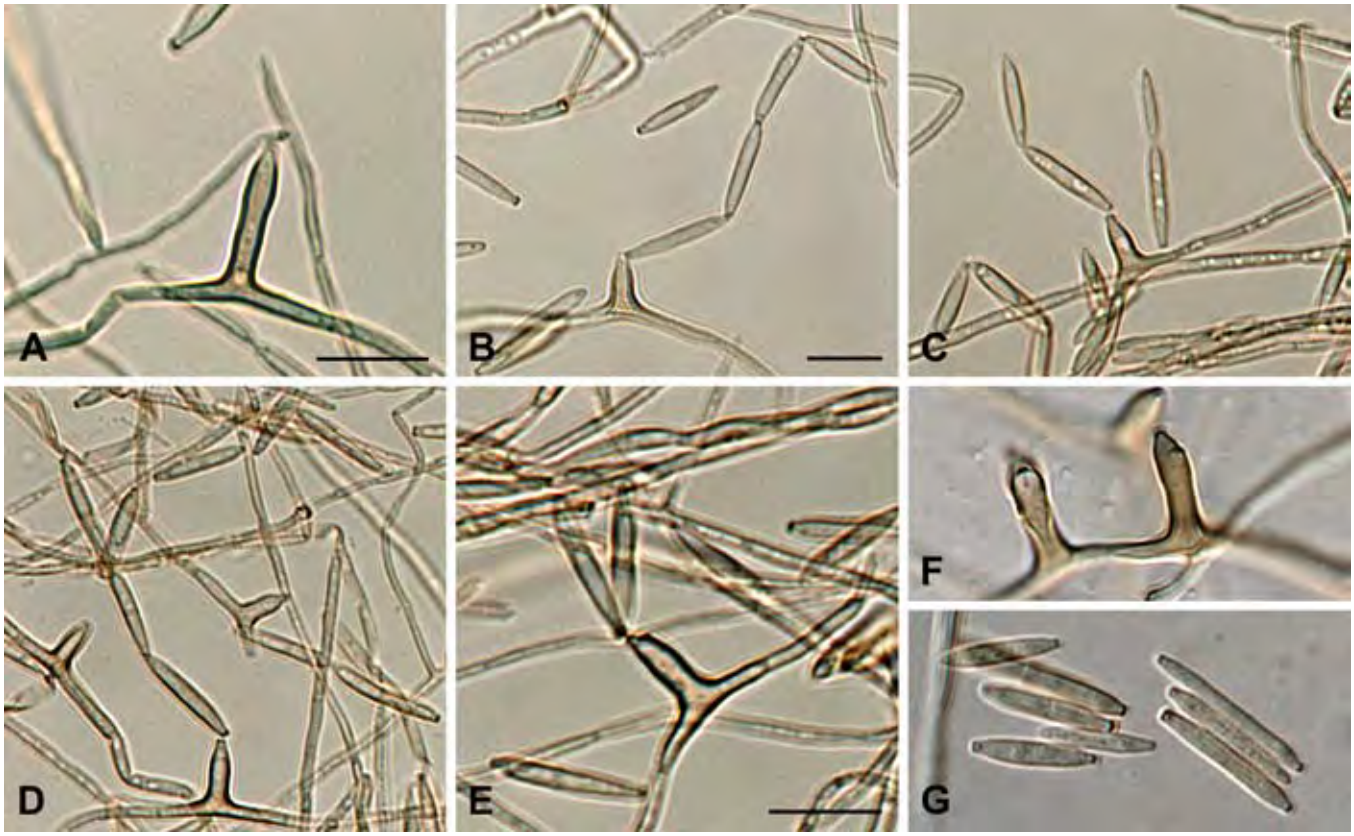

Fig. 24. *Fusicladium pini* (CBS 463.82). A–F. Conidiogenous cells with conidiogenous loci. G. Conidia. Scale bars = 10 µm.

Fusicladium mandshuricum (M. Morelet) Ritschel & U. Braun, Schlechtendalia 9: 62. 2003.
Basionym: *Pollaccia mandshurica* M. Morelet, Ann. Soc. Sci. Nat. Archéol. Toulon Var 45(3): 218. 1993.
= *Pollaccia sinensis* W.P. Wu & B. Sutton, in herb. (IMI).
Teleomorph: ***Venturia mandshurica*** M. Morelet, Ann. Soc. Sci. Nat. Archéol. Toulon Var 45(3): 219. 1993.

In vivo: Schubert *et al.* (2003: 62).

In vitro on OA: *Mycelium* loosely branched, filiform to narrowly cylindrical-oblong, 1–4 µm wide, later somewhat wider, up to 7 µm, septate, sometimes slightly constricted at the septa, sometimes irregular in outline due to small swellings, subhyaline to pale brown, smooth, walls unthickened, sometimes aggregating, forming compact conglomerations of slightly swollen hyphal cells. *Conidiophores* usually reduced to conidiogenous cells, arising terminally or laterally from hyphae, subcylindrical to cylindrical, unbranched, 9–20 × (2.5–)4–5(–6) µm, aseptate, very rarely 1-septate, very pale brown, smooth, walls unthickened, monoblastic, unilocal, determinate, later occasionally becoming percurrent, enteroblastically proliferating, forming a few (up to five) annellations, loci broadly truncate, (2–)3–5 µm wide, unthickened, not darkened. *Conidia* solitary, straight to curved, fusiform to obclavate, distinctly apiculate, 24–45(–57) × (6–)7–9(–10.5) µm, (1–)2–4(–5)-septate, more or less constricted at septa, sometimes up to 85 µm long with up to 7 septa, septa often somewhat darkened, second cell often bulging, pale medium to medium olivaceous-brown or brown, smooth, walls somewhat thickened, somewhat attenuated towards the base, hilum broadly truncate, (2–)3–5 µm wide, unthickened, not darkened; microcyclic conidiogenesis not observed.

Cultural characteristics: Colonies on OA iron-grey to olivaceous-grey due to aerial mycelium and sporulation (surface); reverse iron-grey to black, somewhat velvety; margin glabrous, olivaceous; aerial mycelium sparsely formed, loose, diffuse; sporulating.

Specimens examined: **China**, Liaoning, on *Populus simonii* × *P. nigra*, 17 Jun. 1992, M. Morelet, **holotype** PC (PFN 1466); *P. simonii*, 20 Apr. 1993, **epitype designated here** CBS H-19912, culture ex-epitype CBS 112235 = CPC 3639 = MPFN 307.

Note: Conidiophores are densely fasciculate *in vivo*, forming sporodochial conidiomata, cylindrical to ampulliform, 5–7 × 6–7.5 µm (Schubert *et al.* 2003).

Fusicladium pini Crous & de Hoog, **sp. nov.** MycoBank MB504540. Fig. 24.

Etymology: Named after its host, *Pinus*.

Fusicladio africano similis, sed conidiis minoribus, (6–)10–12(–17) × 1.5–2(–2.5) µm, locis conidiogenis et hilis angustioribus, 0.5–1 µm latis.

Mycelium consisting of smooth, medium brown, branched, 1.5–2 µm wide hyphae, giving rise to solitary, micronematous conidiophores. *Conidiophores* reduced to conidiogenous cells, medium to dark brown, erect, thick-walled, smooth, subcylindrical, widest at the base, tapering to a subtruncate apex, 5–15 × 2–3 µm; scars flat-tipped, somewhat darkened and thickened, one to several in the apical region, somewhat protruding, 0.5–1 µm wide. *Conidia* in branched or unbranched chains of up to 15, medium brown, smooth, subcylindrical, 0–1-septate, widest in the middle, tapering to subtruncate ends, straight to slightly curved, (6–)10–12(–17) × 1.5–2(–2.5) µm; hila somewhat darkened and thickened, not refractive, 0.5–1 µm wide.

Cultural characteristics: Colonies erumpent, with sparse aerial mycelium and smooth margins on PDA, greyish sepia (surface); reverse fuscous-black; on OA patches of greyish sepia and fuscous-black (surface); on SNA umber (surface). Colonies reaching 15 mm diam on PDA after 1 mo at 25 °C in the dark; colonies fertile.

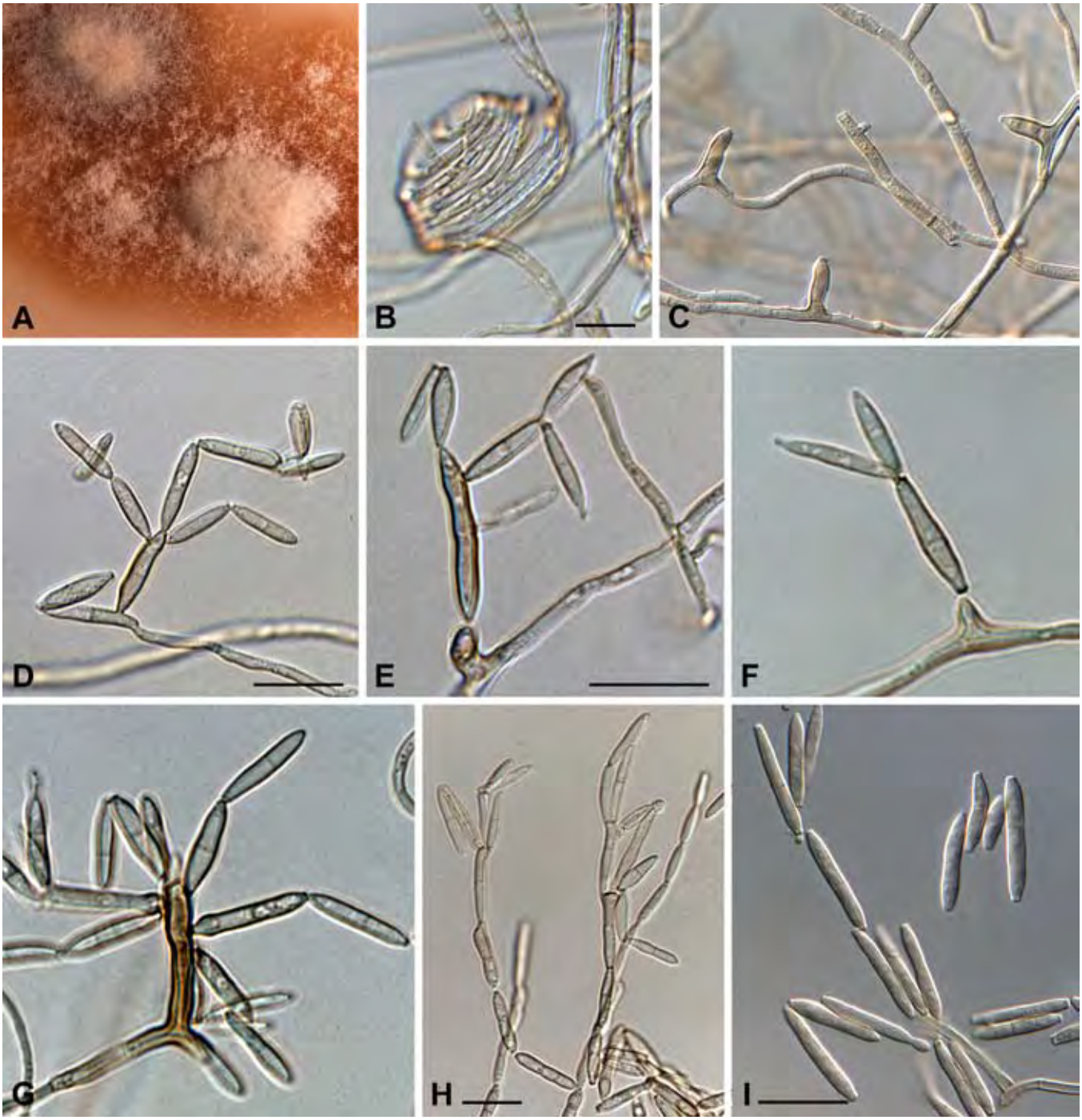

Fig. 25. *Fusicladium ramoconidii* (CBS 462.82). A. Colony on OA. B. Hyphal coil. C–F. Conidiophores reduced to conidiogenous cells. G–H. Conidiophores. I. Conidia. Scale bars = 10 µm.

Specimen examined: **Netherlands**, Baarn, De Vuursche, needle of *Pinus sylvestris* (*Pinaceae*), 12 Apr. 1982, G.S. de Hoog, **holotype** CBS H-1610, culture ex-type CBS 463.82.

Notes: This fungus was originally maintained in the CBS collection as *Anungitea uniseptata* Matsush. In culture, however, only a pseudocladosporium-like state was observed. Conidiophores are reduced to conidiogenous cells, and have several apical loci as in *Fusicladium*, but are not subdenticulate; scars are somewhat darkened and thickened, not refractive. Conidia of *F. africanum* are (8–)11–17(–20) × 2–3(–3.5) µm, thus similar, but somewhat larger than the mean conidial size range (10–12 × 1.5–2 µm) observed in *F. pini*. The conidiogenous loci and conidial hila of *F. africanum* are also somewhat larger. Although the LSU sequence of *F. pini* is identical to that of *F. ramoconidii*, the ITS sequence similarity is 97 % (572/585 nucleotides).

Fusicladium ramoconidii Crous & de Hoog, **sp. nov.** MycoBank MB504541. Figs 25–26.

Etymology: Named after the presence of its characteristic ramoconidia.

Fusicladio brevicatenato similis, sed ramoconidiis minoribus, (12–)15–17(–20) × 2(–3) µm, locis conidiogenis et hilis minoribus, 0.5–1 µm diam.

Mycelium consisting of branched, septate, 1.5–2 µm wide hyphae, pale brown, smooth, frequently with hyphal coils. *Conidiophores* integrated into hyphae, and reduced to small, lateral protruding conidiogenous cells, concolorous with hyphae, or macronematous, dark brown, erect, thick-walled, 10–40 × 3–4 µm, 0–3-septate. *Conidiogenous cells* terminal, integrated, subcylindrical, tapering to a rounded apex, concolorous with hyphae (as hyphal pegs), or dark

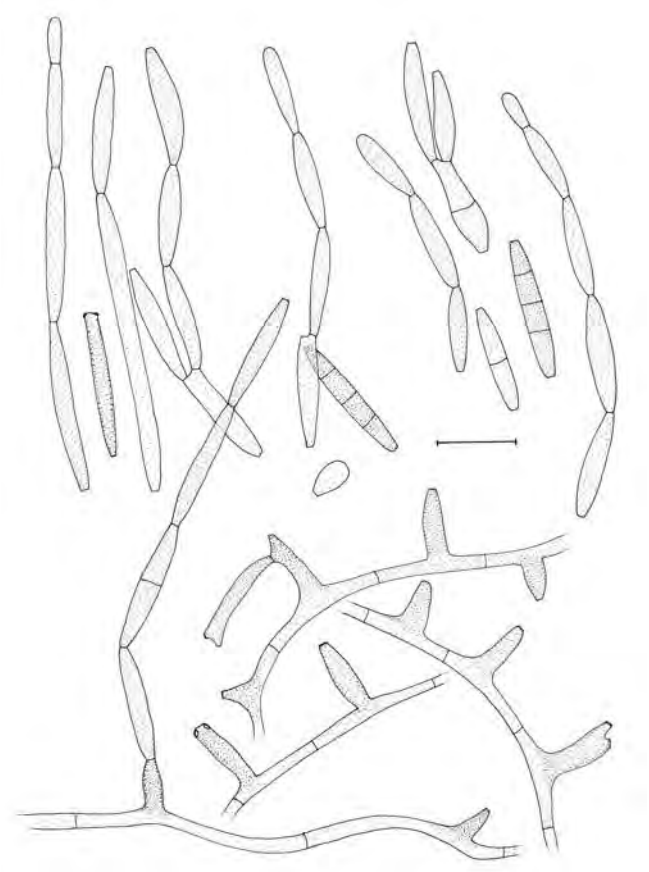

Fig. 26. *Fusicladium ramoconidii* (CBS 462.82). Conidiogenous cells with ramoconidia and conidia. Scale bar = 10 µm.

brown on mononematous conidiophores, smooth, 3–15 × 2–3(–4) µm; proliferating sympodially, loci slightly thickened, darkened and refractive, 0.5–1 µm wide. *Conidia* occurring in branched chains, narrowly ellipsoid to subcylindrical, pale olivaceous, guttulate; ramoconidia (0–)1(–3)-septate, (12–)15–17(–20) × 2(–3) µm; conidia occurring in short chains (–15), 0–1-septate, (8–)10–12(–16) × 2(–3) µm; hila slightly thickened and darkened, not refractive, 0.5–1 µm wide.

Cultural characteristics: Colonies erumpent, with sparse aerial mycelium and smooth margins on PDA, hazel to fawn (surface), with a thin, submerged margin; reverse brown-vinaceous; on OA hazel to fawn (surface) with a wide, fawn, submerged margin. Colonies reaching 25 mm diam on PDA after 1 mo at 25 °C in the dark; colonies fertile.

Specimen examined: **Netherlands**, Baarn, De Vuursche, needle of *Pinus* sp. (*Pinaceae*), 12 Apr. 1982, G.S. de Hoog, **holotype** CBS H-19908, culture ex-type CBS 462.82.

Notes: This strain has been deposited in the CBS collection as *Pseudocladosporium hachijoense* (Matsush.) U. Braun. However, its ramoconidia and conidia are smaller than those cited by Matsushima (1975) (ramoconidia up to 30 µm long, conidia 10–21 × 2–4 µm). Although it clusters with *F. pini* in the LSU phylogeny, there are 13 bp differences in their ITS sequence data. Furthermore, *F. ramoconidii* has ramoconidia which are absent in *F. pini*, and has a faster growth rate, and hazel to fawn colonies, compared to the greyish sepia colonies of *F. pini*. The well-developed, septate conidiophores and ramoconidia are reminiscent of *F. brevicatenatum*, which differs, however, by its longer and wider ramoconidia, up to 30 × 6(–7) µm, as well as larger conidiogenous loci and conidial hila, 1.5–3 µm diam.

Fusicladium rhodense Crous & M.J. Wingf., **sp. nov.** MycoBank MB504542. Fig. 27.

Etymology: Named after the Greek Island, Rhodos, where it was collected.

Fusicladio africano similis, sed locis conidiogenis angustioribus, 1.5–2 µm latis, et differt a F. pini ramoconidiis formantibus.

Mycelium consisting of smooth to finely roughened, medium brown, branched, septate, 1.5–3 µm wide hyphae, frequently forming hyphal coils, giving rise to solitary, micronematous conidiophores. *Conidiophores* reduced to conidiogenous cells that are terminal or lateral on hyphae, medium brown, smooth, subcylindrical, subdenticulate, erect, or more distinct, up to 15 µm tall, 1.5–2 µm wide, mono- to polyblastic; scars flat-tipped, somewhat darkened and thickened, but not refractive. *Conidia* in branched or unbranched chains of up to 15, pale brown in younger conidia, becoming medium brown, smooth, subcylindrical, 0–3-septate, tapering slightly towards the subtruncate ends, straight, but at times slightly curved, (8–)12–16(–20) × (2–)2.5–3(–4) µm; ramoconidia (0–)1(–3)-septate, 12–20 × 3–4 µm; conidia (0–)1-septate, 8–17 × 2–3 µm; hila somewhat darkened and thickened, not refractive, 1–1.5 µm wide.

Cultural characteristics: Colonies spreading, somewhat erumpent, with moderate aerial mycelium and crenate margins on PDA, uneven, greyish sepia (surface), margins fuscous-black; reverse fuscous-black; on OA smooth, spreading, with sparse aerial mycelium and even, regular margins, greyish sepia; on SNA spreading, smooth, even margins, sparse aerial mycelium, greyish sepia (surface). Colonies reaching 9 mm diam on PDA after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **Greece**, Rhodos, on branches of *Ceratonia siliqua* (*Fabaceae*), 1 Jun. 2006, P.W. Crous & M.J. Wingfield, **holotype** CBS H-19910, culture ex-type CBS 121641 = CPC 13156.

Note: *Fusicladium rhodense* has a typical pseudocladosporium-like morphology in culture, with conidial scars that are somewhat darkened and thickened.

Venturia hanliniana (U. Braun & Feiler) Unter., Mycologia 89: 129. 1997.

Basionym: *Capronia hanliniana* U. Braun & Feiler, Microbiol. Res. 150: 90. 1995.

≡ *Caproventuria hanliniana* (U. Braun & Feiler) U. Braun, in Braun, *A Monograph of Cercosporella, Ramularia and Allied Genera (Phytopathogenic Hyphomycetes)* 2: 396. 1998.

Anamorph: ***Fusicladium brevicatenatum*** (U. Braun & Feiler) Crous, U. Braun & K. Schub., **comb. nov.** MycoBank MB504543.

Basionym: *Cladophialophora brevicatenata* U. Braun & Feiler, Microbiol. Res. 150: 84. 1995.

≡ *Pseudocladosporium brevicatenatum* (U. Braun & Feiler) U. Braun, in Braun, *A Monograph of Cercosporella, Ramularia and Allied Genera (Phytopathogenic Hyphomycetes)* 2: 393. 1998.

Venturia hystrioides (Dugan, R.G. Roberts & Hanlin) Crous & U. Braun, **comb. nov.** MycoBank MB504544. Fig. 28.

Basionym: *Capronia hystrioides* Dugan, R.G. Roberts & Hanlin, Mycologia 87: 713. 1995.

≡ *Caproventuria hystrioides* (Dugan, R.G. Roberts & Hanlin) U. Braun, in Braun, *A monograph of Cercosporella, Ramularia and allied genera (Phytopathogenic Hyphomycetes)*. Vol. 2: 396. 1998.

Anamorph: ***Fusicladium*** sp.

Only the anamorph was observed on OA, PDA and SNA in culture.

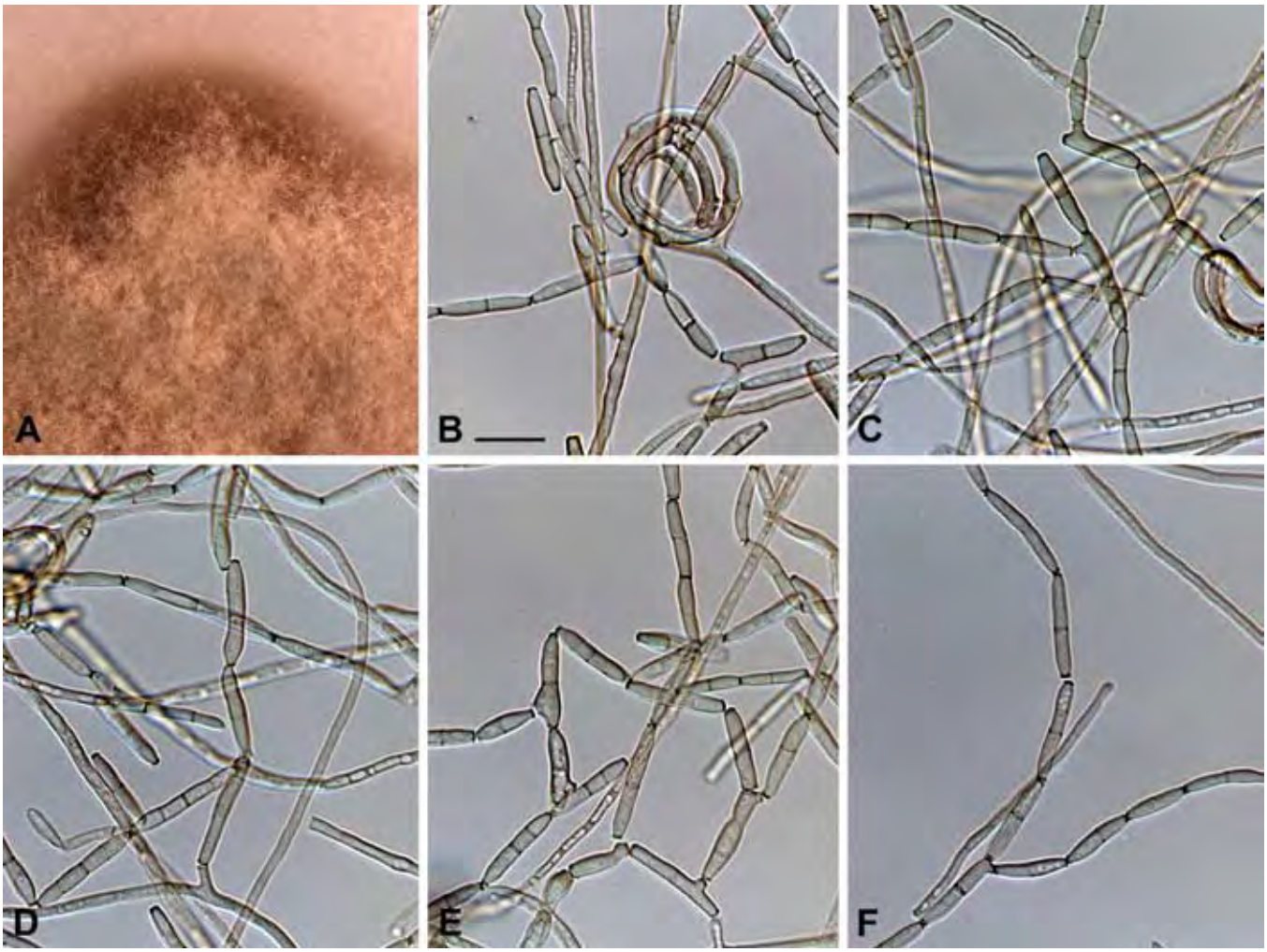

Fig. 27. *Fusicladium rhodense* (CPC 13156). A. Colony on OA. B. Conidial chains and hyphal coil. C–F. Chains of ramoconidia and conidia. Scale bar = 10 µm.

Fig. 28. *Venturia hystrioides* (CBS 117727). A. Conidiophores giving rise to catenulate conidia. B. Ramoconidium giving rise to conidia. C–D. Conidial chains. E. Conidia and conidiogenous cell with conidiogenous loci. F. Ramoconidium. G. Conidia. Scale bars = 10 µm.

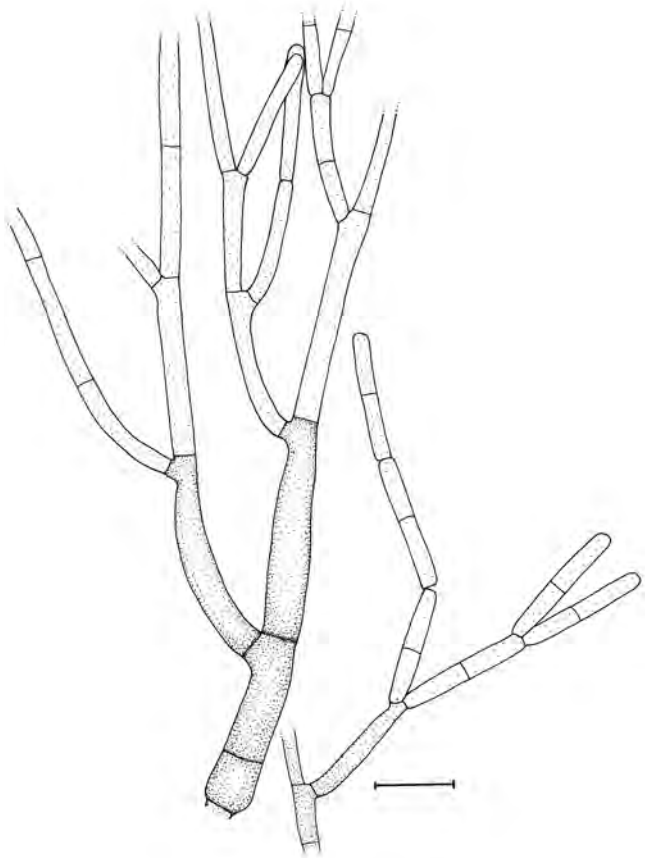

Fig. 29. *Polyscytalum fecundissimum* (CBS 100506). Conidiophores giving rise to catenulate conidia. Scale bar = 10 µm.

Mycelium consisting of branched, septate, smooth, guttulate, 1.5–2.5 µm wide hyphae, pale brown, forming hyphal strands. *Conidiophores* mostly reduced to conidiogenous cells, or if present, micronematous, consisting of a supporting cell, and single conidiogenous cell. *Conidiogenous cells* integrated in hyphae as lateral loci, or terminal, frequently disarticulating, subcylindrical, pale to medium brown, smooth, mono- to polyblastic, loci 1–1.5 µm wide, 2.5 µm tall; conidiogenous cells subcylindrical, up to 40 µm tall, and 2–2.5 µm wide. *Conidia* in long chains of up to 60, branched or not, subcylindrical to narrowly ellipsoid, pale olivaceous to pale brown, smooth; ramoconidia 0–1(–3)-septate, 15–20(–30) × 2–3(–3.5) µm; conidia 0(–1)-septate, 6–8(–12) × 2–3(–3.5) µm; hila 1–1.5 µm wide, inconspicuous to somewhat darkened, subtruncate.

Cultural characteristics: Colonies erumpent, with sparse aerial mycelium on PDA, and smooth, even margins; olivaceous-grey to iron-grey (surface); reverse greenish black; on OA dark mouse-grey (surface), with even, smooth margins. Colonies reaching 40 mm diam after 2 wk at 25 °C in the dark; colonies fertile.

Specimen examined: **U.S.A.**, Washington, Wenatchee, on bing cherry fruit, *Prunus avium* cv. *Bing* (*Rosaceae*), R.G. Roberts, culture ex-type, ATCC 96019 = CBS 117727.

Note: Dugan *et al.* (1995) commented that although similar to *"Phaeoramularia" hachijoensis*, the conidia of this species were predominantly aseptate and somewhat shorter than those described by Matsushima (1975).

Excluded taxa

Polyscytalum fecundissimum Riess, Bot. Zeitung (Berlin) 11: 138. 1853. Fig. 29.

Cultural characteristics: Colonies erumpent, spreading, aerial mycelium sparse, margins smooth; colonies sienna to umber on PDA, with patches of greyish sepia; reverse chestnut-brown; on OA whitish due to moderate aerial mycelium, with diffuse umber pigment in the agar; whitish on SNA. Colonies reaching 15 mm diam on PDA after 3 wk at 25 °C in the dark.

Specimen examined: **Netherlands**, Schovenhorst, leaf litter of *Fagus sylvatica* (*Fagaceae*), 8 Nov. 1997, W. Gams, CBS H-6049, culture CBS 100506.

Notes: *Polyscytalum fecundissimum* is the type species of the genus *Polyscytalum*. Several isolates of this species were investigated here to determine if *Polyscytalum* would be available for taxa that have a pseudocladosporium-like morphology. The clustering of CBS 681.74 within the *Venturiaceae* was surprising. However, this culture proved to be sterile, and therefore its identity could not be confirmed.

Isolate CBS 109882 sporulated profusely. Colonies were grey-olivaceous with olivaceous margins on PDA; conidiophores pale, and not dark brown as depicted for *Polyscytalum* in Ellis (1971); conidial chains were greenish yellow in mass, and pale olivaceous-green under the dissecting microscope, somewhat roughened, polyblastic; on ITS sequence this isolate is identical to U57492, *Cistella acuum* (Alb. & Schwein.) Svrček (*Helotiales*), but the latter species should have a phialidic anamorph, so it is possible that this GenBank sequence is incorrect. The identity of CBS 109882 therefore remains unresolved.

Although isolate CBS 100506 is poorly sporulating, illustrations made *in vitro* when it was collected show this isolate to be authentic for the species and the genus *Polyscytalum*. Based on its LSU sequence, it is allied to *Phlogicylindrium eucalypti* Crous, Summerb. & Summerell (CBS 120080; Summerell *et al*. 2006), and is therefore unrelated to the *Venturiaceae*.

Zeloasperisporium R.F. Castañeda, Mycotaxon 60: 285. 1996, **emend.**

Hyphomycetes. *Mycelium* mostly superficial, hyphae septate, brown to olivaceous. *Hyphopodia* absent. *Conidiophores* differentiated, mononematous, erect, aseptate or septate, brown to olivaceous. *Conidiogenous cells* integrated, terminal, proliferation sympodial, polyblastic, with subdenticulate, somewhat thickened and darkened scars. *Conidia* solitary, fusiform to obclavate or cylindrical, septate, asperulate to verrucose, olivaceous to brown, tips always hyaline, thinner-walled and smooth, forming mucoid appandages, often only visible as a thickened frill. *Synanamorph* present, micronematous. *Conidiogenous cells* short cylindrical, antenna or hyphopodium-like, phialidic, colarette sometimes present, aseptate, subhyaline. *Conidia* solitary, obovoid, ellipsoid, aseptate, brown to olivaceous, verruculose.

Zeloasperisporium hyphopodioides R.F. Castañeda, Mycotaxon 60: 285. 1996. Fig. 30.

In vitro on OA: *Mycelium* internal to superficial, unbranched to sparingly branched, 1.5–3 µm wide, loosely septate, septa almost invisible, pale brown, smooth to asperulate, minutely verruculose, walls unthickened, sometimes inflated at the base of conidiophores.

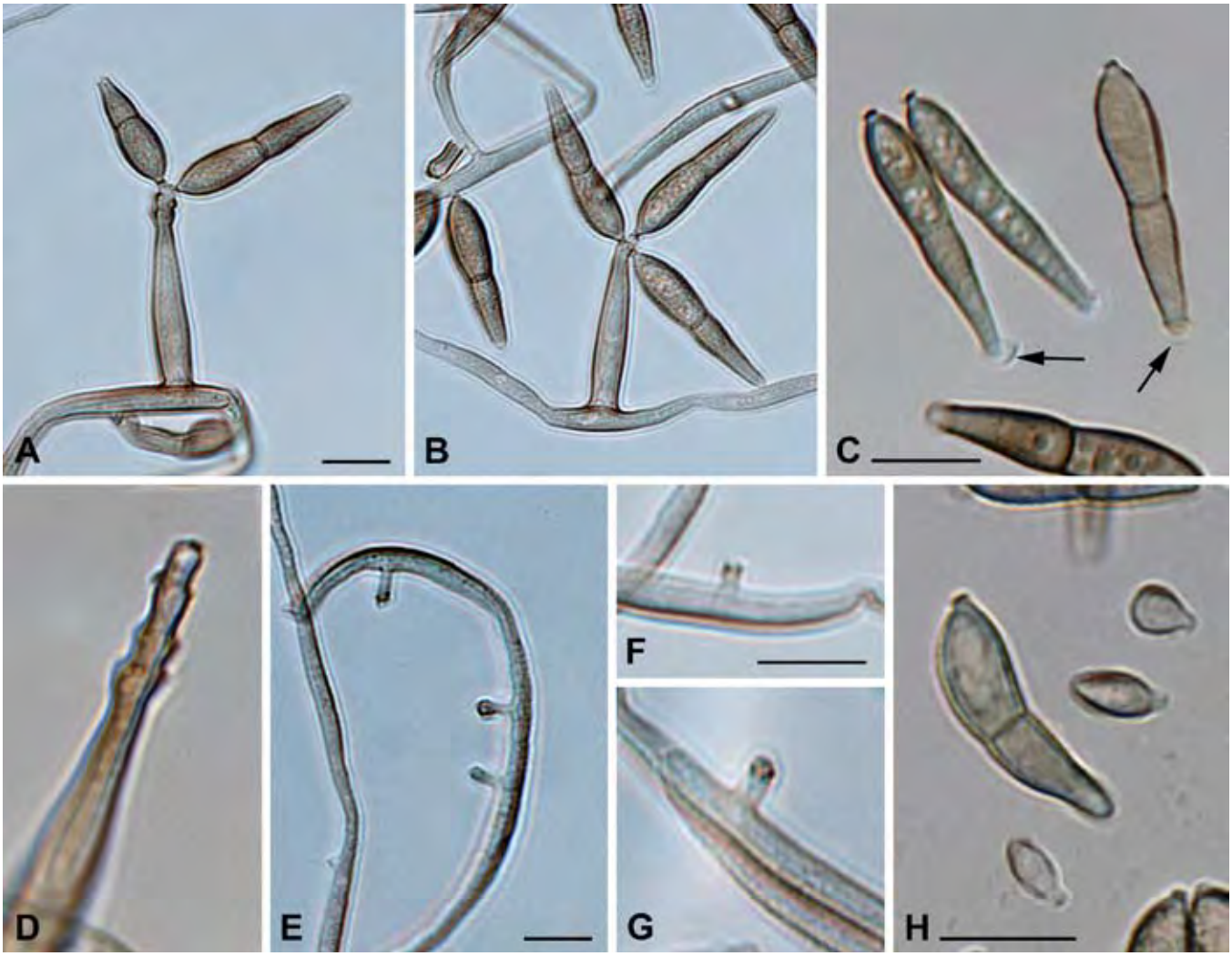

Fig. 30. *Zeloasperisporium hyphopodioides* (CBS 218.95). A–B. Conidiogenous cells. C. Conidia with apical mucoid caps. D. Conidiogenous cell with sympodial proliferation. E–G. Conidiogenous cells of micronematous synanamorph. H. Conidia, and microconidia of synanamorph. Scale bars = 10 µm.

Conidiophores macronematous, arising usually laterally from plagiotropous hyphae, erect, straight, subcylindrical or conical, not geniculate, usually unbranched, rarely branched, 13–45 × 3–4(–5) µm, slightly to distinctly attenuated towards the apex, tapered, aseptate, rarely with a single septum, pale brown to pale medium brown, smooth or minutely verruculose, walls unthickened, often somewhat constricted near the base. *Conidiogenous cells* integrated or conidiophores usually reduced to conidiogenous cells, subcylindrical to conical, proliferation sympodial, with a single or several subdenticulate to denticulate conidiogenous loci mostly crowded at or towards the apex, protuberant, truncate, 0.8–1.2 µm wide, thickened and darkened-refractive. *Conidia* solitary, straight to curved, ellipsoid, fusiform to obclavate, distinctly tapered towards the apex, apiculate, (12–)15–32 × 3.5–5.5 µm, (0–)1–2(–3)-septate, mainly 1-septate, usually constricted at the septa, pale brown to pale medium brown, asperulate to verruculose, walls unthickened or almost so, tips always hyaline, thinner-walled and smooth, forming mucoid appendages, often only visible as a thickened frill, base somewhat rounded or slightly bulbous, hila often situated on short peg-like prolongations, truncate, 0.8–1(–1.2) µm wide, thickened, darkened-refractive; microcyclic conidiogenesis occurring, conidia forming secondary conidiophores.

Synanamorph micronematous. *Conidiophores* reduced to conidiogenous cells, numerous, occurring as short lateral prolongations of hyphae, antenna or telescope-like, cylindrical, unbranched, conidiogenesis unclear, at times appearing phialidic, or having one to two apical scars; up to 5 µm long, 1–1.5 µm wide, aseptate, subhyaline, smooth. *Conidia* of the micronematous anamorph quite different from the conidia formed by the macronematous conidiophores, solitary, obovoid, ellipsoid to somewhat fusiform, 5–9 × 2.5–3 µm, aseptate, pale to pale medium brown, verruculose, somewhat attenuated towards the base, hila flat, unthickened to somewhat thickened, appearing to have the ability to form a slime appendage at the apex.

Cultural characteristics: Colonies on OA iron-grey to olivaceous due to abundant sporulation (surface); reverse black, velvety; margin regular to undulate, feathery; aerial mycelium absent or sparse, sporulation profuse.

Specimen examined: **Cuba**, isolated from air, 2 Oct. 1994, R.F. Castañeda, INIFAT C94/114, **holotype**, CBS-H 5624, H-5639, **isotypes**, culture ex-type CBS 218.95 = INIFAT C94/114 = MUCL 39155 = IMI 367520.

Notes: Within the course of the recent phylogenetic studies in *Herpotrichiellaceae* and *Venturiaceae* the type culture of *Zeloasperisporium hyphopodioides* has been included since it was deposited at the CBS as "*Fusicladium hyphopodioides*". When the culture was re-examined, the described short appressorium-like, inflated hyphopodia with slightly warted to lobed apices (Castañeda *et al.* 1996) could be recognised as conidiogenous cells of a synanamorph forming a second conidial type. In addition,

the conidial tips are hyaline, unthickened and smooth, and have the ability to form mucoid appendages that are often only visible as a thickened frill. These two features, viz., the synanamorph and the conidia with mucoid appendages, easily distinguish this genus from morphologically similar genera such as *Fusicladium*, *Asperisporium* Maubl., and *Passalora* Fr. Phylogenetically *Zeloasperisporium* clusters basal to the *Venturiaceae*.

DISCUSSION

The present paper was initiated to clarify the status of *Cladophialophora* and *Pseudocladosporium* spp., which appear morphologically similar. Confusion occurs when strains with this morphology are identified based solely on microscopic and cultural characteristics. The results clarify that *Cladophialophora* is allied to the *Herpotrichiellaceae* and *Pseudocladosporium* (= *Fusicladium*) to the *Pleosporales* (*Dothideomycetes*). The plant-pathogenic *Cladophialophora* species compose a separate clade within the order (Fig. 1). Another, somewhat remote chaetothyrialean clade contains extremotolerant, rock-inhabiting species around the genus *Coniosporium* Link (Cluster 5 of Haase *et al.* 1999). Both clades are significantly distinct from the prevalently hyperparasitic or oligotrophic, frequently opportunistic species of the remainder of the order (Fig. 1). This remainder includes all *Capronia* teleomorphs sequenced to date, and is thus likely to represent the family *Herpotrichiellaceae*. The ecological trends in each of the main clades of *Chaetothyriales* are thus quite different (Braun 1998).

Several novelties are introduced within the preponderantly plant-associated clade of *Chaetothyriales*, including two new species associated with leaf spots. *Cladophialophora* is distinguished from *Polyscytalum*, which clusters outside the *Herpotrichiellaceae*, and appears allied to *Phlogicylindrium* Crous, Summerb. & Summerell, a recently introduced genus for species occurring on *Eucalyptus* leaves (Summerell *et al*. 2006). Surprisingly *Heteroconium chaetospira* clusters in the *Herpotrichiellaceae*, and is placed in *Cladophialophora* as a distinctively pigmented member of the genus. Some species of *Cladophialophora* and *Exophiala* are newly described from a range of substrates such as fruit juices, drinking water and leaf litter, revealing the potential of these materials as ecological sources of inoculum for taxa associated with opportunistic human and animal infections.

Furthermore, *Pseudocladosporium* belongs to the *Venturiaceae*, and is best treated as a synonym of *Fusicladium*, along with other genera as proposed by Schubert *et al.* (2003) and Beck *et al.* (2005). Although numerous isolates of the *Venturiaceae* were included for study, it was surprising to find relatively little variation within the family, suggesting that previously proposed teleomorph genera such as *Apiosporina*, *Metacoleroa* and *Caproventuria* should be best treated as synonyms of *Venturia*. The *Venturiaceae* is further extended with the inclusion of a novel sister clade of hyphomycetes with a pseudocladosporium-like morphology, which are also referred to as *Fusicladium*, thus widening the generic concept of the latter to encompass all pseudocladosporium-like anamorphs within the family. Some species assigned to *Anungitopsis* proved to cluster within the *Venturiaceae*, but the type species of the latter genus, *A. speciosa*, clustered elsewhere and possesses distinct conidiogenous loci, i.e., *Anungitopsis* cannot be reduced to synonymy with *Fusicladium*. The anamorphs of this sister clade of the main *Venturia* clade are morphologically rather close to taxa assigned to *Anungitea*. However, species of *Anungitea* and *Fusicladium* are morphologically barely distinguishable (Schubert *et al.* 2003), but the true affinity of *Anungitea* depends on its type species of which cultures and sequence data are not yet available.

Several anamorph genera with divergent morphologies were found to cluster together, suggesting that these are either different synanamorphs of the same teleomorph genus, or that they may represent cryptic clades that will diverge further once additional species are added in future studies. Although the *Herpotrichiellaceae* appeared to represent quite a diverse assembledge of morphotypes, the *Venturiaceae* were again surprisingly uniform.

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Molecular analysis and pathogenicity of the *Cladophialophora carrionii* complex, with the description of a novel species

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Abstract: *Cladophialophora carrionii* is one of the four major etiologic agents of human chromoblastomycosis in semi-arid climates. This species was studied using sequence data of the internal transcribed spacer region of rDNA, the partial β-tubulin gene and an intron in the translation elongation factor 1-alpha gene, in addition to morphology. With all genes a clear bipartition was observed, which corresponded with minute differences in conidiophore morphology. A new species, *C. yegresii*, was introduced, which appeared to be, in contrast to *C. carrionii*, associated with living cactus plants. All strains from humans, and a few isolates from dead cactus debris, belonged to *C. carrionii,* for which a lectotype was designated. Artificial inoculation of cactus plants grown from seeds in the greenhouse showed that both fungi are able to persist in cactus tissue. When reaching the spines they produce cells that morphologically resemble the muriform cells known as the "invasive form" in chromoblastomycosis. The tested clinical strain of *C. carrionii* proved to be more virulent in cactus than the environmental strain of *C. yegresii* that originated from the same species of cactus, *Stenocereus griseus*. The muriform cell expressed in cactus spines can be regarded as the extremotolerant survival phase, and is likely to play an essential role in the natural life cycle of these organisms.

Taxonomic novelty: *Cladophialophora yegresii* de Hoog, sp. nov.
Key words: Cactus, chromoblastomycosis, *Cladophialophora*, endophyte, extremotolerance, phylogeny, taxonomy.

INTRODUCTION

Cladophialophora carrionii (Trejos) de Hoog, Kwon-Chung & McGinnis is one of the most frequent etiologic agents of human chromoblastomycosis, a chronic cutaneous disease characterised by verrucose skin lesions eventually leading to emerging, cauliflower-like eruptions. The species is particularly observed in arid and semi-arid climates of e.g. South and Central America (Lavelle 1980) and Australia (Trejos 1954, Riddley 1957). The current hypothesis is that patients suffering from chromoblastomycosis are rural workers who acquire the infection after being pricked by cactus thorns or splinters (Rubin *et al*. 1991, Fernández-Zeppenfeldt *et al*. 1994). A classical case reported by O'Daly (1943) concerns traumatic inoculation with thorns of "guazábara" (*Opuntia caribaea*), a common xerophilic plant in semi-arid Venezuela. This hypothesised traumatic route of infection was later supported by Richard-Yegres & Yegres (Richard-Yegres & Yegres 1987; strain SR3 = CBS 863.96) and Fernández-Zeppenfeldt *et al*. (1994), who isolated strains from *Prosopis juliflora* litter. *Cladophialophora* Borelli has also been detected in association with spines of the common xerophyte *Aloe vera* and of the *Cactaceae*: *Opuntia caribaeae*, *O. caracasana*, *Stenocereus griseus* and *Cereus lanuginosus* (Borelli 1972, Yegres *et al*. 1996). Thorny American cacti are important components of the xerophyte flora of the arid climate of our study area in Falcon State, Venezuela (Richard-Yegres *et al*. 1992). Muriform cells are produced in human skin and represent the supposed pathogenic invasive form of fungi causing chromoblastomycosis (Mendoza *et al*. 1993). Early experiments involving the inoculation of several species of cold-blooded animals have shown the abundant production of the characteristic muriform cells *in vivo* (Trejos 1953).

A similar plant origin of chromoblastomycosis has been supposed for a related agent of chromoblastomycosis, *Fonsecaea pedrosoi* (Brumpt) Negroni. Marques *et al*. (2006) isolated this species from the shells of Babassu coconuts (*Orbignya phalerata*). The habit of local people to sit on these shells might explain the frequent occurrence of lesions on the buttocks (Silva *et al*. 1995). Salgado *et al*. (2004) found the species on the thorns of a *Mimosa pudica* plant which a patient could identify as the source of traumatic onset of his chromoblastomycosis.

Recently, with the development of molecular tools for species identification, doubt has arisen about the correctness of this supposed route of infection. The question whether environmental and clinical strains represent exactly the same species needs to be re-determined. In order to establish this for *C. carrionii*-associated chromoblastomycosis, reference strains from the CBS culture collection, supplemented with a large set of strains from semi-arid Venezuela, have been verified using molecular tools that are currently routinely employed to answer taxonomic questions in black yeasts and their filamentous relatives (de Hoog *et al*. 2003), particularly the internal transcribed spacer (ITS) region of rDNA, the partial β-tubulin gene (BT2), and an intron in the translation elongation factor 1-alpha (EF1). In addition, a series of three experiments has been conducted concerning inoculation into and superficial application onto germlings of *Stenocereus griseus* obtained by cultivation *in vitro*, mature plants of *S. griseus* from the wild, and in spines of *S. griseus* collected in the semi-arid area of study. Our aim is to reveal the role of the cactus *S. griseus* in the life cycle of its associated *Cladophialophora* spp., and to determine whether a link could be made to *C. carrionii* for obtaining a better understanding of human chromoblastomycosis.

MATERIALS AND METHODS

Fungal strains and morphology

Strains studied are listed in Table 1. This list comprises strains which have morphologically been identified as *C. carrionii*. Reference strains from the CBS culture collection, as well as fresh isolates from patients and the environment have been included. Strains were lyophilised and stored in liquid nitrogen soon after deposit at CBS. Stock cultures for transient working collections were grown on slants of 2 % malt extract agar (MEA) and oatmeal agar (OA) at 24 °C. For morphological observation, slide cultures were made of strains grown on potato-dextrose agar (PDA) (de Hoog *et al*. 2000) and mounted in lactophenol cotton blue.

Table 1. Isolation data of *Cladophialophora* strains examined.

Name	CBS nr.	Other reference(s)[1]	GenBank ITS, BT2, EF1	mtDNA* (Kawasaki *et al.* 1993)	Source [human: duration, localization, sex, age (Pérez-Blanco *et al.* 2003)].	Geography
A / I / 1*: C. carrionii*						
	117904	UNEFM 0004-02 = dH 14480	EU137281, –, –		Chromoblastomycosis; 14 y; hip, thigh, leg; male 38	Falcon State, Venezuela
	117891	UNEFM 0002-00 = dH 14475	EU137278, –, EU137222		Chromoblastomycosis; 1 y; male 62	Falcon State, Venezuela
	117906	UNEFM 0014-96 = dH 14504	EU137288, EU137171, EU137231		Chromoblastomycosis; 0.5 y; hand; male 45	Falcon State, Venezuela
	117897	UNEFM 0011-03 = dH 14497	EU137314, –, EU137254		Chromoblastomycosis; 0.5 y; hand; male 42	Falcon State, Venezuela
	859,96	UNEFM 9617 = dH 10703	EU137295, EU137178, EU137237		Dry plant debris, arid zone	Falcon State, Venezuela
	117898	UNEFM 0010-98 = dH 14496	EU137308, –, EU137246		Chromoblastomycosis; 20 y; hand; female 59	Falcon State, Venezuela
	117889	UNEFM 0003-04 = dH 14478	–, EU137190, –		Chromoblastomycosis; 20 y; thigh, leg; female 78	Falcon State, Venezuela
	114392	UNEFM 82267 = dH 13261	EU137267, EU137150, EU137211		Chromoblastomycosis; leg; female	Falcon State, Venezuela
	114394	UNEFM 9803 = dH 13263	EU137307, –, EU137245		Chromoblastomycosis; hand; male 22	Falcon State, Venezuela
	114396	UNEFM 2001/1 = dH 13265	EU137269, EU137152, EU137213		Chromoblastomycosis; arm; male 35	Falcon State, Venezuela
	114399	UNEFM 2003/2 = dH 13268	EU137272, EU137155, EU137216		Chromoblastomycosis; arm; female 64	Falcon State, Venezuela
	114401	UNEFM 9901 = dH 13270	EU137274, EU137157, EU137218		Chromoblastomycosis; arm; female 40	Falcon State, Venezuela
	114402**	UNEFM 9902 = dH 13271	EU137275, EU137158, EU137219		Chromoblastomycosis; arm; female 40	Falcon State, Venezuela
	114403	UNEFM 95195 = dH 13272	EU137276, EU137159, EU137220		Chromoblastomycosis; arm; male	Falcon State, Venezuela
	117899	UNEFM 0010-04 = dH 14495	EU137301, EU137183, EU137241		Chromoblastomycosis; 2 y; hand; male 57	Falcon State, Venezuela
	117901	UNEFM 0009-03 = dH 14492	EU137312, EU137197, EU137252		Chromoblastomycosis; 8 y; arm; female 41	Falcon State, Venezuela
	114393	UNEFM 9801 = dH 13262	EU137268, EU137151, EU137212		Chromoblastomycosis; hand; male 72	Falcon State, Venezuela
	108.97**	UNEFM 9501 = dH 10704	EU137306, EU137188, EU137265		Chromoblastomycosis; skin	Falcon State, Venezuela
	114397	UNEFM 84020 = dH 13266	EU137270, EU137153, EU137214		Chromoblastomycosis; hand, arm; male 54	Falcon State, Venezuela
	114404	UNEFM 95656 = dH 13273	EU137311, EU137196, EU137251		Chromoblastomycosis; arm; male	Falcon State, Venezuela
	117902	UNEFM 0008-03 = dH 14489	EU137283, EU137166, EU137226		Chromoblastomycosis; 3 y; arm; male 42	Falcon State, Venezuela
	117893	UNEFM 0001-00 = dH 14470	EU137316, EU137200, –		Chromoblastomycosis; 2 y; knee; male 19	Falcon State, Venezuela
	117892	UNEFM 0001-02 = dH 14471	EU137277, EU137160, EU137221		Chromoblastomycosis; 8 y; knee; male 52	Falcon State, Venezuela
	117908	UNEFM 0013-04 = dH 14502	–, EU137191, –		Chromoblastomycosis; 6 y; back; male 13	Falcon State, Venezuela
	109.97**	UNEFM 9503 = dH 10706	–, –, –		Chromoblastomycosis; skin	Falcon State, Venezuela
	857.96	UNEFM 9408 = dH 10707	EU137294, EU137177, EU137236		Chromoblastomycosis; skin	Falcon State, Venezuela
	114398	UNEFM 2003/1 = dH 13267	EU137271, EU137154, EU137215		Chromoblastomycosis; arm; female 67	Falcon State, Venezuela
	114400	UNEFM 2003/3 = dH 13269	EU137273, EU137156, EU137217		Chromoblastomycosis; arm; male 50	Falcon State, Venezuela
	117909	UNEFM 0013-00 = dH 14501	EU137287, EU137170, EU137230		Chromoblastomycosis; arm; male	Falcon State, Venezuela
	114395	UNEFM 9802 = dH 13264	EU137299, EU137182, EU137240		Chromoblastomycosis; leg; female 22	Falcon State, Venezuela
	166.54	MUCL 10088	EU137290, EU137173, –		Skin lesion in human	Falcon State, Venezuela
	862.96	UNEFM 9603 = dH 10700	EU137315, EU137199, EU137255		Dry plant debris, semi-arid zone	Falcon State, Venezuela
	863.96**	IFM 41444 = UNEFM SR3 = dH 10699	AB109169 / EU137296, EU137179, EU137238		Dry spine (*Opuntia caribaea*) on soil, semi-arid zone	Falcon State, Venezuela
	861.96	UNEFM 9607 = dH 10701	EU137309, EU137194, EU137249		Dry plant debris, semi-arid zone	Falcon State, Venezuela
	117896	dH 14498	EU137285, –, EU137228		Hand lesion	Falcon State, Venezuela
	114397	UNEFM 84020 = dH 13266	EU137270, EU137153, EU137214		Chromoblastomycosis, hand and arm	Falcon State, Venezuela

117905	dH 14505	EU137300, –, –		Chromoblastomycosis, hand, male	Falcon State, Venezuela
117900	dH 14493	EU137284, –, EU137227		Chromoblastomycosis, hand, male	Falcon State, Venezuela
114392	UNEFM 82267 = dH 13261	EU137267, EU137150, EU137211		Chromoblastomycosis, leg, female	Falcon State, Venezuela
–	FMC 248	AF397181, –, –		Chromoblastomycosis	Venezuela
–	IFM 41807	AB109175, –, –	Group mt-I	–	Venezuela
–	IFM 4812	AB109168, –, –	Group mt-I	–	Venezuela
–	IMTSP 690	AF397180, –, –		Chromoblastomycosis	Brazil
410.96	UAMH 4392 = NCMH 1010 = DUKE 2403	EU137310, EU137195, EU137250		Chromoblastomycosis	–
163.54		EU137304, EU137186, EU137243		Chromoblastomycosis	Australia
117903	dH 14482	EU137282, –, EU137225		Chromoblastomycosis, forearm, male	–
362.70	M.J. Campos 4555 = dH 15806	EU137302, EU137184, EU137242		Human	Mozambique
260.83	CDC B-1352 = FMC 282 = ATCC 44535 (ex-T of *C. ajelloi*)	EU137292, EU137175, EU137234	Group mt-I	Skin lesion in human	Uganda
986.96	UAMH 5717	EU137297, EU137180, –		Clinical material	–
–	IFM 4805	AB087204, –, –		–	–
–	IFM 4811	AB109178, –, –		–	–
–	IFM 41814	AB109176, –, –		–	–
B / II / 2: *C. carrionii*					
160.54	ATCC 16264 = CDC A-835 = MUCL 40053 = IFM 4808 (ex-LT of *C. carrionii*)	AB109177 / EU137266, EU137201, EU137210	Group mt-II	Chromoblastomycosis, human	Australia
–	Todd Pryce 200867 = dH 13218			Human	Australia
406.96	MRL 1114 = UAMH 4366 = dH 15847	EU137317, EU137202, EU137256		Human	Queensland, Australia
100434	ATCC 32279 = dH 10745 = IP 518 = RV 16499	EU137289, EU137172, EU137232		Human	Madagascar
–	IFM 4810	AB109170, –, –		–	–
–	IFM 41446 = DCU 606	AB109171, –, –		–	–
C: *C. carrionii*					
–	IFM 41651	AB109174, –, –	Group mt-I	–	China
–	IFM 41650	AB109173, –, –	Group mt-I	–	China
–	IFM 41641	AB109172, –, –	Group mt-I	–	China
–	IFM 4985	AB109179, –, –		–	–
–	IFM 4986	AB109180, –, –		–	–
D / III / 3: *C. yegresii*					
114406	UNEFM SgSR1 = dH 13275	EU137323, EU137208, EU137263		*Stenocereus griseus* asymptomatic plant	Falcon State, Venezuela
114407	UNEFM SgSR2 = dH 13276	EU137324, –, EU137264		*Stenocereus griseus* asymptomatic plant	Falcon State, Venezuela
114405**	UNEFM SgSR3 = dH 13274 (ex-T of *C. yegresii*)	EU137322, EU137209, EU137262		*Stenocereus griseus* asymptomatic plant	Falcon State, Venezuela

[1]Abbreviations: ATCC = American Type Culture Collection, Manassas, U.S.A.; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CDC = Centers for Disease Control and Prevention, Atlanta, U.S.A.; DCU = Department of Dermatology, School of Medicine, Chiba, Japan; dH = G.S. de Hoog working collection; FMC = Faculdade de Medicina, Caracas, Venezuela; ITMSP = Instituto de Medicina Tropical de São Paulo, São Paulo, Brazil; IFM = Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan; IP = Institut Pasteur, Paris, France; MUCL = Mycotheque de l'Université de Louvain, Louvain-la-Neuve, Belgium; RV = Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; UAMH = The University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada; UNEFM = Universidade Nacional Experimental Francisco de Miranda, Coro, Falcon, Venezuela.
Ex-T = Type strain; ex-LT = Lectotype strain.

*Type I and II groups based on mitochondrial DNA restriction fragment length polymorphism: H-1 and H-2 = restriction patterns 1 and 2, respectively, with *Hae*III enzyme; M-1 and M-2 = restriction patterns 1 and 2, respectively, with *Msp*I enzyme; S-1 and S-2 = restriction patterns 1 and 2, respectively, with *Sau3A*I enzyme.
** Used in plant and mouse inoculation experiments.

Table 2. Results from MrAic using corrected Akaike Information Criterion (AICc).

Fragment/Gene	*Model*	*df**	*lnL**	*AICc**	*wAICc**
rRNA ITS	TrNG	89	-21.840.556	4575.9992"	0.3080
EF-1α	HKYG	88	-19.392.355	41.147.171	0.5242
ß-Tubulin	SYMIG	90	-28.547.745	59.261.933	0.1913

**df* = degrees of freedom; *lnL* = log likelihood; *AICc* = corrected AIC; *wAICc* = weighted corrected AIC.

DNA extraction

Approximately 1 cm^2 mycelium of 30-d-old cultures was transferred to a 2 mL Eppendorf tube containing 300 µL TES-buffer (Tris 1.2 % w/v, Na-EDTA 0.38% w/v, SDS 2 % w/v, pH 8.0) and about 80 mg of a silica mixture (Silica gel H, Merck 7736, Darmstadt, Germany / Kieselguhr Celite 545, Machery, Düren, Germany, 2 : 1, w/w). Cells were disrupted mechanically in a tight-fitting sterile pestle for approximately 1 min. Subsequently 200 µL TES-buffer was added, the mixture was vortexed, 10 µL proteinase K was added and incubated for 10 min at 65 °C. After addition of 140 µL of 5 M NaCl and 1/10 vol CTAB 10 % (cetyltrimethylammoniumbromide) buffer, the material was incubated for 30 min at 65 °C. Subsequently 700 µL SEVAG (24 : 1, chloroform : isoamylalcohol) was mixed to solution, incubated during 30 min on ice water and centrifuged for 10 min at 14 000 rpm. The supernatant was transferred to a new tube with 225 µL 5 M NH_4-acetate, incubated on ice water and centrifuged again for 10 min at 14 000 rpm. The supernatant was transferred to another Eppendorf tube with 0.55 vol isopropanol and spun for 5 min at 14 000 rpm. Subsequently, the pellet was washed with ice cold 70 % ethanol. After drying at room temperature it was re-suspended in 48.5 µL TE buffer (Tris 0.12 % w/v, Na-EDTA 0.04 % w/v) plus 1.5 µL RNAse 20 U/mL and incubated for 15–30 min at 37 °C.

Sequencing and phylogenetic reconstruction

Three loci, namely the internal transcribed spacers (ITS), ß-tubulin (BT2) and translation elongation factor 1-α (EF1), were sequenced. For ITS sequencing, amplification was performed with V9G (5'-TTACGTCCCTGCCCTTTGTA-3') and LS266 (5'-GCATTCCCAAACAACTCGACTC-3'). Sequencing reactions were conducted with ITS1 and ITS4 primers (White *et al*. 1990). For BT2 amplification and sequencing, primers Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') were used (Glass & Donaldson 1995) and for EF1 amplification and sequencing, primers EF1-728F (5'CATCGAGAAGTTCGAGAAGG-3') and EF1-986R (5'-TACTTGAAGGAACCCTTACC-3') (Carbone & Kohn, 1999). Sequences were aligned in BioNumerics v. 4.5 (Applied Maths, Kortrijk, Belgium), exported and converted into Phylip interleaved format (Felsenstein 1993).

Calculation of ILD (incongruence length difference) was performed in PAUP v. 4.0b10 (Swofford 2003). A combined data set of ITS, EF1 and BT2 sequences was created. Optimality criterion was set to parsimony. The total number of characters was 1 263 with equal weight, while 677 characters were constant, and 396 parsimony-informative. Gaps were treated as missing, and tree-bisection-reconnection (TBR) was used as branch-swapping algorithm. Maximum number of trees was set to 100 and left unchanged.

Substitution model testing

The program MrAic (www.abc.se/~nylander/; Nylander 2004) was used to select a substitution model. MrAic is a Perl script for calculating the Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AICc), Bayesian Information Criterion (BIC), and Akaike weights for nucleotide substitution models and model uncertainty. Using an ML algorithm, likelihood scores under different models were estimated using Phyml (http://atgc.lirmm.fr/phyml/). All 56 models implemented in Modeltest (Posada & Crandall 1998) were evaluated. These models were also combined with proportion of invariable sites (I) and/or gamma distribution shape parameter (G). A difference between Modeltest and MrAic is that the latter does not evaluate all models on the same, approximate topology as in PAUP (Swofford 1981). Instead, Phyml was used to try to find the maximum of the likelihood function under all models. This is necessary for finding AIC, AICc, or BIC for the models. The AICc calculation (Table 2) was used to select the right model for the ratio of parameters to characters (Nchar/Nparameters < 40; Burnham & Anderson 2002) for all loci. The substitution matrix of the models is printed next to the trees. Another advantage of using MrAic in combination with Phyml was the obtained accuracy of tree topology and the greater calculation speed (Guindon & Gascuel 2003).

Population genetic analyses

In order to confirm the intraspecific diversity shown in the MP trees, the number of populations in the *C. carrionii* complex was inferred with Structure v. 2.2 (Pritchard *et al.* 2000) using genotype data of the ITS regions of rRNA gene and of the partial EF1 and BT2 genes. Genotypes of these three loci of 43 isolates were sorted on the basis of sequence similarity. Structure is a model-based clustering method for using multilocus genotype data to infer population structure and assign individuals to populations. The parameters were as follows: the length of burn-in period was set to 10^6, number of MCMC repeats after burn-in 30 000; the ancestry model: admixture (individuals have mixed ancestry and is recommended as starting point for most analyses). Uniform prior for ALPHA was set to 1.0 (default) and all allele frequencies were taken as independent among populations with λ set to 1.0 (default). Probability of the data (for estimating K) was also computed (Falush *et al*. 2003). The burn-in period length and number of MCMC repetitions after burn-in were set as 10 000 and 100 000, and admixture model and allele frequencies correlated model were chosen for analysis. The number of populations (K) was assumed from two to four.

Association of multilocus genotypes was screened with the multilocus option in BioNumerics. To test for reproductive mode in each population, index of association (I_A, a measure of multilocus linkage disequilibrium) was calculated with Multilocus v. 1.2.2 (www.bio.ic.ac.uk/evolve/software/multilocus). The null hypothesis for this analysis was complete panmixia. The values of I_A were compared between observed and randomised data sets. The hypothesis would be rejected when $p < 0.05$. Population differentiation (index: theta, θ) was also detected using the same software and a null hypothesis for this analysis is no population differentiation. When observed θ is statistically significantly different from those of random datasets ($p < 0.05$), population differentiation should be considered.

A reticulogram was reconstructed using T-REX (Makarenkov 2001, Makarenkov & Legendre 2004) (www.labunix.uqam.ca/~makarenv/trex.html) on *C. carrionii* / *Cladophialophora* sp. The program first computed a classical additive tree using one of the five available tree reconstruction algorithms. Subsequently, at each step of the procedure, a reticulation (a new edge) was chosen that minimised the least-squares or the weighted least-squares loss function; it was added to the growing reticulogram. Two statistical criteria (Q1 and Q2) were proposed to measure the gain in fit when reticulations were added. The minimum of each of these criteria may suggest a stopping rule for addition of reticulations. With HGT (horizontal gene transfer) reticulogram reconstruction option (Makarenkov 2001) the program mapped the gene tree into the species tree using the least-squares method. Horizontal transfers of the considered gene were then shown in the species tree. The reticulate network was created in the ITS tree, which served as a species tree and compared with a gene tree, EF1. Degrees of recombination or horizontal gene transfer were also visualised using SplitsTree v. 4.8 software (Huson & Bryant 2006). Split decomposition (Bandelt & Dress 1992) was applied on three loci of the entire *C. carrionii* complex. Calculation was done with default settings of characters transformation using uncorrected P-values, equal angles and optimise box iterations set to 1. Star- or brush-like trees indicate clonal development, while reticulation indicates genetic exchange.

Isolation of fungi for inoculation experiments

Nine plants of *Stenocereus griseus*, located within 50 m radius of the house of a patient with chromoblastomycosis due to strain UNEFM 9902 = CBS 114402 (*C. carrionii*) in Sabaneta (Miranda, Falcón State, Venezuela), were analysed. Four fragments of approx. 2 × 3 × 1 cm were excised from each plant at brownish superficial lesions in upper branches. Sampled fragments were soaked in mineral oil for 15 min at 23 °C under agitation at 150 rpm (Fernández-Zeppenfeldt *et al*. 1994). Subsequently four cultivations were made per sample on agar slants. Strains with cultural characteristics and morphology similar to *C. carrionii* (de Hoog *et al*. 2000) were selected. Final identification was made by sequencing of the ITS region, by determining the ability of strains to grow at 35, 37, 38 and 40 °C, and whether they could break down 20 % gelatin (Richard-Yegres & Yegres 1987, Fernández-Zeppenfeldt *et al*. 1994). Environmental strain UNEFM-SgSR3 = CBS 114405 (*Cladophialophora* sp.) and clinical strain UNEFM 9902 = CBS 114402 (*C. carrionii*) were selected for the inoculation experiments.

Inoculum preparation

Approximately 1 cm^2 of a culture on Sabouraud's glucose agar (SGA) was transferred to 50 mL YPG medium (yeast extract 0.5 %, peptone 0.5 %, glucose 2 %) (de Hoog *et al*. 2000), shaken at 150 rpm and incubated for 3 d at 23 °C (Yegres *et al*. 1991). Five mL aliquots of the starter culture were transferred serially every 4 d to 500 mL flasks containing 100 mL synthetic medium (D-glucose 2 %, KH_2PO_4 0.2 %, NH_4SO_4 0.1 %, urea 0.03 %, $MgSO_4$ 0.03 %, $CaCl_2$ 0.003 %; pH 6.2) shaken at 150 rpm at 23 °C. After 4 d the suspensions, which were predominantly conidia, were filtered through sterile gauze, ground in 50 mL 0.85 % saline, centrifuged at 2 000 rpm, and repeatedly washed with saline until a clear supernatant was obtained. The suspensions were adjusted to 5 × 10^6 cells/mL (Yegres *et al*. 1991, Cermeño & Torres 1998). Inocula of 2 mL were checked for viability in lactritmel medium (de Hoog *et al*. 2000).

Experimental cactus germlings

Young cactus plants (*Stenocereus griseus*) were obtained in the laboratory (Clausnitzer 1978) by cultivation from seeds of a single cardon fruit collected near the house of the patient infected with CBS 114402 in the endemic area for chromoblastomycosis in Falcon State, Venezuela. The seeds were rinsed with sterile distilled water, the contents washed by agitation for 10 min at 120 rpm in 250 mL sodium hypochlorite 4 % (v/v), and subsequently with sterile distilled water at 120 rpm for 5 min. The supernatant was decanted, 250 mL HCl 20 % was added, the seeds were incubated for 3 h, decanted and washed repeatedly with sterile distilled water. Seeds were then dried for 24 h on filter paper at 37 °C. Onset of germination was obtained by incubation of the seeds in a moist chamber on filter paper for 15 d under alternately 8 h of continuous white light (26 W) and 16 h of darkness; bud emergence was observed daily. Germlings of 1 cm in length, with green colour and having two leaves were transplanted to 128-container germinators until roots developed. The sterile substrate contained 5 parts Sogemix® and 1 part river soil from the region where the fruit was collected. The daily light regime was as above; plants were watered every 10 d with 5 mL sterile tap water for 1 yr.

Inoculation of *S. griseus* germlings

Fungal suspensions (0.1 mL) were either injected using a syringe (13 × 0.4 mm) at a depth of approximately 5 mm into cortical tissue (Fig. 1A), or superficially applied onto (Fig. 1B) 96 randomly selected 1-yr-old plants: 50 % using clinical strain CBS 114402 (*C. carrionii*) and 50 % using environmental strain CBS 114405 (*Cladophialophora* sp.). The controls were 64 plants which were treated similarly, but using sterile saline (0.85 %). The growth chambers with inoculated plants stayed in the laboratory under the conditions specified above. From day 15 post inoculation onwards every 15th day, six plants of each treatment were sectioned longitudinally from the apex and transversely by means of a hand-held microtome, examined directly in glycerin water (25 %), and cultured in lactritmel medium (Fernández-Zeppenfeldt *et al*. 1994).

Experimental cactus plants

A total of 150 whole *S. griseus* plants ≤ 15 cm tall and without macroscopically visible lesions, were dug from an area within a 50 m radius of the house of the patient with chromoblastomycosis as specified above. Plants were transported to the laboratory and transplanted individually into polyethylene bags with a capacity of 1 kg, using as substrate river soil from the same area. Plants were maintained outside, directly adjacent to the laboratory to adjust at average temperatures of 32 °C and with natural daylight. They were watered with tap water every 15th d for a period of 6 mo.

Scar formation in mature *S. griseus* plants

For inoculation purposes, 150 sharp, wooden toothpicks 4 × 0.3 × 0.2 cm were washed and boiled for 3 min in tap water to eliminate resins (Yegres & Richard-Yegres 2002). This procedure was repeated three times. Three batches of 50 toothpicks each were kept separate in Petri dishes. Plates were incubated for 15 d at 23 °C after inoculating each batch with 1 mL fungal suspension (5 × 10^6 cells/mL) of either strain CBS 114405 or CBS 114402, with sterile water as control. A total of 50 randomly selected plants were inoculated (Fig. 1C) halfway up the shaft with a toothpick colonised with CBS 114402 (*C. carrionii*), CBS 114405 (*Cladophialophora* sp.) or the control (Yegres & Richard-Yegres 2002).

Starting from 2 wk post inoculation, 10 plants were randomly chosen every 15 d, and tissue samples taken at the point of

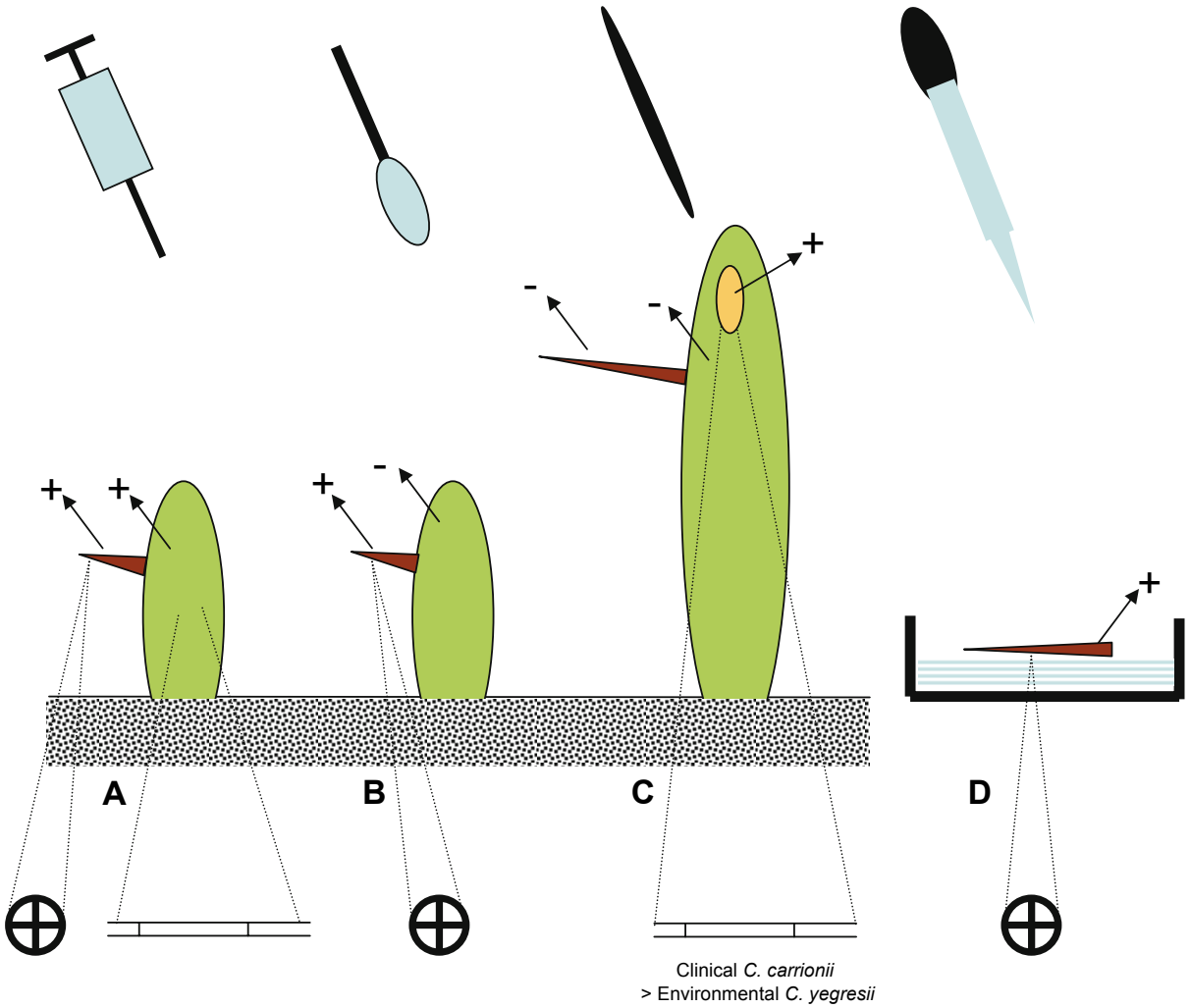

Fig. 1. Diagram of inoculation experiments with results. A. Inoculation of young cactus; B. Superficial application of young cactus; C. Traumatic application of mature cactus, with brown resulting scar; D. Superficial application of mature spines. Indications +/- refer to positive resp. negative results of re-isolated strains. Lower line: circles represent production of muriform cells, filaments represent hyphal growth.

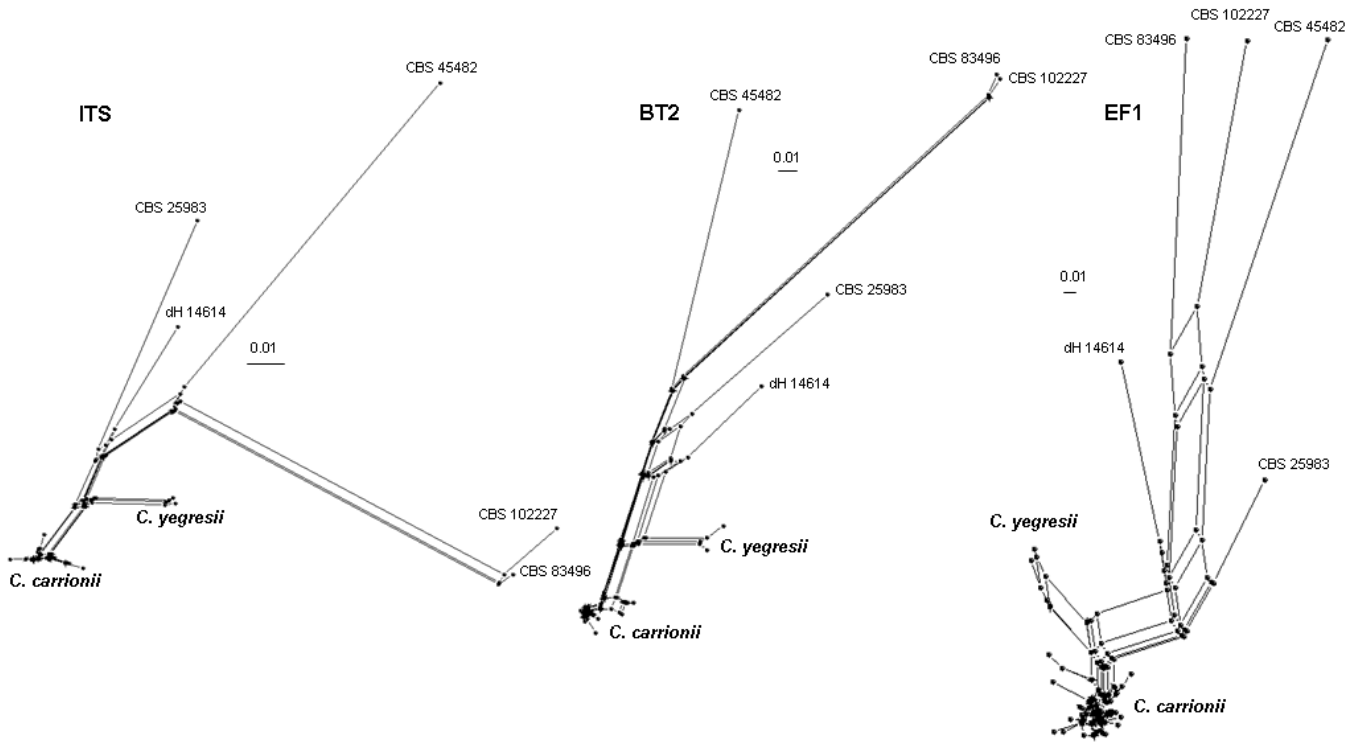

Fig. 2. Split decomposition of the *C. carrionii* complex using SplitsTree with uncorrected (P-value) distances. Nodes are shown only with different genotypes; hence EF1 shows the largest number of nodes. Extensive reticulation is noted in all loci.

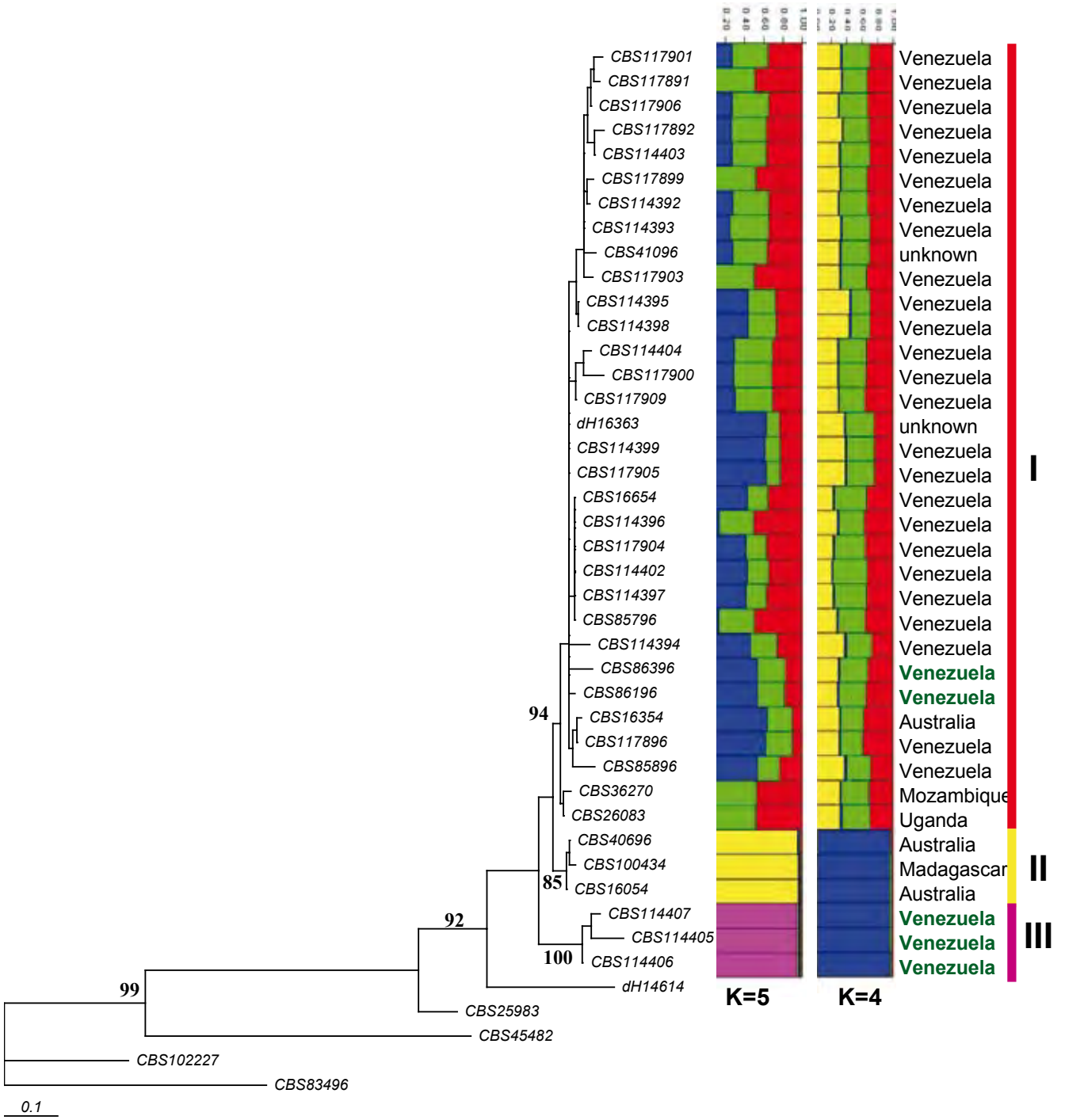

Fig. 3. Phylogenetic tree (Neighbour-joining) of the *C. carrionii* complex based on EF1 (with grouping I–III) using the same strains of Fig. 1, generated using the HKY+G model. The model was calculated using ML in MrAic software. Bootstrap cut-off = 80 %. CBS 834.96 was taken as outgroup. Columns were generated with Structure software hypothesising K = 4 and K = 5, and alleles independent. Geographical origins in black refer to isolates from humans (chromoblastomycosis); origins in green refer to isolates from plant material.

inoculation, and from the thorns directly adjacent to this area. Samples were rinsed with 4 % sodium hypochlorite for 3 min, and subsequently washed in sterile distilled water for re-isolations, and for histological study by means of light microscopy (Fernández-Zeppenfeldt *et al*. 1994).

Experiments with spines of *S. griseus*

Ninety cactus spines of 2.5 cm av. length were collected from a single *S. griseus* plant located near the home of the patient infected with CBS 114402, at approx. 2.5 m height, superficially sterilised, and divided into three groups, of which 30 spines were inoculated with CBS 114402 (*C. carrionii*), 30 with CBS 114405 (*Cladophialophora* sp.) and 30 to be used as control, inoculated with a saline solution (Fig. 1D). A similar series composed of 90 spines of 1.5 cm average length was collected at approx. 1 m height. All spines were incubated in sterile Petri dishes with filter paper (Whatman #1) with 2 mL saline solution; subsequently 0.1 mL fungal suspension was applied. Twenty spines were analysed weekly by means of longitudinal sectioning with a hand-held microtome, cultured as above and observed microscopically until day 75 post incubation.

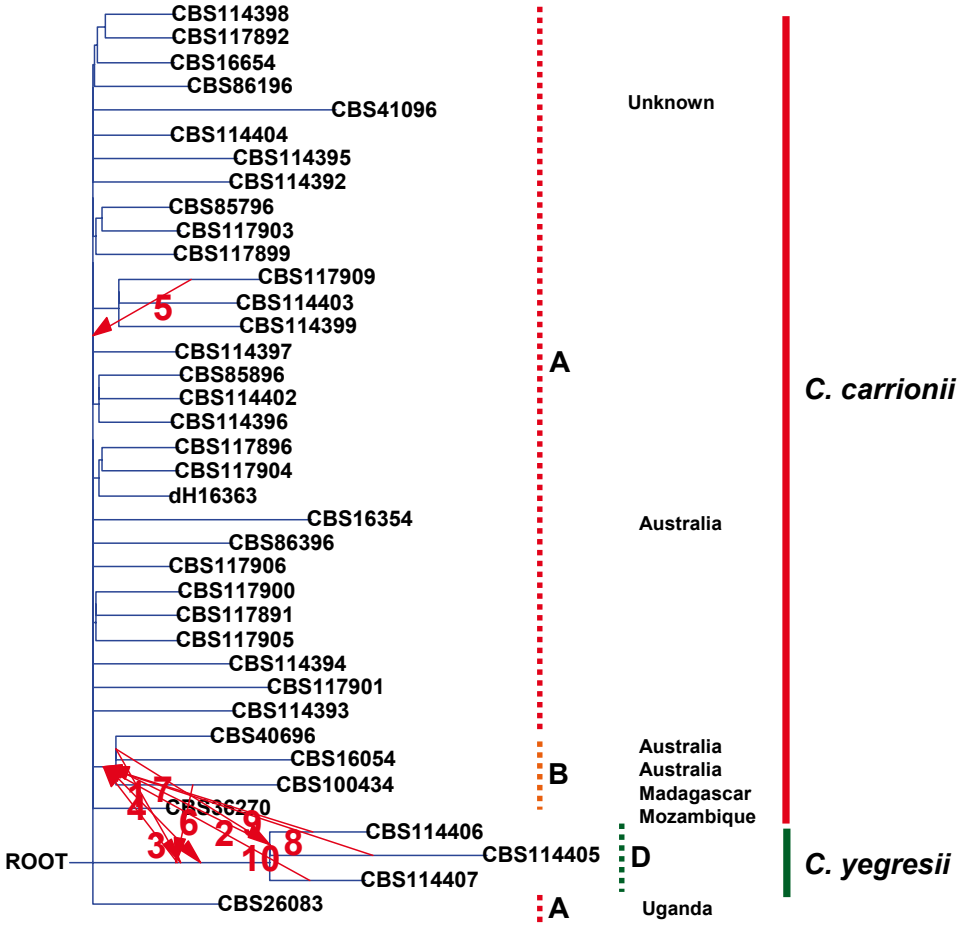

Fig. 4. Reticulogram of South American strains of *Cladophialophora* species and strains from other continents (mentioned) constructed with T-REX software. ITS rDNA (with grouping A, B, D) was used as species tree and compared with the ß-tubulin gene tree. First 10 reticulations are shown with numbers.

Statistics

Survival of the cactus seedlings and collected plants following inoculation were evaluated using the X^2-test (P = 0.05 was considered significant). Stem lesions resulting from inoculations were analysed with Student's T-test (P = 0.01 was considered significant).

RESULTS

The rDNA ITS region was sequenced for 43 strains identified as *C. carrionii* based on morphology. Sequences of 16 additional strains were downloaded from GenBank. Five distantly related, unidentified cladophialophora-like species were added, with CBS 834.96 as outgroup. In *C. carrionii*, 203 positions were compared in ITS1, 158 in the 5.8S rRNA gene and 182 in ITS2 (Table 3). The sequences could be aligned with confidence over their entire lengths. Over the data set, 35 positions were polymorphic, of which 33 were phylogenetically informative (Table 3), the two remaining being variable T-repeats near the ends of ITS1 and 2.

For ITS sequences the AICc selected the TrN+G model (TrNG; Tamura & Nei 1993). The base frequency of ITS: T = 0.2467, C = 0.2897, A = 0.2247, G = 0.2390, TC = 0.5364, AG = 0.4636. The EF1 tree was built with substitution model HKY+G; the base frequency of EF1: T = 0.2990, C = 0.2665, A = 0.2123, G = 0.2221, TC = 0.5655, AG = 0.4345. The best model for BT2 sequences was the SYM+I+G (symmetrical model). The base frequency for BT2: T = 0.2255, C = 0.2953, A = 0.2463, G = 0.2328, TC = 0.5208, AG = 0.4792. Bootstrap values of the EF1 tree were calculated with PAUP using parsimony and with maxtrees set to 500 and 500 replicates (data not shown). Total number of characters was 191 of which 101 were parsimony-informative. Tree length was 365 and had the following indices: Consistency Index = 0.685, Retention Index = 0.542 and Homoplasy Index = 0.315.

The original tree length, L_o was 1 055, the tree length of the combined data, L_c was 1 062. The resulting incongruence length difference $L = (L_c - L_o)$ was 7 (P = 0.24). The observed ILD was not significantly greater than expected by chance and it was concluded that the sequences were congruent and could be used together in a combined analyses.

Split decomposition based on the same alignment generated extensive recombination. The structure found with three loci was robust, with the exception of separation of CBS 834.96 and CBS 102227 with EF1 (Fig. 2).

The core of the network, comprising the strains listed in Table 1, was analysed in more detail. With ITS, four groups were recognised (A–D; Table 1). (A) was the main group with 36 strains / sequences; FMC 248 differed only by a small T-repeat and was regarded as a member of (A). The remaining groups were smaller, differing from group (A) maximally by two consistent positions (Table 3). Group (C) mainly comprised sequences from GenBank and all originated from Abliz *et al*. (2004). One of the strains of group (C), IFM 4808, concerned a subculture of CBS 160.54, which is an original isolate of Trejos (1954) representing *C. carrionii*. Re-sequencing indicated that it was a member of group (B). Analysis of our electropherograms of this isolate was not suggestive of heterothallism. None of the positions characterising groups (A)–(C) were also found to differ in group (D), which deviated in 16 mutations in ITS1 and 8 in ITS2; 17 of the mutations were transitions, 7 were transversions and 7 indels. Group (D) was clearly distinct from the complex of (A)–(C),

Table 3. Nucleotide variability of ITS1-2 ribosomal DNA regions of *Cladophialophora carrionii* (A – C) and *C. yegresii* (D).

rDNA domains (length), with variable nucleotide positions.				
ITS1 (201-203)	***A***	***B***	***C***	***D***
16	C	C	C	T
17	T	C	T	T
19	T	T	T	C
51	A	A	A	G
57	A	A	A	T
90-92	TG-	TG-	TG-	CGT
101	T	T	T	C
103	C	C	C	T
104	G	A	G	G
106	A	A	A	G
114	T	T	T	C
122	T	T	T	C
132	C	C	C	T
137	A	A	A	C
141	C	C	C	T
145	-	-	-	A
163-170	6-10T	6-10T	6-10T	TTGTATCT
180	-	-	-	A
183	G	G	G	A
190	T/A	A	A	A
5.8S (158)	***Monomorphic***			
ITS2 (178-182)	***A***	***B***	***C***	***D***
36	C	T	T	T
48	T	T	G	T
49	T	T	T	C
51	-	-	-	C
114	C	C	C	G
140	A	A	A	G
155	-	-	-	T
178-179	--	--	--	CT

with a total of 27 mutations.

For multilocus analysis with ITS, EF1 and BT2 a smaller set of strains was compared. Sequences of the 205 bp long element of EF1 contained 32 phylogenetically informative mutations. Three entities were distinguished (I–III; Fig. 3). With BT2, three groups with the same composition were recognised. Strains of ITS group (C) were not available for study.

On the basis of multilocus screening in BioNumerics, concordant groups (A)–(D) were tested with the Structure programme. When K was set at 4 or 5, consistent groupings were noted, indicated as I, II and III (Fig. 3), corresponding with ITS groups (A), (B) and (D), respectively in Table 1.

The possibility that group (D) / (III) included a member of another, morphologically similar but phylogenetically unrelated group of fungi was excluded by SSU sequencing. Genera morphologically similar to *Cladophialophora*, such as *Cladosporium* Link*, Devriesia* Seifert & N.L. Nick., *Phaeoramularia* Munt.-Cvetk.*, Pseudocladosporium* U. Braun and *Stenella* Syd. proved to be remote (data not shown).

With T-rex, interaction between groups (B) and (D) was noted, rather than between groups (B) and (A), despite the high sequence similarity of (A) and (B) (Fig. 4).

Morphological observation revealed that representatives of ITS groups (A)–(C) generally had conidiophores that arise at right angles from creeping hyphae (Fig. 5), while those of (D) tend to be ascending, hyphae gradually becoming conidiophore-like. Since slight correspondence was found in independent markers and phenetic criteria, we considered group (D) to represent a separate species, which is described as follows.

Cladophialophora yegresii de Hoog, **sp. nov.** MycoBank MB500208. Figs 6, 7D–F.

Etymology: Named after Francisco Yegres, Venezuelean mycologist.

Coloniae in agaro PDA dicto 22 °C planae, olivaceo-virides, pulverulentae vel velutinae, margine integra; reversum olivaceo-atrum. Hyphae fertiles dilute olivaceo-virides, ascendentes, paulatim in catenas conidiorum concolorium vertentes. Conidiorum catenae ramosae, conidia dilute olivaceo-viridia, levia et tenuitunicata, 4.5–6 × 2.5 µm, faciliter liberata, cicatricibus modice pigmentatis. Chlamydosporae et cellulae zymosae absentes. Synanamorphe phialidica non visa. Teleomorphe ignota.

Holotypus cultura sicca CBS H-18464 in herbarium CBS praeservatur.

Colonies on PDA at 22 °C evenly olivaceous green, powdery to velvety, with entire margin; reverse olivaceous black. Fertile *hyphae* pale olivaceous green, ascending, gradually changing over into concolorous chains of conidia. *Conidial system* profusely branched. *Conidia* pale olivaceous green, smooth- and thin-walled, 4.5–6 × 2.5 µm, detached rather easily, with slightly pigmented scars. *Chlamydospores* and yeast cells absent. Phialidic synanamorph not observed. Teleomorph unknown.

Specimen examined: **Venezuela**, Falcon state, from asymptomatic *Stenocereus griseus* cactus, G. Fernández-Zeppenfeldt, CBS H-18464 **holotype**, culture ex-type CBS 114405 = UNEFM SgSR3.

Notes: Of the 48 dematiaceous isolates obtained from 36 fragments of the cactus *Stenocereus griseus*, four strains originating from four different plants of *S. griseus* presented morphological and physiological key characteristics of *Cladophialophora carrionii* or *C. yegresii* (de Hoog *et al*. 2000, 2006). Gelatin liquefaction was negative in all strains and the maximum growth temperature was 37 °C. After identification to species level using sequence data (de Hoog *et al*. 2006), both *C. carrionii* and *C. yegresii* appeared to be among the strains isolated.

A total of 256 plants obtained at the end of 1 yr from germlings, had ribs, spines, and an average height of 15 cm. The 96 germlings inoculated with fungal suspensions of the test strains CBS 114402 (*C. carrionii*, clinical) and CBS 114405 (*C. yegresii*, environmental) remained without visible external lesions during the year of experimentation. Histological sections of the 96 inoculated plants consistently revealed internal growth of the fungi in their filamentous form. Muriform cells were not observed, neither on the epidermis, nor in the internal tissue, spines or roots. The re-isolated cultures demonstrated the viability of the fungi during the entire experimental process: CBS 114402 (*C. carrionii*) was grown from 26 (54.16 %) of the plants and CBS 114405 (*C. yegresii*) in 23 (47.90 %) of the plants. The X^2 test did not reveal significant differences between the isolates ($X^2c = 0.0729 < X^2t = 3.84$). The 32 control plants remained without external lesions, and in the

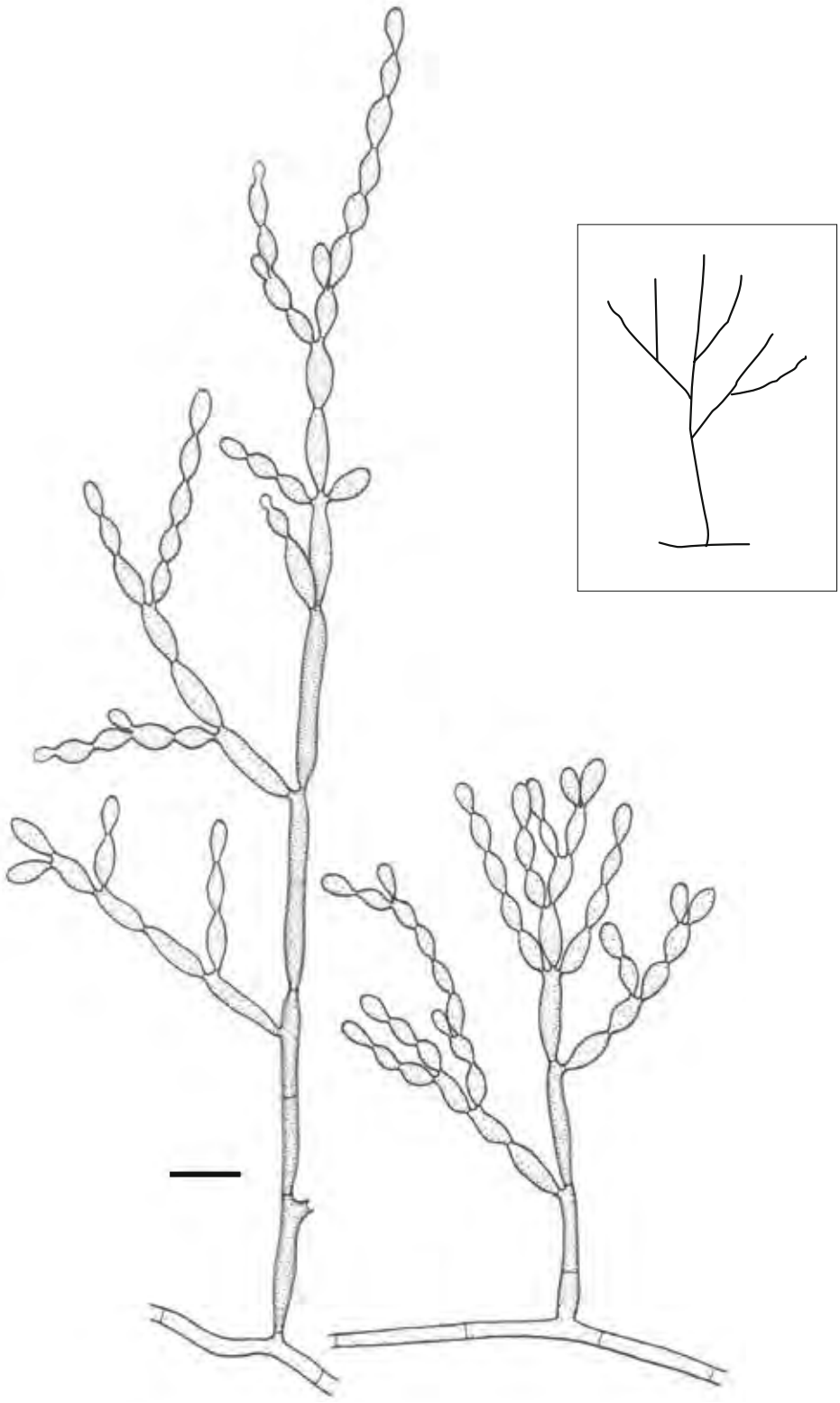

Fig. 5. Microscopic morphology of *C. carrionii*, strain CBS 160.54. Conidiophore erect, i.e. mostly arising at 90° from creeping hypha (sketch). Scale bar = 10 µm.

histological sections no internal or external fungal elements were observed. None of the fungi isolated from the control plants proved to be a species of *Cladophialophora.*

With 96 plants with superficial application of spore suspension (48 plants for each isolate, either clinical or environmental) neither internal nor external lesions were observed. Histological sectioning did not reveal fungal elements in or on plant tissue. Short hyphal elements and meristematic cells were occasionally seen around and inside the outer layers of the spines. The re-isolated strains proved that the fungi survived during the entire experimental procedure: CBS 114402 (*C. carrionii*) was isolated from 32 (66.67 %) plants and CBS 114405 (*C. yegresii*) from 33 (68.75 %). The X^2 test did not detect significant differences in survival rates among the isolates ($X^2c = 0.4375 < X^2t = 3.84$).

Mature plants inoculated using colonised toothpicks showed average scarring of 1.88 cm diam with *C. carrionii* and 1.33 cm diam with *C. yegresii*, around the point of inoculation. In histological sections of 100 plants, dark, septate hyphae with inflated elements were observed at the points of inoculation. Muriform cells were not observed. Re-isolated strains were evidence of isolate viability:

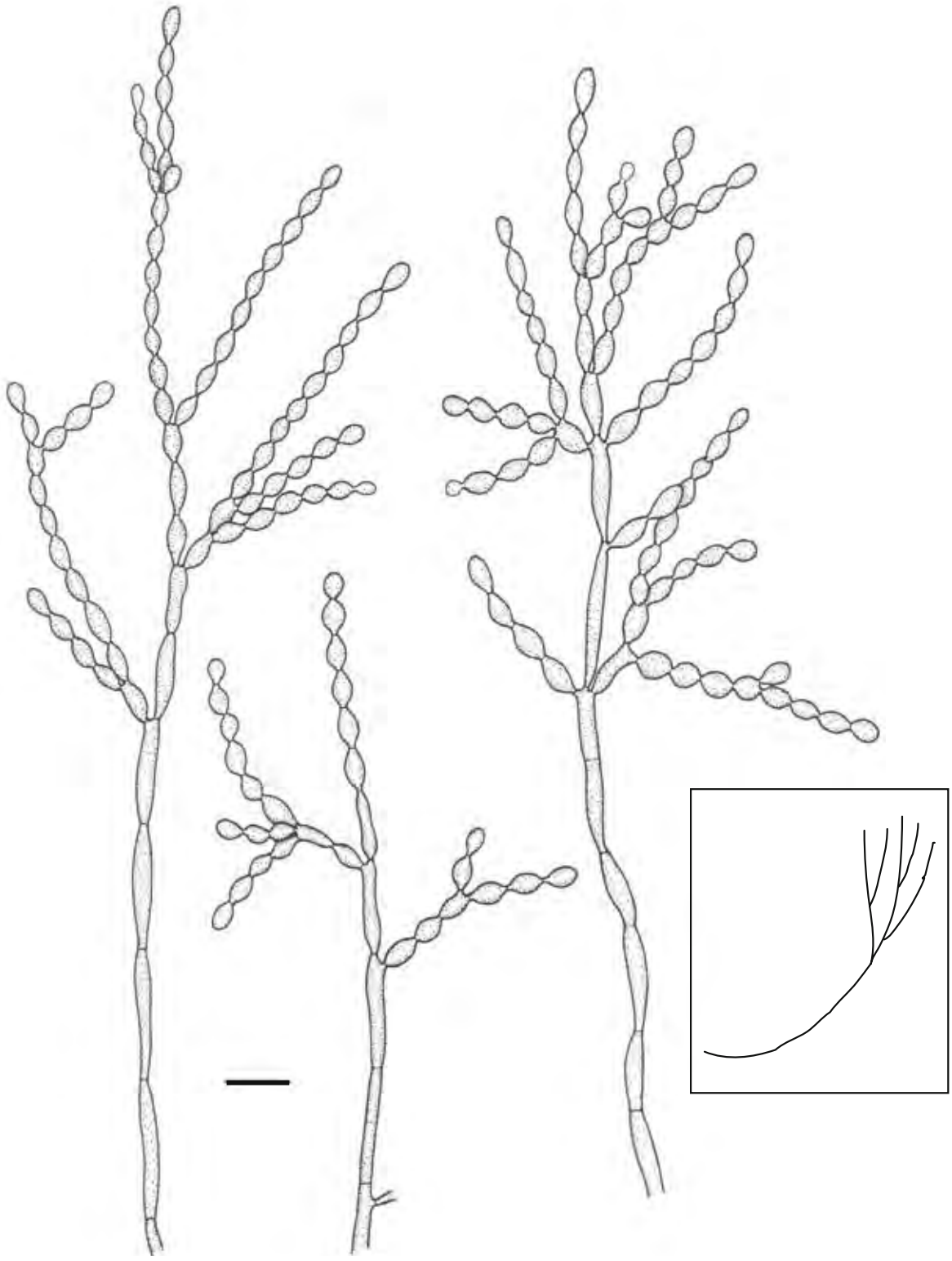

Fig. 6. Microscopic morphology of *C. yegresii*, strain CBS 114405. Conidiophore ascending, i.e. mostly emerging from hyphal end that is gradually growing upwards to become a conidiophore (sketch). Scale bar = 10 µm.

CBS 114402 (*C. carrionii*) was grown from 36 (72 %) plants and CBS 114405 (*C. yegresii*) from 30 (60 %). The fungi could not be isolated from spines. The 50 plants used as controls showed scarring of 1.06 cm diam on average around the point of inoculation. No fungal elements were seen in direct examinations and histological sections of these plants. The retro-cultures were negative. The scarring responses of the plants to the clinical strain, environmental strain and control proved to be highly significant:

Clinical CBS 114402 *vs.* environmental CBS 114405: þ = 0.000832, P = 0.01;

Clinical CBS 114402 *vs.* control: þ = 0.00003128, P = 0.01;

Environmental CBS 114405 *vs*. control: þ = 0.005343, P = 0.01.

Spines 2.5 cm av. in length, seeded with suspensions of CBS 114402 (*C. carrionii*) and CBS 114405 (*C. yegresii*), developed toruloid hyphal elements with some dark, swollen cells similar to muriform cells known in human tissue. The re-isolated strains proved the species to survive during the experimental procedure (< 75 d). Similar results were obtained with the spines 1.5 cm av. in length. No cladophialophora-like fungi were isolated from the controls.

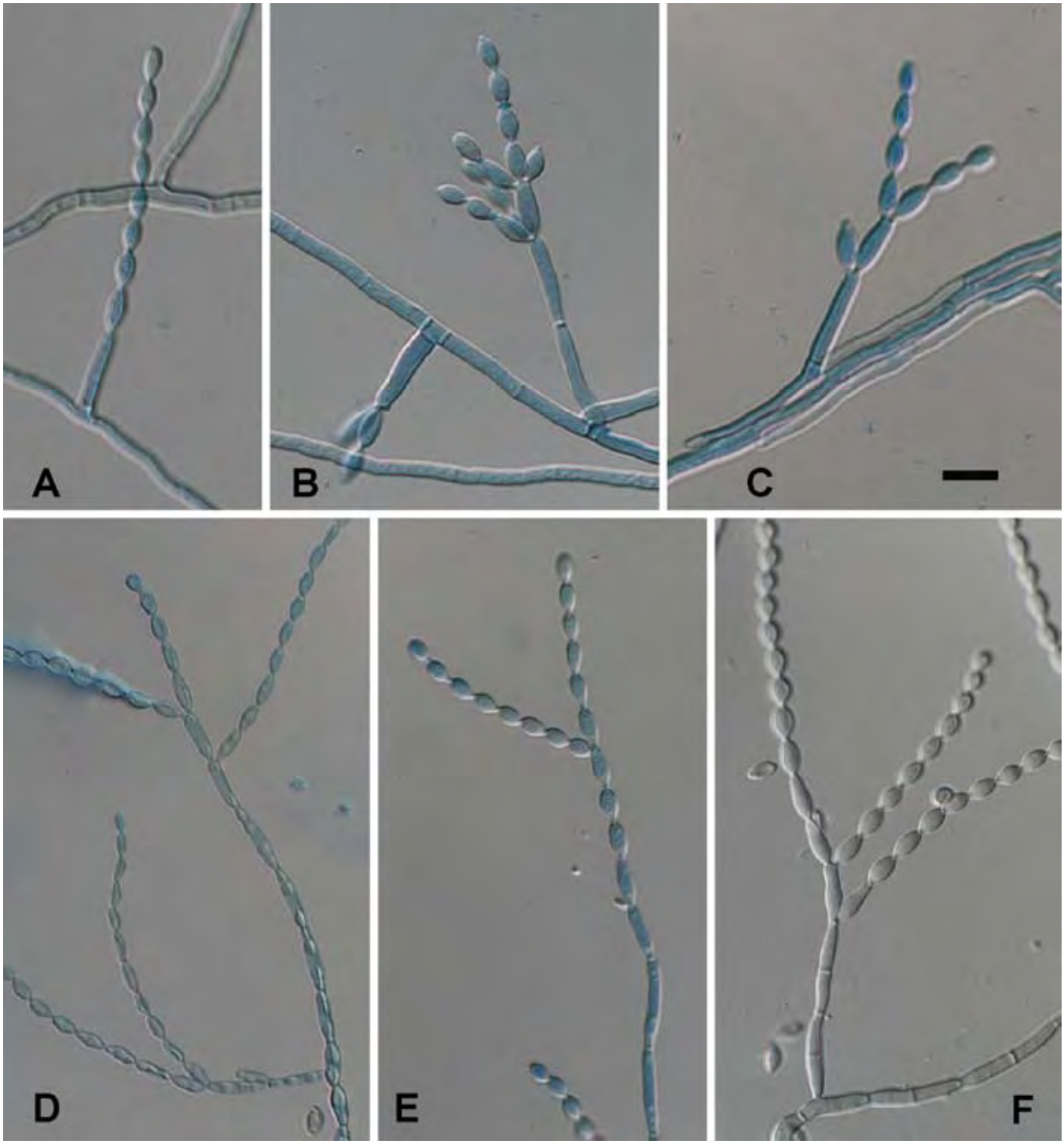

Fig. 7. Conidial morphology in selected branches of (upper row: A–C) *C. carrionii*, strain CBS 260.83; (lower row: D–F) *C. yegresii*, strain CBS 114405. In this respect the two species are identical. Scale bar = 10 μm.

DISCUSSION

Taxonomy of *Cladophialophora*

Judging from SSU rDNA phylogeny data, all *Cladophialophora* species that are consistently associated with pathology to humans belong to the *Herpotrichiellaceae* in the order *Chaetothyriales* (Haase *et al*. 1999). Within this order, the genus *Cladophialophora* is polyphyletic. Conidia of all species are produced in branched chains on poorly differentiated hyphae. This very simply structured conidial system may lead to confusion with morphologically similar but unrelated fungi that are encountered as contaminants in the hospital environment. The genus *Cladosporium* comprises ubiquitous airborne fungi which mostly have erect, more or less differentiated conidiophores, and dark conidial scars. They are associated with *Davidiella* Crous & U. Braun teleomorphs and belong to the *Dothideomycetes*, family *Davidiellaceae* (Braun *et al*. 2003, Schoch *et al*. 2006). *Pseudocladosporium* was introduced by Braun (1998) with three species differing from *Cladophialophora* mainly by intercalary hyphal cells with lateral extensions that bear conidial chains, having *Caproventuria* U. Braun teleomorphs (see Crous *et al*. 2007 – this volume). The group is classified in the *Venturiaceae* and *Mycosphaerellaceae* in the *Dothideales* (Braun *et al*. 2003). The anamorph genus *Devriesia* comprises thermophilic saprobes with a cladophialophora-like appearance and producing

dark, multi-celled chlamydospores alongside the hyphae. Phylogenetically this genus is related to the *Mycosphaerellaceae,* in the *Dothideomycetes* (Seifert *et al*. 2004).

Cladophialophora carrionii was originally introduced by Trejos (1954) on the basis of 46 strains from Venezuela, Australia and South Africa. He did not indicate a holotype. For this reason isolate Trejos 27 = Emmons 8619 = CBS 160.54, the first strain mentioned by Trejos (1954), is selected here as representative for *C. carrionii*. A dried specimen of this strain has been deposited as **lectotype** in the Herbarium of the Centraalbureau voor Schimmelcultures as CBS H-18465.

The ex-type strain of *Cladophialophora ajelloi* Borelli, CBS 260.83, proved to be indistinguishable from *C. carrionii*, which was also known to be able to produce phialides in addition to catenate conidia (Honbo *et al*. 1984). Remarkably, a strain identified as *C. ajelloi* from Samoa (CBS 259.83; Goh *et al*. 1982) proved to be related to but consistently different from all strains of the *C. carrionii* complex. The 43-year-old male patient in otherwise good health carrying this fungus had a 5 × 3 cm erythematous, scaling lesion on his arm. Muriform cells were present in superficial dermis and stratum corneum. This clearly represents yet another agent of human chromoblastomycosis. The name *C. ajelloi* is not available for this taxon, as this is a synonym of *C. carrionii*. The taxon will be formally described in a forthcoming paper.

Members of ITS groups (A)–(D) were shown to be close to each other in SSU phylogeny (data not shown) underlining that all analysed species were correctly assigned to *Cladophialophora*. This genus was defined by melanised acropetal chains of conidia, near absence of conidiophores, and phylogenetic affinity to the order *Chaetothyriales*. Strains (A)–(D) clustered in a clade which contained a mixture of species of *Cladophialophora, Fonsecaea* Negroni and *Phialophora* Medlar. From a point of view of human disease, the species of the clade were known as agents of brain infection [*C. bantiana* (Sacc.) de Hoog *et al., F. monophora* (M. Moore & F.P. Almeida) de Hoog *et al*.], disseminated disease [*C. devriesii* (A.A. Padhye & Ajello) de Hoog *et al.*], cutaneous disease [*C. boppii* (Borelli) de Hoog *et al.*] and particularly chromoblastomycosis (*C. carrionii, Fonsecaea, Phialophora*).

Diversity of *Cladophialophora carrionii* / *C. yegresii*

Infraspecific variability was observed within *C. carrionii.* The groups (A)–(C) were separated on the basis of five mutations in the ITS region, which were supported by mutations in EF1 and BT2, as confirmed by analysis in Structure, where the same separation (K = 5) of entities was observed. Furthermore, K = 4 unites groups (B/II) and (D/III), despite the fact that the sequence of (B) is more close to those of (A). With T-rex software a similar relationship between [(B), *C. carrionii*] and [(D), *C. yegresii*] was noted, suggesting horizontal gene flow between these entities. This is remarkable, since (C) strains predominantly inhabit remote deserts in Madagascar and Australia, while (D) is found in equally remote localities in Venezuela. Extensive reticulation was observed in all genes with SplitsTree. With ITS and BT2, CBS 834.96 and CBS 102227 cluster closely together, while in the more variable EF1 data these are all widely apart, suggesting that in *Cladophialophora* other mechanisms than recombination may occur.

Group (C) contained ITS sequences taken from the public domain, originating from a single study (Abliz *et al*. 2004). Remarkably, strain IFM 4808 found in group (C) on the basis of data from Abliz *et al*. (2004), was the same isolate as CBS 160.54, which was found repeatedly in group (B) in our data set (Table 1). A similar phenomenon was observed with strain IFM 41444 = CBS 863.96, of which GenBank deposition AB109169 consistently deviated from our data in a frequently observed mutation. A possible explanation of these consistent sequence conflicts is heterozygosity. Although most chaetothyrialean fungi are supposed to be haploid (Szaniszlo 2002; Zeng *et al.* 2007), some strains have a double DNA content in yeast cells (Ohkusu *et al*. 1999). Teleomorphs are not known in *Cladophialophora* and related black yeasts, but many species are known to form profuse hyphal anastomoses (de Hoog *et al*. 2006), allowing parasexual processes and mitotic recombination. However, all electropherograms including those from the study of Abliz *et al*. (2004), which were kindly sent by K. Fukushima (Chiba, Japan), were unambiguous, without double peaks. This matched with the observation of preponderant clonality despite frequent anastomoses in *Exophiala* J.W. Carmich. (Zeng *et al*. 2007). An alternative explanation might be the occurrence of paralogous ITS repeats, as reported earlier in *Fusarium* Link (O'Donnell & Cigelnik 1997).

The remaining diversity within *C. carrionii* as confirmed by Structure shows some geographical structuring of populations, in that group (A) does not occur in Asia, group (B) is limited to Australia and Africa, and group (C) has thus far only been reported from Asia. The wide distribution of most genotypes suggests, however, that worldwide occurrence is likely to become apparent when more strains have been analysed. All climate zones where *C. carrionii* was isolated were semi-arid to arid, desert-like. Genotypes were not limited to the endemic semi-arid areas, and thus a relatively rapid vector of dispersal has to be hypothesised enabling the fungus to cross climate zones where the saprobic phase is unable to survive. Kawasaki *et al*. (1993) analysed three further loci in mtDNA using RFLP. Only some of their strains were available for sequencing. These had all identical mtDNA profiles, with the exception of IFM 4808 = CBS 160.54, that differed in two markers (Table 1). If we assume that there is no real separation of ITS groups (A) and (C) (see above), the conclusion is warranted that mtDNA allows distinction of polymorphism at the same level of diversity as detected in this study with ITS, EF1 and BT2.

South America harbours group (D) which represents a second species, *C. yegresii*. This species thus far has not been found on humans, and seems to be restricted to living *Stenocereus* cactus plants. Nishimura *et al*. (1989) published a strain from chromoblastomycosis in China which matched the morphology of strains now classified as *C. yegresii*, but as far as we are aware this strain has not been sequenced.

Ecology and virulence of *Cladophialophora carrionii* / *C. yegresii*

Cladophialophora carrionii was preponderantly found as an agent of human infection and only occasionally on dead plant debris, mainly seceded cactus needles. The only three strains available of *C. yegresii* were isolated from living, asymptomatic *Stenocereus* plants surrounding the cabin of a symptomatic patient from whom *C. carrionii*, CBS 114402 was isolated. Although in some publications convincing evidence was presented that infections originate from puncture by plant material (e.g., Salgado *et al*. 2004), it now becomes clear that the environmental look-alikes of clinical strains do not necessarily belong to the same species (Crous *et al.* 2006, Mostert *et al*. 2006), but may be members of other, related taxa with slightly different ecology; an unambiguous connection between a clinical and an environmental strain still has to be proven.

The endemic area of the two species, *C. carrionii* and *C.*

yegresii, has a semi-arid climate, with average yearly temperatures of 24 ºC, scarce rainfall (up to 600 annual mL) and is located at moderate altitude (up to 500 m) (Borelli 1979, Richard-Yegres & Yegres 1987). The landscape is dominated by large cacti and other xerophytes. *Stenocereus griseus* is a columnar American cactus with a very strong, protective external epidermis that allows the accumulation of water in the shaft and enables tolerance of extreme drought. The species produces ovoidal, thorny fruits of about 5 cm diam, which are commonly eaten by the local population. It has therefore been suggested that patients with chromoblastomycosis acquire their infection by traumatic inoculation with cactus spines, similar to the supposed infection process of *Madurella mycetomatis* (Laveran) Brumpt in the arid climate of Africa (Ahmed *et al*. 2002). The frequent occurrence of 16 / 1 000 for chromoblastomycosis in areas endemic for *Cladophialophora* in Venezuela (Yegres *et al*. 1985; Yegűez-Rodriguez *et al*. 1992) indicates a marked invasive potential for *C. carrionii*. Local goat-keepers are particularly at risk: in 1984, 14 of 18 patients investigated had these occupational characteristics (Yegres *et al*. 1985). Nevertheless, virulence of *C. carrionii* is low when inoculated into the footpads of mice (Yegres *et al*. 1998); also an environmental strain of *C. carrionii* failed to produce lesions in mice and in a volunteer (Richard-Yegres & Yegres 1987).

We performed inoculation experiments with *C. carrionii* and *C. yegresii* using freshly grown, healthy cacti in the greenhouse. The plants were followed over a 1-yr period; during all this time the control plants remained without lesions. Both *Cladophialophora* strains were able to produce infection when syringe-inoculated deep into young cactus tissue. Histopathology showed septate hyphae between host cells, and the shaft was maintained over prolonged periods without causing visible damage. This absence of appreciable destruction would categorise them as endophytes. Cactus tissue is rich in carbohydrates, vitamins and minerals (Vélez & Chávez 1980) which may promote endophyte growth.

In contrast, suspensions applied superficially lead to growth on and in spines only. The absence of infection after superficial application indicates that the fungi are unable to invade healthy plant tissue from the surface and thus they cannot be characterised as obligatory phytopathogens.

The two species differed in the degree of scarring after traumatic inoculation into mature plants: the clinical strain *C. carrionii* was consistently more virulent than *C. yegresii* that originated from the same host plant. Both species showed the same viability in re-isolated cultures. In nature, the fungi are likely to invade only when the integrity of the epidermis is broken, as happens e.g. by goat feeding or transmission by sap-sucking birds or piercing insects. They also show the same transformation to meristematic morphology (González *et al*. 1990) when entering hard spine tissue. A possible trigger for this conversion is the dominance of lignin in the spines. Survival on and in spines is enhanced by their capturing of atmospheric water formed after nightly condensation. The fact that superficial application leads to colonisation around and inside the spines suggests that the spines play a role in mechanic dispersion of the fungi.

A possibly coincidental mechanism of dispersal might be traumatic inoculation into living tissue of humans or animals, where the same muriform cells are formed, defining the skin disease chromoblastomycosis. It may be questioned whether animal/human inoculation plays a role in the evolution of the fungus. ITS differences between the two species are observed in 23 positions, with a ratio of transitions : transversions of 2 : 1 (Table 3). Thus no saturation of mutations has taken place and the diversification can be regarded as an example of recent sympatric speciation. *Cladophialophora carrionii* is widely distributed, and shows a higher degree of diversity than *C. yegresii*. This would be suggestive for a longer evolutionary time span of existence and *C. carrionii* then should be regarded as ancestral to *C. yegresii*, with the latter showing a founder effect due to the absence of polymorphisms. However, such an order of event (a host jump from humans to cactus) is difficult to imagine. It is more likely that *C. yegresii* is the original cactus endophyte exhibiting extremotolerance via its muriform cells. T-REX data suggested a more direct connection of *C. yegresii* with African and Australian rather than Venezuelean strains of *C. carrionii*. We suppose that the low degree of observed variation in *C. yegresii* is not a founder effect, but rather a sampling effect, as living cacti have thus far not been studied outside the framework of our study on the patient with *Cladophialophora* chromoblastomycosis. The difference in virulence may be simply explained by *C. carrionii,* which lives as a saprobe on dead cactus debris for part of its life cycle, and is less adapted to an endophytic life style.

Cladophialophora cf. carrionii is known to occur on lignified materials, such as wood chips of *Eucalyptus crebra* and wooden remains of *Prosopis juliflora* and *Stenocereus griseus* (Riddley 1957, Yegres *et al*. 1985, Fernández-Zeppenfeldt *et al*. 1994). This does not exclude a certain degree of pathogenicity to humans, as also pathogens like *Cryptococcus neoformans* (Sanfelice) Vuill. are known to have an essential part of their life cycle in hollows of *Eucalyptus* trees. *Cryptococcus neoformans* produces diphenol oxidase to degrade lignin, an aromatic polymer in the cell wall of plants and a component of wood (Cabral 1999). Similar degradation pathways are present in *Cladophialophora carrionii* (Prenafeta-Boldú *et al*. 2006).

The natural occurrence of *C. carrionii* and *C. yegresii* in association with xerophytes has been proven, but their environmental route of dispersal is still unknown. As transformation to meristematic cells takes place when the hyphae reach the spines and on dead spines, the muriform cell apparently is the extremotolerant phase of the species. The conidial anamorph can be found sporulating on rotten spines directly after rainfall (Richard-Yegres & Yegres 1987), but as the fungus has thus far never been isolated from outside air, it is still unclear how a new host plant is reached.

The behaviour of *C. carrionii* on humans, provoking the very characteristic disease, chromoblastomycosis, of which the agents are limited to the ascomycete family *Herpotrichiellaceae* (de Hoog *et al*. 2000) is puzzling. In humans, the extremophilic muriform anamorph is expressed rather than hyphae, and thus humans do not seem a natural reservoir of the fungus. Nevertheless some acquired cellular immunity seems to be involved. Albornoz *et al*. (1982) demonstrated that a significant share of the local population of goat keepers (Yegres *et al*. 1985) is asymptomatically infected with *C. carrionii*; Iwatsu *et al.* (1982) detected cutaneous delayed hypersensitivity in rats experimentally-infected with agents of chromoblastomycosis. With murine experimental infection of the related fungus *Fonsecaea pedrosoi*, Ahrens *et al*. (1989) found enlargement and metastasis of lesions in athymic but not in normal mice, or in mice with defective macrophage function. Several authors (Kurita 1979, Nishimura & Miyaji 1981, Polak 1984) observed a significant role of acquired cellular immunity in *F. pedrosoi*, while Cardona-Castro & Agudelo-Flórez (1999) obtained chronic infection in immunocompetent mice when inoculated intraperitoneally. Garcia Pires *et al*. (2002) noted an unbalance between protective Th1 and less efficient Th2 responses. The possible host response leads to different clinical types, referred

to as tuberculoid and suppurative granuloma, respectively. The existence of genetic constitutional factors in susceptibility is underlined by a marked frequency of family relationships among symptomatic individuals (Yegűez-Rodriguez *et al*. 1992). The disease is not observed in local animals such as goats, possibly due to their high body temperature (≈ 39 °C). Nevertheless, hyphal fragments artificially inoculated into goats led to transformation into muriform cells, but the lesions disappeared within 60 d (Martínez *et al*. 2005). Further animal experiments using strains identified according to new taxonomy will be necessary to answer questions on the role of the fungus on warm-blooded animals.

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Taxonomy, nomenclature and phylogeny of three cladosporium-like hyphomycetes, *Sorocybe resinae, Seifertia azaleae* and the *Hormoconis* anamorph of *Amorphotheca resinae*

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Abstract: Using morphological characters, cultural characters, large subunit and internal transcribed spacer rDNA (ITS) sequences, and provisions of the International Code of Botanical Nomenclature, this paper attempts to resolve the taxonomic and nomenclatural confusion surrounding three species of cladosporium-like hyphomycetes. The type specimen of *Hormodendrum resinae*, the basis for the use of the epithet *resinae* for the creosote fungus {either as *Hormoconis resinae* or *Cladosporium resinae*) represents the mononematous synanamorph of the synnematous, resinicolous fungus *Sorocybe resinae*. The phylogenetic relationships of the creosote fungus, which is the anamorph of *Amorphotheca resinae*, are with the family *Myxotrichaceae*, whereas *S. resinae* is related to *Capronia* (*Chaetothyriales, Herpotrichiellaceae*). Our data support the segregation of *Pycnostysanus azaleae,* the cause of bud blast of rhododendrons, in the recently described anamorph genus *Seifertia,* distinct from *Sorocybe;* this species is related to the *Dothideomycetes* but its exact phylogenetic placement is uncertain. To formally stabilize the name of the anamorph of the creosote fungus, conservation of *Hormodendrum resinae* with a new holotype should be considered. The paraphyly of the family *Myxotrichaceae* with the *Amorphothecaceae* suggested by ITS sequences should be confirmed with additional genes.

Key words: *Amorphothecaceae, Cladosporium resinae,* creosote fungus, *Hormoconis resinae,* jet fuel fungus, kerosene fungus, *Myxotrichaceae, Pycnostysanus*, resinicolous fungi.

INTRODUCTION

The ascomycete *Amorphotheca resinae* Parbery (1969) grows in hydrocarbon-rich substrates such as jet fuel, cosmetics and wood preserved with creosote or coal tar. This fungus is widely known by the anamorph name *Hormoconis resinae* (Lindau) Arx & G.A. de Vries or its obligate synonym *Cladosporium resinae* (Lindau) G.A. de Vries. It produces lightly pigmented, warty conidiophores, and branched, acropetally developing chains of lightly pigmented ameroconidia lacking conspicuous scars (Fig. 1B–E). This species is known colloquially as the "creosote fungus", the "kerosene fungus" or the "jet fuel fungus"; to avoid confusion caused by the many heterotypic names with the epithet "*resinae*", in this paper we generally will use the oldest of these informal names, "creosote fungus", when referring to *A. resinae* or its anamorph. This fungus grows in jet fuel contaminated with small amounts of water, and the mycelium clogs fuel lines and corrodes metal parts. Consequently, fuel tanks in airports are monitored for this fungus by private companies using various physiological or biochemical tests.

Sorocybe resinae (Fr.) Fr. produces dark black colonies on conifer resin, comprising dark synnemata and an effuse mononematous synanamorph, both with cladosporium-like conidiogenous cells and conidia. Unlike the anamorph of the creosote fungus, the conidia of *Sorocybe resinae* are dark brown and the lateral walls are conspicuously thicker than the poles (Fig. 2D–G). Colonies with only the mononematous anamorph sometimes occur, and the mononematous anamorph can be sparse on colonies bearing synnemata. However, the conidia of the mononematous anamorph have identical pigmentation and lateral wall thickening to that of the synnematous anamorph. The mononematous anamorph rarely has been referred to by its own binomial name although, as we will show, there is a species epithet available. For the same reasons given above for *Amorphotheca* Parbery, generally we will refer to *Sorocybe resinae* herein as "the resin fungus".

Despite the micromorphological differences noted above, there is disagreement about whether the creosote fungus is conspecific with the mononematous synanamorph of the resin fungus (Parberry 1969). The name for the anamorph of the creosote fungus is based on *Hormodendrum resinae* Lindau (1906). Christensen *et al*. (1942) presented a study of a cladosporium-like fungus commonly isolated from wood impregnated with creosote and coal tar and applied Lindau's name without examining its type. A later ecological study by Marsden (1954) employed the same name for the same fungus. An extra dimension was added to the confusion when de Vries (1952, using the name *Cladosporium avellaneum* G.A. de Vries) described four *formae* for the creosote fungus (differing in the colours of their conidia, the production of setae, or the total absence of conidia), each based on single conidium isolates made from one parent culture. De Vries (1955) and Parberry (1969) examined the holotype of *Hormodendrum resinae* and concluded that it represented the creosote fungus. Hughes (1958), prior to the description of *Amorphotheca* or *Hormoconis* Arx & G.A. de Vries, examined the same specimen and considered it to be the mononematous synanamorph of the resin fungus. If Hughes (1958) is correct, then neither the species *Hormodendrum resinae*, nor the genus that it typifies, *Hormoconis*, can represent the creosote fungus, as intended by Parberry (1969) or von Arx and de Vries (in von Arx 1973).

In this paper, we present micromorphological, cultural and molecular evidence that the resin fungus is a different species from the creosote fungus. Combined with re-examination of the holotype of *Hormodendrum resinae*, this information is used to provide a revised taxonomy and nomenclature for these two species. A third cladosporium-like fungus, *Seifertia azaleae*, is also considered in our discussion of generic concepts.

Historical review

The history of the fungus now known as *Sorocybe resinae* began with Fries (1815), who described *Racodium resinae* Fr. as follows:

"310. *Racodium resinae*, expansum molliusculum dense contextum nigrum, filis inaequalibus.

In resina *Pini Abietis* in silvis Suecia passim.

Habitu et loco natali distinctum. Fila divaricato-ramosa; alia rigidula apice capituli sera, sub miscrosc. *Coremio* Link similia, *Demat. villosum* Schleich. huic simile; sed sub microsc. fila maxime differunt."

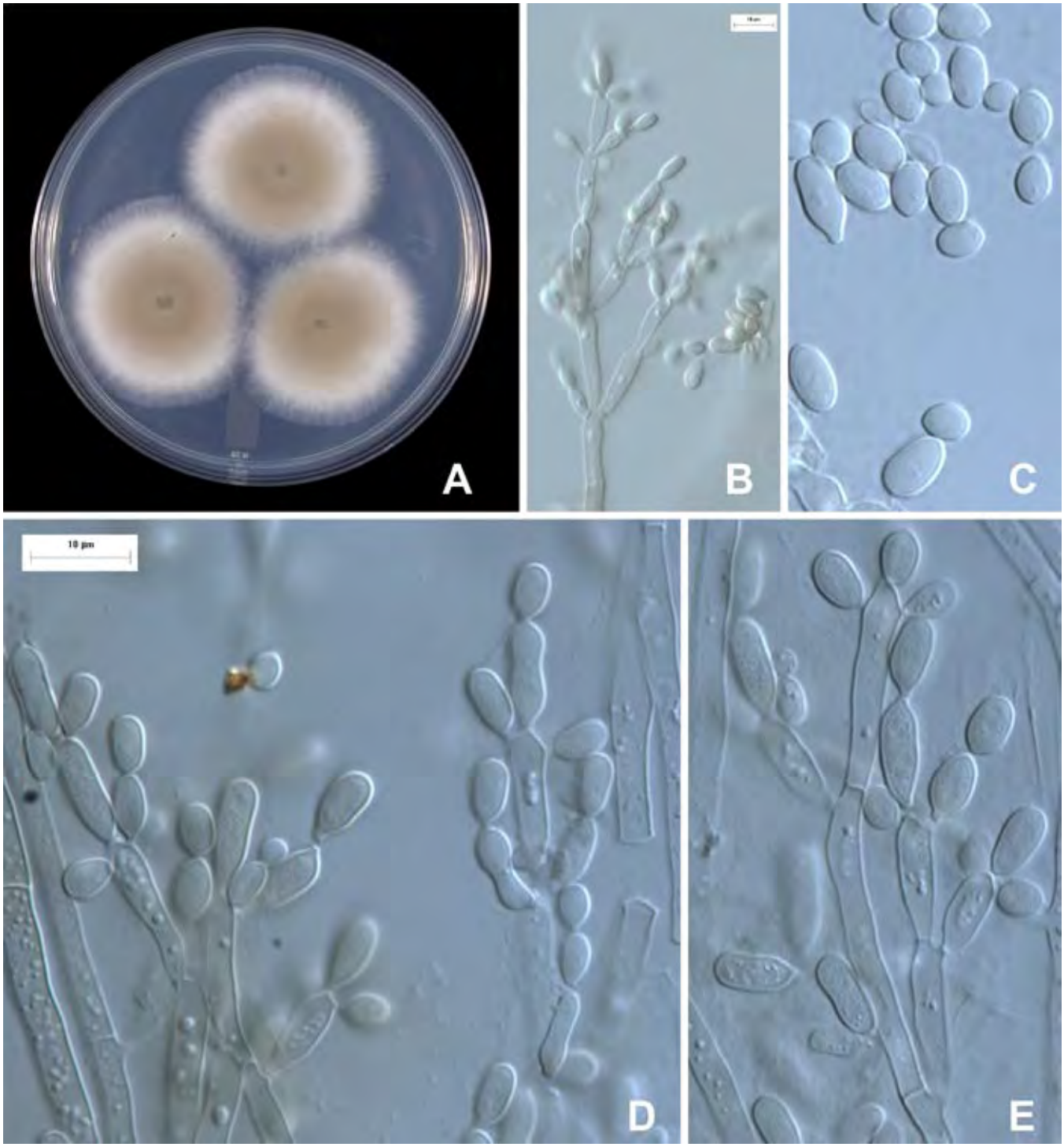

Fig. 1. *Amorphotheca resinae*, colony characters and anamorph micromorphology. A. 10-d-old colony on PDA. B, D–E. Micromorphology of conidiophores, showing acropetal conidial chains, ramoconidia, and conidia. C. Conidia. DAOM 170427; for C, E see scale bar in D.

The comparison with *Coremium* Link indicates the probability of a synnematous fungus, and an authentic specimen of Fries' fungus, which as the only known authentic material we interpret as the holotype, is preserved in Link's herbarium (see below). It represents the synnematous form of the resin fungus[1].

[1]Persoon (1822) described a form of *R. resinae* "β *piceum*". Hughes (1968) examined the holotype of this form, and it represents the mycelium of the ascomycete *Strigopodia resinae* (Sacc. & Bres.) S.J. Hughes. This taxon is thus not relevant to the three species that are the focus of this paper.

Fries (1832) later transferred his species to *Sporocybe* Fr. (1825), a genus then used for relatively conspicuous dark hyphomycetes with dry spores (Mason & Ellis 1953). The 1832 description explicitly stated... "capitulo rotundato inaequali, sporidiis seriatis, stipite aequali simplici." The use of "capitulo" and "stipite" imply what would now be recognised as a synnematous fungus. Fries (1832) further characterised the habit of the fungus as "habitu stipitum Calicii," a further comparison to a group of black, stipitate lichenized fungi classified in *Calicium* Pers., which under a hand lens look similar to a dark synnematous fungus.

Fries (1849) next described the genus *Sorocybe* Fr. for this fungus, as follows:

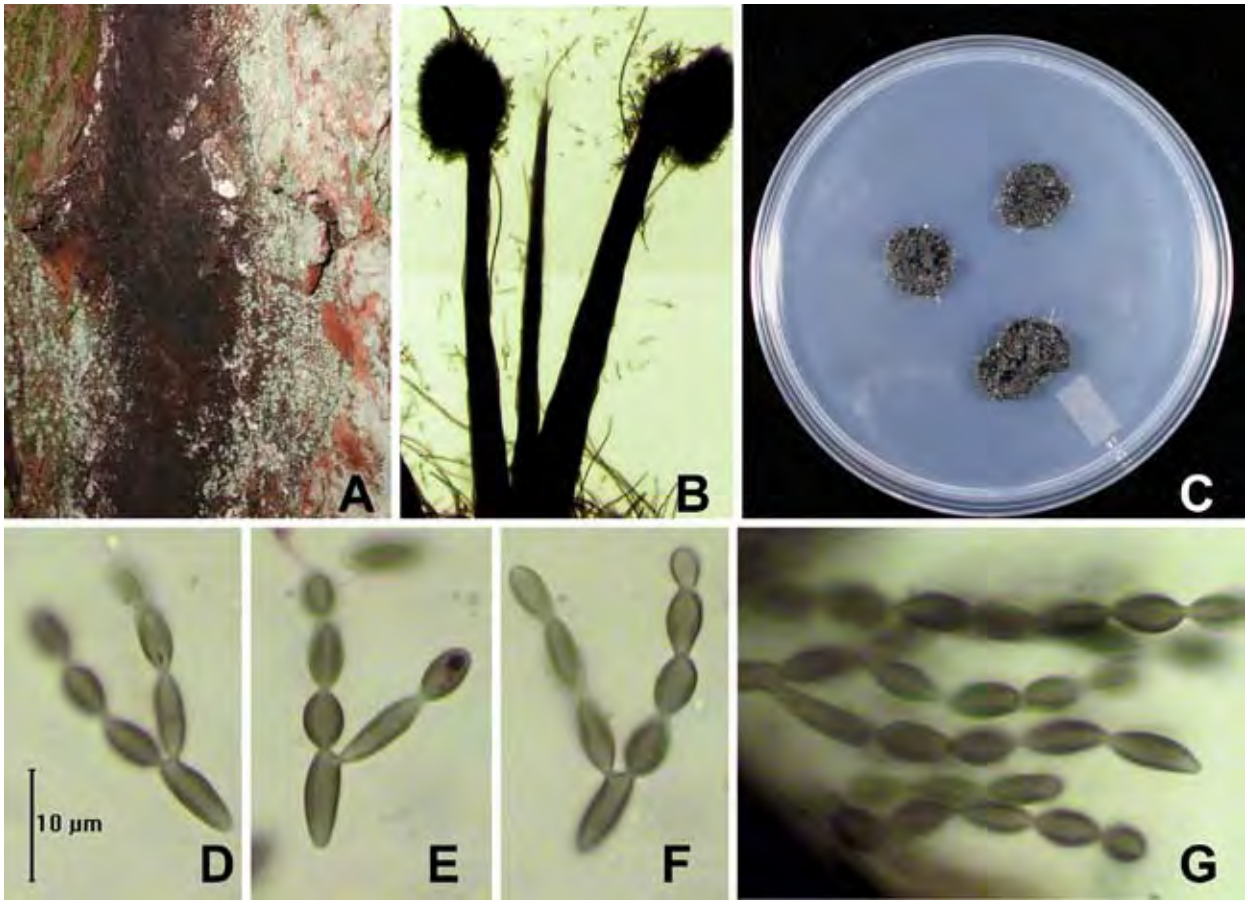

Fig. 2. *Sorocybe resinae*, synnematous form. A. Colony on bark of living, standing conifer. B. Synnemata. C. Four-month-old colony on DG18. D–G. Acropetally developing chains of conidia. Note that the lateral walls are conspicuously thickened; compare with Fig. 3. A, C. DAOM 239134. B, D–G. DAOM 11381.

Sorocybe Fr.
Habitus prioris. sed mycelium floccosum densum, stroma corneo-carbonaceum, sporis moniliformi-concatenatis basi excipulum incompletum praebens.
1. *S. resinae*. Fr. 1–4. at raro fructif. Klotzsch. exs. C. 2.

Because this description explicitly referred to the *Systema*, Fries presumably was segregating the fungus, originally described as *Racodium resinae*, into a new monotypic genus (McNeill *et al.* 2007; Art. 33.3) and this interpretation of *R. resinae* as the basionym generally has been followed in subsequent treatments of *Sorocybe resinae*.

As noted in Table 1, Fries' *Racodium resinae* was placed in several other hyphomycete genera by eighteenth century authors. These diversions need not be reviewed in detail here because the modern status of these other genera, and their lack of similarity with *Sorocybe*, is clear.

Bonorden (1851) described *Hormodendrum* Bonord., with four species originally placed in *Penicillium* Link by Corda (1839); *H. olivaceum* (Corda) Bonord. (≡ *Penicillium olivaceum* Corda 1839) was designated as lectotype by Clements & Shear (1931). This genus was frequently, but incorrectly, spelled *"Hormodendron"*. Bonorden's descriptions and illustrations are of variable quality by modern standards, and his herbarium is unknown (Stafleu *et al*. 1995). Consequently the actual identities of the species Bonorden placed in *Hormodendrum* are unknown and Corda's *Cladosporium olivaceum* (Corda) Bonord. was dismissed in *Penicillium* monographs because the drawing shows branched conidial chains (Thom 1930), although the specimen has apparently not been re-examined. The generic name was used as a segregate for *Cladosporium* Link by some authors (e.g. Kendrick 1961), in particular for species with ameroconidia (de Vries 1952). Although it sometimes has been considered a synonym of *Cladosporium*, it will remain a *nomen dubium* until the type species is properly typified.

Unaware of the resinicolous fungus described by Fries, Lindau described two species growing on conifer resin, *Pycnostysanus resinae* Lindau (1904), the type of this anamorph generic name, and *Hormodendrum resinae* Lindau (1906). The former was clearly illustrated and described as a synnematous species. The protologue of the latter concludes with, "Mit *Pycnostysanus resinae* hat die Art nichts zu tun." Clearly, Lindau observed no synnemata on the specimen of the mononematous fungus and he believed it was a different fungus, rather than what would now be called a synanamorph of the synnematous fungus that he had described previously. Lindau (1910) reproduced the 1904 illustration of *Pycnostysanus resinae* as *Stysanus resinae* (Fr.) Sacc. (1906), thus accepting its identity with the species originally described as *Racodium resinae* Fr. Lindau (1910) made no mention of *Hormodendrum resinae*, indicating he still made no association between the synnematous and mononematous fungi on resin.

De Vries (1952) described a new species, *Cladosporium avellaneum* G.A. de Vries, isolated from cosmetics. Later, he noted the similarities between his *C. avellaneum* and the creosote fungus, and suggested that they were the same species (de Vries 1955), replacing the name of one of his previously described formae, *i.e. viride*, with the forma name *resinae*. He examined Lindau's type of *Hormodendrum resinae* and decided that it provided an earlier epithet for *C. avellaneum*. He transferred the species into *Cladosporium* as *C. resinae* (Lindau) G.A. de Vries, and this name was widely used for the creosote fungus until 1973. This binomial is still commonly employed in non-taxonomic literature, especially commercial publications dealing with the creosote fungus.

Table 1. Nomenclature and synonymies for the creosote fungus and the resin fungus, showing the use of the same basionym for the two fungi. The "false" names and synonymies for the anamorph of the resin fungus are indicated by blue text. The second nomenclatural solution described in the text would have the effect of switching the blue text to black for the creosote fungus, and to simultaneously switch the equivalent black text to blue for the mononematous synanamorph of the resin fungus. Holotypes we have examined, and the herbarium where they are deposited, are marked with exclamation points, and details of these specimens are noted in Materials and Methods.

Creosote fungus
Teleomorph: *Amorphotheca resinae* Parberry, Australian J. Bot. 17: 340. 1969.
Anamorph
Hormodendrum resinae Lindau, in Rabenh. Krypt.-Fl., 2, 1 (Pilze) 8: 699. 1906 (B!).
≡ *Cladosporium resinae* (Lindau) G.A. de Vries, Antonie van Leeuwenhoek 21: 167. 1955.
≡ *Hormoconis resinae* (Lindau) von Arx & G.A. de Vries, in von Arx, Verh. K. Ned. Akad. Wet., Afd. Natuurk. 61: 62. 1973.
= *Cladosporium avellaneum* G.A. de Vries, Contribution to the knowledge of the genus Cladosporium, Uitg. Druk. Hollandia, p. 56, 1952.
Resin fungus
Mononematous synanamorph:
Hormodendrum resinae Lindau, in Rabenh. Krypt.-Fl., 2, 1 (Pilze) 8: 699. 1906 (B!)
≡ *Cladosporium resinae* (Lindau) G.A. de Vries, Antonie van Leeuwenhoek 21: 167. 1955.
≡ *Hormoconis resinae* (Lindau) von Arx & G.A. de Vries, in von Arx, Verh. K. Ned. Akad. Wet., Afd. Natuurk. 61: 62. 1973.
Synnematous anamorph:
Sorocybe resinae (Fr.) Fr., Summa Veg. Scan. 2: 468. 1849.
≡ *Racodium resinae* Fr., Obs. Mycol. 1: 216. 1815 (basionym) (B!).
≡ *Sporocybe resinae* (Fr.) Fr., Syst. Mycol. 3: 341. 1832.
≡ *Dendryphion resinae* (Fr.) Corda, Icon. Fung. 6: 11. 1854.
≡ *Stysanopsis resinae* (Fr.) Ferr., Flora Ital. Crypt., 1 (Fungi, Hyphales), p. 187. 1910.
? = *Dematium nigrum* Link, Mag. ges. naturf. Fr. 3: 21. 1809 (B!).
≡ *Sporotrichum nigrum* (Link) Link, Mag. Ges. naturf. Fr. Berlin 7: 35. 1815.
= *Pycnostysanus resinae* Lindau, Verh. Bot. Ver. Brandenb. 45 : 160. 1904 (B!).
≡ *Stysanus resinae* (Lindau) Sacc., Syll. Fung. 18: 651. 1906.

In his study of type collections of classical hyphomycetes, Hughes (1958) included *Pycnostysanus resinae* Lindau and *Hormodendrum resinae* Lindau as facultative synonyms of *Sorocybe resinae* (Fr.) Fr., with *Racodium resinae* Fr. and several other nomenclatural variants as obligate synonyms (Table 1). The synnematous *Pycnostysanus resinae* was cited as "*Pycnostysanus* state [i.e. synanamorph] of *Sorocybe resinae". Hormodendrum resinae* thus remained to represent the mononematous synanamorph of what was interpreted as a single species.

Parberry (1969) described a cleistothecial ascomycete, *Amorphotheca resinae*, for the teleomorph of the creosote fungus. He also examined the holotype of *Hormodendrum resinae* and agreed with the conclusions of de Vries (1955). He used the epithet *resinae* for the teleomorph to correspond with that of the anamorph. He discounted the possibility that the synnematous *Sorocybe resinae* could be the same fungus as *Hormodendrum resinae* because synnemata never developed in his cultures of the creosote fungus.

Von Arx and de Vries (in von Arx 1973) described the genus *Hormoconis*, typified by *Hormodendrum resinae*, with the new combination *Hormoconis resinae* (Lindau) Arx & G.A. de Vries. Their intention was to erect an anamorph genus for the anamorph of the creosote fungus, which they suggested was improperly classified in *Cladosporium* because it lacked darkened, thickened secession scars on the conidia.

A third cladosporium-like fungus is relevant to this story. *Seifertia azaleae* (Peck) Partridge & Morgan-Jones [until recently known as *Pycnostysanus azaleae* (Peck) E.W. Mason] is a cosmopolitan fungus causing bud blast and twig blight of azaleas and rhododendrons. This species is morphologically similar to *Sorocybe resinae*, but the conidia are paler and lack laterally thickened walls. *Sorocybe* and *Pycnostysanus* have often been considered taxonomic synonyms (Ellis 1976, Carmichael *et al*. 1980); as shown above, both are based on the synnematous form of the resin fungus. Partridge and Morgan-Jones (2002) argued that *Sorocybe resinae* and "*Pycnostysanus azaleae*" are not congeneric, and described the new genus *Seifertia* Part. & Morgan-Jones for the *Rhododendron* fungus. They observed that the connection between conidia in *Seifertia azaleae* is much narrower than in *Sorocybe resinae*, and that minute denticles are visible on the conidiogenous cells of the former fungus. The broader connections between conidia of *Sorocybe resinae* result in broadly protuberant conidiogenous loci on the conidiogenous cells, and more truncate detached conidia.

MATERIALS AND METHODS

Herbarium material and fungal strains

Full details of herbarium material examined are listed below. Cultures and dried herbarium specimens were studied in 90 % lactic acid without stains; preparations of some exsiccate and types were mounted in glycerin jelly. Cultures were grown on potato-dextrose agar (PDA, Difco), oatmeal agar (OA, Samson *et al*. 2004), Blakeslee's malt extract agar (MEA, Samson *et al*. 2004) and dichloran-18 % glycerol agar (DG-18, Samson *et al.* 2004). Colony characters were taken from cultures grown at 25 °C in darkness. Cultures are maintained in the Canadian Collection of Fungal Cultures (DAOM), Agriculture & Agri-Food Canada, Ottawa.

Exsiccati and types

Dematium nigrum [scr. Link]. E. Hbr. Link (23) = *Sporocybe resinae* III. 341 [scr. ?] (herb. Link, B).

Hormodendrum resinae Lindau, n. sp. Fl. v. Hamburg 206, auf Harz an *Picea excelsa*, Sachsenwald, leg. O. Jaap, 29-4-1906. [scr. Lindau]. (DAOM 41888, slide prepared from the **holotype** preserved in B.)

Pycnostysanus resinae Lindau nov. gen. et nov. spec., Kabát et Bubák: Fungi imperfecti exsiccati no. 99. Auf erhärteten Fichtenharz an Brockenweg, am Dreieckigen Pfahl in Harz, Deutschland, leg. G. Lindau, 13.VIII. 1903 (**holotype**, B).

Racodium resinae Fries. E. Hbr. Link, Fries legi, Smol. [scr. Fries]. (DAOM 41890, slide prepared from herb. Link, B). This is the presumed **holotype** of *R. resinae*, the basionym for the resin fungus, *Sorocybe resinae*. The specimen includes dark, decapitated synnemata, brown conidia with laterally thickened walls, and acropetal conidial chains, allowing it to be recognised as the fungus we now know as *S. resinae*. Fries perhaps sent this fungus to Link to see if it could be differentiated from *Coremium* Link. The minimal details, that the fungus was collected by Fries, presumably in Småland (a province of Sweden), match the details in the protologue of this species.

Sorocybe resinae. "Fungi Rhenani Fasc. II, 1863, L. Fuckel, no. 129, ad Abietis resinam, raro Hieme, in sylva Hostrichiensi" (as *Myxotrichum resinae* Fr., DAOM 55543 ex FH). "Flora Suecica, 2956, Ad resinam piceae, Småland: Femsjö, Prostgaidsshogen, 6 Aug. 1929, leg. J.A. Nannfeldt, s.n." (as *Stysanus resinae* (Fr.) Sacc., DAOM 41891 ex UPS). "Flora Suecica, 4709, Ad resinam abietinum, Uppland: Bondkysko sin Valsätra, 9 May 1932, leg. J.A. Nannfeldt" (as *Hormodendrum resinae* Lindau, DAOM 41889 ex UPS). "[on wood scr. Berkeley] J.E. Vize, Hereford 1877" (as *Torula pinophila* Fr., DAOM 113425 ex K). "Sydow, Mycotheca germanica, 350. Auf Fichtenharz… am Brockenweg 30.9.1904, leg. P. Sydow" (DAOM 41893).

Other material examined

Sorocybe resinae. **Canada**, British Columbia: Burnaby, Central Park, on resin of *Tsuga heterophylla*, leg. S. & L. Hughes, 17 Aug. 2000 (DAOM 228572a, 228573a); Cameron Lake, Cathedral Grove, on *Pseudotsuga menziesii*, leg. isol. S.J. Hughes, 21 Aug. 1957 (DAOM 56088a). Ladysmith, Ivy Green Park, on resinous exudates, leg. R.J. Bandoni no. BC-978, 18 Apr. 1960, det. S.J. Hughes (DAOM 70462). North Vancouver, Lynn Valley Conservation Area, leg. det. S.J. Hughes, 1 Jul. 1975 (DAOM 139385); North Vancouver, Lynn Valley Conservation Area, on bark of living conifer (probably *Pseudotsuga menziesii*), leg. isol. K.A. Seifert no. 1574, 26 May 2002 (single conidium isolate, culture and specimen DAOM 239134; ITS GenBank EU030275, LSU GenBank EU030277); Terrace, near Kalum, on *Tsuga heterophylla,* leg. W.G. Ziller no. V-6549, 10 July 1950, det. S.J. Hughes (DAOM 59657); Queen Charlotte Islands, east coast of Moresby Island, north side of Gray Bay, 53°08' N, 131°47' W, on *Picea sitchensis,* leg. I. Brodo, M.J. Schepanek, W.B. Schofield, 28 Sep. 1973, det. S.J. Hughes (DAOM 144757); Queen Charlotte Islands, Graham Island, Tow Hill area, on resin of *Picea sitchensis,* leg. S.A. Redhead no. 4440, 20 Sep. 1982, det. G.P. White (DAOM 184025); Revelstoke, Wigwam, on *Tsuga heterophylla,* leg. W. Ziller V-6567 det. S.J. Hughes, 6 Jun. 1950 (DAOM 59710); Vancouver Island, Cathedral Grove, Cameron Lake, on *Pseudotsuga menziesii*, leg. det. S.J. Hughes, 21 Aug. 1957 (DAOM 56088a); Vancouver Island, Caycuse, on resin of *Pseudotsuga menziesii,* leg. det. S.J. Hughes, 17 Jul. 1972 (DAOM 139355); Vancouver Island, Lake Cowichan, Honeymoon Bay, on resin of *Pseudotsuga menziesii*, leg. J Ginns, det. S.J. Hughes, 29 Oct. 1971 (DAOM 134968); Vancouver Island, Lake Cowichan, Mesachie Lake Forest Experimental Station, leg. det. S.J. Hughes, 5 Jul. 1972 (DAOM 139277a, DAOM 139278) and 6 Jul. 1072 (DAOM 139281). **Czechoslovakia**, Ještěd near Liberec, leg. det. S.J. Hughes, on resin of *Larix europaea*, 10 May 1955 (DAOM 51723). **United States**, Oregon: Andrews' Experimental Forest, Forest Service Rd. no 1553, on resin of *Tsuga heterophylla,* leg. det. S.J. Hughes, 10 May 1969 (DAOM 134565); Andrews' Experimental Forest, Blue River, on resin of conifer, cut wood, leg. det. K.A. Seifert no. 69, 10 Jul. 1981 (DAOM 228203); Oregon, del Norte Co., J. Smith's State Park, on *Tsuga heterophylla*, leg. det. S.J. Hughes, 11 May 1069 (DAOM 134614); Devil's Elbow State Park, Cape Perpetus, on *Picea sitchensis,* leg. det. S.J. Hughes, 6 May 1969 (DAOM 134615); Linn Co., near Cascadia, on *Pseudotsuga menziesii,* leg. R. Fogel, det. S.J. Hughes, 14 May 1969 (DAOM 127885); U.S. Forest Service Rd. no. 126, North fork Cape Creek, on resin of *Abies grandis*, leg. det. S.J. Hughes, 7 May 1969 (DAOM 134852,134563); Willamette National Forest, McKenzie Bridge Camp Grounds, leg. det. S.J. Hughes, 10 May 1969 (DAOM 134564). Washington: Kittitas Co., Wanatchee National Forest, Rocky Run, on *Abies nobilis*, leg. Field Mycology Class 1955, 22 Jul. 1955, det. S.J. Hughes, (mononematous synanamorph only, DAOM 118934 ex WSP 45210, as *Helminthosporium* sp.); Jefferson Co., Olympic National Forest, 10 mi Camp, Sec. 17, T26N, R3W, on *Pseudotsuga mucronata*, leg. Field Mycology Class, 22 Jul. 1955 (DAOM 113801 ex WSP 45212, as *Helminthosporium*); Grays Harbor Co., Twin Harbors Beach State Park, resin of *Picea sitchensis*, leg. W.B. & V.G. Cooke, 24 Jul. 1951, det. S.J. Hughes (DAOM 118970 ex WSP 28432).

Amorphotheca resinae. Isolated from jet fuel by P. Emonds (culture, DAOM 170427 = ATCC 22711, ITS GenBank EU030278, LSU GenBank EU030280). Canada, British Columbia, source unknown, isol. "Mrs. Volkoff", Jul. 1969 (culture, DAOM 194228, ITS GenBank EU030279).

Seifertia azaleae. All on flower buds of *Rhododendron* spp. **Canada**, British Columbia: Burnaby, Central Park, leg. S.J. Hughes, 17 Aug. 2000 (DAOM 228571); Vancouver, Stanley Park, leg. K.A. Seifert no. 1571, 11 May 2002 (culture and specimen, DAOM 239136, LSU GenBank EU030276). **Ireland,** Munter, Kerry, near Glenbeigh (*ca*. N 52° 03' W 9° 54'), leg. K.A. Seifert no. 3197, 26 Sep. 2006 (culture and specimen, DAOM 239135, ITS GenBank EU030273). **Netherlands**, Gelderland, Kröller-Müller Museum, leg. K.A. Seifert no. 1235, 12 May 2000 (DAOM 227136). **United Kingdom,** Wales, Hafod Estate (ca. N 52° 22' W 3° 51'), leg. K.A. Seifert no. 3198, 1 Oct. 2006 (culture and specimen, DAOM 239137, ITS GenBank EU030274).

DNA extraction, amplification and sequencing

DNA was isolated using a FastDNA™ Kit and the FastPrep™ FP120 (BIO 101 Inc.) or an UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, U.S.A.) using mycelium removed from agar cultures. PCR and cycle sequencing reactions were performed on a Techne Genius™ thermocycler (Techne Cambridge Ltd.). PCR reactions were performed using Ready-To-Go™ Beads (Amersham Canada Ltd.) in 25 µL volumes, each containing 20–100 ng of genomic DNA, 2.5 units pure *Taq* DNA Polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µL of each primer (50 µM), and stabilizers including bovine serum albumin. The reaction profile included an initial denaturation for 4 min at 94 °C, followed by 30 cycles of 1.5 min denaturation at 95 °C, 1 min annealing at 56 °C, 2 min extension at 72 °C, with a final extension of 10 min at 72 °C. Amplicons were purified by ethanol/sodium acetate precipitation and resuspended as recommended for processing on an ABI PRISM 3100 DNA Analyzer or an ABI 373 Stretch DNA Sequencer (Applied Biosystems, Foster, CA). Amplification products were sequenced using the BigDye v. 2.0™ Terminator Cycle Sequencing Ready Reaction Kit (ABI Prism/Applied Biosystems) following the manufacturer's directions. An approximately 1 000 bp portion of the large subunit (LSU) ribosomal DNA was amplified and sequenced using primers LR0R and LR6, and cycle-sequenced using primers LR0R, LR3R, LR16 and LR6 (Vilgalys & Hester 1990, Rehner & Samuels 1995; www.biology.duke.edu/fungi/mycolab/primers.htm). The complete ITS and 5.8S rRNA genes were amplified and sequenced using the primers ITS5 and ITS4, with ITS2 and ITS3 primers used for cycle sequencing when necessary (White *et al*. 1990). Some sequences were derived from single PCR amplifications of the ITS5–LR6 region.

Data matrices were subjected to parsimony analysis using heuristic searches in PAUP* v. 4.0b10 (Swofford 2002) with simple stepwise addition of taxa, and tree bisection-reconnection (TBR) branch swapping. Uninformative characters were removed for all analyses. Strict consensus trees were calculated, and the robustness of the phylogenies was tested using full bootstrap analyses (1 000 replications). For all analyses, GenBank accession numbers are given on the tree figures, and the sequences generated in this study are indicated in bold.

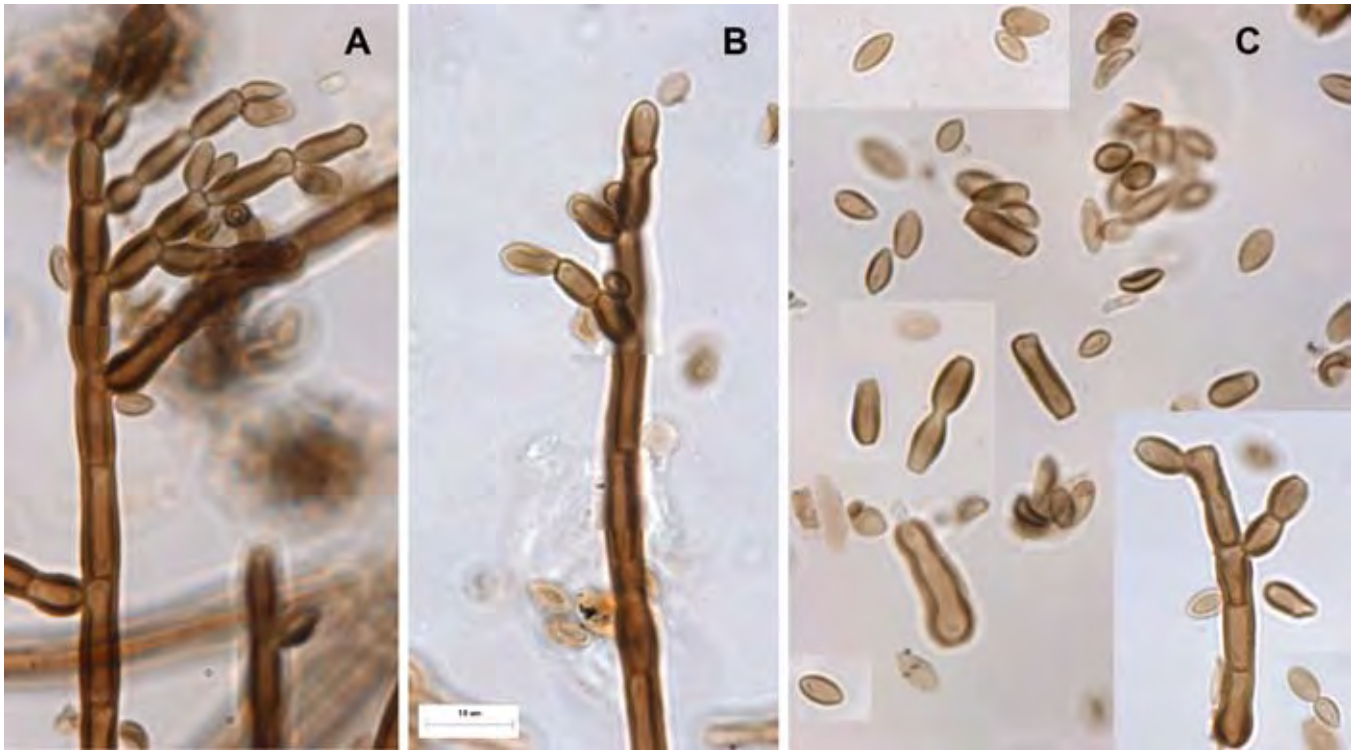

Fig. 3. *Hormodendrum resinae*, A–B. Conidiophores and acropetally developing chains of conidia. C. Conidia. Note that the lateral walls are conspicuously thickened compared to the walls at the poles. From a slide (DAOM 41888) prepared from the holotype (B).

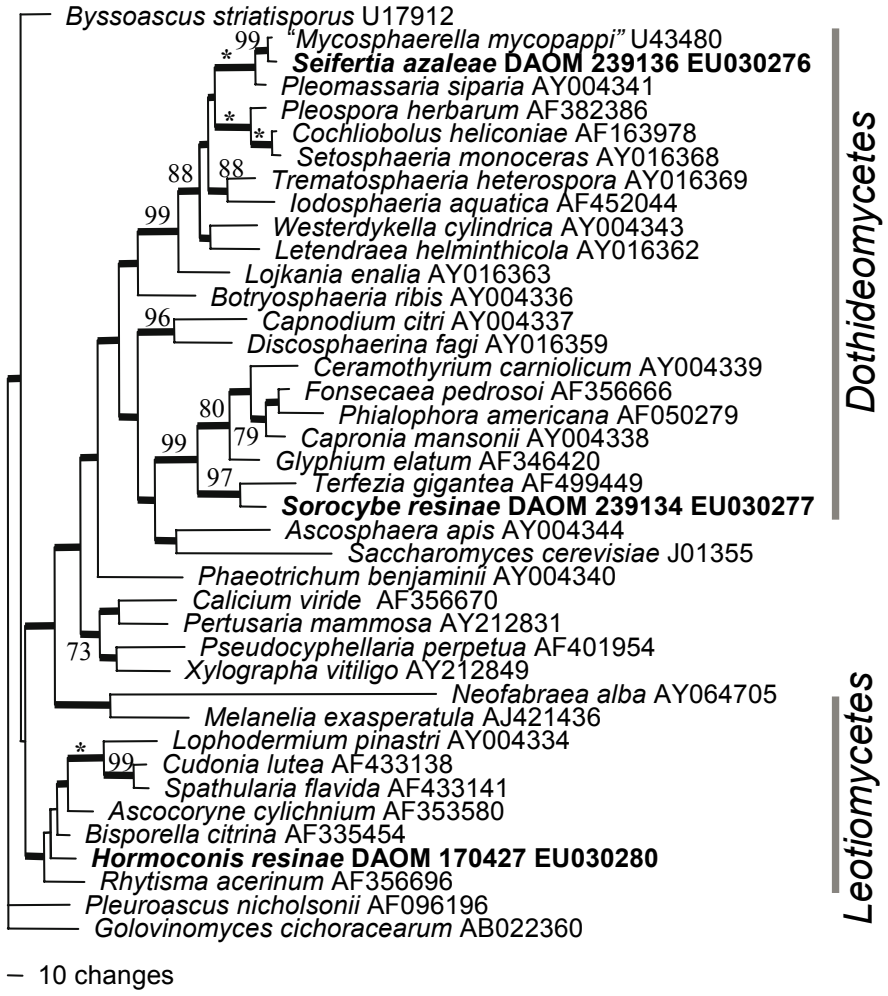

Fig. 4. Parsimony analysis of large subunit sequences, demonstrating the phylogenetic positions of *Amorphotheca resinae*, *Sorocybe resinae* and *Seifertia azaleae* (all shown in bold) in the Ascomycota. One of 12 equally parsimonious trees (1 888 steps, CI = 0.390, RI = 0.554, RC = 0.216, HI = 0.610) with *Golovinomyces cichoracearum* as the outgroup. Bootstrap values above 70 % are shown at the relevant nodes, with an asterisk representing 100 % bootstrap support; branches with thick lines occurred in all equally parsimonious trees.

The large subunit matrix was assembled from the closest BLAST matches using our sequences for the three fungi of interest, *S. resinae, A. resinae* and *S. azaleae*; *Golovinomyces cichoracearum* was added as an out-group to root the tree. Although these sequences were put into a single matrix, there is no implication that this data set represents the diversity of the *Ascomycota.* The alignment was calculated using MAFFT (Katoh *et al.* 2002) and adjusted using Se-Al (Sequence Alignment Program v. 1.d1; http://evolve.zoo.ox.ac.uk/software/Se-Al/main.html) to maximise homology.

The internal transcribed spacers alignment including *Sorocybe resinae* was derived from an alignment of *Capronia* and related anamorphs used by Davey & Currah (2007), originally produced using MAFFT. This data set was modified considerably using Se-Al to maximise homology, but still included several areas where the homology of aligned sequences was difficult to evaluate. ITS sequences of *Amorphotheca resinae* were used to retrieve closely related sequences using a BLAST search of GenBank, and these relevant sequences were added to an alignment of *Oidiodendron* Robak sequences from the study of Hambleton *et al.* (1998), and then adjusted using Se-Al.

We attempted direct PCR from two specimens containing only the putative mononematous synanamorph of *Sorocybe resinae* (DAOM 228772a, 228573a), to allow comparison of sequences obtained from cultures of the synnematous synanamorph. These attempts, using the same methods outlined above, were unsuccessful.

RESULTS

Cultural characters and micromorphology

Most micromorphological characters of the resin fungus *Sorocybe resinae* (Partridge & Morgan-Jones 2002), the creosote fungus *Amorphotheca resinae* (Parbery 1969, de Vries 1952, 1955, Ho *et al.* 1999) and the rhododendron fungus *Seifertia azaleae* (Ellis 1976, Partridge & Morgan-Jones 2002, Glawe & Hummel 2006) are well-described in the literature and will not be repeated here.

The three species are readily distinguished based on growth rates and overall cultural phenotypes. Agar colonies of *Sorocybe resinae* are coal-black, wrinkled, and restricted in growth, no matter what agar medium is employed; even after 3 mo, the colonies are rarely more than 2 cm diam (Fig. 2C). Synnemata did not form in our cultures; *in vivo*, the synnemata produce branched, acropetal chains of conidia with laterally thickened walls (Figs 2D–G). No thickened, refractive or darkened secession scars were evident on individual conidia or ramoconidia. Conidial masses were removed from the mononematous and synnematous parts of a freshly collected specimen (DAOM 56088a) and grown on PDA and sterilised conifer wood. There were no discernable differences between colonies derived from the two types of conidiophores, in all cases yielding restricted black colonies, or in their microscopic characters. Therefore, we conclude that these two types of conidiophores represent synanamorphs of one fungus. An identical conclusion was reached by Partridge & Morgan-Jones (2002). We documented the occurrence of this fungus in California, Oregon, and Washington State, U.S.A. and British Columbia, Canada, on resinous exudates on *Abies nobilis, Picea sitchensis, Pseudotsuga menziesii* and *Tsuga heterophylla.*

Microscopic features from the holotype specimen of *Hormodendrum resinae* Lindau are shown in Fig. 3. Dark, thick-walled conidiophore stipes give rise to branched, acropetally developing conidial chains. The conidia are relatively darkly pigmented, and the lateral walls are more conspicuously thickened and darkened than the polar walls. There are no obvious thickened, refractive or darkened secession scars on any of the cells. Apart from the production of synnemata, the characters of the conidia and conidium ontogeny are identical in Lindau's specimen and the synnematous specimens of *Sorocybe resinae* examined.

In contrast, both the resin fungus and the rhododendron fungus have spreading rather than restricted agar colonies. Cultures of the resin fungus are sandy brown (Kornerup & Wanscher 1989), planar and powdery, growing 4–4.5 cm diam in 10 d on PDA (Fig. 1A). Cultures of the rhododendron fungus are slower, growing 2.5–3.5 cm diam after 21 d on MEA (not shown). They are planar and greyish brown, with an orange-brown reverse. No synnemata were observed in our cultures of the rhododendron fungus on MEA, OA or PDA, but cladosporium-like conidiation occurred in the aerial mycelium.

Phylogeny

The large subunit analysis (LSU) was used to demonstrate the general phylogenetic relationships of the resin fungus *Sorocybe resinae* (DAOM 239134), the creosote fungus *Amorphotheca resinae* (DAOM 170427, 194228) and the rhododendron fungus *Seifertia azaleae* (DAOM 239136), and subsequent analyses of the internal transcribed spacers were used to estimate more precise affinities. Fig. 4 shows the LSU analysis and demonstrates that *Sorocybe resinae* appears to be a member of the *Herpotrichiellaceae, Chaetothyriales*, *A. resinae* is related to the inoperculate discomycetes (*Leotiomycetes*) and *Seifertia azaleae* is most closely related to a sequence labelled *Mycosphaerella mycopappi* A. Funk & Dorworth, which is unrelated to *Mycosphaerella s. str*.

For the ITS alignment of *Sorocybe resinae,* two preliminary parsimony analyses were conducted, one with informative characters from the full alignment, the second with a subset with 179 characters excluded from seven ambiguously aligned regions. The consistency indices (full 0.301, partial 0.324), tree topologies, and bootstrap supports for the two analyses were relatively similar. Therefore, the complete alignment was used for the tree presented here (Fig. 5). The data matrix included 57 taxa, with 352 of 752 characters phylogenetically informative. *Sorocybe resinae* clearly is related to *Capronia* and allied anamorph genera, as suggested by the LSU analysis. In the ITS analysis (Fig. 6) it forms a well-supported clade with *C. villosa* Samuels, that is a well-supported sister group to species now in three different anamorph genera, *Phaeococcomyces nigricans* (M.A. Rich & A.M. Stern) de Hoog, *Ramichloridium cerophilum,* and an undescribed species of *Heteroconium* Petr.

The ITS matrix for *A. resinae* included 42 taxa, with 171 phylogenetically informative characters in the 530 base alignment. The phylogenetic analysis confirmed the relationship of this species with the *Leotiomycetes*, and provided a more precise hypothesis of its family-level relationships (Fig. 6). *Amorphotheca resinae* DAOM 170427 and 194228 had identical ITS sequences to another strain of the same species reported in GenBank (AY251067, from Braun *et al.* 2003), and one bp substitution from a second strain (AF393726 based on the isotype ATCC 200942 = CBS 406.68). These four sequences formed a sister group to two sequences of "*Cladosporium*" *breviramosum* Morgan-Jones (AF393683, AF393684). The well-supported clade of *A. resinae* and *C. breviramosum*, which represent the proposed family *Amorphothecaceae,* was previously noted by Braun *et al.* (2003). The nesting of this clade within two well-supported

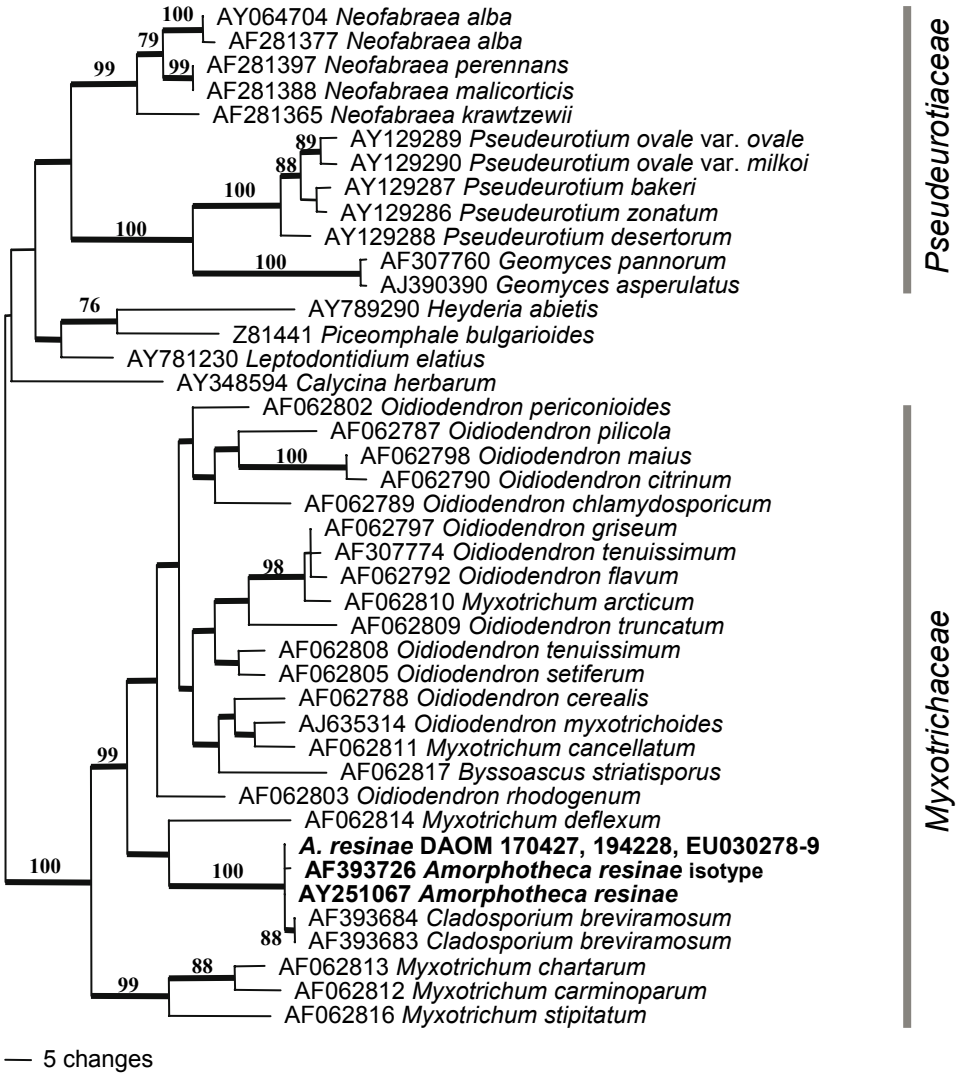

Fig. 5. Parsimony analysis of internal transcribed spacers sequences, demonstrating the position of *Amorphotheca resinae* (shown in bold) in the ascomycete family *Myxotrichaceae*. One of 44 equally parsimonious trees (645 steps, CI = 0.460, RI = 0.758, RC = 0.349, HI = 0.540) with mid-point rooting. Bootstrap values above 70 % are shown at the relevant nodes; branches with thick lines occurred in all equally parsimonious trees.

clades of *Myxotrichum* spp. and the associated anamorph genus *Oidiodendron,* which comprise the family *Myxotrichaceae,* has not been documented previously.

The ITS sequences of two strains of *Seifertia azaleae* were 474 bp and differed by one bp. BLAST searches with these sequences revealed significant homologies only with unidentified fungi, and lower probability matches with various members of the *Dothideomycetes*. Therefore, no taxonomically meaningful phylogenetic analysis can be presented with these ITS sequences. The species does seem to have affinities with the *Dothideomycetes*, but the putative relationship with *Mycosphaerella*, suggested by the LSU analysis, could not be confirmed with the ITS analysis.

DISCUSSION

Micromorphological comparisons, differences in culture characters, and phylogenetic analysis all support the conclusion that the mononematous synanamorph of *Sorocybe resinae*, the resin fungus, is different from the anamorph of *Amorphotheca resinae*, the creosote fungus. Based on ribosomal DNA sequences, the creosote fungus is related to the family *Myxotrichaceae*, the genus *Myxotrichum* and its *Oidiodendron* anamorphs (Fig. 5). In this gene tree, *Myxotrichum* and the *Myxotrichaceae* are paraphyletic, with *Amorphotheca* and the *Amorphothecaceae* nested within them. *Sorocybe* appears to be an additional anamorph genus phylogenetically associated with *Capronia* (*Herpotrichiellaceae, Chaetothyriales*, Fig. 6). The genetic connection between the synnematous and mononematous morphs of *S. resinae* was verified by morphological comparison of polyspore isolates derived from the two synanamorphs. However, the living cultures are no longer available and the connection was not confirmed with single conidium isolations. The type specimen of *Hormodendrum resinae* (Fig. 3) is the basis for the application of the most frequently used anamorph epithet for the creosote fungus. This specimen represents the mononematous synanamorph of *Sorocybe resinae,* not the anamorph of *Amorphotheca resinae*.

It is difficult to understand how these two fungi were confused when their micromorphologies are so different. The conidia are of the same general size and shape, but in both morphs of *Sorocybe resinae* (Figs 2D–G, 3C), the lateral walls are conspicuously thickened, a condition not present in the creosote fungus (Fig. 1C), and the conidia are much darker. In his monograph of *Cladosporium*, de Vries (1952) noted that single conidium isolates of *C. avellaneum* gave rise to four different colony types. In 1955, he extended these observations and decided that the much darker resin fungus was

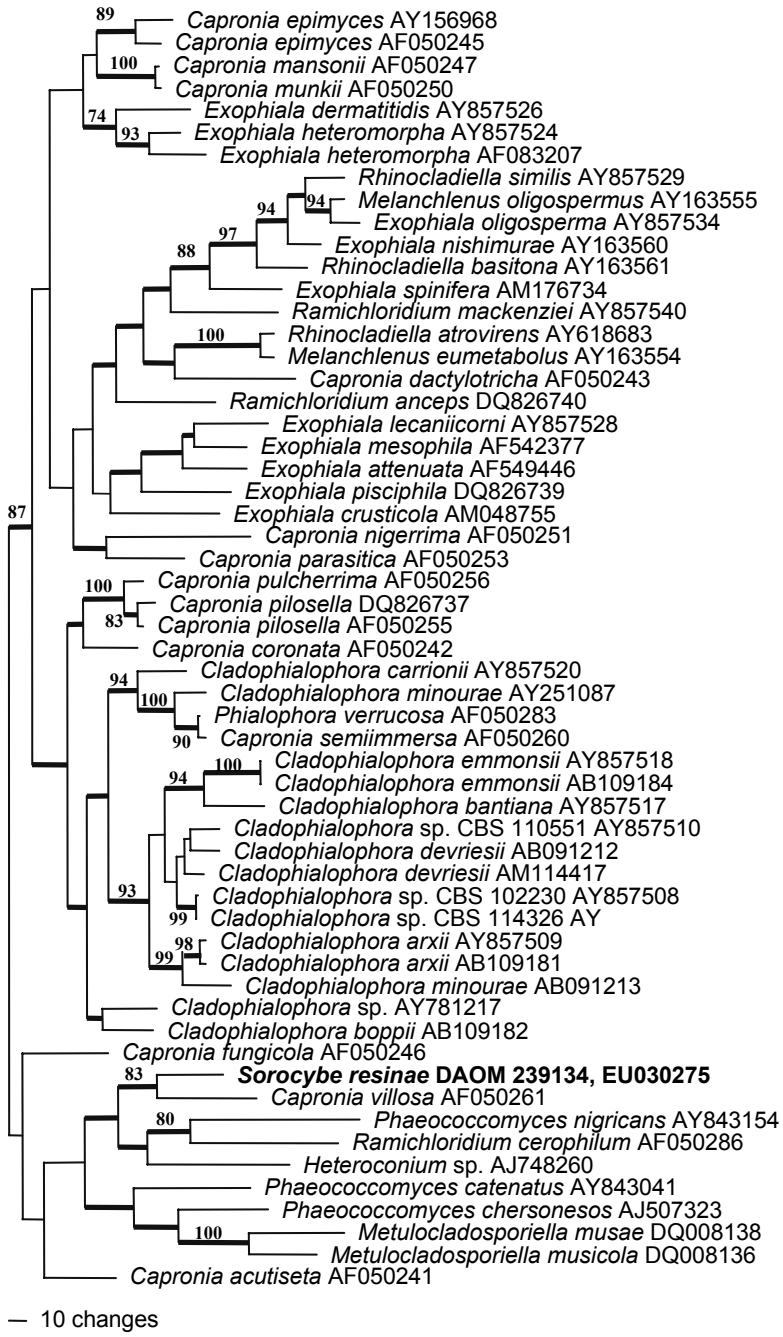

Fig. 6. Parsimony analysis of internal transcribed spacers sequences, demonstrating the position of *Sorocybe resinae* (shown in bold) among species of *Capronia* (*Herpotrichiellaceae*, *Chaetothyriales*) and its associated anamorph genera. One of 34 equally parsimonious trees (2 607 steps, CI = 0.301, RI = 0.506, RC = 0.153, HI = 0.699), with mid-point rooting. Bootstrap values above 70 % are shown at the relevant nodes; branches with thick lines occurred in all equally parsimonious trees.

the same as one of his mutant forms of the creosote fungus, despite never having isolated such a dark spored form from any of his cultures. Parbery (1969) implied that the demonstrated ability of the creosote fungus to grow on a diversity of hydrocarbon-rich substrates favoured the thought that it would be able to grow on conifer resin. If cultures of the true *Sorocybe resinae* had been available, it is unlikely that this confusion would have persisted for so long. *In vitro*, the creosote fungus and the resin fungus are so different (Figs 1A, 2C) that it would difficult to defend the idea that they were mutants of the same fungus. These differences in the cultures are reflected by the disparate phylogenetic affinities of what now are clearly demonstrated to be two different species.

Unfortunately, the name *Hormodendrum resinae* has been misapplied to the creosote fungus, a species of economic importance. Also unfortunately, this species is the type of *Hormoconis*, a generic name that the community concerned with this fungus has been slow to adapt to in the 30 years since its introduction. There are several possible solutions to this problem. The conventional solution would be to apply names based strictly on the type specimens and accept *Hormoconis* as a synonym of *Sorocybe,* or to use it as a generic name for the mononematous synanamorph of the resin fungus. A new anamorph genus would then be described for the creosote fungus, making *Cladosporium avellaneum* G.A. de Vries the basionym for its type. However, the resulting binomial would be unfamiliar to those concerned with the creosote fungus, and the earlier literature citing *H. resinae* would be misleading.

A more parsimonious solution is possible. Article 14.9 of the International Code of Botanical Nomenclature (McNeil *et al*. 2006) allows for conservation of a name with a different type from that

designated by the authors. The name *Hormodendrum resinae* is not otherwise needed because the mononematous synanamorph of the resin fungus is rarely referred to by a Latin binomial, and because *Sorocybe resinae* is based on a different type. Therefore, a new type specimen could be proposed and conserved for *Hormodendrum resinae* Lindau, preferably the holotype of *A. resinae* (MELU 7130). This would make the anamorph-teleomorph connection unequivocal, maintain current species epithets and taxonomic authorities, and ensure that most of the historical literature can be interpreted easily without the need to consult complicated nomenclators (Table 1). However, by perpetuating the use of the epithet "*resinae"*, this change would also perpetuate the misunderstanding that resin is a possible substrate for the creosote fungus. In any case, the use of this epithet for the teleomorph of the creosote fungus, *Amorphotheca resinae*, is legitimate and valid, and unlikely ever to be changed.

A third option would be an intermediate one. The application of the name *Cladosporium avellaneum* G.A. de Vries has never been in doubt, and it would be possible to conserve this species as the type of *Hormoconis*. This has the advantage of maintaining the familiar generic name *Hormoconis*, in combination with a species epithet that has been consistently applied. Furthermore, this solution would allow the confusion about the application and correct author citation around the epithet "*resinae*" for the anamorph of creosote fungus to recede.

The second and third solutions require formal taxonomic proposals to be published in Taxon. We will argue the merits of these possible solutions at more length in that venue.

The phylogenetic position of *A. resinae* raises additional taxonomic problems. This fungus typifies the monotypic family *Amorphothecaceae*, which has been considered *incertae sedis* since its description by Parbery (1969). Our phylogenetic analysis suggests that this family sits within the *Myxotrichaceae. Amorphothecaceae* (1969) is the older name, but *Myxotrichaceae* (1985) is well-entrenched in the mycological literature. As a consequence, the *Myxotrichaceae* are paraphyletic with respect to the *Amorphothecaceae*. The peridium of *A. resinae*, the only species presently placed in this family, lacks the thick-walled appendages that characterise most species of the *Myxotrichaceae*. Furthermore, the acropetal-blastic features of the anamorph of *A. resinae* differ from the thallic-arthric conidiogenesis of the other anamorphs associated with the *Myxotrichaceae*, principally *Oidiodendron*. These morphological differences explain why the affinity of *A. resinae* with the *Myxotrichaceae* was not noted before. A formal proposal to conserve *Myxotrichaceae* as the name for this family might be prudent eventually, but this should await analysis of additional genes to confirm the phylogenetic relationship.

Whether *Cladosporium breviramosum,* originally isolated from discoloured wallpaper, is actually a distinct species from *A. resinae* requires further study. It is clear that this species, if it is distinct, would be a member of *Hormoconis* rather than *Cladosporium*. Apart from the study of additional specimens, it might be fruitful to attempt to induce an *Amorphotheca*-like teleomorph in the two available cultures of *C. breviramosum*, and to compare the morphology with that of *A. resinae*. According to Parbery (1969), *A. resinae* includes both homothallic and heterothallic strains.

Unfortunately, the phylogenetic affinities of *Seifertia azaleae* were not established with certainty in this study. Its closest relative in the LSU analysis is a sequence identified as *Mycosphaerella mycopappi* Funk & Dorworth (U43480, based on the apparent type culture ATCC 64711), but this sequence does not cluster with others representing the family *Mycosphaerellaceae* (data not shown). Similarly, the ITS sequences of the rhododendron fungus did not cluster with the many ITS sequences of *Mycosphaerella* available. Presently, it seems that *Seifertia azaleae* fungus is allied with the *Dothideomycetes*, but its precise affinities are uncertain. It is clear that this fungus should not be classified in *Pycnostysanus* (a taxonomic synonym of *Sorocybe*), and continued recognition of the monotypic genus *Seifertia* seems justified.

ACKNOWLEDGEMENTS

We are grateful to Sarah Hambleton and Marie Davey for providing ITS alignments that served as the basis for analyses in this paper. Walter Gams and Scott Redhead provided expert nomenclatural advice and helpful reviews of this manuscript. We thank Uwe Braun for proposing the third option for resolving the name of the creosote fungus, outlined in the text. The Canadian Collection of Fungal Cultures (DAOM) provided several of the strains for this study. The curators of DAOM and B kindly provided access to critical type specimens.

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INDEX OF FUNGAL NAMES

Arranged according to genus and then epithets.
Synonymised names are italicised and page numbers of main entries are bolded. A superscript asterisk (*) points to pages with illustrations, a superscript c (c) to pages with a cladogram, a superscript t (t) to pages with a table and a superscript k (k) to pages with a key to genera or species.