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A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales)

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ABSTRACT

Partial sequences of nuLSU rDNA were obtained to investigate the phylogenetic relationships of Pyronemataceae, the largest and least studied family of Pezizales. The dataset includes sequences for 162 species from 51 genera of Pyronemataceae, and 39 species from an additional 13 families of Pezizales. Parsimony, ML, and Bayesian analyses suggest that Pyronemataceae is not monophyletic as it is currently circumscribed. *Ascodesmidaceae* is nested within Pyronemataceae, and several pyronemataceous taxa are resolved outside the family. *Glaziellaceae* forms the sister group to Pyronemataceae in ML analyses, but this relationship, as well as those of Pyronemataceae to the other members of the lineage, are not resolved with support. Fourteen clades of pyronemataceous taxa are well supported and/or present in all recovered trees. Several pyronemataceous genera are suggested to be non-monophyletic, including *Anthracobia*, *Cheilymenia*, *Geopyxis*, *Humaria*, *Lasiobolidium*, *Neottiella*, *Octospora*, *Pulvinula*, *Stephensia*, *Tricharina*, and *Trichophaea*. Cleistothecial and truffle or truffle-like ascomata forms appear to have evolved independently multiple times within Pyronemataceae. Results of these analyses do not support previous classifications of Pyronemataceae, and suggest that morphological characters traditionally used to segregate the family into subfamilial groups are not phylogenetically informative above the genus level.

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Introduction

Pyronemataceae is the largest and most heterogeneous family of Pezizales. The most recent circumscription of Pyronemataceae includes 75 genera (Eriksson 2006) that encompass approximately 500 species (Kirk *et al.* 2001), roughly half of the known species within Pezizales. The family is primarily temperate to arctic–alpine in distribution, with a few taxa known from the tropics. Members of the family are diverse in ascomatal form, with sessile to stipitate, cupulate, discoid, pulvinate, or turbinate epigeous apothecia, as well as sub-hypogeous to

hypogeous taxa with closed, folded, or solid ascomata. Apothecia range in size from less than 1 mm up to 12 cm diam. Ecologically, the family displays great diversity with regard to substrate preference, including terricolous, coprophilous, lignicolous, pyrophilous, urinophilic, and bryophilous members. The majority of taxa within the family have traditionally been considered saprotrophic, but the trophic strategies of most species are not well studied and remain undocumented. Several genera have been found to be parasitic on bryophytes (Benkert 1993; Döbbeler 1979), and an increasing number of species are being identified as ectomycorrhizal associates

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(Amicucci et al. 2001; Bidartondo et al. 2001; Fujimura et al. 2005; Hobbie et al. 2001, 2002; Izzo et al. 2005; Smith et al. 2006; Teder-soo et al. 2006; Vrålstad et al. 1998).

The genera of *Pyronemataceae* are not united by any unique combinations of characters, either macro- or microscopically. Rather, the family is distinguished by the lack of characters by which the taxa it comprises can be placed in other families. For example, ascospore cytology has been used to distinguish several groups within *Pezizales*: *Morchellaceae*, *Sarcoscyphaceae* and *Sarcosomataceae* have multinucleate ascospores, and *Pezizaceae* and *Ascobolaceae* have uninucleate ascospores. *Pyronemataceae* also have uninucleate ascospores, but lack the amyloid reaction characteristic of the asci in *Pezizaceae* and *Ascobolaceae*. Many families of *Pezizales* are characterized by distinctive septal pore plug ultrastructure at the base of the asci and in the ascogenous hyphae, but in *Pyronemataceae* at least five distinct types of septal plug structure have been reported thus far (Kimbrough 1994). This lack of uniting characters can be seen in the diverse classification schemes involving *Pyronemataceae*. Some authors have included as few as one or two genera in the family (Arpin 1969; Kimbrough 1970, 1989; Rifai 1968), while others have included from 21 (Eckblad 1968) to 47 (Korf 1972). In more restricted circumscriptions, the families *Otideaceae*, *Humariaceae* and *Aleuriaceae* have been employed to accommodate genera currently placed within *Pyronemataceae*.

Recent phylogenetic studies of *Ascomycota* indicate that *Pezizales*, along with *Orbiliiales*, are early diverging lineages within *Euascomycetes* (Liu & Hall 2004; Liu et al. 1999; Lumbsch et al. 2000; Lutzoni et al. 2004; Platt & Spatafora 2000). Several peizizalean families have been the subject of recent phylogenetic studies, including *Pezizaceae* (Hansen et al. 2001; 2005a), *Sarcoscyphaceae* and *Sarcosomataceae* (Harrington et al. 1999), *Discinaceae*, *Helvellaceae*, *Morchellaceae*, and *Rhiziniaceae* (O'Donnell et al. 1997) and *Tuberaceae* (O'Donnell et al. 1997; Percudani et al. 1999). Landvik et al. (1997) studied the subordinal relationships within *Pezizales* using nuSSU gene sequence data, and resolved three main lineages within the order. The relationships among these three lineages were not resolved with confidence. No other studies have included a significant number of *Pyronemataceae* taxa, and it has remained the least studied family of *Pezizales*. This study presents phylogenetic analyses based on nuLSU gene sequence data with a focus on *Pyronemataceae* and related taxa from lineage C of Landvik et al. (1997).

Taxonomic background

Corda (1842) was the first to recognize a group of taxa centred around the genus *Pyronema*. He erected "*Pyronemeae*" to accommodate *Pyronema* and *Midotis* (a fungus imperfectly known by Corda), within his "*Ordo Ascophori*", characterized by immarginate, horizontally expanding apothecia and simple, one-celled ascospores. Additional, early concepts of the taxa centred around *Pyronema* include those of Schröter (1897) and Boudier (1907). Seaver (1928) treated the small, immarginate discomycetes (i.e. *Pyronemataceae sensu* Corda, Schröter, Boudier) in the all-inclusive family *Pezizaceae*. Clements & Shear (1931) included both operculate and inoperculate discomycetes in the *Pezizales*, and treated *Pyronema* and other pyronemataceous genera in various subfamilies of *Pezizaceae*. Velenovský (1934)

erected the family *Humariaceae* to accommodate *Pyronema* and other pyronemataceous taxa, as well as additional genera currently treated in other families of *Pezizales*.

Incorporating characteristics of the asci, paraphyses and ascospores, in addition to macromorphology, Le Gal (1947) treated the taxa of *Pyronemataceae sensu* Boudier (1907), Schröter (1897) and Corda (1842) in the tribe *Pseudoascoboleae* of the family *Ascobolaceae*. Le Gal employed *Aleuriaceae* (tribe *Otideae*) and *Humariaceae* to account for a large majority of other pyronemataceous genera. Kimbrough & Korf (1967) excluded *Pyronema* from *Pseudoascoboleae* in their proposal to abandon this group in favour of tribe *Thelebolae* (*Pezizaceae*). Dennis (1978) followed Le Gal (1947) in treating *Pyronema*, *Ascodesmis*, *Thecotheus*, and other genera in tribe *Pseudoascoboleae*. However, Dennis (1978) indicated that the tribe was heterogeneous, noting that *Pyronema* had pigmentation suggestive of *Humariaceae*, which in his treatment was composed almost exclusively of genera currently treated in *Pyronemataceae*. Rifai (1968) restricted *Pyronemataceae* to include only *Pyronema*. Like Dennis (1978), Rifai indicated the close relationship of *Pyronema* to taxa of *Humariaceae*, stating that *Pyronema* should likely be treated in a tribe of the family. However, due to the polyphyletic nature of *Humariaceae*, Rifai (1968) retained *Pyronemataceae* as a monotypic family rather than expand an already problematic group.

Citing the inability to satisfactorily subdivide the family on the basis of common characters, Eckblad (1968) greatly expanded the limits of the group centred around *Pyronema* in his emended *Pyronemaceae* [sic]. In addition to *Pyronema*, the family included many taxa that had been referred to *Humariaceae* of previous investigators, most of which are characterized by the presence of carotenoid pigments. The family thus included genera representing a high degree of variation in such characters as excipulum structure, spore ornamentation, and type of excipular hairs. Eckblad (1968) erected the family *Otideaceae*, for genera that produce larger, sometimes stipitate apothecia, most of which typically lack bright orange to red colouration (*Ascosparrassis*, *Geopyxis*, *Otidea*, *Sowerbyella*, *Tarzetta*).

Arpin (1969) followed a pattern similar to Rifai (1968), treating *Pyronema* in a distinct monotypic family awaiting more precise chemical analyses of the carotenoid pigments. He erected a new family, *Aleuriaceae*, based on *Aleuria*, in the sense that the genus is recognized today (i.e., type species *A. aurantia*), and corresponding largely to carotenoid containing members of the *Ciliarieae* and *Humariaceae* of Le Gal (1947). Arpin (1969) also emended Eckblad's (1968) *Otideaceae* to include only taxa lacking carotenoid pigments. Kimbrough (1970), following Rifai (1968) and Arpin (1969), restricted *Pyronemataceae* to the single genus *Pyronema*. Kimbrough also followed Arpin (1969) in treating those genera referred to *Humariaceae* by previous investigators (Boudier, 1907; Le Gal, 1949; Dennis, 1978; Rifai 1968) in the families *Aleuriaceae* and *Otideaceae*, adding several additional genera to each.

Korf (1972) emended *Pyronemataceae* to be even more encompassing than that Eckblad (1968) presented. *Humariaceae*, *Otideaceae*, and *Aleuriaceae* of previous workers were subsumed in a single, large, heterogeneous *Pyronemataceae*. The family was subdivided into five subfamilies and 11 tribes, containing 48 genera. Trappe (1979) emended *Pyronemataceae*

sensu Korf by transferring hypogeous taxa into the family from the *Tuberales* as he abandoned that polyphyletic order.

Citing ultrastructural, cytological and cytochemical studies, Kimbrough (1989) once again argued for restricting the limits of *Pyronemataceae*. He erected the suborder *Pyroneminae* to accommodate eugymnohymenial to paragymnohymenial *Pezizales* characterized by excipula that are highly reduced or lacking, and hemispherical septal pore plugs with radiating tubules at the base of the asci. Within the suborder, Kimbrough recognized two families, *Pyronemataceae*, restricted to *Pyronema* and *Coprotus*, characterized by smooth, non-pigmented spores and asci in a hymenial layer, and *Ascodesmidaceae* composed of *Ascodesmis*, *Eleutherascus*, and *Amauroascus*, characterized by pigmented, ornamented spores, and single to loosely clustered asci. In their preliminary discomycete flora of Macaronesia, Korf & Zhuang (1991a) recognized the restricted *Pyronemataceae* sensu Kimbrough. The remaining taxa, previously referred to *Pyronemataceae* sensu Korf (1972), were treated in subfamilies *Otideoideae* and *Scutellinioideae* of the family *Otideaceae*.

The annual *Outline of the Ascomycetes*, published since 1982 (Eriksson 1982), reflects both restricted and broad concepts of *Pyronemataceae*, employing the family *Otideaceae* for the remaining pyronemataceous taxa when necessary. Molecular phylogenetic studies by Landvik et al. (1997) suggest that *Pyronema* is part of a lineage composed primarily of taxa that had been treated in *Otideaceae*. These authors point out that *Pyronemataceae* is the earliest available name for this group. In addition, the results of Landvik et al. indicate that both *Ascodesmis* (*Ascodesmidaceae*) and *Glaziella* (*Glaziellaceae*) are closely related to *Pyronemataceae*. Eriksson (2006) incorporated these findings and recognized *Pyronemataceae*, containing *Pyronema* and those genera previously referred to *Otideaceae*. *Ascodesmidaceae*, composed of *Ascodesmis*, *Eleutherascus*, and *Lasiobolus*, and a monotypic *Glaziellaceae*, are currently still recognized.

Materials and methods

Taxon sampling

Sequence data were collected for 162 species from 51 genera that are broadly representative of the taxonomic, morphological and ecological diversity of *Pyronemataceae*. Every effort was made to sample as many pyronemataceous genera as possible, and to use material representing the type species of each genus when available. Of the 51 pyronemataceous genera sampled, 37 are represented by type species. To assess the monophyly of *Pyronemataceae* and determine relationships of the family to the remainder of *Pezizales*, an additional 39 species were sampled, representing 36 genera, and 13 out of 15 currently recognized families of *Pezizales* (Eriksson 2006). Two outgroup taxa from *Neolectales*, *Neolecta vitellina* and *N. irregularis*, were included for rooting purposes based upon the results of previous phylogenetic analyses (Landvik 1996; Landvik et al. 1997, 2001, 1993; Liu et al. 1999), which suggest that *Neolecta* is basal to the other fruit body-producing ascomycetes. All specimens included in this study are listed in Table 1.

Molecular techniques

Sequences of the 5' end of the nuLSU gene, spanning domains D1 and D2, were analysed. For several taxa, more than one specimen was sampled to verify nuLSU sequences. Of the 226 sequences used in this study, 185 are previously unpublished. Of these, 174 represent taxa from *Pyronemataceae*, and 11 represent taxa from other families of *Pezizales*. The remaining sequences used were obtained from GenBank (Table 1).

Genomic DNA was extracted from ascomata of pre-existing herbarium specimens, fresh material from recent collections stored in 1% SDS extraction buffer, and live cultures. Laboratory techniques typically followed those outlined in Hansen et al. (1999), except that a number of specimens were processed in a FastPrep instrument using a FastDNA Kit (BIO 101 Systems, Carlsbad, CA). The nuLSU region was amplified using primers LROR and LR5, LR3 or LR7 (Moncalvo et al. 2000). Sequencing primers included those used for PCR, as well as LR3R (Moncalvo et al. 2000). Reactions were purified using an ethanol-magnesium chloride solution (1 ml 70% EtOH: 1 µl 0.5 M MgCl₂). Samples were suspended in 74 µl of the solution, mixed, and allowed to sit at room temperature in the dark for 20 min, and then centrifuged for 30 min at 3000 g (~5300 rev min⁻¹) to pellet the sequencing product. The supernatant was poured or drawn off and the samples were allowed to air dry in the dark for approximately 30 min. Cleaned sequencing reactions were then suspended in 30 µl of purified water or formamide, and visualized on an ABI 3100 or 3730 Genetic Analyser capillary sequencer (Applied Biosystems, Foster City, CA).

Sequences were edited and assembled using Sequencher 3.0 and 4.0 (GeneCodes Corp., Ann Arbor, MI). Sequences were aligned manually using MacClade 4 (Maddison & Maddison 2000), Se-AL, version 2 (Rambaut 1996, Se-AL: Sequence Alignment Editor. Available at <http://evolve.zoo.ox.ac.uk/>), and the editor window of PAUP version 4 (Swofford 2003). To reduce the size of the aligned dataset and resulting topologies, 25 redundant sequences were removed before the final analyses. Several regions of the alignment, totaling 80 nucleotides, were deemed to be ambiguously aligned and therefore excluded from all phylogenetic analyses. Additionally, several hundred nucleotides were trimmed from the end of the alignment before analyses to account for missing data in several taxa. Edited sequences have been deposited in GenBank (Table 1), and the aligned dataset is available via TreeBASE (<http://www.treebase.org>).

Phylogenetic analysis

All parsimony analyses were conducted using PAUP version 4 (Swofford 2003). Searches were conducted using heuristic search methods with random stepwise sequence addition, tree bisection-reconnection (TBR) branch swapping, collapse of zero length branches, and equal weighting of all characters. Due to the large size of the dataset, searches followed a two-step strategy. First, 5 K random sequence addition replicates were performed saving no more than five trees per replicate. Second, with MaxTrees set to 15 K, the most parsimonious trees resulting from the first step were used as starting trees and the analysis was allowed to swap to completion. The equally most parsimonious trees from the second step were summarized

Table 1 – Caption for issues

Species	Collection/Isolate no.	Geographic Origin	Year and Collector	GenBank accession nos.
<i>Acervus epispertius</i>	s.n. (FH)	USA, New York	1984, A. Bessette	DQ220305
<i>A. flavidus</i>	DHP PR98.2 (FH)	Puerto Rico	1998, D.H. Pfister	DQ220306
<i>Aleuria aurantia</i>	BAP 426 (FH)	USA, Massachussets	2001, D.H. Pfister	DQ220307
<i>A. bicucullata</i>	s.n. (C)	Denmark	1978, D.H. Pfister, H. Dissing	DQ220308
<i>Aleurina imaii</i>	CUP-CH 233 (CUP)	China	1981, R-y. Zheng, R.P. Korf	AF335112
<i>Amylascus tasmanicus</i>	Trappe 18084 (C, dupl. OSC)	Australia	1996, J. Trappe	AF335113
<i>Anthracobia macrocystis</i> (1)	DED 7355 (SFSU)	USA, California	2002, D.E. Desjardin	DQ220310
<i>A. macrocystis</i> (2)	BAP 429 (FH)	USA, California	2002, F. Stevens, M. Wood, D.E. Desjardin	DQ220311
<i>A. subatra</i>	TL-3650 (C)	Denmark	1994, T. Læssøe	DQ220313
<i>A. tristis</i>	Carolina Biol. Supply (as <i>A. muelleri</i>)	—	—	DQ220314
<i>Anthracobia</i> sp.	DED 6287 (SFSU)	USA, California	1995, N. Wilson, N. Andresen	DQ220312
<i>Arpinia inops</i>	C F-54586/HD Rana75.082 (C)	Norway	1975, H. Dissing	DQ220315
<i>Ascobolus lineolatus</i>	NRRL A23604	—	—	AF133159
<i>Ascodesmis nigricans</i>	CBS 389.68	Netherlands	1986, G. Tichelaar	DQ168335
<i>Barssia oregonensis</i>	RF 533 (OSC)	USA, Oregon	-	U42684
<i>Boubovia luteola</i>	C F-54043/HD Rana75.056 (C)	Norway	1975, H. Dissing	DQ220316
<i>Byssonectria terrestris</i> (1)	C F-29819/JV93-036 (C)	Denmark	1993, J. Vesterholt, V. Sünksen	DQ220317
<i>B. terrestris</i> (2)	KS-94-04 (C)	Denmark	1994, K. Hansen, S.K. Sandal	AY500531
<i>Caloscypha fulgens</i>	DED 6107 (SFSU)	USA, California	1994, D.E. Desjardin	DQ220318
<i>C. fulgens</i>	KH-97-6 (FH)	USA, California	1997, K. Hansen	DQ220319
<i>Cheilymenia crucipila</i> (1)	KH.03.63 (FH)	Norway	2003, K. Hansen	DQ220320
<i>C. crucipila</i> (2)	C F-55437/KS-94-044A (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220321
<i>C. fimilicola</i>	BAP 432 (FH)	USA, California	2002, B.A. Perry	DQ220322
<i>C. stercorea</i>	BAP 440 (FH)	USA, California	2002, B.A. Perry	DQ220323
<i>C. theleboides</i>	KH.03.115 (FH)	Norway	2003, K. Hansen	DQ220324
<i>C. vitellina</i>	KH.01.32 (C)	Denmark	2001, K. Hansen	DQ220325
<i>Choiromyces venosus</i>	JMT 7014 (OSC)	USA, Oregon	J. Trappe	U42688
<i>Chorioactis geaster</i>	S. Kurogi s. n. (FH)	Kyushu, Japan	1997, S. Kurogi	AY307945
<i>Cookeina tricholoma</i>	1D-D5 (FH)	Venezuela	1997, K. Samuels	AY945860
<i>Coprobria granulata</i>	C-F-55284/KS-94.30 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220327
<i>Coprobria</i> sp.	DHP 1276 (FH)	Puerto Rico	1973, D.H. Pfister, B. Pfister	DQ220326
<i>Desmazierella acicola</i>	RK 95.11 (FH)	Norway	1995, R. Kristiansen	DQ220328
<i>Discina macrospora</i>	NSW 4498 (MICH)	USA, Michigan	N.S. Weber	U42678
<i>Donadinia</i> sp.	mh 669 (FH)	USA, New York	1996, F. Harrington & D. Potter	DQ220329
<i>Eleutherascus lectardii</i>	CBS 626.71	France	1968, P. Lectard	DQ168334
<i>E. peruvianus</i>	CBS 101.75	Peru	1975, L. H. Huang	DQ220330
<i>Gelinipes</i> sp. (gen. ined.)	Trappe 24315 (FH, dupl. OSC)	Australia, New South Wales	1999, J. Trappe	DQ220331
<i>Genabea cerebriformis</i>	src637 (OSC)	USA, California	2003, M. E. Smith	DQ206864
<i>Genea arenaria</i>	Trappe 17288 (FH, dupl. OSC)	USA, California	1980, J. Graham	DQ220332
<i>G. harknessii</i> (1)	Trappe 13313 (FH, dupl. OSC)	USA, Washington	1994, A. & D. Claridge	DQ220334
<i>G. harknessii</i> (2)	Trappe 11775 (FH, dupl. OSC)	USA, Washington	1991, J. Trappe	DQ220335
<i>G. hispidula</i> EcM	C40	Estonia	—	AJ534926
<i>Genea</i> sp.	Trappe 26253 (FH, dupl. OSC)	Argentina, Neuquen	2001, L. Dominquez	DQ220333
<i>Geopora arenicola</i> (1)	KS-94-173 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220336
<i>G. arenicola</i> (2)	KS-94-95 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220337
<i>G. cf. cervina</i>	KH.03.61 (FH)	Norway	2003, K. Hansen, C. Lange	DQ220344
<i>G. clausa</i>	Trappe 5715 (FH, dupl. OSC)	USA, California	1980, J. Trappe	DQ220339
<i>G. cooperi</i> (1)	s.n. (FH)	USA, California	1981, R. Trial	DQ220340
<i>G. cooperi</i> (2)	HDT 52489 (SFSU)	USA, Wyoming	1989, J. Ammarati	DQ220341
<i>G. cooperi</i> f. <i>gilkeyae</i>	Trappe 18034 (FH, dupl. OSC)	USA, California	1996, E. Cázares	DQ220342

Table 1 (continued)

Species	Collection/Isolate no.	Geographic Origin	Year and Collector	GenBank accession nos.
<i>G. pellita</i>	DHP 297 (FH)	USA, Michigan	1969, D.H. Pfister	DQ220343
<i>Geopora</i> sp. A.	KH.01.29 (C)	Denmark	2001, S.A. Elborne	DQ220338
<i>Geopora</i> sp. B	KH.03.109 (FH)	Norway	2003, K. Hansen	DQ220345
<i>Geopyxis carbonaria</i> (1)	DED 7357 (SFSU)	USA, California	2002, D.E. Desjardin	DQ220346
<i>G. carbonaria</i> (2)	BAP 460 (FH)	USA, Oregon	2003, B.A. Perry, N.S. Weber	DQ220347
<i>G. carbonaria</i> (3)	C F-49793 (C)	Denmark	1982, T. Laessøe	DQ168336
<i>G. vulcanalis</i> (1)	BAP 434 (FH)	USA, California	2002, B.A. Perry	DQ220348
<i>G. vulcanalis</i> (2)	BAP 501 (FH)	USA, California	2003, A. Wilson	DQ220349
<i>G. vulcanalis</i> (3)	DED 6280 (SFSU)	USA, California	1995, D.E. Desjardin	DQ220350
<i>Geopyxis</i> sp.	KH.04.48 (FH, dupl. DBG)	USA, Colorado	2004, K. Hansen, V. Evenson	DQ062985
<i>Gilkeya compacta</i>	src718 (OSC)	USA, California	2003, M. E. Smith	DQ206862
<i>Glaziella aurantiaca</i>	PR-5954 (FH)	Puerto Rico	1998, N.C. Clum, D.J. Lodge	DQ220351
<i>Gyromitra esculenta</i>	NRRL 22213/CBS 335.73	Finland	—	U42675
<i>Helvella lacunosa</i>	NSW 6373	USA, Oregon	N.S. Weber	U42681
<i>Humaria hemisphaerica</i> (1)	BAP 320 (FH)	China, Tibet	2000, B.A. Perry	DQ220352
<i>H. hemisphaerica</i> (2)	KH.03.100 (FH)	Norway	2003, K. Hansen	DQ220353
<i>H. hemisphaerica</i> EcM	O35	Estonia	—	AJ534927
<i>H. velenovskyi</i>	HK 24-IX-1975 (C)	Denmark	1975, H. Knudsen	DQ220354
<i>Hydnotrya cerebriformis</i>	NSW 6494	USA, Oregon	N.S. Weber	U42676
<i>Jafnea fuscarpa</i>	DHP-148 (FH)	USA, NY	1968, Riedel, D.H. Pfister, Dixon	DQ220355
<i>Kotlabaea deformis</i> (1)	C F-53177/HD Alta 00.014 (C)	Norway	2000, H. Dissing	DQ220356
<i>K. deformis</i> (2)	Finn 00.06 (C)	Norway, Finnmark	2000, H. Dissing, S. Sivertsen	DQ220357
<i>Labyrinthomyces varius</i>	JMT 14825	Australia	J. Trappe	U42689
<i>Lamprospora ascoboloides</i>	KH.03.54 (FH)	Norway	2003, K. Hansen	DQ220358
<i>L. dictydiola</i>	C F-52716/HD Gr.83.002 (C)	Greenland	1983, H. Dissing	DQ220359
<i>L. miniata</i>	BAP 472 (FH)	USA, Oregon	2003, B.A. Perry, N.S. Weber	DQ220360
<i>Lamprospora</i> sp. A	KH.03.131 (FH)	Norway	2003, K. Hansen	DQ220361
<i>Lamprospora</i> sp. B	KH.03.150 (FH)	Norway	2003, K. Hansen	DQ220362
<i>Lasiobolidium orbiculoides</i>	CBS 344.73	USA, California	1953, G. L.Benny	DQ062995
<i>L. spirale</i>	CBS 782.70	USA, Wyoming	1964, R. F. Cain	DQ220363
<i>Lasiobolus ciliatus</i>	C F- 55257/KS-94-05 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ167411
<i>L. cuniculi</i>	C F-54526/ HD Rana76.053 (C)	Norway	1976, H. Dissing	DQ168338
<i>Lazuardia lobata</i>	AAU 43756 (C)	Ecuador	1983, T. Laessøe	DQ220364
<i>Leucoscypha leucotricha</i>	C F- 55460/KS-94-174 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220365
<i>Marcelleina tuberculisporea</i>	ALL-94-8 (C, holotype)	Denmark	1994, K. Hansen, S.K. Sandal	AF335120
<i>Melastiza contorta</i>	KH.01.06 (C)	Sweden	2001, B.T. Olsen	AY500539
<i>M. cornubiensis</i> (1)	Griffith 7.15.2000 (FH)	USA, Vermont	2000, K. Griffith	DQ220366
<i>M. cornubiensis</i> (2)	KH.01.017 (C)	Denmark	2001, K. Hansen	DQ220367
<i>M. flavorubens</i> (1)	KS-94-075 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220368
<i>M. flavorubens</i> (2)	DHP 04.570 (FH)	Iceland	2004, D.H. Pfister	DQ220369
<i>Microstoma floccosum</i>	Weinstein 45 (FH)	Mexico, Tlaxcala	1998, K. Griffith	DQ220370
<i>Miladina lecithina</i> (1)	KH.03.156 (C)	Sweden	2003, C. Lange	DQ220371
<i>M. lecithina</i> (2)	KH.04.22 (FH)	USA, New Mexico	2004, K. Hansen, B.A. Perry, N. Weber	DQ220372
<i>Moravecia hvaleri</i>	RK 97.44 (Herb. Roy Kristiansen, Holotype)	Norway, Østfold	1997, R. Kristiansen	DQ220373
<i>Morchella elata</i>	NRRL 25405	USA, Michigan	—	U42667
<i>Nanoscypha tetraspora</i>	mh PR61 (FH)	Puerto Rico	1996, D.H. Pfister, F. A. Harrington	DQ220374
<i>Neolecta irregularis</i>	JP 176	—	—	AF279401
<i>N. vitellina</i>	NSW 6359	USA, Oregon	N.S. Weber	U42695
<i>Neottiella albocincta</i>	C F-53559/HD Finn.99.18 (C)	Norway	1999, H. Dissing	DQ220375
<i>N. aphanodictyon</i>	C F-53531/HD Finn.86.35 (C)	Norway	1986, H. Dissing	DQ220376
<i>N. rutilans</i>	KH.03.55 (FH)	Norway	2003, K. Hansen, C. Lange	DQ220377
<i>Neourmula pouchetti</i>	NSW 6435 (OSC)	USA, Oregon	1991, N.S. Weber	AY307940
<i>Octospora axillaris</i>	C F-55450/KS-94-187 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220378
<i>O. hygrophynophila</i>	KH.03.30 (FH)	Norway	2003, K. Hansen	DQ220379
<i>O. leucoloma</i>	C F-52723/HD Gr.83.016 (C)	Greenland	1983, HFG, H. Dissing	DQ220380
<i>O. lilacina</i>	KS-94-204 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220381
<i>O. phagospora</i>	C F-55452/KS-94-224A+B+C (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220382

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Table 1 (continued)

Species	Collection/Isolate no.	Geographic Origin	Year and Collector	GenBank accession nos.
<i>O. rubens</i>	C F-55444/KS-94-99 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220383
<i>Octospora</i> sp.	KH.03.136 (FH)	Norway	2003, K. Hansen	DQ220384
<i>Orbicula parietina</i>	C F-24441 (C)	Denmark	1988, U. Søchting	DQ062988
<i>Otidea alutacea</i>	HDT 53088 (SFSU)	USA, California	1990, H.D. Thiers	DQ220385
<i>O. concinna</i>	NSW 7574 (OSC)	—	N.S. Weber	AF086593
<i>O. leporina</i>	s.n. 21 August 1983 (FH)	USA, Maine	1983, J. Hrbek	DQ220386
<i>O. onotica</i>	s.n. 13 February 1998 (FH)	USA, California	1998, D.S. Otte, R. Roper	DQ220387
<i>O. rainierensis</i>	OSC 56745 (OSC)	—	—	AF086599
<i>O. umbrina</i>	KH.01.09 (C)	Denmark	2001, C. Lange	AY500540
<i>Otidea</i> sp.	T. Laessoe 6236 (C)	Malaysia	1999, T. Laessoe	AF335111
<i>Pachyella babingtonii</i>	KH-99-09 (C)	USA	1999, K. Hansen, D. H. Pfister	AF335123
<i>Parascutellinia carneosanguinea</i>	KH.03.34 (FH)	Norway	2003, K. Hansen, C. Lange	DQ220388
<i>Paratrachophaea boudieri</i>	BAP 481 (FH)	USA, California	2003, B.A. Perry	DQ220402
<i>Paurocotylis pila</i>	Trappe 12583 (FH, dupl. OSC)	New Zealand, South Island	1993, M. Amaranthus	DQ168337
<i>Peziza varia</i>	KH-99-04 (C)	USA, New Hampshire	1999, Z.-L. Yang	AF335151
<i>Pezizales</i> sp. A	B48	Estonia	—	AJ534928
<i>Pezizales</i> sp. B	d334	USA, California	M. Bidartondo	AF266707
<i>Phillipsia crispata</i>	T. Læssøe AAU-44895a (C)	Ecuador, Napo	1983, T. Læssøe	AY945845
<i>Plectania nannfeldtii</i>	KH-97-16 (FH)	USA, California	1997, K. Hansen	AY945853
<i>Pseudaleuria quinaultiana</i>	NSW 7107	USA, Oregon	N. S. Weber	DQ220389
<i>Pseudombrophila guldeniae</i> (1)	Kongsv. 85.10B (C)	Norway, Oppdal	1985, H. Dissing, S. Sivertsen	DQ062993
<i>P. guldeniae</i> (2)	s.n. (FH, part in C and TRH)	Norway, Oppdal	1985, S. Sivertsen, I. Dissing, H. Dissing	DQ062994
<i>P. merdaria</i> (1)	s.n. (FH)	USA, Maine	1994, D.H. Pfister	DQ062990
<i>P. merdaria</i> (2)	s.n. (FH)	USA, Vermont	1979, M. Shemluck	DQ062991
<i>P. merdaria</i> (3)	s.n. (FH)	USA, Iowa	T.J. Farrell	DQ062992
<i>P. theioleuca</i>	C F-70057 (C)	Denmark	1982, H. Knudsen	DQ062989
<i>Pseudophyella minuscula</i>	mh 675 (FH)	USA, California	1997, F.A. Harrington	AY945849
<i>Pseudoplectania nigrella</i>	KH-97-28 (FH)	USA, California	1997, K. Hansen	AY945852
<i>Psilopezia deligata</i>	KH-99-13 (FH)	USA, Vermont	1999, K. Griffith	DQ220390
<i>P. juruensis</i>	T. Læssøe AAU 44912 (QCA, C, FH)	Ecuador, Orellana	1983, T. Læssøe	DQ220391
<i>Pulvinula archeri</i>	BAP 458 (FH)	USA, Oregon	2003, B.A. Perry, N.S. Weber	DQ220392
<i>P. constellatio</i>	KH.03.64 (FH)	Norway	2003, K. Hansen, C. Lange	DQ062987
<i>P. convexella</i>	KH.01.20 (C)	Denmark	2001, K. Hansen	DQ062986
<i>P. globifera</i>	DHP DR-104 (FH)	Dominican Republic	2002, D.H. Pfister et al.	DQ220393
<i>P. ovalispora</i> (1)	BTO 95206/C F-34031 (C)	Denmark	1995, B.T. Olsen	DQ220394
<i>P. ovalispora</i> (2)	KH.03.65 (FH)	Norway	2003, K. Hansen, C. Lange	DQ220395
“ <i>Pustularia patavina</i> ”	KH.03.73 (FH)	Norway	2003, K. Hansen, C. Lange	DQ220396
<i>Pyronema domesticum</i>	AFTOL (CBS 666.88)	Netherlands	1988, H.A. van der Aa	DQ247805
<i>P. omphalodes</i> (1)	TL-11685 (QCNE, C)	Ecuador, Carchi	2004, K. Hansen et al.	DQ220397
<i>P. omphalodes</i> (2)	BAP 490 (FH)	USA, California	2003, B.A. Perry, M. Wood	DQ220398
<i>Pyronemataceae</i> sp. A	KH.03.125 (FH)	Norway	2003, C. Lange	DQ220462
<i>Pyronemataceae</i> sp. B	KH.04.21 (FH)	USA, New Mexico	2004, N. Weber, K. Hansen, B.A. Perry	DQ220399
<i>Pyronemataceae</i> sp. nov. (1)	HDT 53173 (SFSU)	USA, California	1990, H.D. Thiers	DQ220400
<i>Pyronemataceae</i> sp. nov. (2)	DHP & HDT 5.18.86 (FH)	USA, California	1986, H.D. Thiers, D.H. Pfister	DQ220401
<i>Pyronemataceae</i> sp. nov. (3)	BAP 492 (FH)	USA, California	2003, J. Laws	DQ220403
<i>Pyropyxis rubra</i> (1)	DAOM 178733/K. Egger 289 (DAOM)	Canada, Ontario	1979, K.N. Egger	DQ220404
<i>P. rubra</i> (2)	DAOM 178736/K. Egger 323 (DAOM)	Canada, Ontario	1979, K.N. Egger	DQ220405
<i>Ramsbottomia asperior</i> (1)	C F-53681/HD Finn00.07 (C)	Norway	2000, H. Dissing, S. Sivertsen	DQ220406
<i>R. asperior</i> (2)	DHP 30.8.2000 (FH)	USA, Vermont	2000, D.H. Pfister	DQ220407
<i>R. asperior</i> (3)	KH.03.79 (FH)	Norway, Nordland	2003, K. Hansen	DQ220408
<i>Ramsbottomia</i> sp.	NSW 7417	USA, Oregon	1994, N. S. Weber	DQ220409
<i>Rhizina undulata</i>	KH.02.44 (FH)	Norway, Østfold	2002, D.H. Pfister, B.A. Perry, K. Hansen	DQ220410
<i>Rhodoscypa ovilla</i> (1)	C F-54650/HD Rana79.060 (C)	Norway, Nordland	1979, H. Dissing	DQ220411

Table 1 (continued)

Species	Collection/Isolate no.	Geographic Origin	Year and Collector	GenBank accession nos.
<i>R. ovilla</i> (2)	C F-55007/HD Rana81.089 (C)	Norway, Nordland	1981, H. Dissing	DQ220412
<i>Rhodotarzetta rosea</i> (1)	KH.03.107 (FH)	Norway, Nordland	2003, K. Hansen	DQ220413
<i>R. rosea</i> (2)	HD Rana 75.081 (C)	Norway, Nordland	1975, H. Dissing	DQ220414
<i>Sarcoscypha coccinea</i>	KH.04.78 (C)	Denmark	2004, H. Knudsen	AY945847
<i>Scutellinia barlae</i>	KH.01.023 (C)	Denmark	2001, T. Læssøe	DQ220415
<i>S. blumenaviensis</i>	KH.02.55 (FH)	Costa Rica	2002, K. Hansen	DQ220416
<i>S. cf. erinaceus</i>	KH.03.15 (FH)	Norway, Nordland	2003, K. Hansen	DQ220417
<i>S. geneospora</i>	R.P. Korf and S.C. Gruff. <i>Discomycetes exsiccati</i> #70 (FH)	Japan, Yaku Island	1961, Y. Kobayasi, K. Tubaki, R.P. Korf	DQ220418
<i>S. hyperborea</i>	ALL-94-14 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220419
<i>S. pennsylvanica</i>	DHP 105 (FH)	USA, New York	1968, M. Riedel, D.H. Pfister	DQ220420
<i>S. scutellata</i>	C F-55466/KS-94-035H (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220421
<i>S. subhirtella</i>	C F-55440/KS-95-059A (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220423
<i>S. trechispora</i> (1)	C F-55441/KS-94-093 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220424
<i>S. trechispora</i> (2)	KH.01.37 (C)	Denmark	2001, K. Hansen, J.H. Petersen	DQ220425
<i>Scutellinia</i> sp.	BAP 427 (FH)	USA, Massachusetts	2001, D. Hewitt	DQ220422
<i>Smardaea amethystina</i>	KH-97-132 (C)	Denmark	1997, C. Lange, K. Hansen	AF335176
<i>S. reticulosperma</i>	Part of isotype (herb. Roy Kristiansen)	France	1984, G. Rioussset	AY500532
<i>Sowerbyella imperialis</i> (1)	C F-29814/JV91-810 (C)	Denmark	1991, D. Boertman, J. Vesterholt	DQ220426
<i>S. imperialis</i> (2)	CL2004-105 (C)	Denmark	2004, C. Lange	DQ220427
<i>S. radiculata</i> (1)	KH.04.30 (FH)	USA, New Mexico	2004, B. Chapman, K. Hansen	DQ220428
<i>S. radiculata</i> (2)	C F-54211/TL-6854 (C)	Denmark	2001, T. Læssøe	DQ220429
<i>S. radiculata</i> (3)	C F-38457/TL-5355 (C)	Denmark	1998, T. Læssøe	DQ220430
<i>S. rhenana</i>	DED 6693 (SFSU)	USA, California	1997, D.E. Desjardin	DQ220309
<i>Sphaerospora brunnea</i> (1)	C F-55022/HD Rana81.104 (C)	Norway	1981, H. Dissing	DQ220431
<i>S. brunnea</i> (2)	DHP DR.02.16 (FH)	Dominican Republic	2002, D.H. Pfister et al.	DQ220432
<i>S. brunnea</i> (3)	KH.03.04 (FH)	USA, Massachusetts	2003, K. Hansen.	DQ220433
<i>Spooneromyces laeticolor</i>	C F-48310/HFG 88.013 (C)	Denmark	1988, H.F. Gøtzsche	DQ220434
<i>Stephensia bombycina</i>	Trappe 3268 (FH, dupl. OSC)	Mexico, Mexico City	1972, J. Trappe	DQ220435
<i>S. shanorii</i>	OSC 80635 (FH, dupl. OSC)	USA, Illinois	1960, D.D. McLain	DQ220436
<i>Strobiloscypha keliae</i>	NSW 6387 (OSC)	USA, Oregon	1991, K. Kuykendall	DQ220437
<i>Tarzetta catinus</i>	C F-55260/KS-94-10A (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ062984
<i>T. gaillardiana</i>	C F-55462/ALL-94-09 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220439
<i>T. pusilla</i>	KH.03.66 (FH)	Norway, Nordland	2003, K. Hansen, C. Lange	DQ062983
<i>T. spurcata</i>	AMNH-44124/F-14527 (AMNH)	Iceland	1993, G. G. Eyjólfssdóltir	DQ220441
<i>Tricharina gilva</i> (1)	C F-55212/HD Rana81.118 (C)	Norway	1981, H. Dissing	DQ220442
<i>T. gilva</i> (2)	DED 7356 (SFSU)	USA, California	2002, D.E. Desjardin	DQ220443
<i>T. gilva</i> (3)	BAP 431 (FH)	USA, California	2002, B.A. Perry	DQ220444
<i>T. ochroleuca</i>	C F-53062/HD Gr83.107 (C)	Greenland	1983, H. Dissing	DQ220445
<i>Tricharina</i> sp. A	TL-10051 (C, QCA)	Ecuador	2003, J. Salazar, T. Læssøe	DQ220447
<i>Tricharina</i> sp. B	Barr 5907 (FH)	USA, Massachusetts	1971, M. E. Barr	DQ220446
<i>Trichophaea abundans</i> (1)	CBS 348.76	Finland	1976, V. Hintikka	DQ220448
<i>T. abundans</i> (2)	KH.01.036 (C)	Denmark	2001, K. Hansen	DQ220449
<i>T. abundans</i> (3)	CBS 250.31	—	1931, H.C.I. Gwynne-Vaughan	DQ220450
<i>T. hemisphaerioides</i> (1)	C F-55283/KS-94-57 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220456
<i>T. hemisphaerioides</i> (2)	KH.97.31 (FH)	USA, California	1997, K. Hansen	DQ220457
<i>T. hybrida</i> (1)	DHP 30.VIII.2000 (FH)	USA, Vermont	2000, D.H. Pfister	DQ220453
<i>T. hybrida</i> (2)	KH.04.39 (FH, dupl. DBG)	USA, Colorado	2004, K. Hansen, V. Evenson	DQ220454
<i>T. hybrida</i> (3)	AMNH-49682/F-17491 (AMNH)	Iceland	2003, G. G. Eyjólfssdóltir	DQ220455
<i>T. minuta</i>	CBS 236.57	Canada	1953, R. F. Cain, N. A. Hastings	DQ220452

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Table 1 (continued)

Species	Collection/Isolate no.	Geographic Origin	Year and Collector	GenBank accession nos.
<i>T. saccata</i>	CBS 804.70	England	1968, H. Evans	DQ220451
<i>T. woolhopeia</i> (1)	C F-55285/KS-94-63 (C)	Denmark	1994, K. Hansen, S.K. Sandal	DQ220458
<i>T. woolhopeia</i> (2)	KH.01.033 (C)	Denmark	2001, K. Hansen	DQ220460
<i>T. woolhopeia</i> (3)	BAP 453 (FH)	Norway	2002, D.H. Pfister, B.A. Perry	DQ220459
<i>Trichophaeopsis bicuspis</i>	NSW 8316 (OCS)	USA, Oregon	1988, N. S. Weber	DQ220461
<i>T. tetraspora</i>	C F-47525 (C)	Denmark	1974, H. Dissing	DQ220463
<i>Tuber cf. gibbosum</i>	Trappe 12396 (FH, dupl. OSC)	USA, Oregon	1992, J. Toledo	DQ220464
<i>Unicava</i> sp. (<i>gen. ined.</i>)	Trappe 18483 (FH, dupl. OSC)	Australia, Victoria	1996, A. Jumpponen, J. Trappe	DQ220465
<i>Unicava</i> sp.	Trappe 19051 (FH, dupl. OSC)	Australia, Victoria	1996, J. Trappe	DQ220466
<i>Urnula craterium</i>	DHP 04.511 (FH)	USA, North Carolina	2004, D.H. Pfister	AY945851
<i>Verpa conica</i>	NRRL 20856/CBS 407.81	Netherlands	—	U42671
<i>Warcupia terrestris</i>	CBS 891.69	Canada	1966, J.W. Paden	DQ220467
<i>Wilcoxina mikolae</i>	WS 36 (SFSU)	USA, Wyoming	1995, W. Stoll	DQ220468
<i>W. rehmi</i>	—	USA, California	—	AF266706
<i>Wilcoxina</i> sp.	ITS RFLP RPC-10	USA, California	—	AF156926
<i>Wolfina aurantiopsis</i>	RPK 4337 (CUP)	USA, Ohio	1976, S.J. Mazzer	AY307941

by a strict consensus tree. Support of individual clades was assessed by parsimony BS (PB) analyses (Felsenstein 1985) using 1 K heuristic replicates, each consisting of ten random addition sequences replicates, TBR branch swapping, and keeping no more than five trees per replicate.

ML searches employed a modified 'ratchet' method to explore tree space (Nixon 1999; Vos 2003) using PAUP version 4 (Swofford 2003). Starting trees were built via the NJ method. Each starting tree was calculated using a pre-specified amount of data (e.g., 10, 20, and 30 % of the data randomly removed). The complete data were then restored to perform TBR branch swapping. One hundred iterations were performed on each search (10, 20, and 30 % of data excluded from starting trees, respectively), swapping trees for no more than 5 min per iteration. Best trees found at each iteration were saved to an output file. A final tree search was then performed, swapping on the pool of trees saved at each iteration. The model of sequence evolution was determined using the Akaike Information Criterion as calculated in the program Modeltest version 3.7 (Posada & Crandal 1998). All searches were performed using a GTR + I + G model of sequence evolution with parameters fixed to values calculated from one of the equally most parsimonious trees recovered in the parsimony analyses described above. Clade support was assessed by non-parametric ML BS (MLB) analyses as implemented in the program PhyML (Guindon & Gascuel 2003) and consisted of 1 K replicates using a GTR + I + G model of sequence evolution, with all parameters estimated by the program.

Bayesian analyses were performed using Metropolis-coupled MCMC (MCMCMC) methods as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), using a GTR + I + G model as determined above. Analyses consisted of two parallel searches, run for 40 M generations and initiated with random starting trees. The chains were sampled every 2 K generations for a total of 20 K trees each, sampled from the posterior distribution. Those trees sampled before the chains reaching a split deviation frequency of 0.03 were discarded from the sample as the 'burn-in', while the

remaining trees were used to calculate the Bayesian PPs (BPPs) of the clades. The incremental heating scheme for the analyses used the default settings in MrBayes (i.e., three heated chains and one cold chain). The default settings were also used to set unconstrained branch length [unconstrained:exponential (10.0)] and uninformative topology (uniform) priors.

Based upon the results of the phylogenetic analyses, constrained topology analyses were conducted in PAUP version 4 (Swofford 2003) to evaluate the significance of alternative tree topologies in which specific families were constrained to be monophyletic or resolved in different regions of the phylogeny. Constraint topologies were manually specified in PAUP and heuristic searches of 1 K replicates, saving only trees in agreement with the constraint, were conducted using the same settings as the parsimony searches described above. Resulting trees were sorted by likelihood score under GTR + I + G model of sequence evolution, with parameters estimated from a single, unconstrained parsimony tree. For each analysis, the ten most likely trees were compared using the Shimodaira & Hasegawa (1999) test as implemented in PAUP, with the resampling estimated log-likelihood (RELL) method and 1 K BS replicates. Constrained topologies tested included: (1) forcing all pyronemataceous taxa to form a monophyletic group; (2) forcing the representative taxa of *Ascodesmidaceae* to group outside of the main clade of *Pyronemataceae*; and (3) forcing the sole representative of *Glaziellaceae*, *Glaziella aurantiaca*, to group within the main clade of *Pyronemataceae* (essentially the same topology as the ML tree).

Results

Alignment

For most specimens, approximately 900–1000 bp of nuLSU sequence were obtained, but for *Aleuria bicucullata*, *Moravecia hvaleri*, and *Psilopezia juruensis* only 580–614 bp were obtained using

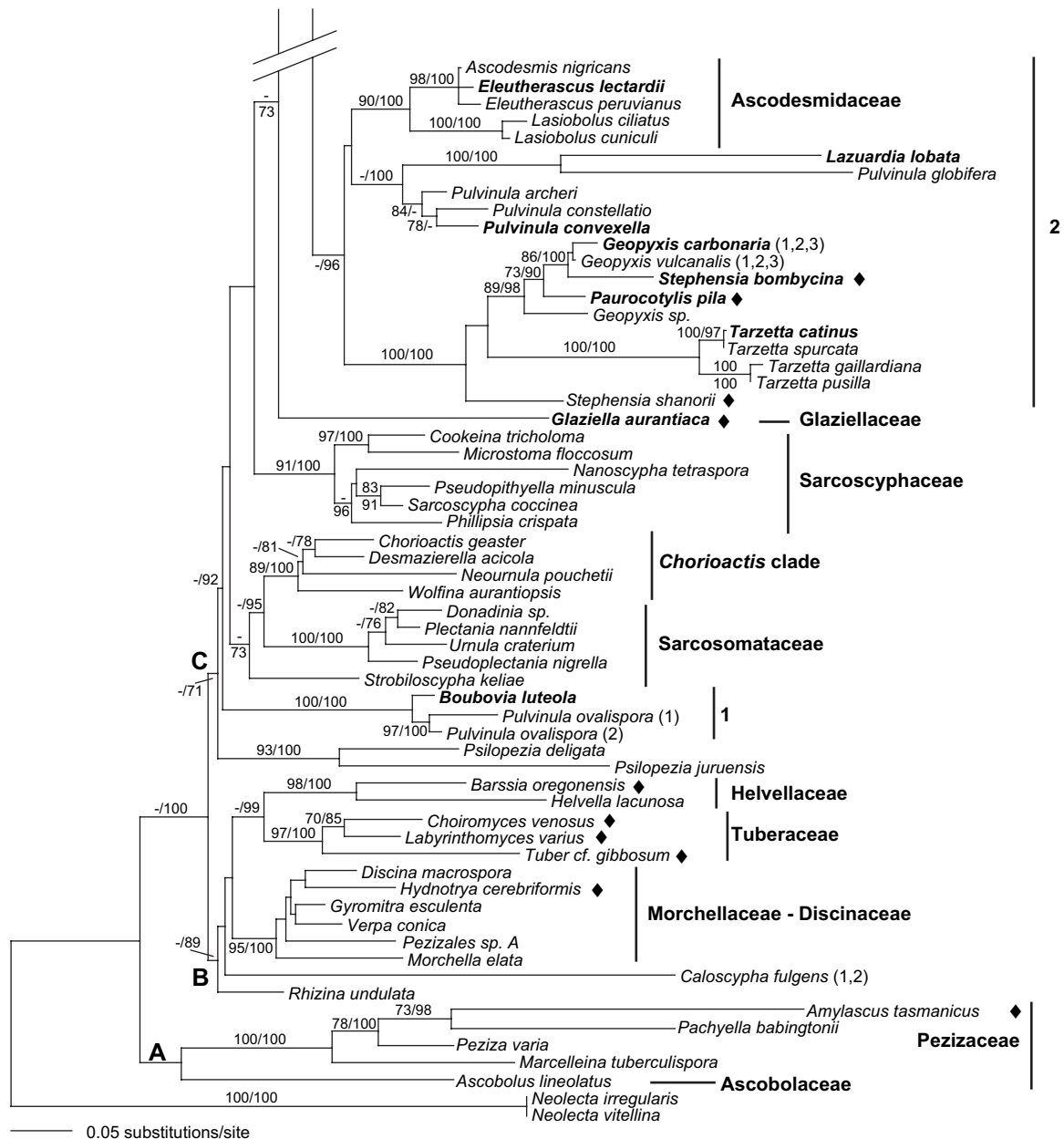


Fig 1 – ML tree ($-\ln L = 24294.87727$) of the Pezizales based on nuLSU sequence data. Numbers separated by, or above and below branches represent nonparametric ML BS proportions and Bayesian PPs greater than 70 %, respectively (- designates a value lower than 70 %). Parsimony BS proportions correspond closely to the ML BS values, and have been omitted due to space constraints. An asterisk is used to denote values above 95 %/95 % on short branches. Clade numbers correspond to groups of Pyronemataceae taxa treated in the discussion. Type species of genera from Pyronemataceae, Ascodesmidaceae and Glaziellaceae are highlighted in bold. Additional families sampled are indicated, and the three main lineages of Pezizales resolved are labelled as A, B and C. *Caloscypha fulgens* and *Rhizina undulata*, respectively. (◆) Truffle or truffle-like taxa; (◆) taxa with cleistothecial ascomata.

the primer combination LROR/LR3. The final size of the aligned data set consists of 842 bp for 198 taxa, and contains 474 variable positions, including 390 that are parsimony informative.

Phylogenetic analyses

Parsimony analyses of the nuLSU data set produced 15 K equally most parsimonious trees (5233 steps, $CI = 0.187$,

$RI = 0.621$). Despite the high number of trees recovered, the strict consensus of these trees (not shown) is highly resolved. The ML analyses recovered a single tree ($-\ln L = 24294.87727$; Fig 1). MLB values are very similar to those obtained via parsimony bootstrapping. Bayesian analyses reached an average standard deviation of split frequencies below 0.03 after approximately 6 M generations, and the first 3 K trees were excluded as the 'burn-in.' PB and MLB values, and

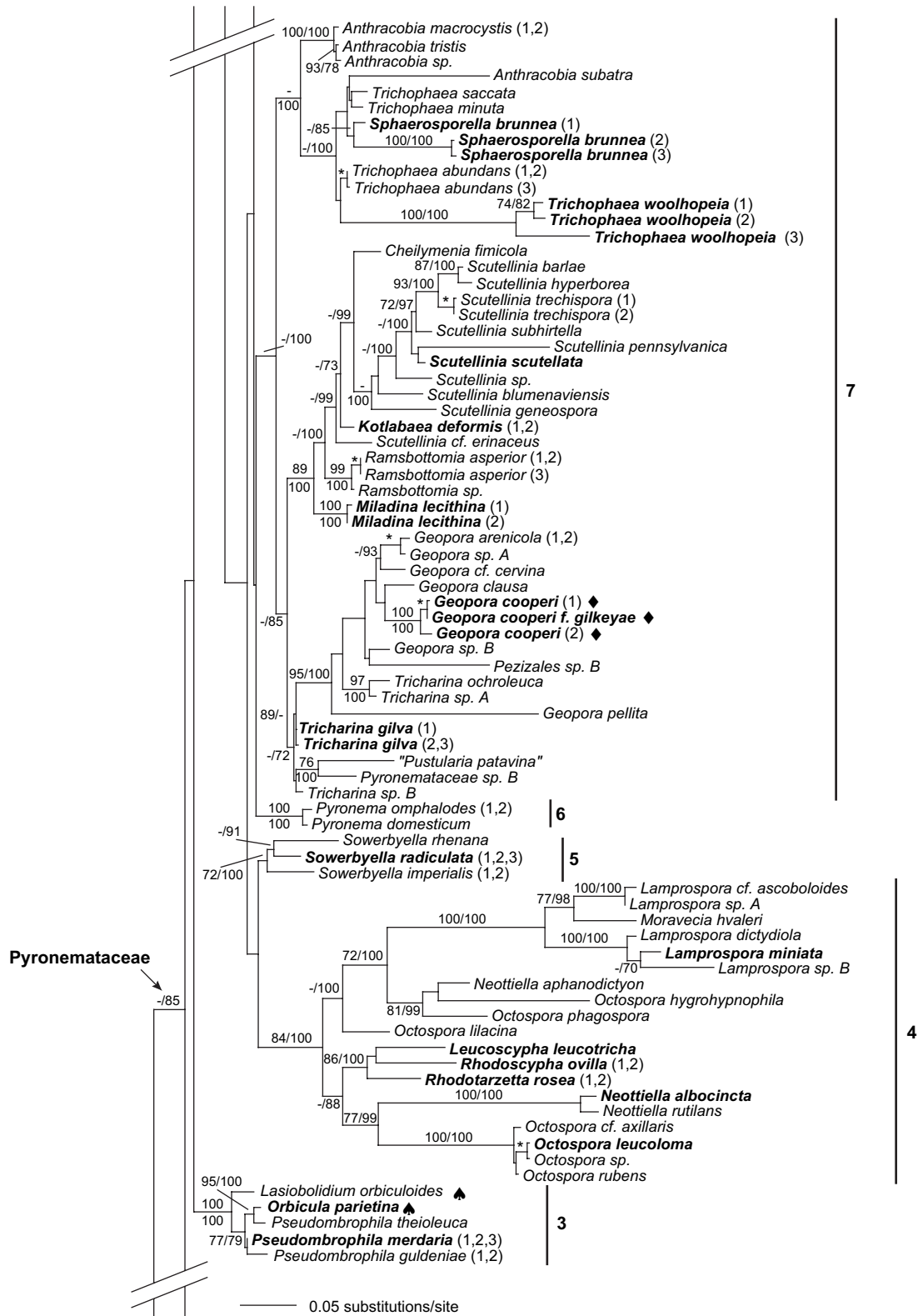


Fig 1 - (Continued)

BPPs, support many of the terminal relationships in the phylogeny, but fail to resolve the deeper nodes with support.

The ML and Bayesian analyses produced topologies similar to those of the parsimony analyses, differing mainly in the

resolution of the families and genera towards the base of the phylogeny, as well as the relationships of the major clades in the main grouping of pyronemataceous taxa. In the ML topology (Fig 1), three major lineages of Pezizales are recovered

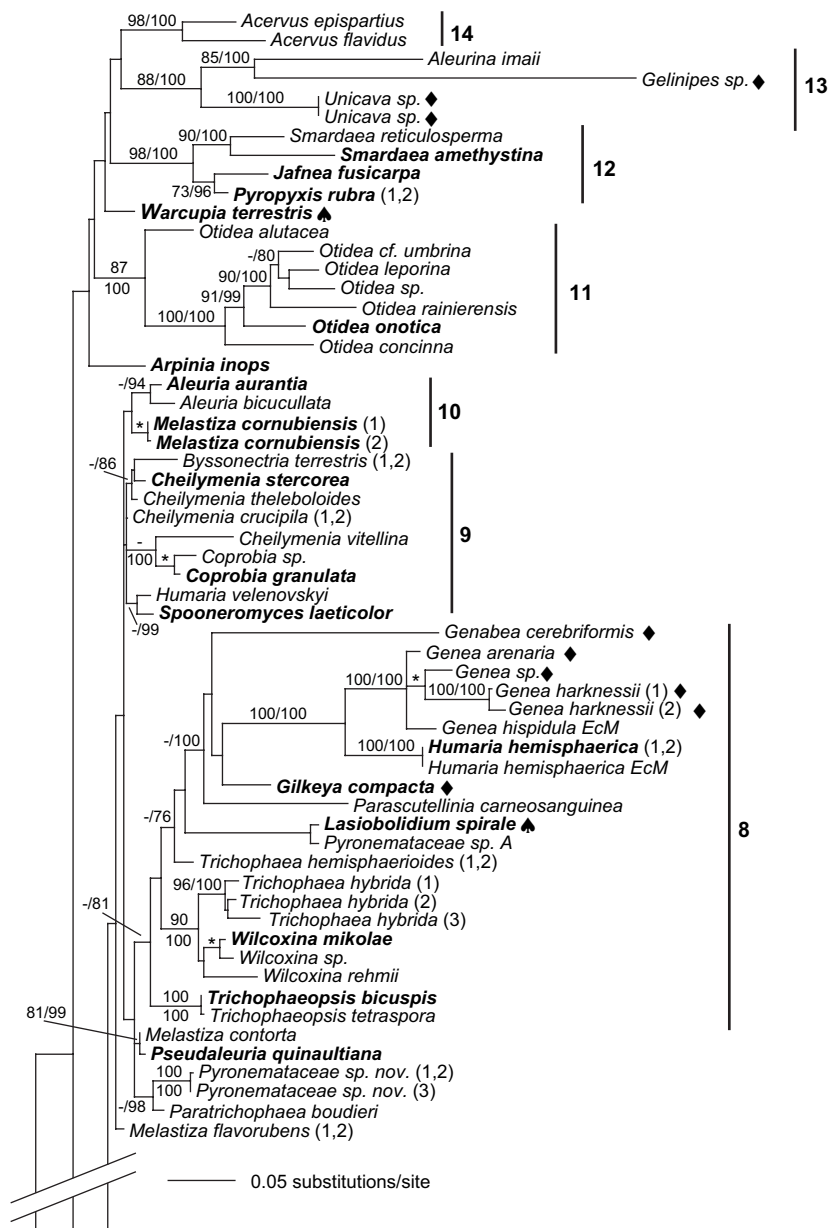


Fig 1 – (Continued)

that correspond to the lineages resolved in the analyses of Landvik et al. (1997). Lineage A is composed of Pezizaceae and Ascobolaceae, while lineage B is composed of members of Rhiziniaceae, Caloscyphaceae, Morchellaceae–Discinaceae, Tuberaceae, and Helvellaceae. Lineage C, the focus of this study, is composed of taxa from Sarcosomataceae, Sarcoscyphaceae, Glaziellaceae, Ascodesmidaceae, Pyronemataceae, and a group of taxa centred on *Chorioactis geaster* (herein referred to as the *Chorioactis* clade). All three major lineages received PB and MLB values below 50 %, and low BPP. Lineage A is resolved as the sister group to lineages B and C, but this relationship is not strongly supported. Lineages B and C are, however, strongly supported as sister lineages (BPP 100 %).

All analyses indicate that *Pyronemataceae*, in its broad circumscription, does not form a monophyletic group. Although

the majority of *Pyronemataceae* taxa sampled form a weakly supported (PB <50 %/MLB <50 %/BPP 85 %) clade (herein referred to as *Pyronemataceae*), several pyronemataceous taxa fall outside this large group (Fig 1). In the ML tree, *Psilopezia deligata* and *P. juruensis* together are resolved outside *Pyronemataceae*, as a sister group to the remainder of lineage C. *Boubovia luteola*, and two specimens of *Pulvinula ovalispora*, also form an isolated clade outside *Pyronemataceae* (clade 1). Members of *Ascodesmidaceae*, *Ascodesmis nigricans*, *Eleutherascus lectardii*, *E. peruvianus*, *Lasiobolus ciliatus* and *L. cuniculi*, form a well-supported clade (83 %/90 %/100 %) within *Pyronemataceae*. Among the pyronemataceous taxa, 14 primary clades were identified that are moderately to strongly supported by PB, MLB and BPP values, and/or are present in all trees recovered from the parsimony, ML and Bayesian analyses

Table 2 – Shimodaira & Hasegawa (1999) likelihood test values comparing trees resulting from constrained and unconstrained parsimony analyses

Topology	Steps	-ln L	Diff. -ln L	P*
Unconstrained MP analysis	5233	24199.12422	3.69613	0.790
'Pyronemataceae' + Glaziellaceae	5240	24195.42809	–	best
'Pyronemataceae' - Ascodesmidaceae	5243	24219.91192	24.48382	0.150
Pyronemataceae monophyletic	5242	24196.82991	1.40182	0.898

The most likely tree according to likelihood score is provided for each analysis. No constrained trees can be rejected based on the results of the S-H test ($P < 0.05$).

(Fig 1). Although there is BS and BPP support for many of the terminal groupings of species within *Pyronemataceae*, the relationships among most of the genera are not resolved with high levels of support. In the ML analyses *Glaziellaceae* is resolved as sister to *Pyronemataceae*, while in the parsimony and Bayesian analyses this family groups with *Psilopezia* or other members of the C lineage. None of these relationships are supported.

Parsimony trees resulting from constrained analyses forcing all pyronemataceous taxa to form a monophyletic group, including *Ascodesmidaceae*, were not rejected (Table 2). Similarly, those trees recovered from analyses forcing *Ascodesmidaceae* out of *Pyronemataceae*, as well as those forcing *Glaziella aurantiaca* into the family, were also not rejected (Table 2).

Discussion

Delimitation and relationships of *Pyronemataceae*

These analyses suggest that *Pyronemataceae* is not monophyletic as it is currently circumscribed (Eriksson 2006). Although the majority of pyronemataceous taxa sampled fall out in a single, weakly supported clade (Fig 1), three genera are resolved outside *Pyronemataceae* among the other families of lineage C. The relationships of these outlying taxa to other families of Pezizales, however, remain uncertain. *Ascodesmidaceae* is resolved as monophyletic, but nested within *Pyronemataceae* as part of a moderately supported clade (Fig 1, clade 2). Although our results suggest that several pyronemataceous taxa should be treated outside *Pyronemataceae*, and that the taxa of *Ascodesmidaceae* should be transferred to *Pyronemataceae*, the lack of support for the deeper nodes of the phylogeny, and the results of the constrained analyses, render such emendations unwarranted until these relationships can be demonstrated with greater confidence. In agreement with previous results (Landvik et al. 1997, Harrington et al. 1999), the restricted families *Sarcosomataceae* and *Sarcoscyphaceae*, and a clade composed of *Chorioactis geaster*, *Desmazierella acicola*, *Neournula pouchetii* and *Wolfina aurantiopsis* are supported as distinct, but closely related sister groups of pyronemataceous taxa.

Our data fail to clearly delimit *Pyronemataceae* or resolve its relationships, due to the lack of sufficient phylogenetic information within the nuLSU data. Nevertheless, our phylogeny represents the largest taxonomic sampling of *Pyronemataceae* to date and gives new insight into the relationships among the taxa of this family. Fourteen clades of pyronemataceous taxa are present in all trees recovered in our analyses. To

facilitate discussion of the large number of taxa represented in this study, each of these clades will be treated individually below.

Clade 1

This small clade and subtending branches represent pyronemataceous taxa that are resolved outside the family. *Boubovia luteola* and *Pulvinula ovalispora* are strongly supported sister taxa (100%/100%/100%), as are two species of *Psilopezia* Berk. (95%/93%/100%) on a subtending branch. *Pulvinula* is not monophyletic; the other *Pulvinula* species sampled are nested within clade 2. As discussed by Pfister (1976), *P. ovalispora* is unique due the presence of ellipsoid spores, whereas the remaining taxa of the genus have globose spores. Korf & Zhuang (1984, 1991b) subsequently described two additional ellipsoid- to subglobose-spored species within the genus, *P. ascoboloides* and *P. subprolata*, and noted that these taxa in addition to *P. ovalispora*, differed from the other species of the genus in the presence of asci that develop thickened walls during the early stages of ascospore delimitation. Both Pfister (1976) and Korf & Zhuang (1984) suggested that *Pulvinula* occupies an isolated position within *Pyronemataceae*, but refrained from erecting yet another tribe within an already heterogeneous family. Yao & Spooner (1996a) recognized the similarity of the thickened ascus walls and ellipsoid to subglobose spores of these *Pulvinula* species to the genus *Boubovia*, and transferred both *P. ascoboloides* and *P. subprolata*. These authors did not have the opportunity to examine the type material of *P. ovalispora*, but felt this species was also a member of *Boubovia*, and perhaps even conspecific with *B. nicholsonii*. Our results support the transfer of the ellipsoid- to subglobose-spored *Pulvinula* species to *Boubovia*.

Korf (1972) treated *Psilopezia* in tribe *Otideae* of *Pyronemataceae* due to the lack of carotenoids, asci that are non-bluing in iodine, uninucleate spores, and anatomical similarity of the genus to species of *Otidea* (Pfister 1973). Our results indicate that these genera are not closely related, and that *Psilopezia* is not a member of *Pyronemataceae*. Both *Psilopezia* and *Boubovia* (including *Pulvinula ovalispora*) are isolated on separate branches that subtend the remaining taxa and families of the C lineage, and their relationships to the other genera of this lineage remain unclear based upon the nuLSU data.

Clade 2

This clade contains representative taxa of *Ascodesmidaceae* and several globose-spored species of *Pulvinula*, that together form the sister group to species of *Tarsetta*, *Geopyxis*, and the truffle-like genera *Paurocotylis* and *Stephensia*. As discussed

above, the placement of *Ascodesmidaceae* within *Pyronemataceae* renders the latter family paraphyletic. The placement of ascodesmidaceous taxa within *Pyronemataceae*, however, is not a novel idea. *Obrist (1961)* indicated that the spore structure of *Ascodesmis* suggests a close relationship to species of *Humariaceae*, tribe *Humarieae sensu Le Gal (1947)*. *Merkus (1974)* concluded that *Ascodesmis* differs from members of *Pyronemataceae sensu Eckblad* in the development of ascospore ornamentation, but is similar to these taxa in the sense that both the endo- and epispore differentiate within the primary wall. Based on similarities in the structure of the ascus apical apparatus, *Samuelson (1978)* suggested that *Ascodesmis* was most closely related to members of *Otidea* and *Aleuriaceae sensu Kimbrough (1970)*. *Korf (1972)* erected a subfamily, *Ascodesmidoideae*, in his emended *Pyronemataceae* to accommodate a heterogeneous assemblage including: *Ascodesmis*, *Sphaerozone*, *Aleurina* Masee (as *Jafneadelphus*), and *Marcelleina* (as *Pulparia*). Other investigators have treated *Ascodesmis* and related taxa in the family *Ascobolaceae (Brummelen 1967; Eckblad 1968; Dennis 1978)*. *Brummelen (1981)* felt that *Ascodesmis* held an isolated position within the *Pezizales*, and reinstated the family *Ascodesmidaceae sensu Schröter* to accommodate the genus. Based upon similarities in ultrastructure and development of the asci and ascospores of *Eleutherascus* to those of *Ascodesmis*, *Brummelen (1989)* later emended the family to include this genus. The molecular analyses of *Landvik et al. (1997)* indicated a close relationship between *Ascodesmis* and *Pyronemataceae*, but were ambiguous as to whether *Ascodesmidaceae* should be retained as a separate family. *Landvik et al. (1997)* suggested a possible relationship between *Ascodesmis* and other small, fimicolous members of *Pyronemataceae* with protruding asci such as *Lasiobolus*, and later (*Landvik et al. 1998*) demonstrated such a relationship based on additional nuSSU sequence data. Our results agree with the findings of *Landvik et al. (1997, 1998)* indicating a close relationship between *Ascodesmidaceae* and *Pyronemataceae*, and confirm the close relationships of *Ascodesmis*, *Eleutherascus* and *Lasiobolus*.

Sister to *Ascodesmidaceae* is a clade comprising four *Pulvinula* species and *Lazuardia lobata*. Our results indicate that *Pulvinula* is not monophyletic (see discussion of clade 1). *Lazuardia lobata* forms a sister group to *Pulvinula globifera*, likely due to long branch attraction (*Felsenstein 1978; Hendy & Penny 1989*). Parsimony analyses excluding *P. globifera* resolved the three remaining *Pulvinula* species as a well-supported, monophyletic group sister to *Ascodesmidaceae*, with *L. lobata* forming a weakly supported sister group to the *Ascodesmidaceae–Pulvinula* clade (results not shown). *Eriksson & Hawksworth (1988)* felt that the separation of the monotypic *Lazuardia* Rifai from *Marcelleina* by *Rifai (1988)* was not fully warranted, but retained it as a distinct genus in *Pyronemataceae* awaiting further information from other investigators. Our results, as well as those of *Hansen et al. (2001)*, indicate that *Marcelleina* is a member of *Pezizaceae*.

The sister group to *Ascodesmidaceae–Pulvinula* is a strongly supported group composed of *Geopyxis*, *Tarzetta*, and three truffle-like species from the genera *Stephensia* and *Paurocotylis* (99%/100%/100%). Within the clade, only *Tarzetta* is monophyletic. *Geopyxis* is rendered paraphyletic by the nesting of *Stephensia bombycina* and *Paurocotylus pila* among the three members of *Geopyxis* sampled. *Stephensia shanorii* is

isolated on a subtending branch, rendering *Stephensia* paraphyletic. *Trappe (1979)* transferred both *Stephensia* and *Paurocotylis* to *Pyronemataceae*, and felt that *Stephensia* was derived from genera within *Korf's (1972)* tribe *Mycolachneae*, while *Paurocotylis* fit well within tribe *Aleuriaceae* due to the red pigmentation of the apothecia. Our analyses support the inclusion of these genera in *Pyronemataceae*, but do not agree with *Trappe's* hypotheses regarding their generic relationships. However, there are few morphological characters to indicate a close relationship between *Geopyxis* and either of these truffle-like genera. *Landvik et al. (1997)* suggested a possible relationship between *Paurocotylis* and *Glaziella*, which grouped together in their analyses along with other pyronemataceous genera including *Geopyxis* and *Tarzetta*. Our results agree with the findings of *Landvik et al. (1997)* regarding a close relationship of *Paurocotylis* to *Geopyxis* and *Tarzetta*, but they do not support a close relationship of this genus to *Glaziella* (Fig 1).

Although *Geopyxis* and *Tarzetta* are similar in morphology (i.e., often stipitate, deeply cupulate, ellipsoid spores, etc.) a close relationship between these genera has not been suggested in many of the previous classification schemes. *Le Gal (1947)* treated *Tarzetta* (as *Pustularia*) in *Aleuriaceae*, presumably due to the lack of carotenoid pigments and 'true' hairs, and *Geopyxis*, which contains carotenoids, in *Humariaceae*. *Arpin (1969)* and *Kimbrough (1970)* similarly treated *Tarzetta* (as *Pustularia* and *Pustulina* respectively) in *Otidea* *Eckblad emend.* *Arpin*, and *Geopyxis* in *Aleuriaceae sensu Arpin*. *Korf (1972)* also kept the genera separate, treating them in different subfamilies of *Pyronemataceae* due to the biguttulate ascospores of *Tarzetta*, and the non-guttulate ascospores of *Geopyxis*. Our analyses indicate a very close relationship between these genera, and highlight the limited phylogenetic value of characters such as carotenoids and guttules at higher taxonomic levels. The inclusion of *Paurocotylis pila* and *Stephensia* species in the clade suggests that the truffle-like form has arisen one or more times within this group.

Clade 3

This well-supported clade (97%/100%/100%) represents the apothecial genus *Pseudombrophila* and species from two cleistothecial genera, *Orbicula parietina* and *Lasiobolidium orbiculoides*. Both cleistothecial taxa have been treated in *Eotterfeziaceae (Benny & Kimbrough 1980; Malloch & Cain 1971)*, and *Theleboleae sensu Korf* of *Pyronemataceae (Jeng & Krug 1976)*. *Dennis (1978)* treated *Orbicula* in the *Eurotiaceae (Plectascales)*, while *Arx (1981)* treated the genus in the *Pezizales*. *Malloch (in Dissing & Schumacher 1994)* suggested that both *Lasiobolidium* *Malloch & Cain* and *Orbicula*, with their more pezizalean characteristics, might be more appropriately placed within *Pyronemataceae* or included in *Pezizales incertae sedis*. In the present analyses, as well as those of a previous study by the current authors (*Hansen et al. 2005b*), *O. parietina* is resolved as a sister taxon to *Pseudombrophila theioleuca*, with *L. orbiculoides* sister to the remainder of the *Pseudombrophila* lineage. Included here is the type species of *Lasiobolidium*, *L. spirale*, which falls out in *Pyronemataceae* quite distant from *L. orbiculoides* (clade 8). These results support the evolutionary origins of *Orbicula* and *Lasiobolidium* within *Pyronemataceae* and indicate that the cleistothecial form, with loss of active spore

discharge, has arisen at least once within the *Pseudombrophila* lineage.

Clade 4

This moderately supported clade represents the only assemblage of parasitic taxa identified to date within *Pyronemataceae*. Species of *Octospora*, *Neottiella*, and *Lamprospora* form obligate associations with numerous bryophytes, which have been interpreted as parasitic in nature (Benkert 1993; Döbbeler 1979). In a few instances, the apothecia of these fungi are borne directly upon their bryophyte hosts, while most species fruit on soil near the host and the relationship is therefore not directly apparent. The majority of species are associated with acrocarpous mosses, although a small number of taxa are also parasitic on liverworts. The group has a complex taxonomic history, and diverging concepts of the genera remain in use (Khare 2003; Yao & Spooner 1996b). Several authors recognize *Octospora*, *Neottiella*, and *Lamprospora* as distinct genera on the basis of differences in spore shape and ornamentation, excipular structure, and the presence or absence of excipular hairs (Benkert & Brouwer 2004; Dissing 2000; Korf & Zhuang 1991b), while others have synonymized *Neottiella* with *Octospora* (Dennis & Itzerott 1973; Yao & Spooner 1996b), *Lamprospora* with *Octospora* (Le Gal 1969), or both genera with *Octospora* (Cailliet & Moyne 1980) citing the lack of distinguishing features. Rifai (1968) and Eckblad (1968) treated *Neottiella* as a synonym of *Leucoscypha*, a non-bryophilous genus represented in this clade, but recognized *Lamprospora* and *Octospora* as distinct genera. Several non-bryophilous genera have been segregated from *Octospora sensu lato*. Rifai (1968) erected *Inermisia* (syn. *Byssonectria*) to accommodate *O. fuispora*, a taxon that differs from other *Octospora* species in the structure of the apothecial excipulum and margin, while Svrček (1969) erected *Kotlabaea* to accommodate *O. deformis*, differing in its smooth, eguttulate ascospores. In our analyses *Lamprospora* is isolated from both *Octospora* and *Neottiella*, supporting the recognition of this genus as distinct. Interestingly, *Moravecia hvaleri* is nested within the highly supported *Lamprospora* lineage. Benkert et al. (1987) introduced *Moravecia* to accommodate a single species, *O. calospora*, distinguished from *Octospora*, *Lamprospora* and *Neottiella* by eguttulate ascospores and a non-bryophilous habit. Khare (2003) suggested that this genus is closely related to *Aleuria* due to similarities in spore ornamentation. However, our results indicate that *Moravecia*, or at least *M. hvaleri*, represents an eguttulate, ellipsoid-spored member of *Lamprospora* that does not form apparent bryophilous relationships. The type species of *Moravecia*, *M. calospora*, is not included in our analyses, and any taxonomic decisions regarding the genus will have to await inclusion of this taxon.

Our results suggest that *Octospora* and *Neottiella* are not monophyletic. Several of the taxa sampled from these genera form a distinct moderately supported sub-clade (79%/77%/99%) sister to *Leucoscypha leucotricha*, *Rhodotarzetta rosea* and *Rhodoscypa ovilla*, while the remaining taxa are grouped as successive sister taxa to the *Lamprospora*–*Moravecia* lineage. The *Octospora* species forming this sub-clade, *O. cf. axillaris*, *O. leucoloma*, *O. rubens* and *Octospora* sp., are characterized by smooth ascospores, whereas those of the species grouping more closely to the *Lamprospora*–*Moravecia* lineage are ornamented. This result indicates that spore ornamentation is

likely a valuable taxonomic character within *Octospora*, and may provide a means by which to divide the genus into phylogenetically meaningful subgroupings or even distinct genera. *O. leucoloma*, designated the type of the genus (Korf 1954), is present in this sub-clade of smooth-spored species.

The placement of *Leucoscypha*, *Rhodotarzetta*, and *Rhodoscypa* among *Octospora* and *Neottiella* is somewhat surprising due to the non-bryophilous nature of these genera. However, *L. leucotricha*, has been reported to form endotrophic infections of the ectomycorrhizae of *Russulaceae* (*Basidiomycota*) on *Fagus sylvatica* (Brand 1990), indicating *Leucoscypha* species may be able to form other biotrophic associations. Khare & Tewari (1978) disregarded the taxonomic importance of the bryophilous habit in their treatment of *Octospora*, and more recently Khare (2003) has reported that several terrestrial *Octospora* species have been collected in the absence of any associated bryophytes. However, most authors treat the bryophilous habit as a distinguishing character of *Octospora*, *Neottiella*, and *Lamprospora* (Benkert, 1993; Dennis & Itzerott, 1973). Our results indicate that the ability or need to form a bryophilous association has either been gained once and lost twice within this clade (and family), or gained once and lost once. This clade represents a taxon-rich group, estimated at well over 100 species (Kirk et al., 2001). A more thorough sampling of taxa will be necessary to understand the evolutionary history and taxonomic implications of the bryophilous habit and to fully evaluate the disparate generic concepts currently in use for *Octospora*, *Lamprospora*, and *Neottiella*.

Several non-bryophilous genera previously recognized as close relatives of *Octospora*, *Neottiella*, and *Lamprospora*, are suggested to be distantly related. *Ramsbottomia* was erected by Buckley (1923) for a single species, morphologically similar to *Lamprospora*, but differing in the presence of hyphoid excipular hairs. Our analyses suggest that *Ramsbottomia* is more closely related to *Scutellinia* (see clade 7 below). *Kotlabaea* and *Byssonectria*, which were merged again with *Octospora* as subgenera by Khare & Tewari (1978), are resolved as closely related to *Scutellinia* and *Cheilymenia*, respectively (in clades 7 and 9).

Clade 5

This clade includes three species of *Sowerbyella*, and supports the transfer of *Aleuria rhenana* to this genus by Moravec (1986). As pointed out by Moravec, this taxon shares many characters with taxa of *Sowerbyella*, including stipitate apothecia, reticulate, non-apiculate ascospores, long excipular hairs, and hooked paraphyses. Moravec (1985, 1988) indicated that *Sowerbyella* and the genera of tribe *Sowerbyelleae* Le Gal emend. Korf should not be placed in the subfamily *Scutellinioideae* Clements emend. Korf, and suggested that the tribe fit more naturally in subfamily *Otideoideae* Seaver emend. Korf. The placement of *Sowerbyella* is weakly supported (<50% for all methods), and the position of the genus within the family remains uncertain.

Clade 6

The generic relationships of *Pyronema* are not resolved in our analyses. Although more than 55 combinations have been proposed in the genus, the work of Moore & Korf (1963) indicated that only two species, *P. omphalodes* and *P. domesticum*, are common and widely distributed. Kimbrough (1989) restricted the limits of *Pyronemataceae* to include only *Pyronema*

and *Coprotus*, and placed the family along with *Ascodesmidaceae* in the new suborder *Pyronemineae*. Brummelen (1994) suggested that *Coprotus*, along with several other coprophilous genera, be transferred to *Thelebolaceae*. However, in a later study Brummelen (1998) determined, using TEM, that *C. lacteus* has the *Octospora*-type ascus, characteristic of *Pyronemataceae* in the broad sense. *Coprotus* is currently treated with *Thelebolus* and other coprophilous genera in *Thelebolaceae* of the order *Thelebolales* (Eriksson 2006). Unfortunately, we were unable to obtain material of *Coprotus* to include in our analyses to determine the relationship of this genus to *Pyronema* and the remainder of *Pezizales*.

Clade 7

This clade represents the largest assemblage of species that is present in all trees. With the exception of *Miladina* and *Kotlabaea*, all the taxa represented in this clade are characterized by the presence of excipular hairs, which typically have some degree of brown pigmentation. This character is not unique to the taxa of this clade, however, but rather is present in numerous genera throughout the family. Within this large assemblage three sub-clades are resolved. In the first of these, both *Anthracobia* and *Trichophaea* are resolved as non-monophyletic. *Anthracobia subatra* is isolated from the remaining *Anthracobia* species sampled, on a long branch as part of a sister group composed of *Trichophaea* species. As indicated by Yao *et al.* (1998), *A. subatra* is unique within the genus due to the very dark brown to black hymenium, but otherwise fits quite well based on other characters. Korf (1972) suggested a possible relationship between *Anthracobia* and *Trichophaea* (including *Sphaerosporella*) due to the pyrophilous habit shared by species of both genera. Although our results support this relationship, the boundaries between these genera remain unclear.

In addition to *A. subatra* grouping within *Trichophaea*, *Sphaerosporella brunnea* is nested within the genus, and several additional *Trichophaea* species are resolved distantly in clade 8. The placement of *Sphaerosporella* among species of *Trichophaea* is not unexpected, as many authors have treated the species of this genus as globose-spored members of *Trichophaea* (Hennebert 1973; Korf 1972, 1973; Larsen 1980). Wu & Kimbrough (1994) determined that the ultrastructure of spore ontogeny in *S. brunnea* (as *T. brunnea*) is the same as that observed in *T. abundans* and *T. woolhopeia*, and concluded that the presence of globose spores and minor excipular differences are not sufficient to warrant the recognition of *Sphaerosporella* as a separate genus. Based on the ultrastructure of spore ontogeny in additional species of *Trichophaea*, Wu & Kimbrough (1996) also suggested that the taxa currently treated in the genus are not congeneric, and that the taxonomic limits of *Trichophaea* need to be re-examined. These authors concluded that the smooth-spored species examined differed significantly in spore ontogeny from the rough-spored *T. paludosa*. Wu & Kimbrough (1996) pointed out that the smooth-spored species all share a pyrophilous habit and form *Dichobotrys* anamorphic states, whereas the rough-spored species are not pyrophilic and are not known to form an anamorph. Our analyses support these findings. All the *Trichophaea* species contained in the current clade, including *Sphaerosporella*, have smooth spores and most produce a *Dichobotrys* anamorph (*T. abundans*, *T. saccata*, *T. minuta*, *S. brunnea*), whereas the species

grouping in clade 8 (*T. hybrida* and *T. hemisphaerioides*) have verruculose spores and are not known to produce anamorphic states. However, the pyrophilous habit does not follow a similar pattern. Both smooth and rough-spored taxa occur on burned substrates. *T. woolhopeia*, the type of the genus, is the only *Trichophaea* species in the current clade for which an anamorphic state has not been reported. It should be noted that a *Dichobotrys* anamorph has also been reported for *Pyropyxis rubra* (Egger 1984), resolved in clade 12. The inclusion of the type of *Trichophaea* within this clade suggests that the species with ornamented spores will need to be segregated into a separate genus.

The remaining taxa of clade 7 are resolved in two additional sub-clades, which together form the sister group to *Anthracobia* and *Trichophaea*. The first of these sub-clades is composed of brightly pigmented taxa, primarily *Scutellinia* species, and members of *Cheilymenia*, *Ramsbottomia*, *Miladina* and *Kotlabaea*. With the exception of *S. cf. erinaceous*, the species of *Scutellinia* sampled form a monophyletic group. *S. cf. erinaceous* is isolated on a subtending branch, separated from the remaining species of the genus by *Cheilymenia fimicola* and *Kotlabaea deformis*. However, the support for these branches is weak, and both *S. cf. erinaceous* and *K. deformis* tend to shift positions in the parsimony and Bayesian topologies. The close relationship between *C. fimicola* and *Scutellinia* resolved in our analyses is not unexpected, as many investigators have considered these genera closely related (Le Gal 1953). However, the remaining *Cheilymenia* taxa sampled are resolved quite distantly from *Scutellinia* (clade 9).

Aside from the yellow to orange colouration of the apothecia, there are few morphological characteristics to explain the resolution of *Kotlabaea deformis* in this clade. *Kotlabaea* has traditionally been treated as closely related to the bryophilous genus *Octospora* due to morphological similarities. Species of *Ramsbottomia*, which are also resolved in this clade, similarly have been placed in *Lamprospora* (Seaver 1928) or been considered closely related to the other bryophilous genera (Buckley 1923). However, neither *Kotlabaea* nor *Ramsbottomia* are known to form bryophilous associations, and their exclusion from the primarily bryophilous clade suggests that the presence or absence of a bryophilous habit may be taxonomically informative. *Ramsbottomia* does share some morphological characters with genera of the current clade, including yellow to orange colouration, brown hyphoid hairs on the outer excipulum and apothecial margin, and often spherical, ornamented ascospores with numerous guttules, a condition also present in the spores of *Scutellinia*. Yao & Spooner (1995a) stated that the delimitation of *Ramsbottomia* and *Lamprospora* requires further investigation, and Rifai (1968) tentatively listed the genus as a synonym of *Lamprospora*. Our analyses suggest that the presence of brown excipular and marginal hairs in *Ramsbottomia*, as well as a one-layered excipulum and ascospores with multiple guttules, are sufficient to delimit this genus from *Lamprospora* as well as *Octospora* and *Neottiella*.

In addition to the yellow to orange colouration of the apothecia, the excipular structure in *Miladina* is similar to that observed in species of *Scutellinia* (i.e. medullary excipulum of *textura intricata*, ectal excipulum of *t. angularis* to *t. globulosa*). The spores of *Miladina*, which are marked with small, occasionally anastomosing warts and are single to multi-guttulate or

spumose, and the habit of fruiting on wood, are also similar to those observed in species of *Scutellinia*. The main difference between these genera is the lack of excipular hairs in *Miladina* and the aquatic habitat of this genus. Yao & Spooner (1995b) reported the presence of hyaline, thin-walled obtuse hairs on the excipulum of several collections of *Miladina* collected in the UK, but these are quite distinct from those present in *Scutellinia*, which are rooting, septate, dark pigmented and pointed.

The final, weakly supported clade in this large assemblage is composed of species of *Geopora* and *Tricharina*, as well as '*Pustularia patavina*' and an unidentified ectomycorrhizal root isolate (*Pezizales* sp. B). The *Geopora* species sampled form a well-supported (96%/95%/100%) group that also includes two species of *Tricharina*, *T. ochroleuca* and an undetermined *Tricharina* species, and the mycorrhizal isolate. In the ML analyses (Fig 1), *G. pellita* is isolated from the other *Geopora* species, but support values are quite low for these branches and *Geopora* is resolved as monophyletic in the parsimony and Bayesian analyses (not shown). The inclusion of the mycorrhizal isolate within *Geopora* in our analyses agrees with previous molecular investigations confirming the status of the genus as ectomycorrhizal (Fujimura et al. 2005; Gehring et al. 1998; Izzo et al. 2005; Tedersoo et al. 2006). Korf (1972) indicated that *Geopora* appears closely related to *Humaria*, differing mainly in the presence of smooth ascospores and flexous hairs, and by large, deeply cupulate apothecia. In our analyses *H. hemisphaerica* is quite distant, being closely related to *Genea* (clade 8). The closest taxa to *Geopora* in our analyses are species of *Tricharina*.

The placement of *Tricharina* in this clade in the absence of *Wilcoxina* is unexpected (see clade 8). Furthermore, *Tricharina* is not resolved as monophyletic. Two species are nested in the well-supported sub-clade with *Geopora*, while the remaining taxa sampled are in various weakly supported positions subtending this group along with '*Pustularia patavina*' and an undescribed taxon designated *Pyronemataceae* sp. B. '*Pustularia patavina*' has been considered to belong in *Leucoscypha* (Pant & Tewari 1977; Svrček 1974), and more recently has been keyed out with species of *Tarsetta* (Dissing 2000). *Pustularia* (syn. *Pustulina*) represents a synonym of *Tarsetta*, but the name '*P. patavina*' was never formally combined in the genus. Pant & Tewari (1970) investigated the type material of this species and concluded that it does not belong in the genus (*Pustulina*). The apothecia of *Tarsetta* are cup to urn-shaped with an outer surface that is delicately scurfy to tomentose. '*Pustularia patavina*' is characterized by bi-guttulate spores, and hyaline hairs that cover the outer surface of the apothecia. Our results do not support the treatment of this taxon as a species of *Leucoscypha*, and agree with the conclusion of Yao & Spooner (2002) that the generic placement of this taxon requires further investigation.

Clade 8

This clade contains the largest group of hypogeous taxa resolved in our analyses, as well as another large assemblage of epigeous taxa that are characterized by the presence of excipular hairs. The hypogeous genera *Genea*, *Genabea*, and *Gilkeya* are resolved in a weakly supported sub-clade with the epigeous *Humaria hemisphaerica* and two ectomycorrhizal root isolate sequences (*H. hemisphaerica* EcM and *Genea hispidula* EcM, Tedersoo et al. 2006). Several authors have treated *Genabea* as a synonym of *Genea* (Korf 1973; Pfister 1984; Zhang 1991),

while others have recognized them as distinct, closely related genera (Castellano et al. 1989; Gilkey 1954a,b, 1961; Montecchi & Sarasini 2000; Pegler et al. 1993). Trappe (1979) transferred *Genea* and *Genabea* to the *Pezizales* as part of his abandonment of *Tuberales*, but retained the genera in the hypogeous family *Geneaceae* because he felt the relationships of *Genea* to other *Pezizales* had not been adequately established. Pfister (1984), recognizing similarities in the excipular construction, pigmentation, and ascospore ornamentation of *Genea* and the epigeous genus *Jafneadelphus* (syn. *Aleurina*), abandoned *Geneaceae* and treated *Genea* (including *Genabea*) in *Pyronemataceae*. Similarly, Li & Kimbrough (1994) studied the ascospore ontogeny and septal pore ultrastructure of *Genea gardnerii* and concluded that the genus is related to epigeous members of *Otidea* (syn. *Pyronemataceae*). Pfister (1984) also noted that some of the tomentose members of *Genea* appeared anatomically more similar to *Humaria* than to *Jafneadelphus*, and Li & Kimbrough (1994) found that the ascospore ontogeny of *G. gardnerii* was very similar to that observed in *Humaria hemisphaerica* (as *Mycolachnea hemisphaerica*). In our analyses *Genea* and *Genabea* are resolved as distinct genera, with the species of *Genea* sampled forming a well-supported (94%/100%/100%) monophyletic group. *Humaria hemisphaerica* is highly supported (100%/100%/100%) as the epigeous sister group to *Genea*, confirming the observations of Li & Kimbrough (1994) and Pfister (1984). However, our results do not support a close relationship of *Genea* and *Aleurina* as suggested by Pfister (1984). Although potentially an artefact of our sampling, the placement of *H. hemisphaerica* within this otherwise hypogeous lineage suggests that the epigeous habit of this taxon may be a secondarily derived condition. The resolution of two EcM root isolates within this clade agrees with recent molecular investigations confirming the status of *Genea* (Smith et al. 2006; Tedersoo et al. 2006) and *Humaria* (Tedersoo et al. 2006) as ectomycorrhizal associates. The ectomycorrhizal status of *Genabea cerebriformis* has also been confirmed by sequencing of ITS from colonized root tips (Izzo et al. 2005).

Smith et al. (2006) recently described the genus *Gilkeya* to accommodate a single species, *G. compacta*, previously treated in *Genea* as *G. intermedia*. The erection of this segregate genus is based upon morphological differences, namely globose ascospores, vinaceous colouration of the peridium, and the lack of a basal mycelial tuft in *Gilkeya*, as well as the results of phylogenetic analyses of nuLSU sequence data (Smith et al. 2006). Our results agree with those of Smith et al. (2006), resolving *Gilkeya* outside the monophyletic group of *Genea* species sampled and further indicate, albeit weakly, a sister relationship of *Gilkeya* to the *Genea*–*Humaria* lineage.

The cleistothecial genus *Lasiobolium* is not monophyletic in our analyses. *Lasiobolium spirale*, the type of the genus, is a member of the current clade, whereas *L. orbiculoides* is resolved as sister to the *Pseudombrophila*–*Orbicula* lineage (clade 3). *L. orbiculoides* differs from *L. spirale* in producing wavy to irregular ascocarp appendages and oblate ascospores that are uniseriate in cylindrical asci (Malloch & Benny 1973). *L. spirale* produces distinctly coiled appendages and ellipsoid spores born loosely in clavate asci (Malloch & Cain 1971). With the exception of *L. aegypticum* Moustafa & Ezz-Eldin, which is reported to be morphologically intermediate between these two taxa (Moustafa & Ezz-Eldin 1989), the remaining species

recognized in the genus are characterized by the presence of clavate asci and ellipsoid ascospores, and thus appear morphologically to be more closely related to the type species. Interestingly, the sister taxon to *L. spirale* in our analyses is an undetermined species with excipular structure similar to that of *Trichophaeopsis*. Two species of *Trichophaeopsis*, the type species *T. bicuspis*, and *T. tetraspora*, are resolved in this clade, but form a weakly supported sister group to the remaining taxa.

The rough-spored species of *Trichophaea* nested within this clade do not form a monophyletic group. *T. hybrida*, which as sampled here may represent a species complex (Dissing 2000), forms a highly supported group with *Wilcoxina*, whereas *T. hemisphaerioides* falls out separately on a branch subtending *Lasiobolium*, *Humaria*, *Genea*, and *Genabea*. The smooth-spored species of *Trichophaea* are resolved in clade 7 (see discussion above).

The species of *Wilcoxina* included in our analyses are resolved as a monophyletic group, and unexpectedly, are not closely related to *Tricharina*. *Wilcoxina* was erected by Yang & Korf (1985a) to accommodate *T. mikolae* and several additional species, which differ from *Tricharina* in the arrangement of the excipular hairs, excipulum structure, proportion of the asci occupied by spores, mode of ascospore germination, and the presence of anamorphs referable to *Complexipes* Walker. *Tricharina* is characterized by the production of an *Ascorhizoctonia* anamorph. However, *Tricharina* and *Wilcoxina* are morphologically similar, and have been considered to form a complex of closely related taxa (Egger 1996; Yang & Korf 1985b). Egger (1996) investigated the phylogenetic relationships between these genera using nu-rDNA (ITS1 and partial 18S and 28S), and concluded that although related, they should be maintained as separate genera. In Egger's (1996) analyses no other pyronemataceous taxa were included in the ingroup, and therefore the relationships of *Tricharina* and *Wilcoxina* to other members of the family were not assessed.

Yang & Wilcox (1984) determined that *Wilcoxina mikolae* (as *T. mikolae*) is one of the fungal species responsible for forming the ectendomycorrhizal, or E-strain, infections first reported from pine roots by Mikola (1966) and later from other genera of *Pinaceae* (Laiho 1966). Before the true identity of this fungus was known, Wilcox et al. (1974) observed that the E-strain fungus described by Mikola (1966) produces characteristic chlamyospores, and Walker (1979) erected the genus *Complexipes*, tentatively placed in *Endogonaceae* (*Zygomycota*), to accommodate the species as *C. moniliformis* C. Walker. Based on studies of the hyphae and septa, Danielson (1982) determined that *C. moniliformis* is an ascomycetous anamorph and suggested that a teleomorph should be sought among species of *Geopora*, *Trichophaea*, *Sphaerosporella* and *Humaria*. Although both *S. brunnea* and *Geopora* have been demonstrated to form ectendomycorrhizae (Egger & Paden 1986; Fujimura et al. 2005), the former taxon is characterized by producing a *Dichobotrys* rather than a *Complexipes* anamorph (Hennebert 1973), and neither of these genera are closely related to *Wilcoxina* in our analyses. *Trichophaea hybrida*, recently identified as a mycorrhizal associate (Teder-soo et al., 2006), forms a sister group to *Wilcoxina*. Interestingly *T. hybrida*, along with *Geopora* and *Wilcoxina* spp., have been observed to occasionally colonize the cortical cells of *Pinus* species in the analyses of Teder-soo et al. (2006), suggesting that this species may also form

ectendomycorrhiza. The other *Trichophaea* species present in this clade, *T. hemisphaerioides*, was shown by Egger & Paden (1986) to penetrate the cortex cells of *Pinus contorta* in monoxenic culture experiments, and was interpreted by these authors to be potentially mutualistic under some conditions.

Parascutellinia carneosanguinea is also nested in the current clade, subtending the lineage composed of *Humaria* and the hypogeous genera. Superficially this genus is similar to the other epigeous apothecial taxa of this clade in the presence of stiff, brown, multi-septate excipular hairs, but differs in the presence of a distinctly red to red-blue hymenium (Dissing 1982). Unfortunately, we were unable to obtain material of the type species, *P. violacea*, for inclusion in our analyses.

Subtending this clade are several taxa that tend to shift position in the parsimony and Bayesian topologies. Of these, three collections forming a sister group to *Paratrachophaea boudieri* represent an undescribed genus and species based upon our morphological observations and molecular results, and have been addressed in a separate publication (Perry & Pfister 2007). *Melastiza contorta* and *Pseudaleuria quinaultiana* are moderately supported as sister species. The monotypic *Pseudaleuria* appears to be a rare genus, endemic to Oregon and Washington, and known only from a few localities (Castellano et al. 1999; Lusk 1987). As the name implies, *Pseudaleuria* was considered to be most closely related to *Aleuria* (Lusk 1987), differing primarily in the presence of interwoven, hyphoid excipular hairs and smooth ascospores. Although *Pseudaleuria* is not resolved with species of *Aleuria* in our analyses, the genus is sister to *Melastiza contorta*. *Melastiza* has been treated as a synonym of *Aleuria* by Moravec (1994). Unfortunately, the nuLSU data do not resolve the relationships of *Aleuria* or *Melastiza*, with both genera tending to occupy short, unsupported branches (see clade 10).

Clade 9

Our results do not support *Cheilymenia* as monophyletic (see *C. fimicola* in clade 7). The *Cheilymenia* species resolved in the current clade are part of a weakly supported polytomy with species of *Coprobia* and *Byssonectria*, as well as *Spooneromyces laeticolor* and *Humaria velenovskyi*. In his treatment of *Cheilymenia* from North America, Denison (1964) discussed the high degree of variation that exists among species of the genus in regard to features such as excipulum structure, ascospore ornamentation, and the shape and origin of excipular hairs. Denison (1964) recognized three distinct subgroups within the genus, two of which he felt had affinities with *Scutellinia* and *Coprobia*, but chose not to recognize these as infrageneric taxa due to the then small size of the genus. Rifai (1968) recognized the infrageneric groupings identified by Denison (1964), but refrained from giving them formal status pending a more thorough understanding of the specific characters of all *Cheilymenia* species. Moravec (1989) expressed the opinion that *Cheilymenia* represents a complex of several distinct genera, but later (Moravec 1990) chose to treat the group as a single genus (including *Coprobia*) due to a large number of intermediate forms, which he felt inhibited delimitation into smaller generic groupings. Moravec (1987) proposed the transfer of *C. theleboides* to *Coprobia* due to the superficial, hyphoid excipular hairs in this taxon, which are also characteristic of *Coprobia*, but chose to merge these genera in his later treatment of

Cheilymenia (1990). Our results support a close relationship of *Cheilymenia* species to *Coprobia*, and of *C. fimicola* to *Scutellinia*, and suggest that the infrageneric relationships within *Cheilymenia*, and the generic boundaries between these genera, are in need of further investigation. Although our sampling of the genus is limited, our results do not support the recognition of infrageneric groups within *Cheilymenia* based upon the presence of specific excipulum, hair or ascospore characteristics.

Humaria velenovskyi and *Spooneromyces laeticolor* are also resolved as sister taxa (PP 99 %) in this clade. *Spooneromyces* was erected by Schumacher & Moravec (1989) to accommodate *Peziza laeticolor*, which had previously been described as a new taxon, *Melastiza asperula* (Spooner 1981). Schumacher (1988), realizing the synonymy of *M. asperula*, recombined this taxon under the older name *M. laeticolor*. Due to the stiff, multiseptate, superficial hairs characteristic of this species, however, Schumacher & Moravec (1989) felt it did not fit well within *Melastiza* or any other existing genera of *Pyronemataceae*, and warranted a new genus. These authors noted the similarity of *Spooneromyces* to *Scutellinia* and *Cheilymenia*, and placed the genus in tribe *Scutellinieae* of Korf (1972). Like *Spooneromyces*, *Humaria velenovskyi* is characterized by stiff, multi-septate, superficial hairs, warted ascospores and a brightly pigmented hymenium due to the presence of carotenoids. *Humaria hemisphaerica*, the other *Humaria* species sampled here, is resolved quite distantly as part of the *Genea* lineage (clade 8). Although we have not had the opportunity to examine the holotype of *H. velenovskyi*, our morphological observations of the material sampled in this study agree well with the description of the taxon given by Korf & Sagara (1972), and indicate that the species is congeneric with *Spooneromyces*. A formal proposal to transfer this species will have to await confirmation of our findings by examination of additional specimens and comparison with the type material. The results of our molecular and morphological analyses also suggest a close relationship between *Spooneromyces* and *Cheilymenia* as indicated by Schumacher & Moravec (1989). Ecologically, the majority of taxa included in this clade are similar in their occurrence on dung, urinophilic habitats, and manured or otherwise enriched soils.

Clade 10

In this clade the species of *Aleuria* sampled, *A. aurantia* and *A. bicucullata*, are monophyletic and sister to two specimens of *Melastiza cornubiensis*. A close relationship between *Aleuria* and *Melastiza* has been indicated by many investigators (Le Gal 1963) due to similar ascospore ornamentation, excipulum structure, colouration, and habit. The main character distinguishing these genera is the presence of appressed, pale to dark brown excipular hairs in *Melastiza*. Moravec (1994) felt that the presence of such hairs did not warrant generic segregation, and treated *Melastiza* as a subgenus of *Aleuria*. Although our results do indicate a close relationship between *Aleuria* and *M. cornubiensis*, *M. contorta* is resolved as the sister taxon to *Pseudaleuria quinaultiana* (clade 8), and *M. flavorubens* is isolated on a branch subtending clades 8, 9 and 10. However, the nuLSU data do not provide enough information to resolve the relationships among these genera with confidence; the branches separating these taxa are quite short, weakly supported, and collapse in the strict consensus of the parsimony and Bayesian trees.

Clade 11

This moderately supported clade (81%/87%/100%) represents the morphologically distinct genus *Otidea*, characterized by large, typically ear-shaped apothecia. The majority of *Otidea* species sampled form a well-supported sub-clade (100%/100%/100%), isolated from the single remaining species, *O. alutacea*, possibly due to our limited sampling of the genus. Although Kirk et al. (2001) estimate 15 species in the genus, Dissing (2000) indicates a minimum of 15 species occurring in the Nordic countries alone, and Kanouse (1949) described an additional four species from the United States. Of particular interest in future analyses is the inclusion of two species segregated from *Otidea* into the new genus *Flavoscypha* Harmaja (1974) on the basis of unique excipular structure, bright yellow colouration and other morphological characters.

Nannfeldt (1937, 1938, 1966) suggested a close relationship between *Otidea*, *Tarzettia* (as *Pustularia*), and *Helvella*, stating that these genera form a natural group (*Pezizaceae* tribe *Acetabuleae* sensu Nannfeldt). Eckblad (1968) felt that the helvelloid-morchelloid families (*Helvellaceae*, *Morchellaceae* and *Rhiziniaceae* of lineage B) represent evolutionarily advanced groups polyphyletically derived from pezizoid ancestors, and stated that *Otidea*, *Tarzettia*, and *Sowerbyella* (treated in *Otideaaceae*) may well be derived from the same origin due to the similar nature of the excipulum, spores and paraphyses. The idea of a close relationship between *Otidea* and *Helvella* is no longer entertained, and molecular phylogenetic studies (Fig 1) (Harrington et al. 1999; Landvik et al. 1997), do not support a common origin of the helvelloid-morchelloid families and *Pyronemataceae*. Our analyses do not support a close relationship between *Otidea* and either *Tarzettia* or *Sowerbyella*.

Clade 12

The placement of *Pyropyxis rubra* in this well-supported clade rather than close to *Geopyxis* (clade 2) is unexpected. Egger (1984) described *Pyropyxis* based upon the type material of *Peziza rubra*, and personal collections made from burn sites in eastern Ontario, tentatively assigned to *Geopyxis*. Pfister (1979) had previously investigated the type material of *P. rubra* and synonymized the taxon with *G. carbonaria*. Egger distinguished *Pyropyxis* from *Geopyxis* on the basis of differences in spore guttulation, pigment distribution, and the presence of moniliform excipular hairs and a *Dichobotrys* anamorph in *Pyropyxis*. These two taxa are very similar in their smooth, ellipsoid ascospores, excipulum structure and pyrophilous habit (Egger 1984). Egger tentatively placed *Pyropyxis* in tribe *Aleurieae* sensu Korf (1972, 1973), but indicated a close relationship with *Geopyxis* and potentially *Pulvinula*, *Aleuria*, and *Rhodotarzettia*. Our results do not support a close relationship of *Pyropyxis* with any of these genera. Morphologically and ecologically, however, there is little to support the grouping of *Pyropyxis* with the other genera represented in clade 12. *Smardaea* is characterized by the presence of purple pigments in all parts of the ascomata, including the ascospore walls, and most species of *Smardaea*, and all *Jafnea*, are characterized by highly ornamented ascospores.

Clade 13

This small, moderately supported clade (81%/88%/100%) includes a single species of *Aleurina*, and representative taxa

from two provisional genera, *Unicava* Trappe *gen. ined.* and *Gelinipes* Trappe *gen. ined.*, both based on Australian collections (James M. Trappe, pers. com.). Although the relationships of this clade may be a result of long-branch attraction (Felsenstein 1978; Hendy & Penny 1989), analyses excluding either *Gelinipes* or *A. imaii* resulted in well-supported relationships between the remaining taxon and the *Unicava* sp. (data not shown). Morphologically, there is little to explain the grouping of *A. imaii* with *Gelinipes* and *Unicava*. *Aleurina* forms epigeous, sessile, discoid apothecia, whereas *Gelinipes* forms hypogeous ascomata characterized by a thick, gelatinous base and exposed hymenium, and *Unicava* forms hypogeous truffle-like ascomata with a hollow interior, which produce a small opening at maturity (James M. Trappe, pers. comm.).

Clade 14

The species of *Acervus* sampled form a well-supported (99%/98%/100%) monophyletic group. The genus is characterized by asci and ascospores that are quite small for Pezizales. The type species was originally thought to be inoperculate and was treated in *Dermateaceae* (Kanouse 1938) and *Helotiaceae* (Seaver 1942). Korf (1963), in his investigation of the holotype material concluded that the asci were suboperculate and placed the genus in *Sarcoscyphaceae*, but later (1973) reconsidered this position, and moved *Acervus* to tribe *Sowerbyelleae* of *Pyronemataceae*. Pfister (1975) emended the genus to include *Phaedropezia*, and indicated that the placement of the genus in the Pezizales remained problematic. Le Gal (1969) treated *Acervus* (as *Phaedropezia*) in tribe *Sowerbyelleae* of *Pyronemataceae* along with *Sowerbyella* and *Caloscypha*, believing that *Acervus* and *Sowerbyella* would be shown to have the same type of carotenoids as those found in *Caloscypha* by Arpin (1969). Kimbrough & Curry (1986a) investigated the septal structures of *Acervus epispertius* and *Caloscypha fulgens*, and indicated that these taxa had septa very similar to those they had previously observed in *Aleuria* (Kimbrough & Curry 1986b). Although our analyses support the placement of *Acervus* within *Pyronemataceae*, they do not resolve the genus as closely related to either *Sowerbyella* or *Aleuria*. *Caloscypha*, recently made the type of the monotypic family *Caloscyphaceae* (Harmaja 2002), occupies an isolated position within lineage B (Fig 1) (Landvik et al. 1997).

Classification of Pyronemataceae

The long and complex taxonomic history of the taxa currently treated in *Pyronemataceae* is reflected in the variable concepts of the family put forth by numerous investigators. The previous classifications of these taxa into one or several families have relied upon morphological, microchemical, ultrastructural, and developmental characteristics of the species and genera. In particular, the presence or absence of carotenoid pigments and excipular hairs, spore ornamentation, and excipulum structure have played a large role in the delimitation of taxonomic units at various levels in many of the earlier classifications. More recently, ultrastructural studies, especially those of the asci and septal pore apparatus, as well as developmental analyses, have been used to delimit suborders and families and propose a restricted concept of *Pyronemataceae* (Kimbrough 1989). Our analyses, as well as those of Landvik et al. (1997), do not support the segregation of carotenoid

containing taxa into a separate family in the sense of Arpin's *Aleuraceae* (1969). Our results indicate that carotenoids occur in many distantly related taxa throughout *Pyronemataceae*, as well as *Sarcoscyphaceae* in lineage C, and *Caloscypha fulgens*, resolved in lineage B. Likewise, excipular hairs, spore ornamentation, spore guttulation and excipulum structure are also phylogenetically uninformative at higher taxonomic levels based on our analyses. Although these characters are undoubtedly valuable at the generic level and below, their use in the delimitation of families, subfamilies, or tribes (Le Gal 1947; Rifai 1968; Eckblad 1968; Korf 1972; Arpin 1969; Dennis 1978; Kimbrough 1970) is not supported.

Pyronema is nested within a large assemblage of species representing *Otideaceae*, *Humariaceae*, *Aleuraceae*, and *Pyronemataceae* of previous investigators, as well as *Ascodesmidaceae*. Although we were unable to include *Coprotus* in our analyses, our results suggest that the characters used by Kimbrough (1989) to segregate this genus and *Pyronema* into a restricted *Pyronemataceae* are not phylogenetically meaningful at this level. Our analyses do not support Korf's (1972) subdivision of *Pyronemataceae* into subfamilies and tribes, but they do agree with a broader concept of the family proposed by Korf (1972) and Eckblad (1968), and adopted in the most recent 'Outline of Ascomycota' (Eriksson 2006). In the analyses of Landvik et al. (1997) *Glaziella* is nested within *Pyronemataceae* among taxa corresponding to clade 2 of this investigation. In our ML analyses *Glaziella* is isolated on a branch subtending *Pyronemataceae*, and is resolved as sister to *Psilopezia* and other members of the C lineage in our parsimony and Bayesian analyses (not shown). However, the branches resolving *Glaziella* are weakly supported in our analyses, and we are unable to reject constraint topologies forcing *Glaziellaceae* to group within *Pyronemataceae*. Until the relationships of *Glaziella* can be resolved with more confidence, we feel this genus should be retained in a separate, monotypic *Glaziellaceae*.

Ascocarp evolution

Our analyses indicate that cleistothecial ascomata have arisen independently at least three times within *Pyronemataceae*. Cain (1956a,b, 1961) and Malloch (1979, 1981) were among the first investigators to suggest that certain cleistothecial forms are derived from apothecial and perithecial ancestors. Malloch (1981) hypothesized the independent derivation of cleistothecial ascomata in multiple ascomycete families as an evolutionary trend of 'increasing simplification'. The loss of a distal opening in these ascomata appears to be followed by the loss of forcibly discharged ascospores, and the loss of a distinct hymenial layer (Malloch 1981). The cleistothecial species *Orbicula parietina* and *Lasiobolidium orbiculoides* are members of the apothecial *Pseudombrophila* lineage (clade 2). Two additional cleistothecial taxa, *Warcupia terrestris* and *Lasiobolidium spirale*, are also resolved in *Pyronemataceae*, subtending clade 12 and nested within clade 8, respectively.

A similar situation to that of the cleistothecial ascomata is the evolution of multiple hypogeous to subhypogeous (truffle or truffle-like) forms within *Pezizales*. These fungi are also characterized by closed ascomata, and with the exception of *Geopora cooperi*, the loss of forcible spore discharge. Morphological and molecular evidence suggests that these truffle

and truffle-like forms have evolved multiple times independently within Pezizales (Burdall 1968; Hansen et al 2001; Landvik et al. 1997; O'Donnell et al. 1997; Percudani et al. 1999; Trappe 1979 (Fig 1), supporting the hypothesis that the ascomycetous truffles are all derived from epigeous, apothecial ancestors (Trappe 1979). In Pyronemataceae, our results suggest that the truffle or truffle-like form has arisen at least five times independently, within clades 2, 7, 8 and 13.

In conclusion, this study makes a major contribution toward understanding the evolutionary relationships of Pyronemataceae, and gives direction for, or clarifies, the delimitations of many genera in the family. For the first time a close relationship between the truffle genus *Stephensia* and apothecial species of *Geopyxis* is suggested. Likewise, a close relationship of these taxa to the truffle-like *Paurocotylis* and the apothecial *Tarzetta*, is strongly indicated. Several species are suggested to be taxonomically misplaced, and should be treated in other genera; the ellipsoid-spored *Pulvinula ovalispora* belongs in *Boubovia*, *Aleuria rhenana* in *Sowerbyella*, *Humaria velenovskyi* in *Spooneromyces*, and *Moravecia hvaleri* in *Lamprospora*. The tropical, monotypic genus *Lazuardia* appears to be a distinct taxon within Pyronemataceae. *Trichophaea* should be restricted to include only smooth-spored species, most of which are known to produce *Dichobotrys* anamorphic states, and the rough-spored *Trichophaea* species that lack *Dichobotrys* anamorphs should be segregated into a separate genus. *Sphaerosporella* should be included in *Trichophaea* s. str. *Wilcoxina* and *Tricharina*, long considered sister genera based on morphology, are resolved for the first time as distantly related. Similarly, *Pyropyxis* and *Geopyxis*, also thought to be closely related or congeneric, are indicated as distantly related. *Octospora* and other bryophilous taxa are restricted to a single, well-supported lineage that appears quite divergent within Pyronemataceae. The non-bryophilous genera *Leucoscypha*, *Rhodotarzetta* and *Rhodoscypha* are closely related and nested within the bryophilous lineage, while other non-bryophilous genera (*Ramsbottomia*, *Kotlabea* and *Byssonectria*), thought to be closely related to the bryophilous taxa, appear distantly related.

The 14 clades resolved within Pyronemataceae in all analyses provide a framework from which we can begin to evaluate the evolutionary patterns and taxonomic characters within this family. Unfortunately, the nuLSU data do not provide the phylogenetic information necessary to reconstruct the evolutionary history of this group with significant support. To propose a phylogenetic classification for Pyronemataceae, multiple gene analyses are likely necessary to resolve the deeper nodes of the family and order, and are currently being undertaken by the authors. These analyses, coupled with renewed studies addressing such topics as morphological, microchemical, ultrastructural, and developmental characteristics, and especially the trophic strategies of the species and genera of Pyronemataceae, will provide information to further understand the evolutionary history of these fungi and the larger role they play in ecological communities.

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