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A phylogeny of the highly diverse cup-fungus family Pyronemataceae (Pezizomycetes, Ascomycota) clarifies relationships and evolution of selected life history traits

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ABSTRACT

Pyronemataceae is the largest and most heterogeneous family of Pezizomycetes. It is morphologically and ecologically highly diverse, comprising saprobic, ectomycorrhizal, bryosymbiotic and parasitic species, occurring in a broad range of habitats (on soil, burnt ground, debris, wood, dung and inside living bryophytes, plants and lichens). To assess the monophyly of Pyronemataceae and provide a phylogenetic hypothesis of the group, we compiled a four-gene dataset including one nuclear ribosomal and three protein-coding genes for 132 distinct Pezizomycetes species (4437 nucleotides with all markers available for 80% of the total 142 included taxa). This is the most comprehensive molecular phylogeny of Pyronemataceae, and Pezizomycetes, to date. Three hundred ninety-four new sequences were generated during this project, with the following numbers for each gene: RPB1 (124), RPB2 (99), EF-1α (120) and LSU rDNA (51). The dataset includes 93 unique species from 40 genera of Pyronemataceae, and 34 species from 25 genera representing an additional 12 families of the class. Parsimony, maximum likelihood and Bayesian analyses suggest that Pyronemataceae is paraphyletic due to the nesting of both Ascodesmidaceae and Glaziellaceae within the family. Four lineages with taxa currently classified in the family, the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages, form a monophyletic group with Ascodesmidaceae and Glaziellaceae. We advocate the exclusion of these four lineages in order to recognize a monophyletic Pyronemataceae. The genus *Coprotus* (Thelebolales, Leotiomycetes) is shown to belong to Pezizomycetes, forming a strongly supported monophyletic group with *Boubovia*. Ten strongly supported lineages are identified within Pyronemataceae *s. str*. Of these, the *Pyropyxis* and *Otidea* lineages are identified as successive sister lineages to the rest of Pyronemataceae *s. str*. The highly reduced (gymnohymenial) *Monascella* is shown to belong to Pezizomycetes and is for the first time suggested to be closely related to the cleistothecial *Warcupia*, as a sister group to the primarily apothecial *Otidea*. None of the lineages of pyronemataceous taxa identified here correspond to previous families or subfamily classifications. Ancestral character state reconstructions (ASR) using a Bayesian approach support that the ancestors of Pezizomycetes and Pyronemataceae were soil inhabiting and saprobic. Ectomycorrhizae have arisen within both lineages A, B and C of Pezizomycetes and are suggested to have evolved independently seven to eight times within Pyronemataceae *s. l.*, whereas an obligate bryosymbiotic lifestyle has arisen only twice. No reversals to a free-living, saprobic lifestyle have happened from symbiotic or parasitic Pyronemataceae. Specializations to various substrates (e.g. burnt ground and dung) are suggested to have occurred several times in mainly saprobic lineages. Although carotenoids in the apothecia are shown to have arisen at least four times in Pezizomycetes, the ancestor of Pyronemataceae *s. str*., excluding the *Pyropyxis* and *Otidea* lineages, most likely produced carotenoids, which were then subsequently lost in some clades (– and possibly gained again). Excipular hairs were found with a high probability to be absent from apothecia in the deepest nodes of Pezizomycetes and in the ancestor of Pyronemataceae *s. str*. True hairs are restricted to the core group of Pyronemataceae *s. str*., but are also found in *Lasiobolus* (Ascodesmidaceae), the *Pseudombrophila* lineage and the clade of Chorioactidaceae, Sarcoscyphaceae and Sarcosomataceae. The number of gains and losses of true hairs within Pyronemataceae *s. str*., however, remains uncertain. The ASR of ascospore guttulation under binary coding (present or absent) indicates that this character is fast evolving and prone to shifts.

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1. Introduction

Pezizomycetes, commonly known as cup-fungi or operculate discomycetes, are among the earliest diverging lineages of Pezizomycotina (the largest subphylum of the Ascomycota) along with Orbiliomycetes (Spatafora et al., 2006; Schoch et al., 2009). The class currently includes 1684 species (Kirk et al., 2008), classified in 16 families and a single order, Pezizales. With the exception of two families with several members in the tropics, cup-fungi are most diverse in temperate regions or at high elevations. They are saprobic, mycorrhizal/symbiotic and a few are plant parasitic. A shared derived character, the operculate ascus (a lid-like structure at the apex of the asci that opens at spore discharge), characterizes Pezizomycetes. Pyronemataceae is the largest and most heterogeneous family of Pezizales, with 78 genera that encompass ca. 660 currently recognized species (Kirk et al., 2008). The family is highly diverse both morphologically and ecologically. Species produce fruitbodies (ascomata) that are epigeous, sessile to stipitate or rooting, disc-, cup- or ear-shaped (apothecia) and with active spore dispersal, or sub-hypogeous to hypogeous, closed, folded to solid and without active spore dispersal (i.e., truffles) (Fig. 1). The fruitbodies range in size from 300 μm to 12 cm in diam. Until recently, most epigeous fruiting Pyronemataceae have been considered primarily saprobic and rarely plant pathogenic, but an increasing number of species are being identified as ectomycorrhizal associates using molecular techniques (e.g. Smith et al., 2007; Tedersoo et al., 2006, in press), i.e. they live in a mutualistic symbiosis with plant roots; they gain photosynthetic sugars from their plant hosts, which in turn benefit from fungus-mediated uptake of mineral nutrients. Furthermore several groups of Pyronemataceae have been found as orchid associates (Tešitelov et al., 2012; Waterman et al., 2011), or as foliar endophytes and endolichenic (U'Ren et al., 2010), i.e. they live within asymptomatic aboveground living tissues such as plant leaves and lichen thalli (close to the photobiont), respectively. Species of Pyronemataceae occur in a broad range of habitats and many are substrate specialists, fruiting on all types of soil, including burnt ground, on dung, decaying leaves, needles, wood and living mosses. The majority of the soil-inhabiting species have a preference for high pH and a low content of organic matter (Petersen, 1985) and often produce ascomata in disturbed habitats. The family is primarily temperate to arctic-alpine in distribution, but a few strictly tropical taxa are known.

No shared derived characters define Pyronemataceae. It has been a default family for pezizalean taxa with uninucleate spores and iodine negative asci that lack distinguishing anatomical characters by which they could be segregated into natural families. Most other families of Pezizomycetes are now considered quite well delimited and are recognized, or have been refined, primarily by using characters such as: the number of nuclei in the ascospores; ascus apical ultrastructure and histochemistry; ultrastructure of the septal pore plug located at the base of the asci and in the ascogenous hyphae; and molecular data (see van Brummelen, 1994; Kimbrough, 1994; Hansen and Pfister, 2006; Læssøe and Hansen, 2007; Pfister et al., 2008; Hansen et al., 2008). Pyronemataceae share the presence of uninucleate spores with Ascobolaceae and Pezizaceae, but lack the blueing reaction of the asci in iodine solutions unique to these two families. Distinctive septal pore plugs in the asci and ascogenous hyphae characterize most families of Pezizomycetes, but within Pyronemataceae at least five distinct types have been reported (Kimbrough, 1994). The lack of clear synapomorphies is reflected in the concept of Pyronemataceae that has varied widely among contemporary mycologists. Some authors included only one or two genera (Arpin, 1969; Rifai, 1968; Kimbrough, 1970) while others included from 21 (Eckblad, 1968) to 49 genera (Korf, 1972, 1973). When the Pyronemataceae is used in a restricted sense, the families Aleuriaceae *sensu* Arpin (1969), Otideaceae, Humariaceae and Ascodesmidaceae have been employed to accomodate the remaining taxa.

When the concept of Pyronemataceae was first expanded, the inability to satisfactorily subdivide the family on common characters was indicated (Eckblad, 1968). Consequently it was accepted as a taxon characterized by very wide, but continuous patterns of variation in a few characters, such as spore ornamentation, excipulum structure and type of excipular hairs. Three distinct phylogenetic lines were delineated, but these were recognized by a gradual transition of the characters from one line to the other (Eckblad, 1968). Carotenoid pigments in the apothecia were regarded as the principal character of the family, although several genera with hyaline paraphyses (*Humaria*, *Leucoscypha*, *Geopora*, *Pseudombrophila*, *Sphaerosporella*, *Tricharina*, *Trichophaea*) were regarded as derived within the family. The family Otideaceae was erected for genera that produce larger, sometimes stipitate apothecia, most of which typically lack bright orange to red coloration: *Ascosparassis*, *Geopyxis*, *Otidea*, *Sowerbyella* and *Tarzetta* (Eckblad, 1968).

One formal subfamily level classification for a broadly defined Pyronemataceae has been proposed (Korf, 1972). It includes five subfamilies, Pyronematoideae, Ascophanoideae, Ascodesmidoideae, Otideoideae and Scutellinioideae, and eleven tribes. The divisions into subfamilies (Korf, 1972, 1973) or families (Arpin, 1969; Kimbrough, 1970) were based primarily on the pigmentation of the apothecia (±carotenoids), spore pigmentation and guttulation, and the presence or absence of a subiculum. The divisions into tribes (Korf, 1972) were further based on characters such as presence or absence, origin and pigmentation of apothecial hairs (±rooting, ±pigmented). Hypogeous taxa (truffles) were transferred to Pyronemataceae *sensu* Korf, when the strictly hypogeous, polyphyletic order Tuberales, was abandoned (see Læssøe and Hansen, 2007).

Based on ascomatal ontogeny, type of operculum development and distinctive striated septal pore plugs in the base of the asci, Kimbrough (1989, 1994) argued for a restricted concept of the Pyronemataceae, including only *Pyronema* and *Coprotus* (Fig. 1a and b). Following this, Korf and Zhuang (1991) placed numerous other taxa once treated in the family (not including Ascodesmidaceae and Thelebolaceae) in two subfamilies of the Otideaceae, Otideoideae and Scutellinioideae, using the presence or absence of carotenoids and hairs of the apothecia. Molecular phylogenetic studies (Hansen and Pfister, 2006; Liu and Zhuang, 2006; Perry et al., 2007) have not supported a segregation of *Pyronema* from the Otideaceae. Nevertheless, LSU and SSU rDNA sequences of *Pyronema* appeared to be somewhat divergent, as did sequences of such genera as *Otidea* and *Sowerbyella*, and the sister group relationships of *Pyronema* is still unresolved. For a further review of the taxonomic history of Pyronemataceae see Perry et al. (2007).

Molecular phylogenetic analyses have identified three main lineages within Pezizomycetes. The Pyronemataceae in all analyses is nested within a strongly supported C lineage (e.g. Landvik et al., 1997; Hansen and Pfister, 2006; Marek et al., 2009), along with the families Ascodesmidaceae, Chorioactidaceae, Glaziellaceae, Sarcoscyphaceae and Sarcosomataceae. In all of these previous analyses, the relationships among the families within the C lineage were not recovered with strong support. In our previous molecular phylogenetic study of Pyronemataceae (Perry et al., 2007), we included a significant number of taxa (201 Pezizomycetes species) and employed the large subunit nuclear ribosomal RNA gene (LSU-rDNA), spanning domains D1 and D2. The results indicate that Pyronemataceae, in its broad circumscription, does not represent a monophyletic family, due to the nesting of Ascodesmidaceae within, and the resolution of several pyronemataceous taxa outside, the family. Additionally, placement of the monotypic Glaziellaceae within Pyronemataceae could not be rejected. Fourteen primary clades of pyronemataceous taxa were identified, but the relationships among these were not supported using only LSU

rDNA sequences. Therefore, in the present study we considerably expand the number of molecular characters, through the addition of sequence data from three protein-coding genes: the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2) and translation elongation factor 1-alpha (EF-1α). This enlarged sampling represents a combined dataset of 132 distinct Pez-

izomycetes species and 4437 bp (with all four markers available for 80% of the taxa), constituting the most comprehensive molecular phylogenetic dataset for Pyronemataceae and Pezizomycetes to date. With these data, our main goals were to (1) provide a robust phylogenetic hypothesis for Pyronemataceae and resolve the relationships to its closest relatives, (2) further test the monophyly of the genera, in light of our previous results (Perry et al., 2007) that suggest several genera to be non-monophyletic, and (3) to trace the evolution of life history and morphological traits, and understand their implications for classification of the family. At the same time we wanted to: (1) explore and compare the utility of partial sequences from the three protein-coding genes at the family and generic level within Pezizomycetes, especially that of RPB1 and EF-1α, which have not previously been employed at this level within the class; and (2) explore the impact of third-codon positions (which are prone to saturation) on the phylogenies constructed under different methods of phylogenetic inference.

2. Material and methods

2.1. Taxon sampling

A data matrix containing 93 unique species of Pyronemataceae was constructed with sequences from LSU, RPB1, RPB2 and EF-1α genes (Table 1), representing 40 of 78 genera currently treated in the family. Thirty-eight genera are not sampled in our analyses, due to the lack of fresh or recent material. Sixteen of these were included in our previous analyses using LSU (Perry et al., 2007), but the protein-coding gene regions failed to amplify due to either poor quality DNA (from old or poorly dried collections) or possible primer mismatches. Most of the genera that were not included are small, often monotypic, and rare. Twenty-eight pyronemataceous genera are represented by type species.

To assess the monophyly of Pyronemataceae, an additional 34 species were sampled representing 25 genera encompassing all families of the class (Hansen and Pfister, 2006), except for Karstenellaceae and Tuberaceae. To determine if species of the genus *Coprotus* (currently placed in Thelebolaceae, Leotiomycetes by Kirk et al. (2008) and Lumbsch and Huhndorf (2010)) belong to Pezizomycetes, *Coprotus ochraceus* was included. To determine the exact placement of the anamorph *Cephaliophora* within Pezizomyetes, *C. irregularis* and *C. tropica* were included. Likewise *Monascella botryosa* was included to determine the relationships of this monotypic genus. It has been suggested, based on neighbor-joining analysis of limited sampling of ITS sequences, to belong to Pyronemataceae (Stchigel et al., 2001). *Monascella* was tentatively placed in the family by Kirk et al. (2008).

Four outgroup taxa from Taphrinomycotina; *Neolecta* spp., *Schizosaccharomyces pombe*, *Taphrina deformans* and *T. wiesneri* (Neolectomycetes, Schizosaccharomycetes and Taphrinomycetes), were included for rooting purposes based upon previous results which support these as basal to the Pezizomycotina, and thus, basal to the early diverging Pezizomycetes (e.g. Spatafora et al., 2006).

2.2. Molecular techniques

DNA was isolated from fresh ascomata (from recent collections stored in 1% SDS extraction buffer), or in a few cases from dried ascomata or live cultures (obtained from CBS), and extracted as in Hansen et al. (1999) with the exception of fresh and live material, which was ground directly in an Eppendorf tube. Dried material was shaken in a Fastprep FP120 Cell Disrupter (Qbiogene, Inc., Carlsbad, CA, USA). Two dilutions of DNA (1:10, 1:100) were used for PCR amplification. The following four gene regions were amplified: the 5′ end of the nLSU rDNA, spanning domains D1 and D2 (c. 900 bp), part of the nuclear genes that encode the two largest subunits of RNA polymerase II (RNA polymerase I (RPB1), A–C region, c. 700 bp (Matheny et al., 2002); and RNA polymerase II (RPB2), 6–11 region, c. 1700 bp) (Liu et al., 1999; Hansen et al., 2005a), and nearly the complete coding region of translation elongation factor 1-alpha (EF-1α) (c. 1000–1500 bp) (Rehner and Buckley, 2005). PCR and sequencing primers are listed in Table 2. The sequence spanning RPB2 regions 6–11 was amplified as one piece, or two pieces when required. The primer RPB2-Pb7F was used to amplify and sequence the overlapping piece between regions 6–7 and 7–11 in some cases. In a few instances, where the regions 6–7 did not successfully amplify, the regions 5–7 were amplified (Denton et al., 1998; James et al., 1991). The EF-1α region was amplified in one or two pieces using different primer combinations. For PCR and cycle sequencing procedures see Suppl. Appendix A. Electrophoresis and data collecting were done on an ABI PRISM® 3100 Genetic Analyzer (ABI, Foster City, CA).

2.3. Sequence alignment

Sequences were edited and assembled using Sequencher 4.0 and 4.9 (Gene Codes Corp., Ann Arbor, MI) and have been deposited in GenBank (Table 1). Nucleotide sequences were aligned manually using Se-Al, version 2.0a11 (Rambaut, 2002). The combined alignment (LSU, RPB1, RPB2 and EF-1α) is available from TreeBASE as accession no. S13710. Each alignment of the protein-coding genes was translated to amino acids in MacClade 4.05 (Maddison and Maddison, 2000) for examination and refinement of the nucleotide alignment. Only the exons of RPB1, RPB2 and EF-1α were included in analyses; introns differed in length and were too variable to align among species. To explore the reliability of the variable LSU alignment, the LSU sequences were additionally aligned using the Q-INS-i algorithm in the online version of the program MAFFT ver. 6 (Katoh et al., 2002; Katoh and Toh, 2008), with or without ambiguous regions as identified by Gblocks ver. 0.91b (Castresana, 2000) eliminated. The following relaxed parameters were used in Gblocks: minimum number of sequences for a conserved position = 72 ($n \times 0.5$), minimum number of sequences for a flanking position = 72 ($n \times 0.5$), maximum number of contiguous non-con-

Fig. 1. Ascomata and ecological diversity in Pyronemataceae, showing exemplar character states reconstructed. (a and b) Small, gymnohymenial apothecia. (a) *Pyronema omphalodes* on burnt ground, +carotenoids (JHP-05.303, C). (b) *Coprotus granuliformis* on dung (KH.10.317, S). (c–f) Ectomycorrhizal species on the undisturbed forest ground. (c) *Tarzetta* cf. *spurcata* (KH.10.270, S). (d) *Otidea mirabilis* ear-shaped apothecia (KH.09.188, S). (e) *Humaria hemisphaerica*, +superficial, long, pointed, stiff hairs (KH.10.172, S), insert shows margin densely set with hairs. (f) *Genea arenaria* truffle-like, closed ascomata. g. Bryosymbiotic *Octospora phagospora* + carotenoids (KH-94-227, C). (h, i, k) +carotenoids. (h) *Melastiza cornubiensis* on disturbed ground, +superficial, short, appressed, blunt hairs (KH.08.19, S), insert shows margin densely set with short, dark hairs. (i) *Sowerbyella imperialis* with rooting stipe (KH.09.222, S). (j) *Genea* ectomycorrhizal root tip; fungal mantle covering the root and emanating hyphae. (k) Close-up of *Scutellinia* cf. *scutellata* margins with rooting, stiff hairs (KH.12.95, S). (l and m) True hairs. (l) Rooting hairs, i.e. originating deep within the sterile tissue, near the junction of the outer and medullary excipulum, *Scutellinia trechispora* (KH.11.79, S). (m) Superficial hairs, i.e. originating from the outermost sterile cells, *Spooneromyces laeticolor* (M. Karström 0939). (n and o) Paraphyses, i.e. sterile elements between asci, +carotenoids in lipid globules. (n) *Scutellinia* sp. (KH.10.142, S). (o) *Pulvinula* sp. (KH.10.166, S). (p and q) Ascospores with one large guttule in *Lamprospora* cf. *arvensis* (KH.08.34, S) and multiple guttules in *Scutellinia* cf. *heterosculpturata* (KH.08.37, S). (r) *Cheilymenia sclerotiorum* ascospores without guttules, central nucleus seen (KH.08.29, S). Photos: (a) and insertions on (e and h) ©Jens H. Petersen/Mycokey; (f) Matthew E. Smith, (j) Leho Tedersoo; (b–e, g–i, k–q) Karen Hansen.

Table 1
Species examined, sequenced and used in the molecular phylogenetic study, with voucher information and GenBank accession numbers. Numbers in parentheses following species names indicate multiple collections of a single taxon. For 10 species not all gene regions could be obtained from a single collection and these are therefore represented by two or three collections (indicated by a /). Sequences generated in the present study are in bold. NA are sequences not obtained.

Species	Collection no. (Herbarium)/ Culture collection no.	Geographic origin, year and collector	LSU	RPB1	RPB2	EF-1α
Aleuria aurantia	KH.04.81 (FH)	Sweden, 2004, K. Hansen	**KC012661**	**JX943715**	**JX943815**	**KC109217**
Aleuria bicucullata	BAP 526 (FH)	USA, MA, 2005, B.A. Perry, D.H. Pfister	**KC012662**	**JX943716**	**JX943816**	**KC109218**
Anthracobia macrocystis /	KH.01.35 (C)	Denmark, 2001, K. Hansen	**KC012663**	NA	**JX943777**	–
Anthracobia macrocystis	OSC 100026 (OSC)	AFTOL-ID 73	–	NA	–	FJ238388[a]
Anthracobia sp.	TL-11709 (QCNE, dupl. C)	Ecuador, 2004, K. Hansen et al.	**KC012664**	**JX943670**	**JX943778**	**KC109219**
Ascobolus carbonarius	KH.00.008 (C)	Denmark, 2000, K. Hansen	AY500526[b]	**JX943623**	AY500459[b]	NA
Ascobolus crenulatus	KH.02.005 (C)	USA, MA, 2002, K. Hansen	AY500527[b]	DQ471132[a]	AY500462[b]	DQ471061[a]
Ascodesmis nigricans /	CBS 428.91	Germany, 1981, M. Wellacher	**KC012665**	**JX943653**	NA	**KC109220**
Ascodesmis nigricans	CBS 389.68	Netherlands, 1986, G. Tichelaar	DQ168335[e]	**JX943654**	**JX943761**	**KC109221**
Boubovia nicholsonii[k]	KH.03.65 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220395[e]	**JX943646**	**JX943755**	**KC109222**
Byssonectria terrestris	KS-94-04 (C)	Denmark, 1994, K. Hansen, S. Sandal	AY500531[b]	**JX943704**	AY500504[b]	NA
Caloscypha fulgens (1)	KH-97-17 (FH)	USA, CA, 1997, K. Hansen	**KC012666**	**JX943631**	**JX943746**	**KC109202**
Caloscypha fulgens (2)	KH.06.02 (FH)	USA, VT, 2006, K. Griffith	**KC012667**	**JX943632**	**JX943747**	**KC109203**
Cephaliophora irregularis	CBS 218.62	USA, VA, 1962, R.D. Goos	**KC012668**	**JX943655**	**JX943762**	**KC109223**
Cephaliophora tropica	CBS 133.33	1933, W.H. Weston	**KC012669**	**JX943656**	**JX943763**	**KC109224**
Cheilymenia granulata	KH.08.66 (S)	Sweden, 2008, K. Hansen	**KC012670**	**JX943709**	**JX943809**	**KC109225**
Cheilymenia sclerotiorum (1)	KH.03.115 (FH)	Norway, 2003, K. Hansen	DQ220324[e]	**JX943706**	NA	**KC109226**
Cheilymenia sclerotiorum (2)	KH.08.32 (S)	Sweden, 2008, K. Hansen, J. Santos	**KC012671**	**JX943707**	**JX943807**	**KC109227**
Cheilymenia stercorea /	BAP 440 (FH)	USA, CA, 2002, B.A. Perry	DQ220323[e]	**JX943705**	–	–
Cheilymenia stercorea	OSC 100034 (OSC)	AFTOL-ID 148	–	–	EF080826[a]	DQ471052[a]
Cheilymenia vitellina	KH.01.32 (C)	Denmark, 2001, K. Hansen	DQ220325[e]	**JX943708**	**JX943808**	**KC109228**
Chorioactis geaster /	DHP 02.497 (FH)	USA, TX, 2002, F. Mims	**KC012672**	NA	–	**KC109211**
Chorioactis geaster	s.n. (FH)	USA, TX, 1992, H.W. Keller, K.C. Rudy	–	NA	DQ017609[f]	–
Coprotus ochraceus	JHP-06.121 (C)	Sweden, 2006, J.H. Petersen	**KC012673**	NA	**JX943764**	**KC109229**
Disciotis venosa	OSC 100045 (OSC)/ NRRL 22213	AFTOL-ID 179	AY544667[a]	DQ471131[a]	DQ470892[a]	DQ471060[a]
Eleutherascus lectardii	CBS 626.71	France, 1968, P. Lectard	DQ168334[e]	**JX943651**	**JX943759**	**KC109230**
Eleutherascus peruvianus	CBS 101.75	Peru, 1975, L.H. Huang	DQ220330[e]	**JX943652**	**JX943760**	**KC109231**
Galiella rufa /	DHP 05-600 (FH)	USA, MA, 2005, L. Millman	**KC012674**	**JX943642**	–	**KC109213**
Galiella rufa	mh 101 (FH)	USA, GA	–	–	DQ017594[f]	–
Gelinipes sp. (gen. ined.)	Trappe 24315 (FH, dupl. OSC)	Australia, NSW, 1999, J. Trappe	DQ220331[e]	**JX943701**	NA	**KC109232**
Genea arenaria	src872 (OSC)	USA, CA, 2005, M.E. Smith	**KC012675**	**JX943728**	**JX943827**	**KC109233**
Genea gardneri	src867 (OSC)	USA, CA, 2005, M.E. Smith	**KC012676**	**JX943729**	NA	**KC109234**
Genea harknessii group	src865 (OSC)	USA, CA, 2005, M.E. Smith	**KC012677**	**JX943727**	**JX943826**	NA
Geopora cf. cervina	KH.03.61 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220344[e]	**JX943679**	**JX943785**	**KC109235**
Geopora cooperi	BAP 517 (FH)	USA, NM, 2004, New Mexico Mycological Society	**KC012678**	**JX943681**	**JX943787**	**KC109236**
Geopora sp. A	KH.01.29 (C)	Denmark, 2001, S.A. Elborne	DQ220338[e]	**JX943677**	**JX943783**	**KC109237**
Geopora sp. B	KH.03.109 (FH)	Norway, 2003, K. Hansen	DQ220345[e]	**JX943680**	**JX943786**	**KC109238**
Geopora sp. C	KH.08.52 (S)	Sweden, 2008, K. Hansen	**KC012679**	**JX943678**	**JX943784**	**KC109239**
Geopyxis vulcanalis	KH.04.37 (FH, dupl. DBG)	USA, CO, 2004, K. Hansen, V. Evenson	**KC012680**	**JX943661**	**JX943770**	**KC109240**
Geopyxis sp.	KH.04.48 (FH, dupl. DBG)	USA, CO, 2004, K. Hansen, V. Evenson	DQ062985[c]	JX943660	JX943769	KC109241
Glaziella aurantiaca /	PR 6376 (FH)	Puerto Rico, 2006, D.J. Lodge	**KC012681**	**JX943645**	**JX943754**	**KC109242**
Glaziella aurantiaca	PR-5954 (FH)	Puerto Rico, 1998, N.C. Clum, D.J. Lodge	–	–	–	**KC109243**
Gyromitra californica	OSC 100068 (OSC)	AFTOL-ID 176	AY544673[a]	DQ471130[a]	DQ470891[a]	DQ471059[a]
Helvella leucomelaena	KH.06.01 (FH)	USA, MA, 2006, K. Hansen, G. Lewis-Gentry	**KC012682**	**JX943637**	**JX943751**	**KC109207**
Helvella sp.	KH.03.21 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ191678[h]	**JX943638**	**JX943752**	**KC109208**
Humaria hemisphaerica (1)	KH.03.100 (FH)	Norway, 2003, K. Hansen	DQ220353[e]	JX943725	JX943824	KC109244
Humaria hemisphaerica (2)	KH.03.10 (FH)	Norway, 2003, K. Hansen, C. Lange	KC012683	**JX943726**	**JX943825**	NA
Hydnotrya cubispora	DHP 05-605 (FH)	Canada, NS, 2002, C. Pfister	DQ200845[h]	**JX943636**	**JX943750**	**KC109209**
Kotlabaea deformis	HD Alta 00.014 (C)	Norway, 2000, H. Dissing	DQ220356[e]	**JX943690**	**JX943795**	**KC109245**
Lamprospora ascoboloides	KH.03.54 (FH)	Norway, 2003, K. Hansen	DQ220358[e]	**JX943741**	**JX943840**	**KC109246**
Lamprospora norvegica	KH.03.138 (FH)	Norway, 2003, K. Hansen	**KC012684**	**JX943745**	**JX943842**	**KC109247**
Lamprospora sp. B	KH.03.150 (FH)	Norway, 2003, K. Hansen	DQ220362[e]	JX943742	JX943841	KC109248
Lamprospora sp. C	TL-11703 (QCNE, dupl. C)	Ecuador, 2004, K. Hansen et al.	**KC012685**	**JX943743**	NA	NA
Lamprospora sp. D	TL-11753 (QCNE, dupl. C)	Ecuador, 2004, K. Hansen, T. Læssøe	**KC012686**	**JX943744**	NA	NA
Lasiobolidium orbiculoides	CBS 344.73	USA, CA, 1953, G.L. Benny	DQ062995[c]	**JX943649**	**JX943757**	**KC109313**
Lasiobolidium spirale	CBS 782.70	USA, WY, 1964, R.F. Cain	DQ220363[e]	**JX943702**	**JX943804**	**KC109249**
Lasiobolus cf. papillatus	KH.08.30 (S)	Sweden, 2008, K. Hansen, J. Santos	**KC012687**	**JX943650**	**JX943758**	**KC109314**
Marcelleina persoonii	TL-5696 (C)	Denmark, 1999, T. Læssøe	AY500537[b]	**JX943625**	AY500464[b]	**KC109196**
Marcelleina pseudoanthracina	KH.02.15 (FH)	Norway, 2002, K. Hansen et al.	AY500538[b]	**JX943626**	AY500509[b]	**KC109197**
Melastiza contorta	KH.01.06 (C)	Sweden, 2001, B.T. Olsen	AY500539[b]	**JX943713**	AY500505[b]	**KC109250**
Melastiza cornubiensis (1)	KH.01.17 (C)	Denmark, 2001, K. Hansen	DQ220367[e]	**JX943710**	JX943810	**KC109251**
Melastiza cornubiensis (2)	KH.03.43 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ646524[d]	**JX943711**	**JX943811**	**KC109252**

(*continued on next page*)

Table 1 (*continued*)

Species	Collection no. (Herbarium)/ Culture collection no.	Geographic origin, year and collector	LSU	RPB1	RPB2	EF-1α
Melastiza flavorubens	DHP 04-570 (FH)	Iceland, 2004, D.H. Pfister	DQ220369[e]	**JX943712**	**JX943812**	**KC109253**
Miladina lecithina (1)	KH.03.156 (FH)	Sweden, 2003, C. Lange	DQ220371[e]	**JX943687**	**JX943792**	**KC109254**
Miladina lecithina (2)	KH.04.22 (FH)	USA, NM, 2004, K. Hansen, B.A. Perry, N. Weber	DQ220372[e]	**JX943688**	**JX943793**	**KC109255**
Monascella botryosa	CBS 233.85; Type of *Monascella botryosa*	Spain, 1985, J. Guarro	**KC012688**	**JX943733**	**JX943831**	**KC109256**
Morchella esculenta	OSC 100041 (OSC)	AFTOL-ID 60	AY544664[a]	DQ471117[a]	DQ470880[a]	DQ471046[a]
Neolecta irregularis /	DAH-3 (FH)	USA, NH, 2002, Griffith et al.	–	–	–	DQ471109[a]
Neolecta vitellina /	KH.04.34 (FH)	USA, NM, 2004, K. Hansen, B.A. Perry	FJ171879	–	–	–
Neolecta vitellina	NSW 6359	USA, OR, –, N.S. Weber	–	EF014375	AF107786	–
Neottiella rutilans	KH.03.55 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220377[e]	**JX943736**	**JX943835**	**KC109257**
Octospora hygrohypnophila	KH.03.30 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220379[e]	**JX943737**	**JX943836**	**KC109258**
Octospora melina	DHP 04-552 (FH)	Iceland, 2004, D.H. Pfister	**KC012689**	**JX943738**	**JX943837**	**KC109259**
Octospora roxheimii	KH.05.01 (FH)	USA, CA, 2005, E.C. Vellinga	**KC012690**	**JX943739**	**JX943838**	NA
Octospora sp.	KH.03.136 (FH)	Norway, 2003, K. Hansen	DQ220384[e]	**JX943740**	**JX943839**	**KC109260**
Otidea alutacea	KH.09.170 (S)	Sweden, 2009, K. Hansen, I. Olariaga	**KC012691**	**JX943732**	**JX943830**	**KC109261**
Otidea bufonia	KH.07.37 (S)	Denmark, 2007, K. Hansen	JN941098	JQ012829	JN993552	**KC109262**
Otidea onotica	KH.09.132 (S)	Norway, 2009, K. Hansen, I. Olariaga	**KC012692**	**JX943730**	**JX943828**	**KC109263**
Otidea unicisa	KH.06.06 (FH)	USA, MA, 2006, L. Millman	**KC012693**	**JX943731**	**JX943829**	**KC109264**
Pachyella babingtonii	KH-99-09 (C)	USA, 1999, K. Hansen, D.H. Pfister	AF335123[g]	**JX943630**	AY500467[b]	**KC109198**
Parascutellinia carneosanguinea (1)	KH.03.34 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220388[e]	**JX943722**	**JX943823**	**KC109265**
Parascutellinia carneosanguinea (2)	DHP 04-530 (FH)	Iceland, 2004, D.H. Pfister	**KC012694**	**JX943723**	NA	**KC109266**
Parascutellinia carneosanguinea (3)	KH.04.65 (FH)	USA, CO, 2004, K. Hansen	**KC012695**	**JX943724**	NA	NA
Paratrichophaea boudieri	BAP 481 (FH)	USA, CA, 2003, B.A. Perry	DQ220402[e]	**JX943719**	**JX943820**	**KC109267**
Peziza gerardii	TL-5693 (C)	Denmark, 1999, T. Læssøe	AY500546[b]	**JX943624**	AY500513[b]	**KC109199**
Peziza lobulata	KH.03.157 (FH)	USA, WA, 2002, J.D. and B.S. Rogers	AY500548[b]	**JX943627**	AY500495[b]	**KC109200**
Peziza michelii	TL-5692 (C)	Denmark, 1999, T. Læssøe	AY500549[b]	**JX943628**	AY500494[b]	**KC109201**
Peziza succosa	KH-98-07 (C)	Denmark, 1998, A. Storgaard	AF335166[g]	**JX943629**	AY500487[b]	NA
Peziza vesiculosa /	TL-6398 (C)	Denmark, 2000, T. Læssøe	AF378367[g]	DQ471140[a]	–	DQ471052[a]
Peziza vesiculosa	JV95-652 (C)	Denmark, 1995, S. Hansen, J. Vesterholt	–	–	AY500489[b]	–
Plectania nannfeldtii	KH-97-16 (FH)	USA, CA, 1997, K. Hansen	AY945853[f]	**JX943641**	DQ017592[f]	**KC109214**
Pseudombrophila theioleuca (1)	C F-70057 (C)	Denmark, 1982, H. Knudsen	DQ062989[c]	**JX943647**	NA	**KC109268**
Pseudombrophila theioleuca (2)	DHP 3498 (FH)	USA, MA, 2003, D.H. Pfister	**KC012696**	**JX943648**	**JX943756**	**KC109269**
Pseudopithyella minuscula /	mh 675 (FH)	USA, CA, 1997, F.A. Harrington	AY945849[f]	NA	DQ017600[f]	–
Pseudopithyella minuscula	OSC 100022 (OSC)	AFTOL-ID 69	–	NA	–	FJ238387[a]
Psilopezia deligata	KH-99-13 (FH)	USA, VT, 1999, K. Griffith	DQ220390[e]	**JX943635**	EF494071[i]	**KC109204**
Psilopezia cf. nummularialis	TL-11785 (QCNE, dupl. C)	Ecuador, 2004, T. Læssøe	EU722509[i]	**JX943634**	**JX943749**	**KC109205**
Pulvinula archeri	BAP 458 (FH)	USA, OR, 2003, B.A. Perry, N.S. Weber	DQ220392[e]	**JX943662**	**JX943771**	**KC109270**
Pulvinula constellatio	KH.03.64 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ062987[c]	NA	**JX943773**	**KC109271**
Pulvinula convexella (1)	KH.01.20 (C)	Denmark, 2001, K. Hansen	DQ062986[c]	JX943663	JX943772	KC109272
Pulvinula convexella (2)	KH.03.19 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ062986[e]	**JX943664**	NA	**KC109273**
Pulvinula globifera	DHP DR-104 (FH)	Dominican Republic, 2002, D.H. Pfister et al.	DQ220393[e]	**JX943665**	**JX943774**	**KC109274**
"Pustularia patavina"	KH.03.73 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220396[e]	**JX943685**	NA	**KC109275**
Pyronema domesticum	CBS 666.88	Netherlands, 1988, H.A. van der Aa	DQ247805[a]	DQ471166[a]	DQ247795[a]	DQ471093[a]
Pyronema omphalodes	TL-11685 (QCNE, C)	Ecuador, 2004, K. Hansen et al.	DQ220397[e]	**JX943700**	EU360915	**KC109309**
Pyronemataceae sp. A (1)	KH.03.125 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220462[e]	**JX943703**	**JX943805**	**KC109276**
Pyronemataceae sp. A (2)	TL-11605 (QCNE, dupl. C)	Ecuador, 2004, K. Hansen et al.	**KC012697**	NA	**JX943806**	NA
Pyronemataceae sp. B	KH.04.21 (FH)	USA, NM, 2004, N. Weber, K. Hansen, B.A. Perry	DQ220399[e]	**JX943686**	**JX943791**	**KC109277**
Pyropyxis rubra (1)	K. Egger 323 (DAOM)	Canada, Ontario, 1997, K.N. Egger	DQ220405[e]	**JX943666**	**JX943775**	**KC109310**
Pyropyxis rubra (2)	K. Egger 289 (DAOM)	Canada, Ontario, 1997, K.N. Egger	DQ220404[e]	**JX943667**	**JX943776**	**KC109311**
Ramsbottomia asperior	KH.03.79 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220408[e]	**JX943691**	**JX943796**	**KC109278**
Ramsbottomia crec'hqueraultii /	DHP 04-565b (FH)	Iceland, 2004, D.H. Pfister	–	–	NA	**KC109279**
Ramsbottomia crec'hqueraultii	DHP 06.608 (FH)	USA, MA, 2006, D.H. Pfister	**KC012698**	**JX943692**	NA	–
Rhizina undulata	KH.02.44 (FH)	Norway, 2002, D.H. Pfister, B.A. Perry, K. Hansen	DQ220410[e]	**JX943633**	**JX943748**/ +DQ470911[a]	**KC109206**
Rhodoscypha ovilla	KH.08.007 (S)	Sweden, 2008, J. Santos, K. Hansen	**KC012699**	NA	**JX943834**	**KC109280**
Rhodotarzetta rosea	KH.03.107 (FH)	Norway, 2003, K. Hansen	DQ220413[e]	**JX943735**	**JX943833**	**KC109281**
Saccobolus dilutellus	CBS 472.80	Spain, J. Guarro	FJ176870[a]	FJ238436[a]	FJ238353[a]	NA
Sarcoscypha occidentalis /	DAH-12 (FH)	USA, MA, 2003, D. Hewitt, G. Riner, D. Chou	–	–	DQ017596[f]	–
Sarcoscypha sp.	TL-11692 (C)	Ecuador, Carchi, 2004, on cf. *Myrcianthes* branch in the litter, K. Hansen et al.	**KC012700**	**JX943644**	–	**KC109210**
Sarcosoma globosum	KH.07.04 (S)	Sweden, 2007, H. Kaufmann	FJ499393[j]	**JX943640**	**JX943753**	**KC109215**
Schizosaccharomyces pombe	972 / 972h		Z19136	X56564	D13337	CU329670
Scutellinia cf. cejpii	TL-11751 (QCNE, dupl. C)	Ecuador, Carchi, 2004, K. Hansen et al.	**KC012701**	**JX943695**	**JX943799**	**KC109282**

Table 1 (*continued*)

Species	Collection no. (Herbarium)/ Culture collection no.	Geographic origin, year and collector	LSU	RPB1	RPB2	EF-1α
Scutellinia crucipila	KH.03.63 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220320[e]	**JX943699**	**JX943803**	**KC109283**
Scutellinia hyperborea	KH.03.116 (FH)	Norway, 2003, K. Hansen	**KC012702**	**JX943697**	**JX943801**	**KC109284**
Scutellinia scutellata	KH.03.26 (FH)	Norway, 2003, K. Hansen, C. Lange	**KC012703**	**JX943696**	**JX943800**	**KC109285**
Scutellinia cf. subhirtella	KH.03.117 (FH)	Norway, 2003, K. Hansen	**KC012704**	**JX943698**	**JX943802**	**KC109286**
Scutellinia trechispora	KH.01.37 (C)	Denmark, 2001, K. Hansen, J.H. Petersen	DQ220425[e]	**JX943694**	**JX943798**	**KC109287**
Scutellinia sp. A	KH.03.15 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ220417[e]	**JX943689**	**JX943794**	**KC109288**
Scutellinia sp. B	TL-11648 (QCNE, dupl. C)	Ecuador, Carchi, 2004, K. Hansen et al.	**KC012705**	**JX943693**	**JX943797**	**KC109289**
Smardaea amethystina	KH-97-132 (C)	Denmark, 1997, C. Lange, K. Hansen	AF335176[g]	**JX943669**	NA	NA
Smardaea reticulosperma	Part of isotype (herb. Roy Kristiansen)	France, 1984, G. Riousset	AY500532[b]	**JX943668**	NA	**KC109312**
Sowerbyella densireticulata	KH.08.133 (S)	Sweden, 2008, K. Hansen, J. Santos	**KC012706**	NA	**JX943819**	NA
Sowerbyella imperialis	CL2004-105 (C)	Denmark, 2004, C. Lange	DQ220427[e]	**JX943717**	**JX943817**	**KC109290**
Sowerbyella radiculata	KH.04.30 (FH)	USA, NM, 2004, B. Chapman, K. Hansen	DQ220428[e]	**JX943718**	**JX943818**	**KC109291**
Spooneromyces helveticus	JS080.30 (S)	Sweden, 2008, K. Hansen, J. Santos	**KC012707**	**JX943714**	**JX943813**	**KC109292**
Spooneromyces laeticolor	HFG 88.013 (C)	Denmark, 1988, H.F. Gøtzsche	DQ220434[e]	NA	**JX943814**	**KC109293**
Taphrina deformans /	CBS 356.35	AFTOL-ID 1234	DQ470973[a]	DQ471170[a]	–	DQ471097[a]
Taphrina deformans	NRRL T-857		–	–	AY485633	–
Taphrina wiesneri	IAM 14515	AFTOL-ID 265	AY548292[a]	DQ471134[a]	AY548298[a]	DQ479936[a]
Tarzetta catinus	KS-94-10A (C)	Denmark, 1994, K. Hansen, S.K. Sandal	DQ062984[c]	NA	**JX943768**	NA
Tarzetta cupularis	KH.04.64 (FH, dupl. DBG)	USA, CO, 2004, K. Hansen	**KC012708**	**JX943657**	**JX943765**	**KC109294**
Tarzetta pusilla	KH.03.66 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ062983[c]	**JX943659**	**JX943767**	**KC109295**
Tarzetta sp.	KH.03.102 (FH)	Norway, 2003, K. Hansen	**KC012709**	**JX943658**	**JX943766**	**KC109296**
Tricharina gilva	DED 7356 (SFSU)	USA, CA, 2002, D.E. Desjardin	DQ220443[e]	**JX943684**	**JX943790**	**KC109297**
Tricharina praecox	KH.03.101 (FH)	Norway, 2003, K. Hansen, C. Lange	DQ646525[d]	**JX943682**	**JX943788**	**KC109298**
Tricharina sp.	TL-10051 (QCA, dupl. C)	Ecuador, 2003, on litter amongst moss under an *Espeletia* trunk, T. Læssøe & J. Salazar	DQ220447[e]	**JX943683**	**JX943789**	**KC109299**
Trichophaea abundans /	KH.01.36 (C)	Denmark, 2001, K. Hansen	DQ220449[e]	**JX943673**	**JX943780**	**KC109300**
Trichophaea abundans	CBS 250.31	1931, H.C.I. Gwynne-Vaughan	DQ220450[e]	**JX943674**	–	**KC109301**
Trichophaea brunnea	KH.03.04 (FH)	USA, MA, 2003, K. Hansen	DQ220433[e]	**JX943671**	**JX943779**	**KC109302**
Trichophaea hemisphaerioides	KH.04.54 (FH)	USA, CO, 2004, K. Hansen	**KC012710**	**JX943720**	**JX943821**	**KC109303**
Trichophaea hybrida	KH.04.39 (FH, dupl. DBG)	USA, CO, 2004, K. Hansen, V. Evenson	DQ220454[e]	**JX943721**	**JX943822**	**KC109304**
Trichophaea minuta	CBS 236.57; Isotype of *Sphaerospora minuta*	Canada, 1953, R.F. Cain, N.A. Hastings	DQ220452[e]	**JX943675**	**JX943781**	**KC109305**
Trichophaea saccata	CBS 804.70; Type of *Sphaerospora saccata*	England, 1968, H.C. Evans	DQ220451[e]	**JX943676**	**JX943782**	**KC109306**
Trichophaea woolhopeia	KH.01.33 (C)	Denmark, 2001, K. Hansen	DQ220460[e]	**JX943672**	NA	**KC109307**
Urnula craterium	DHP 04-511 (FH)	USA, NC, 2004, D.H. Pfister	AY945851[f]	**JX943639**	DQ017595[f]	**KC109216**
Warcupia terrestris	CBS 891.69	Canada, 1966, J.W. Paden	DQ220467[e]	**JX943734**	**JX943832**	**KC109308**
Wolfina aurantiopsis	DHP 04-599 (FH)	USA, NC, 2003, L.F. Grand, C.S. Vernia	AY945859[f]	**JX943643**	DQ017605[f]	**KC109212**

[a] Sequences from the AFTOL project obtained from GenBank.
[b] Sequences from Hansen et al. (2005a).
[c] Sequences from Hansen et al. (2005b).
[d] Sequences from Hansen and Pfister (2006).
[e] Sequences from Perry et al. (2007).
[f] Sequences from Pfister et al. (2008).
[g] Sequences from Hansen et al. (2001).
[h] Sequences from Tedersoo et al. (2006).
[i] Sequences from Marek et al. (2009).
[j] Sequences from Hansen et al. (2008).
[k] To avoid making a superfluous combination of *Pulvinula ovalispora* in *Boubovia*, we use here the name *Boubovia nicholsonii* for a species that has commonly been referred to as *Pulvinula ovalispora* in the Nordic countries, until it has been clarified if one or two species exists. Judging from the original descriptions these two species differ in spore size, but a study of the type material (Korf and Zhuang, 1984) gives a much broader range in spore size of *P. ovalispora*, overlapping with *B. nicholsonii* (Yao and Spooner, 1996a).

served positions = 50, minimum length of a block = 5, and allowed gap positions = "with half."

2.4. Phylogenetic analyses

Individual and combined parsimony analyses of the RPB1, RPB2, EF-1α and LSU were performed using PAUP 4.0b10 for Unix (Swofford, 2003). All gene regions were analyzed using the nucleotide data. Parsimony (MP) analyses with heuristic searches consisted of 1000 random sequence addition replicates, TBR branch swapping, MULPARS in effect, and saving all equally most parsimonious trees (MPTs). All characters were equally weighted and unordered. In the analysis of the LSU dataset a two-step search was performed (due to the exceedingly large number of trees generated). First, 1000 heuristic searches were performed with random sequence addition and TBR branch swapping, with MAXTREES unrestricted, keeping only 15 trees per replicate. Second, exhaustive swapping was performed on all of the MPTs discovered during the first step, with MAXTREES set to 15,000. Support for individual branches was assessed by parsimony bootstrap (MP-BP) analyses, using 500 bootstrap replicates, each consisting of a heuristic search with 10 random addition sequence replicates, TBR branch swapping and keeping no more than 100 trees per replicate.

Prior to combined analyses the congruence among the four single-gene datasets was determined by visually comparing the bootstrap values resulting from analysis of the individual alignments. Phylogenetic hypotheses resulting from different loci were considered incongruent if they displayed conflict between well-supported (BP > 85%) clades (e.g. Mason-Gamer and Kellogg, 1996; Wiens, 1998).

Table 2
RPB1, RPB2 and EF-1α degenerate primers and general fungal primers for LSU rDNA (5–3′)[a].

Locus	Primer	Reference	Sequence	PCR	Sequencing
RPB1	gRPB1-A	B. Hall unpubl.	GARTGYCCDGGDCAYTTYGG	X	X
	fRPB1-C rev	Matheny et al. (2002)	CCNGCDATNTCRTTRTCCATRTA	X	X
	RPB1-PyrC rev	This study	TTCGCRCGRATRATRTCTCC	X	X
RPB2	RPB2-P5F	Hansen et al. (2005a)	GAYGACAGAGATCACTTYGG	X	
	RPB2-P6Fa	Hansen et al. (2005a)	TGGGGRYTKGTBTGYCCKGCHGA	X	X
	RPB2-P7Fa	Hansen et al. (2005a)	ATGGGYAARCARGCNATGGG	X	X
	RPB2-P7Ra	Hansen et al. (2005a)	CCCATNGCYTGYTTRCCCAT	X	X
	RPB2-Pb7F	Hansen et al. (2005a)	TGYGARATYCAYCCNAGTATGA	X	X
	RPB2-Pyr6Fb	This Study	GTDTGYCCBGCNGARACYCCNGA	X	X
	RPB2-Pyr7R	This Study	CCCATHGCYTGYTTVCCCATRGC	X	X
	RPB2-Pyr7F	This Study	GCYATGGGBAARCARGCDATGGG	X	X
	fRPB2-11aR	Liu et al. (1999)	GCRTGGATCTTRTCRTCSACC	X	X
EF1	526F	S. Rehner unpubl.[b]	GTCGTYGTYATYGGHCAYGT	X	X
	EF-df	S. Rehner unpubl.[b]	AAGGATGGHCAGACYCGYGARCAYGC	X	X
	1567R	S. Rehner unpubl.[b]	ACHGTRCCRATACCACCRATCTT	X	X
	2218R	Rehner and Buckley (2005)	ATGACACCRACRGCRACRGTYTG	X	X
	EF1-2F	S. Rehner unpubl.[b]	AACATGATSACTGGTACYTCC	X	X
	PyrEF1-df	This Study	AAGGATGGHCAGACYCGYGARCAYGC	X	X
	PyrEF1-4AF	This Study	TCAARTCBGTBGARATGCAYC		X
	PyrEF1-3AR	This Study	AGYTGYTCGTGRTGCATYTC	X	X
LSU	LR5	Moncalvo et al. (2000)	TCCTGAGGGAAACTTCG	X	X
	LROR	Moncalvo et al. (2000)	ACCCGCTGAACTTAAGC	X	X
	LR3	Moncalvo et al. (2000)	CCGTGTTTCAAGACGGG		X
	LR3R	Moncalvo et al. (2000)	GTCTTGAAACACGGACC		X

Primers designed in this study for RPB1, RPB2 and EF1 are modified for Pyronemataceae; for location of most of these see Matheny et al. (2002) for RPB1, Liu et al. (1999) for RPB2 and S. Rehner at http://www.aftol.org/pdfs/EF1primer.pdf for EF1-α.
[a] Follow the international nomenclature for degenerate positions: R = G or A, K = G or T, S = G or C, W = A or T, M = A or C, Y = T or C, B = G, T or C, H = A, T or C, N = G, A, T or C.
[b] See http://www.aftol.org/pdfs/EF1primer.pdf.

Relationships were likewise reconstructed using Metropolis-coupled MCMC (MCMCMC) methods as implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003; Altekar et al., 2004) and maximum likelihood (ML) as implemented in RAxML ver. 7.2.6 as mpi (Stamatakis, 2006). These analyses were run on the freely available Bioportal, University of Oslo (Kumar et al., 2009). To determine which model of nucleotide substitution was most appropriate for each dataset, hierarchical likelihood ratio tests were performed using the program jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). Using the AIC criterion the GTR+I+G model was selected for all datasets among 88 models, including models with equal or unequal base frequencies (+F), with the possibility of a proportion of invariable sites (+I) and allowing gamma distributed rate heterogeneity between sites (+G) modeled by four discrete categories and using full ML optimization. For the combined four-gene Bayesian analysis, each codon position in the protein-coding genes, as well as the LSU, was specified as a distinct partition. Thus, the dataset was analyzed with 10 partitions using the GTR+I+G model for each as determined above and with all parameter values, except branch lengths and tree topologies, unlinked. Site-specific rates were allowed to vary across partitions. Analyses consisted of eight parallel searches, initiated with random starting trees, each with four chains. The analysis was diagnosed for convergence every 100,000 generations, and was set to end automatically when the average standard deviation between runs descended below 0.01 (e.g. Millanes et al., 2011). The chains were sampled every 100 generations from the posterior distribution. Posterior probabilities (PP) were calculated using the last 50% of the trees sampled from the posterior distribution. 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 and uninformative topology (uniform) priors.

An additional Bayesian analysis, excluding third codon positions of the three protein-coding genes (RPB1, RPB2 and EF-1α) of the combined four-gene dataset, was performed under the same settings as specified above, with seven data partitions (comprising the protein-coding genes divided by first and second codons, and the LSU region), and the chains sampled every 250 generations. A MP and MP-BP analysis were also conducted with third codon positions excluded from the combined dataset, under the same settings as specified above for the parsimony analyses.

ML analysis of the four-gene data set used a 10 data-partition scheme (as given for the Bayesian analyses), assigning a GTR-GAMMA model with four rate categories and all free model parameters estimated by the program. For the ML bootstrap analysis (ML-BP), 1000 rapid bootstrapping replicates from distinct starting trees were performed on eight nodes, followed by a subsequent ML search similarly using 1000 replicates.

2.5. Character state coding and analyses of character evolution

We traced the evolution of three characters over the phylogeny, trophic strategy (trait 1), habitat/substrate for ascomata production (trait 2), and carotenoids (trait 3). We consider these to be of high biological and evolutionary importance and the current knowledge of these traits is reasonably advanced. In addition, we traced two morphological characters, hairs (trait 4) and ascospore guttulation (trait 5), which have been used previously in the classification of Pyronemataceae. Trophic strategies in Pezizomycetes include saprobic (assigned state 0), ectomycorrhiza (1), parasitic (2) and bryosymbiotic (3). We follow the basic concept of ectomycorrhiza as a balanced reciprocal parasitism between plant roots and fungi, where the symbiosis is beneficial (i.e. mutualistic) to both partners in natural conditions (e.g. Egger and Hibbett, 2004) – and in distinguishing the symbiosis morphologically from other putative mutualistic plant-fungus interactions by the presence of a fungal mantle covering the root tips (Fig. 1j) and a Hartig net. Habitat/substrate for formation of ascomata can be various types of soil (0), burnt ground (1), dung (2), on or among mosses (3), wood or woody debris (4), on plant debris and where animals have urinated (5) and on living plants (6). Carotenoids in the ascomata

are treated as absent (0) or present (1). Ascomatal hairs are treated as absent (0), hyphoid, i.e. hyaline, discrete, thin-walled, projecting, blunt hairs (1) or true hairs, defined as specialized structures that are thick-walled, often brown, and developing either from the outermost excipular cells (i.e. superficial true hairs), being blunt (2) or pointed (3) (Fig. 1m), or originating deep in the tissue at the junction of the outer and medullary excipulum (i.e. rooting hairs) and pointed (4) (Fig. 1l). Several types of hairs are present in some species and we have here coded those with the most distinctive type (considered to be true, rooting hairs). Oil guttules in the ascospores are coded as absent (0) or present (1). Information on trophic strategies for coding individual taxa are from our own or previously published studies (e.g. Amicucci et al., 2001; Davidson, 1950; Döbbeler, 1979, 2002; Egger, 1984; Egger and Paden, 1986; Ginns, 1968; Paden et al., 1978; Tedersoo et al., 2006, 2010, in press). The coding of presence or absence of carotenoids is based either directly on Arpin (1969), Arpin et al. (1971) and Korf (1972), or otherwise on the assumption that only brightly colored red, yellow or orange ascomata contain carotenoids. Habitat/substrate for fruiting, hairs, and presence or absence of guttules, are based primarily on our own observations, substantiated by the literature (e.g. Dissing, 2000; Moravec, 2005; Schumacher, 1990). Observations of guttules are made from fresh material in H_2O. Taxa with uncertain or unknown states were coded as "missing" (?).

Ancestral character state reconstructions (ASR) were performed in SIMMAP ver. 1.5.2 (Bollback, 2006) using a Bayesian approach. This version of SIMMAP reconstructs states at all ancestral nodes in the phylogeny using the Mk+Γ model integrating uncertainty over observed character states with the MCMC posterior distribution of trees and with estimated parameters (including model parameters, trees and branch lengths taken from the original four-gene Bayesian analysis; see Section 2.4). Prior values were calculated using a two-step approach (Bollback, 2009). First, overall evolutionary rate values (a discrete gamma prior; α and β) with 60 categories were estimated for each of the characters separately, and in addition a bias value (a discrete symmetrical beta prior; α) with 31 categories was estimated for the two-state characters (carotenoids and guttulation), in SIMMAP using an MCMC approach (Schultz and Churchill, 1999). The MCMC analysis for the priors used the fifty percent majority rule consensus tree from our combined MrBayes analyses (Section 2.4), 100,000 cycles, sampling every 200 generations, using a burnin of 10,000 and an upper rate bound of 1000. All characters were unordered. Second, using the samples from the posterior distribution of these parameters, the best fitting gamma and beta distribution were estimated in the R statistical package version 2.15.2 (R Foundation for Statistical Computing), script available in SIMMAP. For multi-state characters the MCMC analyses were conducted twice, with an equal (1/k) and empirical bias prior, respectively, to explore the impact of these two options on the results. Marginal posterior probabilities for ancestral character states were calculated using 8000 post burn-in trees (using the last 1000 trees from each of the eight runs from the combined MrBayes analysis; Section 2.4), with 10 samples for each tree and 10 priors drawn (using the priors as determined above). Plotting of the SIMMAP results were performed using the R script "PlotSimMap.R" (available at http://github.com/nylander/PlotSimMap).

3. Results

3.1. Nucleotide sequences and introns

Three hundred ninety-four sequences were newly generated in this study (Table 1), including 124 RPB1, 120 EF-1α, 99 RPB2 and 51 LSU sequences. These were analyzed together with 19 RPB2 and 85 LSU sequences from our previous studies (e.g. Hansen et al., 2005a,b; Perry et al., 2007) and 43 sequences obtained from GenBank (including 35 sequences determined by the AFTOL project). Four datasets were produced of LSU, RPB1, RPB2 and EF-1α from 142 taxa. Of the 142 taxa included in the combined dataset, 10 taxa lack RPB1, 13 taxa lack RPB2 and 14 taxa lack EF-1α (Table 1). In the combined dataset, sequences for all four markers were available for 80% of the taxa. For those taxa with missing data, at least two of the four DNA markers were available.

The RPB1 alignment consisted of 663 bp and the EF-1α of 1095 bp (both excluding introns). The nearly complete coding region of EF1-α was obtained for most collections, but for six collections only the first 920–996 bp segment spanning the first 2/3 of the 5′ end was obtained, and for another seven collections, only the first 614–797 bp were obtained. The RPB2 alignment consisted of 1624 bp (excluding introns). Complete sequences spanning regions 6–11 were obtained for most collections, but for 21 only the 6–7 region was obtained (ca. 720 bp; including sequences from our previous studies). For *Genea harknessii* and *Scutellinia* cf. *subhirtella*, only the 7–11 region (784 and 906 bp, respectively) was obtained. An ca. 900 bp region of LSU was sequenced for most collections. See Table 3 for proportions of variable and parsimony informative characters for the different data partitions.

Spliceosomal intron positions in the protein coding nuclear genes were recognized by sequence comparisons and the conserved dinucleotide sequences at the intron ends (GT or very rarely GC at start and AG at end). The introns ranged in length from 42 to 99 bp. The A–C region of RPB1 contained two closely spaced spliceosomal introns at the 5′ end of the gene, whose combined length was 161 bp. The first intron occupies a phase 1 insertion with respect to the reading frame, while the second intron has a phase 0 insertion. The two introns were present in all Pezizomycetes. The RPB1 region included four indels in Pezizomycetes, the first varying between groups from a 3 to 30 bp deletion; the second a 3 bp insertion in the *Scutellinia–Kotlabaea* clade (not present in *Scutellinia* sp. A); and the third a 3 bp insertion in the A lineage, except for the *P. gerardii–Marcelleina* clade.

The 6–11 region of RPB2 contained four spliceosomal introns within Pezizomycetes whose combined length was 311 bp. The first intron was located between the 6–7 and 7–11 regions, and the last three were located towards the 3′ end of the 7–11 region. The first two introns have phase 0 insertions with respect to the reading frame, and introns 3 and 4 have phase 1 and 2 insertions, respectively. Introns 1 and 2 are shared by most Pezizomycetes, whereas introns 3 and 4 are present only in some families or lineages. Although intron positions appear lineage-specific, homoplasy is evident. For example, the *Pyronema*, *Lasiobolidium* and *Pseudombrophila*-lineages, the *Genea–Humaria* clade (Pyronemataceae), and the families Glaziellaceae, Discinaceae–Morchellaceae and Sarcoscyphaceae all lack intron 2. Intron 3 is present only in lineage A (except for *Pachyella babingtonii*), Caloscyphaceae, Helvellaceae and Rhizinaceae; and intron 4 only in Pezizaceae (except *P. babingtonii*), Helvellaceae and Rhizinaceae. The RPB2 6–7 region included three small indels within Pezizomycetes that varied from 3 to 12 bp, being restricted to a smaller number of taxa, but only the third appearing clade specific: a 3 bp insertion in the Discinaceae–Morchellaceae clade.

The EF1-α contains 16 spliceosomal introns within Pezizomycetes, placed throughout the region. Their combined length is 946 bp. Eight of the introns (nos. 3, 4, 6, 8, 11, 12, 14 and 16) occupy phase 0 insertions with respect to the reading frame, and 6 of the introns (nos. 2, 5, 7, 9, 13 and 15) phase 1 insertions, while only intron 10 has a phase 2 insertion. Intron 1 was placed in the beginning of the sequences and was not sequenced in its full length, and therefore the position of the insertion could not be determined. Introns 1 and 2 are present in all Pezizomycetes sequenced. Introns 9 and

Table 3
Phylogenetic information of LSU, RPB1, RPB2 and EF1 individual and combined data sets, based on parsimony analyses.

Data sets	No. of taxa	Total characters included	Constant characters	Parsimony informative characters	Percent informative characters[a,b] (%)	No. MP trees	MPT length	CI	RI	No. of pezizomycetous clades ⩾75%/⩾85% MP-BP
LSU rDNA	142	1055	526	425	40.28	>15,000	4289	0.242	0.603	60/50
LSU using MAFFT	142	1088	555	420	38.60	1104	4471	0.236	0.599	57/53
LSU using MAFFT and Gblock	142	757	451	247	32.63	>15,000	1870	0.281	0.644	51/39
RPB1, all sites	132	663	166	456	68.78	4	8605	0.129	0.569	64/54
RPB1, 1 and 2 codons		442	153	250	54.82					
RPB1, 3 codons		221	13	206	45.18					
RPB2, all sites	129	1624	615	928	57.14	32	16,841	0.122	0.523	72/58
RPB2, 1 and 2 codons		1083	594	413	44.50					
RPB2, 3 codons		541	21	515	55.50					
EF1, all sites	129	1095	514	494	45.11	4	6740	0.157	0.497	39/35
EF1, 1 and 2 codons		730	504	142	28.74					
EF1, 3 codons		365	10	352	71.26					
LSU, RPB1, RPB2, EF1	142	4437	1821	2303	51.90	4	36,916	0.143	0.533	98/91 Excl. 3rd codons 98/88

[a] For data sets including all sites: percent informative characters out of total number of characters for individual data sets.
[b] For data sets per codon positions: percent informative characters out of total number of informative characters in individual data sets including all sites.

12 are present in all taxa of lineage C, except the *Pyronema* and *Pyropyxis* lineages lack both introns; Chorioactidaceae, Sarcoscyphaceae, Sarcosomataceae, *Lasiobolidium orbiculoides*, *Lasiobolus papillatus* lack intron 9; and the *Lasiobolidium* lineage, *Pyronema omphalodes* and *Lasiobolus papillatus* lack intron 12. The Chorioactidaceae, Sarcoscyphaceae, Sarcosomataceae share a unique intron position (intron 8), with the spliceosomal site differing only 1 bp from intron 9, and we consider this (a novel) position to be caused by intron sliding (relocation of a preexisting intron within a gene) (Lehmann et al., 2010). Introns 3 and 7 are present in the B-lineage, Caloscyphaceae and Rhizinaceae; intron 15 only in the B-lineage; intron 13 in Helvellaceae and Discinaceae; and intron 14 only in Rhizinaceae. Introns 4 and 10 are only present in Pezizaceae; and intron 5 only in Ascobolaceae (*A. crenulatus*). Intron 16 is present in Pezizaceae and Caloscyphaceae. Introns 6 and 11 are present only in a single species *Lasiobolus papillatus*. The EF1-α included only a 3 bp insertion in Pezizomycetes (also present in *Neolecta*) restricted to Pyronemataceae *s. str.* (as defined in the current paper), except it was present also in *Peziza gerardii*, *Rhizina undulata* and *Pseudombrophila theioleuca* (DHP 3498).

3.2. Congruence and phylogenetic signal among data partitions

The phylogenies derived from the separate LSU, RPB1, RPB2 and EF-1α datasets did not possess any strongly supported incongruence and were therefore combined. The RPB2 exons account for the greatest number of putative parsimony informative characters (PIC) within the combined dataset (40.30%), followed by the EF-1α (21.45%) and RPB1 exons (19.80%), and the LSU (18.45%). Nevertheless, RPB1 exhibits 1.5–1.7 times more phylogenetic signal than EF-1α and LSU per sequenced base pairs, and 1.2 times more than the RPB2 (Table 3), which is in agreement with the profile of phylogenetic informativeness found for these genes across relative dates of all of the Ascomycota (Schoch et al., 2009). Also the RPB1 recovered almost as many pezizomycetous clades with MP-BP ⩾ 85% as the RPB2 (54 vs. 58 clades) despite the much shorter region sequenced (663 vs. 1624 bp). All three protein-coding genes exhibit higher phylogenetic signal than the LSU based on number of PIC, although the EF-1α only 1.1 times more than the LSU region. The LSU recovered more pezizomycetous clades with MP-BP ⩾ 85% than the EF-1α (50 vs. 35 clades). MP analyses of the individual RPB1 and EF-1α alignments resulted in four MPT's, the RPB2 in 32 MPTs, but the LSU in more than 15,000 MPTs. The CI and RI suggest that there is less homoplasy and a higher amount of synapomorphies in the LSU data set compared to the other data sets (Table 3). However, the fact that no data are missing in the LSU data set likely affects these figures. LSU sequences are highly variable across Pezizomycetes and therefore the reliability of the alignment was further explored using MAFFT and Gblocks. The original LSU alignment obtained by manual adjustment in Se–Al contains 1055 bp with 425 PIC; using MAFFT the alignment contains 1088 bp with 420 PIC; and using MAFFT with the elimination of ambiguous regions as identified with Gblocks, only 757 bp with 247 PIC. The topology from MP analysis of the LSU alignment obtained using MAFFT and Gblocks was similar, but less resolved and with fewer highly supported nodes as measured by MP-BP (see Table 3). Similarly, in combined analyses of the four-gene dataset, including the LSU alignment obtained using MAFFT and Gblocks, the topology was identical to that recovered using the LSU alignment obtained by manual adjustment in Se–Al, except the strict consensus tree was slightly less resolved (eight MPTs were recovered, compared to four MPTs using the Se–Al alignment) and the MP-BP were lowered for a majority of the clades. The number of clades receiving MP-BP ⩾ 75% and ⩾85% were lowered from 98 and 92, to 97 and 89, respectively. Based on this, we conclude that the LSU alignment obtained by manual adjustment and including all characters is reliable. Although this alignment may be introducing some noise into the dataset, it adds more phylogenetic signal than when excluding nearly half of the putative PIC by using Gblocks. The LSU MAFFT alignment including all characters recovered a topology very similar to the topology recovered from the manual alignment, with similar levels of support, CI and RI values (Table 3).

Third codon positions of each of the protein-coding genes provided the most PIC, with the most pronounced percentage in the EF-1α region (71%) compared to the RPB2 and the RPB1 (56% and 45%, respectively). The combined four-gene dataset included 2303 PIC, with 1073 (47%) provided by third codon positions.

3.3. The combined four-gene phylogeny

Parsimony analyses of all four genes yielded four equally parsimonious trees (Table 3) (length 36,916 steps; CI: 0.143; RI: 0.533).

The strict consensus tree is nearly completely resolved; the four MPTs differ only in minor arrangements between closely related species of *Tarzetta*, and among *Lasiobolidium spirale* and two collections of Pyronemataceae sp. A (Suppl. Fig. 1). In the searches with RAxML the four-gene alignment had 3436 distinct patterns with a proportion of gaps and undetermined characters of 20.71%. The partitioned ML analyses recovered a single best scoring tree of $-\ln L = 14{,}5202.28$ (Suppl. Fig. 2). The Bayesian analyses of the combined dataset halted after 28,000,000 generations, the last half of each tree sample being stationary. A majority rule consensus tree was constructed from the 1,120,008 trees sampled from the 8 runs, each consisting of 140,001 trees sampled from the stationary tree distribution (Fig. 2).

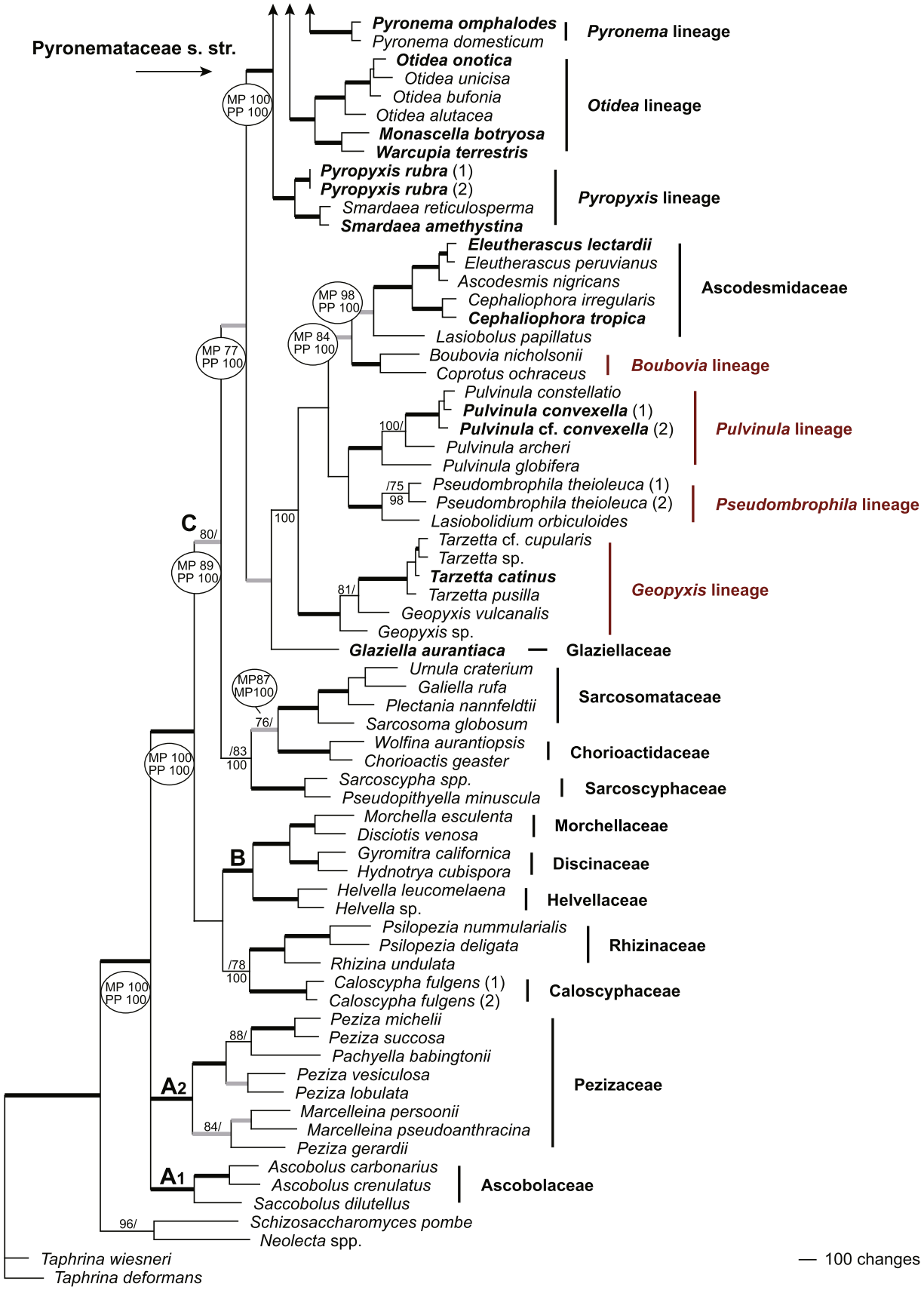
Parsimony analysis of the four-gene dataset excluding third codon positions yielded six MPTs (length 10,886; CI: 0.251; RI: 0.647). The supported topology (MP-BP $\geqslant$ 75%) did not differ from the supported topology recovered by MP analysis including all characters, except for a few shallow branches that were collapsed or unsupported when third codon positions were excluded. The MP-BP values for all the deeper branches rose however, when third-codon positions were excluded (Fig. 2 and Suppl. Fig. 1). In the Bayesian analysis of the combined data excluding all third codon positions, the average standard deviation between runs never descended below 0.01. The analyses were run for 40 M generations and the average standard deviation between runs only reached 0.0237. A majority rule consensus tree was constructed from the 640,008 trees sampled from the eight runs, each containing 80,001 trees of the stationary tree distribution (the first 50% discarded as the burn-in). The supported (PP $\geqslant$ 95%) topology recovered differed only in a few shallow branches of the phylogeny, among taxa within the 10 lineages identified within Pyronemataceae *s. str.*, and in the placement of Caloscyphaceae and Rhizinaceae, compared to the topology recovered from Bayesian analysis including all positions (Fig. 2). This tree will therefore only be mentioned in relation to these different groupings. The number of pezizomycetous clades with PP $\geqslant$ 95% was lowered from 116 to 112 clades in Bayesian analysis without third codon positions.

The ML and Bayesian analyses produced topologies with basal branching patterns similar to those recovered in the parsimony analyses, but differed in the relationships among the major lineages of Pyronemataceae. These relationships are, however, resolved with low support. The molecular phylogeny presented here is based on the most character intensive sampling (4437 bp) of Pyronemataceae and sister families to date. It resulted in 88 strongly supported internodes (PP = 100, ML- and MP-BP $\geqslant$ 85%) and an additional 17 internodes with high support from the model-based analyses (PP = 100, ML-BP $\geqslant$ 85%), indicated as thick black and gray internodes, respectively, in Fig. 2. Ten of the latter internodes were also strongly supported by MP-BP in analyses without third codon positions (Fig. 2). Three main lineages are identified by ML analyses that correspond to the A, B and C lineages resolved previously (see Hansen and Pfister, 2006) (Suppl. Fig. 2). In the Bayesian and MP analyses Ascobolaceae and Pezizaceae (lineage A) are not resolved as a monophyletic group, but rather form a trichotomy with a strongly supported clade containing the rest of the Pezizomycetes (Fig. 2). The B and C lineages are each highly supported in nearly all analyses, the exception being the C lineage, which received only moderate MP support (MP-BP 80%) when third codon positions were included. In MP analyses excluding third codon positions, the MP-BP for the C lineage rose to 89%. All analyses resolve Rhizinaceae and Caloscyphaceae as part of the B lineage (but without support), except Bayesian analyses excluding third codon positions that support the Caloscyphaceae–Rhizinaceae as a distinct sister lineage to the B and C lineages (PP 99%). Rhizinaceae and Caloscyphaceae are supported as a monophyletic group in Bayesian and ML analyses (PP = 100, ML-BP = 78%). Confirming our previous results (Hansen and Pfister, 2006; Marek et al., 2009), two species of *Psilopezia* (previously Pyronemataceae) are resolved within *Rhizinaceae* (PP 100%, ML-BP 97%, MP-BP 86%; Fig. 2). Sarcoscyphaceae, Sarcosomataceae and Chorioactidaceae form an early diverging monophyletic group within lineage C using model-based analyses (PP 100%, ML-BP 83%). Support for this group is lowered in Bayesian analysis without third codon positions (PP 89%). In parsimony analyses excluding third codon positions, a highly supported Chorioactidaceae–Sarcosomataceae clade (MP-BP 87%), and the Sarcoscyphaceae, are resolved as successive sister groups to the remainder of lineage C, which are supported by MP-BP 77% (Fig. 2 and Suppl. Fig. 1).

All analyses suggest that Pyronemataceae is paraphyletic, as both Ascodesmidaceae and Glaziellaceae are nested within the larger family. A core group of taxa representing a restricted Pyronemataceae is identified with strong support in all analyses, and herein referred to as Pyronemataceae *s. str.* (Fig. 2). Four strongly supported lineages of taxa currently classified in Pyronemataceae, the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages, form a monophyletic group with Ascodesmidaceae in all analyses, plus Glaziellaceae in model-based analyses with high support (PP 100%, ML-BP 92%) and in MP analyses excluding third codon positions, although without support (Table 4). Glaziellaceae is strongly supported as a distinct sister lineage to the rest of the group in Bayesian analyses (PP 100%). The *Boubovia* lineage is recovered as a sister group to Ascodesmidaceae in all analyses, and with support from model-based analyses (PP 100%, ML-BP 91%), and parsimony analyses excluding third codon positions (MP-BP 84%) (Fig. 2). *Coprotus*, currently classified in Thelebolaceae (Leotiomycetes), forms a strongly supported monophyletic group with *Boubovia* (currently Pyronemataceae) in all analyses. The anamorphic genus *Cephaliophora* is nested within Ascodesmidaceae, forming a strongly supported group with species of *Ascodesmis* and *Eleutherascus* (100% support in all analyses). The relationships among the *Geopyxis*, *Pseudombrophila*, *Pulvinula* and Ascodesmidaceae-*Boubovia* lineages are resolved with low support and differ between the MP and model-based analyses. In Bayesian and ML analyses the *Pulvinula* and *Pseudombrophila* lineages are resolved as a monophyletic group, with the Ascodesmidaceae-*Boubovia* and *Geopyxis* lineages as successive sister groups (Fig. 2 and Suppl. Fig. 2), whereas in MP analyses the *Pulvinula* and *Geopyxis* lineages are resolved as a monophyletic group, with the *Pseudombrophila* and Ascodesmidaceae-*Boubovia* lineages as successive sister groups (Suppl. Fig. 1). Table 4 summarizes the support values from the different gene regions and combined analyses for lineage C, Pyronemataceae *s. str.*, the four lineages of pyronemataceous taxa, Ascodesmidaceae and Glaziellaceae.

Ten lineages are identified within Pyronemataceae *s. str*. with strong support in all analyses. To facilitate results and discussion, we have named these as indicated on Fig. 2. *Byssonectria terrestris*, *Gelinipes* sp. and *Paratrichophaea boudieri*, represented by single collections, constitute separate lineages with uncertain placement. Several of the supported lineages are identical to those obtained in previous analyses of LSU sequences including a larger sampling of taxa (Perry et al., 2007), but some are new or otherwise more strongly supported. Relationships among two of the lineages are strongly supported in the model-based analyses; the *Pyropyxis* and *Otidea* lineages are shown as early diverging within Pyronemataceae *s. str.* (both PP 100%; ML-BP 95%, MP-BP 78% and ML-BP 99%, MP-BP 69%, respectively). This placement of the *Pyropyxis* and *Otidea* lineages is also highly supported in parsimony analyses excluding third codon positions (MP-BP 95% and 96%, respectively; Fig. 2). The *Pyropyxis* lineage includes the monotypic *Pyropyxis* and two species of *Smardaea*, *S. amethystina* and *S. reticulosperma* (syn.

Greletia reticulosperma). The *Otidea* lineage includes a broad sampling of *Otidea* spp. and the two monotypic genera *Monascella* and *Warcupia*. The relationships among the other eight lineages within Pyronemataceae *s. str.* are still without support.



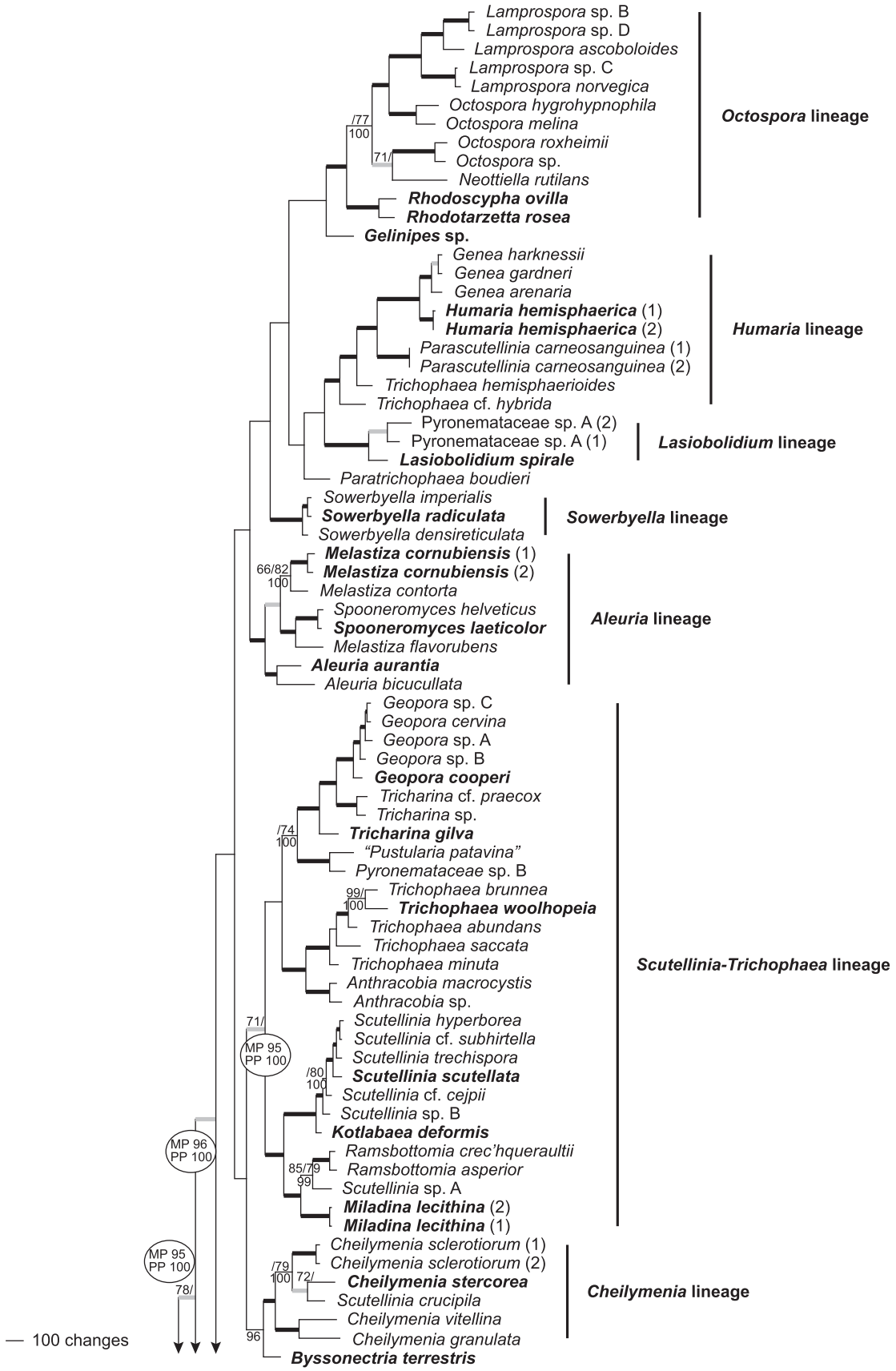


Fig. 2. (*continued*)

Table 4
Parsimony bootstrap support for selected lineages in separate and combined analyses of the LSU, RPB1, RPB2 and EF1 datasets (lineages not present in the strict consensus and bootstrap trees are marked with a dash (–)). Parsimony excluding third codon positions, Bayesian PP and ML-BP are given for the combined dataset.

Lineages	LSU	RPB1	RPB2	EF1	MP combined	Combined excl. 3rd codons	Bayesian PP combined	ML BP combined
Lineage C (±*Glaziella*)	<50 (−*Glaziella*)	–	70 (−*Glaziella*)	–	80 (+*Glaziella*)	89 (+*Glaziella*)	100 (+*Glaziella*)	100 (+*Glaziella*)
Pyronemataceae *s. str.*[a]	–	80	76	–	99	100	100	100
Ascodesmidaceae, *Pulvinula–Geopyxis–Pseudombrophila–Boubovia* (±*Glaziella*)	–	<50 (+*Glaziella*)	<50 (−*Glaziella*)	–	<50 (−*Glaziella*)	55 (+*Glaziella*)	100 (±*Glaziella*)	92 (+*Glaziella*)
Geopyxis lineage	100	–	89	–	100	100	100	100
Pseudombrophila lineage	96	95	100	74	100	100	100	100
Pulvinula lineage	94	89	93	53	100	96	100	100
Geopyxis–Pulvinula lineages	<50	–	82	–	90	52	–	–
Ascodesmidaceae + *Cephaliophora*; – *Lasiobolus*	95	100	100	82	100	100	100	100
Ascodesmidaceae + *Cephaliophora* and *Lasiobolus*	89	–	–	–	67	98	100	100
Ascodesmidaceae + *Cephaliophora*, *Lasiobolus* and *Boubovia* lineage	–	–	–	–	–	84	100	91

Missing taxa from the individual datasets with regard to Ascodesmidaceae and the *Pulvinula*, *Geopyxis*, *Pseudombrophila* and *Boubovia* lineages: LSU: All taxa included; RPB1: *Coprotus ochraceus*, *Tarzetta catinus*, *Pulvinula constellatio;* RPB2: *Pseudombrophila theioleuca* (1) and *Pulvinula* cf. *convexella* (2); EF1: *Tarzetta catinus*.
[a] Pyronemataceae excluding the *Boubovia–Geopyxis–Pulvinula–Pseudombrophila* lineages.

3.4. Character mapping and ancestral state reconstructions

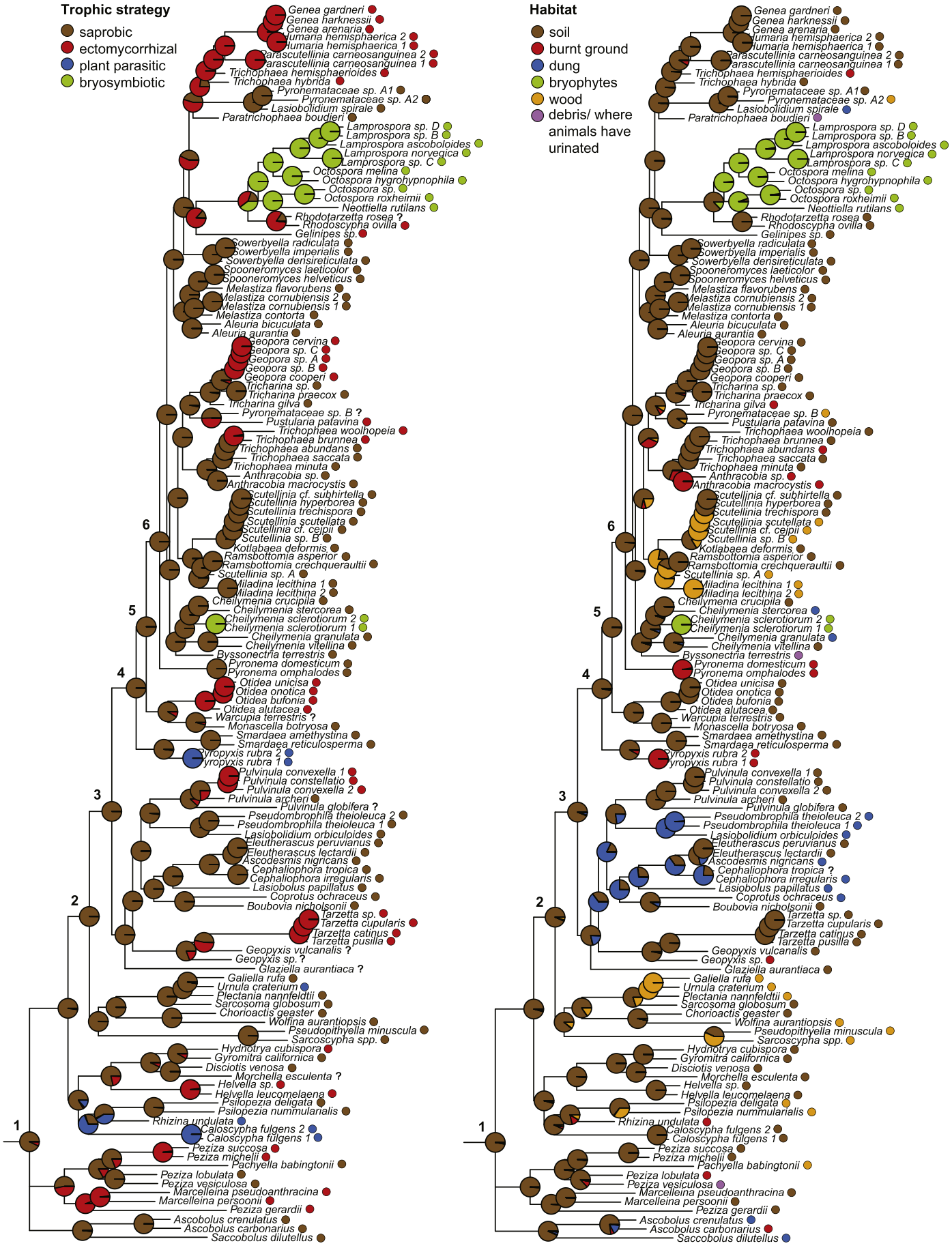
Ancestral states are shown for all nodes, plotted on the fifty percent majority rule consensus tree from the Bayesian analysis as pie charts (Figs. 3 and 4 and Suppl. Fig. 3). The total values of pie charts are the number of Bayesian trees with that node present out of a possible 8000 post burn-in trees. Six selected nodes that were significantly supported by Bayesian PP and bootstrap are summarized in Table 5. The Bayesian ASR of trophic strategies (trait 1) found a high probability that the ancestral state for all deeper nodes in Pezizomycetes and in nodes 3–6, was saprobic (Fig. 3, nodes 1–6). Ectomycorrhizae are shown to have arisen within both lineages A, B and C. Within Pyronemataceae *s. l.* ectomycorrhizae are suggested to have evolved 7–8 times: once in each of the *Geopyxis*, *Pulvinula* and *Otidea* lineages; three times in the *Scutellinia–Trichophaea* lineage; and once or twice in the *Octospora–Humaria* clade (the node uniting these two lineages are without support in our separate analyses and unreliable). Obligate bryosymbiotism has originated only twice within Pyronemataceae *s. str.*: in the *Octospora* lineage and once in the genus *Cheilymenia*. Plant pathogenicity has originated only once in Pyronemataceae *s. str.* (in the *Pyropyxis* lineage), and in the ancestral node of the Rhizinaceae–Caloscyphaceae clade and in *Urnula craterium* (Sarcosomataceae). Transitions to ectomycorrhizal, plant pathogenic and bryosymbiotic modes appear in all cases to have been from a saprobic ancestor. A single exception remains uncertain – that is the most recent ancestor of the *Octospora* lineage. It is equally likely to have been ectomycorrhizal ($P = 0.4069$) or bryosymbiotic ($P = 0.4004$) (the node uniting the *Octospora* lineage with *Gelinipes* is without support in separate analyses and therefore disregarded).

Reconstruction of the habitat (here considered substrate on which ascomata are produced) found a high probability that the ancestral state for the deepest nodes of Pezizomycetes and nodes 3–6 was soil (Fig. 3). The results show that a specialization to burnt ground (a pyrophilous lifestyle) has happened multiple times. A switch to dung (a coprophilous lifestyle) is shown to have happened at least three times within Pyronemataceae: in the Ascodesmidaceae-*Boubovia–Pseudombrophila*, *Cheilymenia* and *Lasiobolidium* lineages. Also a transition to wood as the sole habitat has happened at least three times in Pyronemataceae: in the ancestor of the *Scutellinia–Miladina* clade, in Pyronemataceae sp. B, and in the *Lasiobolidium* lineage. It may then subsequently have been reverted to soil twice within the *Scutellinia–Miladina* clade: in *Ramsbottomia* and within *Scutellinia*. A transition to mosses as substrate has happened at least twice within Pezizomycetes.

Carotenoids in the ascomata were found with high probability to be absent in all of the deepest nodes of Pezizomycetes (nodes 1–4, Fig. 4). The probability for carotenoids in node 5 is still low ($P = 0.3650$), whereas the probability for carotenoids in node 6 is high ($P = 0.9955$). The results suggest that carotenoids have been gained at least four times in Pezizomycetes: in *Caloscypha*(ceae), Sarcoscyphaceae, in the clade including *Pulvinula* and *Glaziella*(ceae), and in Pyronemataceae *s. str.* It is likewise suggested that carotenoids have been lost at least twice within Pyronemataceae *s. str.*: in the *Scutellinia–Trichophaea* lineage and in the *Humaria–Lasiobolidium* lineages – and possibly regained: in *Anthracobia*.

Hairs were found with high probability to be absent from ascomata in the deepest nodes of Pezizomycetes, and in the ancestor of the *Pulvinula*-Glaziellaceae clade (Fig. 4). The ancestor of Pyronemataceae *s. str.* (node 4) also likely produced ascomata without hairs, although the probability for this is lower ($P = 0.7865$). Hyphoid hairs are shown to have arisen several times in Pyronemataceae *s. l.* The exact number of gains and losses of true hairs within Pyronemataceae *s. str.* remains uncertain, because the relationships among the 10 lineages in our separate analyses are unresolved (Fig. 2). Nevertheless, true hairs (superficial or rooting) are restricted to Pyronemataceae *s. str.* excluding the *Pyropyxis* and *Otidea* lineages (node 6), and *Lasiobolus* (Ascodesmidaceae), the *Pseu-*

Fig. 2. Phylogenetic relationships of Pyronemataceae among members of Pezizomycetes based on Bayesian analysis of combined RPB1, RPB2, EF-1α and LSU sequences (4437 bp dataset). Fifty percent majority rule consensus tree of 1,120,008 trees obtained by MCMCMC under the GTR+I+G model applied across ten partitions. Thick black branches received high support in all three analyses conducted (Bayesian posterior probabilities (PP) = 100%, maximum likelihood bootstrap (ML-BP) and parsimony bootstrap (MP-BP) values ⩾85%), thick gray branches received high support in the model-based analyses but not under parsimony. Support values below 70% are not shown, other values are MP-BP/ML-BP above branches and PP below. Bar indicates the nucleotide substitutions per site. Support values from parsimony and Bayesian analyses excluding third codon positions shown in circles (MP-BP and PP). Type species of genera in Ascodesmidaceae, Glaziellaceae and Pyronemataceae are highlighted in bold. Additional families sampled are indicated, and the three main lineages of Pezizomycetes are labeled as A, B and C. Ten strongly supported lineages within Pyronemataceae *s. str.* are indicated for discussion in the text. Four lineages of pyronemataceous members resolved outside Pyronemataceae *s. str.* and with uncertain placement are highlighted in red.


Trophic strategy
saprobic
ectomycorrhizal
plant parasitic
bryosymbiotic
Habitat
soil
burnt ground
dung
bryophytes
wood
debris/ where animals have urinated
Genea gardneri
Genea harknessii
Genea arenaria
Humaria hemisphaerica 2
Humaria hemisphaerica 1
Parascutellinia carneosanguinea 2
Parascutellinia carneosanguinea 1
Trichophaea hemisphaerioides
Trichophaea hybrida
Pyronemataceae sp. A1
Pyronemataceae sp. A2
Lasiobolidium spirale
Paratrichophaea boudieri
Lamprospora sp. D
Lamprospora sp. B
Lamprospora ascoboloides
Lamprospora norvegica
Lamprospora sp. C
Octospora melina
Octospora hygrohypnophila
Octospora sp.
Octospora roxheimii
Neottiella rutilans
Rhodotarzetta rosea ?
Rhodoscypha ovilla
Gelinipes sp.
Sowerbyella radiculata
Sowerbyella imperialis
Sowerbyella densireticulata
Spooneromyces laeticolor
Spooneromyces helveticus
Melastiza flavorubens
Melastiza cornubiensis 2
Melastiza cornubiensis 1
Melastiza contorta
Aleuria bicuculata
Aleuria aurantia
Geopora cervina
Geopora sp. C
Geopora sp. A
Geopora sp. B
Geopora cooperi
Tricharina sp.
Tricharina praecox
Tricharina gilva
Pyronemataceae sp. B ?
Pustularia patavina
Trichophaea woolhopeia
Trichophaea brunnea
Trichophaea abundans
Trichophaea saccata
Trichophaea minuta
Anthracobia sp.
Anthracobia macrocystis
Scutellinia cf. subhirtella
Scutellinia hyperborea
Scutellinia trechispora
Scutellinia scutellata
Scutellinia cf. cejpii
Scutellinia sp. B
Kotlabaea deformis
Ramsbottomia asperior
Ramsbottomia crechqueraultii
Scutellinia sp. A
Miladina lecithina 1
Miladina lecithina 2
Cheilymenia crucipila
Cheilymenia stercorea
Cheilymenia sclerotiorum 2
Cheilymenia sclerotiorum 1
Cheilymenia granulata
Cheilymenia vitellina
Byssonectria terrestris
Pyronema domesticum
Pyronema omphalodes
Otidea unicisa
Otidea onotica
Otidea bufonia
Otidea alutacea
Warcupia terrestris ?
Monascella botryosa
Smardaea amethystina
Smardaea reticulosperma
Tyropyxis rubra 2
Tyropyxis rubra 1
Pulvinula convexella 1
Pulvinula constellatio
Pulvinula convexella 2
Pulvinula archeri
Pulvinula globifera ?
Pseudombrophila theioleuca 2
Pseudombrophila theioleuca 1
Lasiobolidium orbiculoides
Eleutherascus peruvianus
Eleutherascus lectardii
Ascodesmis nigricans
Cephaliophora tropica ?
Cephaliophora irregularis
Lasiobolus papillatus
Coprotus ochraceus
Boubovia nicholsonii
Tarzetta sp.
Tarzetta cupularis
Tarzetta catinus
Tarzetta pusilla
Geopyxis vulcanalis ?
Geopyxis sp. ?
Glaziella aurantiaca ?
Galiella rufa
Urnula craterium
Plectania nannfeldtii
Sarcosoma globosum
Chorioactis geaster
Wolfina aurantiopsis
Pseudopithyella minuscula
Sarcoscypha spp.
Hydnotrya cubispora
Gyromitra californica
Disciotis venosa
Morchella esculenta ?
Helvella sp.
Helvella leucomelaena
Psilopezia delgata
Psilopezia nummularialis
Rhizina undulata
Caloscypha fulgens 2
Caloscypha fulgens 1
Peziza succosa
Peziza michelii
Pachyella babingtonii
Peziza lobulata
Peziza vesiculosa
Marcelleina pseudoanthracina
Marcelleina persoonii
Peziza gerardii
Ascobolus crenulatus
Ascobolus carbonarius
Saccobolus dilutellus
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dombrophila lineage, the Chorioactidaceae–Sarcoscyphaceae–Sarcosomataceae clade and some taxa of Tuberaceae (not included). True, rooting hairs are unique to Pyronemataceae *s. str.*

For the majority of nodes, the absence or presence of oil guttules in the ascospores was recovered with equivocal character states in the ASR, highlighting that this character is fast evolving and prone to shifts (Suppl. Fig. 3). States of even the most common ancestors of several closely related species or genera in Pyronemataceae *s. l.* were not reconstructed with high confidence. The ancestors of Pyronemataceae *s. str.* (node 4), and nodes 5–6 were suggested to have spores with guttules ($P = 0.7993$; $P = 0.8688$; $P = 0.7828$), and guttules have likely been lost several times within the family.

4. Discussion

4.1. Usefulness of RPB1, RPB2, EF-1α and LSU rDNA in Pezizomycetes phylogenetics

In this study we added sequences from portions of three protein-coding genes (RPB1, RPB2 and EF-1α) to our LSU dataset for Pezizomycetes and Pyronemataceae, increasing the number of nucleotides in the final data set from 842 to 4437 bp. Although the number of taxa sampled is lower in the current study, 93 pyronemataceous species (40 genera) compared to 140 species (55 genera) (Perry et al., 2007), the analyses answers several of the questions we were left with previously and identifies a much more robust phylogeny, especially in the deeper branches of the tree (Fig. 2). Consistent with prior studies, the RPB1, RPB2 and EF-1α contribute a high percentage of putative PIC per sequenced base pair. The variable region of RPB1 (A–C) and RPB2 (6–11) appear highly informative. The combination of all four loci produces the most resolved phylogeny and the highest number of strongly supported clades (Fig. 2, Table 3) within Pezizomycetes to date. We conclude that the third codon positions are not markedly saturated across Pezizomycetes, because the topologies retrieved from Bayesian analyses of the four-gene dataset with or without third codon positions are identical in the deep divergences, except for one node, and posterior probabilities were lower for a few nodes without third codons. In the unweighted parsimony analyses, which do not account for differences in substitution rates, the impact from third codon positions was striking. This is not unexpected, given that these positions are prone to saturation and constitute 47% of the putative PIC in our four-gene dataset. The MP-BP support values rose for all deeper nodes in the combined phylogeny when excluding third codon positions. At the same time, the total number of clades with high support stayed the same or was lowered slightly (Table 3). Support for more shallow branches was reduced when excluding third codon positions. Similar results with increases in parsimony bootstrap values for deeper nodes without third codons, in weighted and/or unweighted pasimony, have been noted in other studies within the Ascomycota (e.g. Schoch et al., 2006; Zhang et al., 2006) and Basidiomycota (Matheny et al., 2007). Third codon positions in the RPB2 and EF-1α, across the Basidiomycota, were found to be saturated and were recommended to be excluded at higher taxonomic levels (Matheny et al., 2007). The EF-1α in our data appears to be most prone to saturation. It holds 71% of the PIC at third codons positions and recovers a low number of well-supported pezizomycetous clades compared to the other gene regions, despite a relatively high number of putative PIC (21% of the PIC in the four-gene dataset). In a previous study of Pezizomycetes (Hansen et al., 2005a) we discovered an even higher percentage of PIC in third codons (82%) in a region of the β-tubulin. This region showed strong indication of third codon saturation among the most divergent taxa, with erroneous placements and inability to resolve relationships among lineages. The EF-1α in the current dataset resolved much fewer clades with moderate and strong bootstrap support compared to the other gene regions (Table 3), but did not recover obviously erroneous branches. In a study of the Lecanoromycetes, exploring RPB1 (A–F region) and RPB2 (7–11 region), most of the phylogenetic signal came from third codon positions that were found not to be saturated (Hofstetter et al., 2007). In the current study it appears that although the largest amount of putative PIC are third codons, a large majority of the signal still comes from first and second codons, especially at the deeper divergences. Our best estimate of the deeper branches of the phylogeny is the total character model-based trees, and the parsimony tree from analyses excluding third codon positions. For the more recent divergences (shallow branches) the total character trees of all analyses are credible (third codons adding more information, also under parsimony).

The Pezizomycetes protein-coding gene sequences align easily and have few indels, but in agreement with other studies of fungi (e.g. Liu et al., 1999; Matheny et al., 2007) positions of introns are highly variable across RPB2 and EF-1α at broad taxonomic levels. Liu et al. (1999) reported three introns in *Peziza quelepidotia* in the RPB2 gene and we reported one additional in a previous study primarily on Pezizaceae (Hansen et al., 2005a); the four RPB2 introns we report here correspond to those. The introns in RPB2 are conserved by position, but appear to have been lost or gained too frequently over the course of evolution to be useful as phylogenetic markers. Twenty-two unique spliceosomal intron positions were recorded in the EF-1α, and seven in RPB2, across the Basidiomycota (Matheny et al., 2007). We report 16 different spliceosomal intron sites in the EF-1α region sequenced within the Pezizomycetes. Several of the introns within EF-1α appear to be lineage specific, and despite the occurrence of homoplasy, intron positions may provide a source of molecular evidence to delineate lineages at different taxonomic levels within the class. For example, the EF-1α introns 3 and 7 support the placement of Caloscyphaceae and Rhizinaceae in the B-lineage, which is in agreement with a recent study across all of the Ascomycota (Schoch et al., 2009). Intron 8 supports a monophyletic Chorioactidaceae–Sarcosomataceae–Sarcoscyphaceae clade. Since the placement of these families previously lacked strong support, these intron positions add significant information. Introns 9 and 12 give support to lineage C, although it appears that these introns were lost in a few clades or species, and intron 15 supports lineage B. In addition several introns were restricted to families (Ascobolaceae, Pezizaceae and Rhizinaceae). A denser taxon sampling from families other than Pyronemataceae however, is required to more fully understand the pattern of the EF-1α introns across Pezizomycetes. Matheny et al. (2007) found intron positions in EF-1α and RPB2 conserved enough to distinguish and characterize several higher taxonomic groups of Basidiomycota, and Landvik et al. (2001) used intron gains and losses in the β-tubulin gene as a phylogenetic character within the Ascomycota.

Fig. 3. Reconstruction of ancestral states of trophic strategies and substrate for ascomata production in Pyronemataceae and sister families, using a Bayesian approach as implemented in SIMMAP ver. 1.5. Posterior probabilities for each character state are given as pie charts, plotted on the Bayesian consensus tree from the combined four-gene analysis (presented in Fig. 2). Character states for each species are indicated after species names. Uncertain state for a taxon is given as "?". Six nodes (1–6) are labeled for discussion and the posterior probabilities for these are given in Table 5. Outgroup taxa not shown.

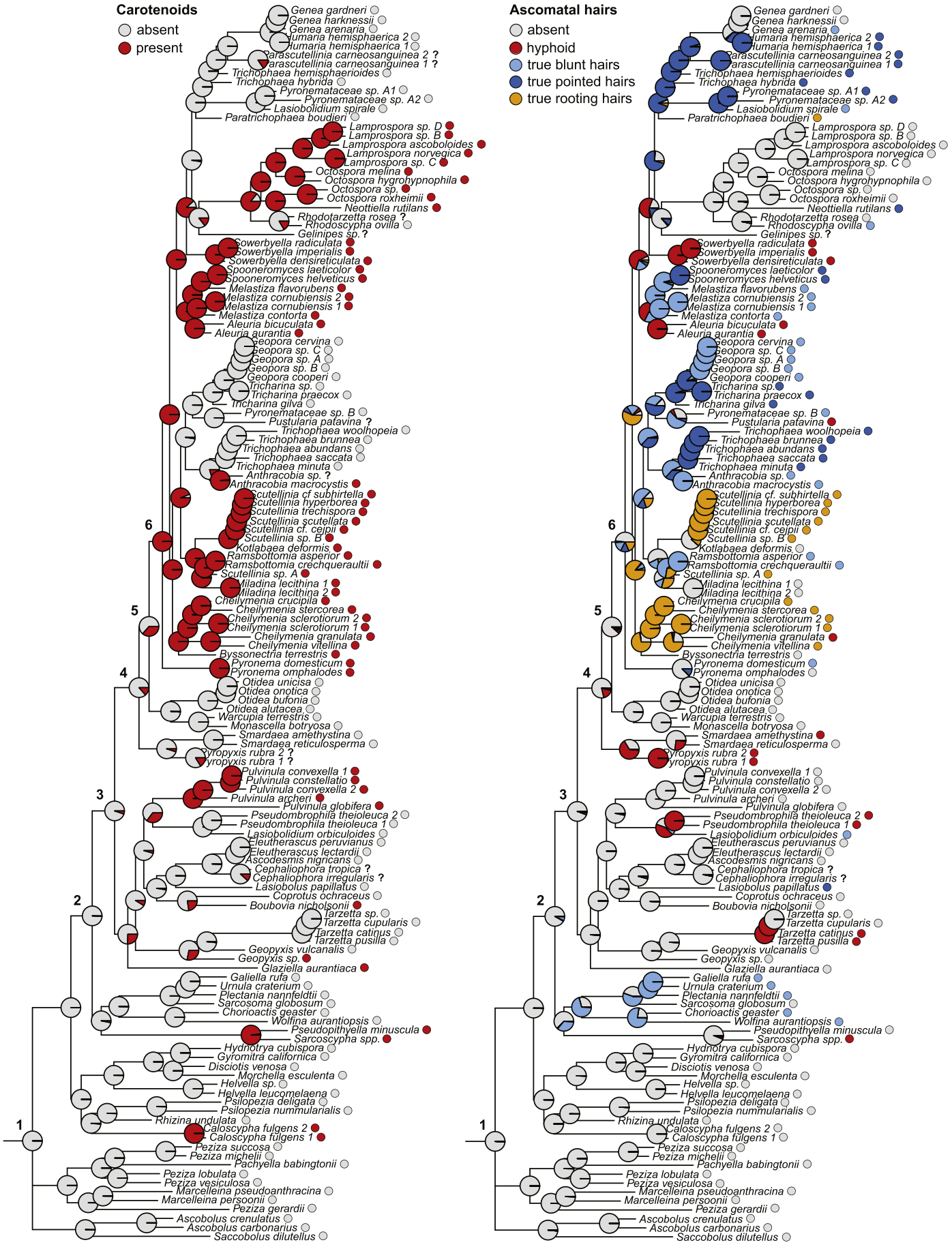
Carotenoids
absent
present
Ascomatal hairs
absent
hyphoid
true blunt hairs
true pointed hairs
true rooting hairs
Genea gardneri
Genea harknessii
Genea arenaria
Humaria hemisphaerica 2
Humaria hemisphaerica 1
Parascutellinia carneosanguinea 2 ?
Parascutellinia carneosanguinea 1 ?
Trichophaea hemisphaerioides
Trichophaea hybrida
Pyronemataceae sp. A1
Pyronemataceae sp. A2
Lasiobolidium spirale
Paratrichophaea boudieri
Lamprospora sp. D
Lamprospora sp. B
Lamprospora ascoboloides
Lamprospora norvegica
Lamprospora sp. C
Octospora melina
Octospora hygrohypnophila
Octospora sp.
Octospora roxheimii
Neottiella rutilans
Rhodotarzetta rosea ?
Rhodoscypha ovilla
Gelinipes sp. ?
Sowerbyella radiculata
Sowerbyella imperialis
Sowerbyella densireticulata
Spooneromyces laeticolor
Spooneromyces helveticus
Melastiza flavorubens
Melastiza cornubiensis 2
Melastiza cornubiensis 1
Melastiza contorta
Aleuria bicuculata
Aleuria aurantia
Geopora cervina
Geopora sp. C
Geopora sp. A
Geopora sp. B
Geopora cooperi
Tricharina sp.
Tricharina praecox
Tricharina gilva
Pyronemataceae sp. B
Pustularia patavina ?
Trichophaea woolhopeia
Trichophaea brunnea
Trichophaea abundans
Trichophaea saccata
Trichophaea minuta
Anthracobia sp. ?
Anthracobia macrocystis
Scutellinia cf subhirtella
Scutellinia hyperborea
Scutellinia trechispora
Scutellinia scutellata
Scutellinia cf. cejpii
Scutellinia sp. B
Kotlabaea deformis
Ramsbottomia asperior
Ramsbottomia crechqueraultii
Scutellinia sp. A
Miladina lecithina 1
Miladina lecithina 2
Cheilymenia crucipila
Cheilymenia stercorea
Cheilymenia sclerotiorum 2
Cheilymenia sclerotiorum 1
Cheilymenia granulata
Cheilymenia vitellina
Byssonectria terrestris
Pyronema domesticum
Pyronema omphalodes
Otidea unicisa
Otidea onotica
Otidea bufonia
Otidea alutacea
Warcupia terrestris
Monascella botryosa
Smardaea amethystina
Smardaea reticulosperma
Pyropyxis rubra 2 ?
Pyropyxis rubra 1 ?
Pulvinula convexella 1
Pulvinula constellatio
Pulvinula convexella 2
Pulvinula archeri
Pulvinula globifera
Pseudombrophila theioleuca 2
Pseudombrophila theioleuca 1
Lasiobolidium orbiculoides
Eleutherascus peruvianus
Eleutherascus lectardii
Ascodesmis nigricans
Cephaliophora tropica ?
Cephaliophora irregularis ?
Lasiobolus papillatus
Coprotus ochraceus
Boubovia nicholsonii
Tarzetta sp.
Tarzetta cupularis
Tarzetta catinus
Tarzetta pusilla
Geopyxis vulcanalis
Geopyxis sp.
Glaziella aurantiaca
Galiella rufa
Urnula craterium
Plectania nannfeldtii
Sarcosoma globosum
Chorioactis geaster
Wolfina aurantiopsis
Pseudopithyella minuscula
Sarcoscypha spp.
Hydnotrya cubispora
Gyromitra californica
Disciotis venosa
Morchella esculenta
Helvella sp.
Helvella leucomelaena
Psilopezia delgata
Psilopezia nummularialis
Rhizina undulata
Caloscypha fulgens 2
Caloscypha fulgens 1
Peziza succosa
Peziza michelii
Pachyella babingtonii
Peziza lobulata
Peziza vesiculosa
Marcelleina pseudoanthracina
Marcelleina persoonii
Peziza gerardii
Ascobolus crenulatus
Ascobolus carbonarius
Saccobolus dilutellus
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Table 5
Ancestral state reconstructions of six selected nodes for the five characters: trophic strategies, habitat for formation of ascomata, carotenoids, ascomatal hairs and ascospore guttulation. For each character the states are indicated and the marginal posterior probability of each state at the particular node (marked in Figs. 3 and 4 and Suppl. Fig. 3) reconstructed using Bayesian inference. Marginal posterior probabilities greater than 0.75 are indicated in bold. Bayesian posterior probabilities (PP) / maximum likelihood bootstrap (ML-BP) for each node from separate analyses are indicated underneath the node number.

Node no.	1 100/100	2 100/100	3 100/100	4 100/100	5 100/95	6 100/99
Trophic strategies						
0 = saprobic	**0.9284**	**0.9962**	**0.9979**	**0.9913**	**0.9838**	**0.9995**
1 = ectomycorrhizal	0.0699	0.0016	0.0014	0.0063	0.0159	0.0005
2 = plant parasitic	0.0014	0.0022	0.0007	0.0023	0.0002	0.0000
3 = bryosymbiotic	0.0003	0.0000	0.0000	0.0001	0.0000	0.0000
Habitat for formation of ascomata						
0 = soil	**0.9709**	**0.9330**	**0.9376**	**0.9649**	**0.9895**	**0.9861**
1 = burnt ground	0.0092	0.0112	0.0137	0.0311	0.0093	0.0113
2 = dung	0.0064	0.0082	0.0410	0.0020	0.0002	0.0003
3 = bryophytes	0.0016	0.0008	0.0007	0.0004	0.0001	0.0002
4 = wood	0.0118	0.0468	0.0069	0.0016	0.0009	0.0020
5 = debris or where animals have urinated	0.0001	0.0000	0.0000	0.0000	0.0000	0.0001
6 = on living plants	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Carotenoids						
0 = absent	**0.9982**	**0.9914**	**0.9483**	**0.8656**	0.6350	0.0045
1 = present	0.0018	0.0086	0.0517	0.1344	0.3650	**0.9955**
Hairs						
0 = absent	**0.9887**	**0.9174**	**0.9428**	**0.7865**	**0.8840**	0.4830
1 = hyphoid	0.0015	0.0127	0.0341	0.1755	0.0386	0.0274
2 = true, superficial, blunt	0.0056	0.0637	0.0158	0.0163	0.0289	0.1667
3 = true, superficial, pointed	0.0034	0.0048	0.0055	0.0135	0.0230	0.1398
4 = true, rooting, pointed	0.0008	0.0013	0.0019	0.0082	0.0255	0.1831
Guttules						
0 = absent	0.3403	0.3356	0.2529	0.2007	0.1312	0.2172
1 = present	0.6597	0.6644	0.7471	**0.7993**	**0.8688**	**0.7828**

4.2. Relationships of the plant parasitic Caloscyphaceae and Rhizinaceae

The Caloscyphaceae and Rhizinaceae are for the first time moderate to strongly supported as a monophyletic group (PP 100%, ML-BP 78%, MP-BP excluding third codons 55%). A close relationship between these two families is interesting, considering both contain plant parasitic species (*Caloscypha fulgens*, in Caloscyphaceae and *Rhizina undulata* and *Phymatotrichopsis omnivora* in Rhizinaceae), that otherwise only are known within Pezizomycetes in Sarcosomataceae (*Urnula craterium*) (e.g. Marek et al., 2009). *Pyropyxis* is also suggested as facultatively parasitic, and species of *Neottiella*, *Lamprospora* and *Octospora* form obligate associations with numerous bryophytes, which have been interpreted as parasitic (see Section 4.6). The relationships of Caloscyphaceae and Rhizinaceae to the rest of the Pezizomycetes have been uncertain (Hansen and Pfister, 2006; Hansen et al., 2008; Marek et al., 2009), but multigene, large-scale studies across the Pezizomycotina (Spatafora et al., 2006) and Ascomycota (Schoch et al., 2009) strongly suggest these belong to the B-lineage. All our analyses corroborate this, although without support (Fig. 2 and Suppl. Figs. 1 and 2).

4.3. Higher level relationships within lineage C

Our best estimate of the data presented here suggests that the Chorioactidaceae–Sarcosomataceae and Sarcoscyphaceae are early diverging families within lineage C. It is also very likely that these families constitute a monophyletic group (Fig. 2 and Suppl. Fig. 2), contrary to previous molecular results (e.g. Harrington et al., 1999; Hansen and Pfister, 2006). The multi-nucleate ascospores present in all three families lends additional support to this relationship, although cytological data are not available for all members. Multi-nucleate spores are also present in the B-lineage, in Morchellaceae, and thus appear to have arisen at least two times within Pezizomycetes. The monophyly of the three families is also supported by similarity in ascus structure in that all members have thick-walled asci. Most members of the Chorioactidaceae, Sarcosomataceae and Sarcoscyphaceae have been considered closely related based on morphology and were at one point placed in a single family (with two tribes; Le Gal, 1953) or in a suborder, Sarcoscyphineae (Rifai, 1968).

4.4. Defining a monophyletic Pyronemataceae

4.4.1. Excluding four early diverging lineages

The four-gene analyses suggest that Pyronemataceae as currently circumscribed (Lumbsch and Huhndorf, 2010; Kirk et al., 2008) is paraphyletic, confirming our previous results (Hansen and Pfister, 2006; Perry et al., 2007). The *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages are strongly suggested to represent separate lineages closely related to Ascodesmidaceae and Glaziellaceae (Fig. 2). The results presented here suggest two possible treatments: (1) members of Ascodesmidaceae and Glaziellaceae could be transferred to Pyronemataceae, or (2) the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages could be treated in two or more separate families. We advocate the latter and recognize a restricted Pyronemataceae (Fig. 2). But, given the conflicting and non-supported relationships among the three lineages and

Fig. 4. Reconstruction of ancestral states of ascomata carotenoids and hairs in Pyronemataceae and sister families, using a Bayesian approach as implemented in SIMMAP ver. 1.5. Posterior probabilities for each character state are given as pie charts, plotted on the Bayesian consensus tree from the combined four-gene analysis (presented in Fig 1). Character states for each species are indicated after species names. Uncertain state for a taxon is given as "?". Six nodes (1–6) are labeled for discussion and the posterior probabilities for these are given in Table 5. Outgroup taxa not shown. True hairs are either superficial, blunt or pointed, or rooting and pointed.

the Ascodesmidaceae-*Boubovia* lineage (Table 4) from different analyses, we postpone the formal descriptions of new families for these lineages. None of the previous restricted classifications of Pyronemataceae correspond to the results presented here. Likewise, none of the phylogenetic lines, subfamilies and tribes recognized in the broadly inclusive Pyronemataceae (Eckblad, 1968; Korf, 1972) reflect the relationships revealed here regarding these early diverging lineages. It is noteworthy that *Geopyxis*, *Pseudombrophila* (incl. *Fimaria*) and *Coprotus* (along with *Lasiobolus*) were placed apart from the major groups of pyronemataceous taxa, in the subfamily Ascophanoideae and the tribes Geopyxideae, Pseudombrophileae and Theleboleae, respectively (Korf, 1972). *Ascodesmis* was placed in the subfamily Ascodesmidoideae, but along with genera such as *Smardaea* and *Aleurina* (as *Pulparia* and *Jafneadelphus*) (Pyronemataceae *s. str.* in our analyses). *Tarzetta* and *Pulvinula* were placed in the more inclusive subfamilies Otideoideae (tribe Jafneeae) and Scutellinioideae (tribe Aleurieae). *Pulvinula*, nonetheless, has been considered a discrete taxon and its position in Aleurieae unsatisfactory, but without clues to its real relationships (Pfister, 1976; Korf and Zhuang, 1984). The genus *Boubovia* (Svrcek, 1977) was recognized for species of *Pulvinula* with ellipsoid spores and thick-walled asci at the early stages of ascospore formation (Yao and Spooner, 1996a). *Boubovia* is represented here by *B. nicholsonii*, based on our previous analyses (as *Pulvinula ovalispora*), which confirmed it is a species of *Boubovia;* it formed a strongly supported monophyletic group with the type species, *Boubovia luteola* (Perry et al., 2007). For the first time *Boubovia* is suggested to be closely related to *Coprotus* (PP and ML-BP 100%, MP-BP 99%) and Ascodesmidaceae.

4.4.2. Developmental and ultra-structural characters

Species of *Coprotus*, *Ascodesmis* and *Eleutherascus* share several developmental and ultrastructural characters (Kimbrough, 1989) that are considered to be of importance at higher levels within Pezizomycetes. They produce simple, gymnohymenial apothecia (that is, the asci are exposed throughout development), with excipula that are highly reduced or lacking, normally developing from clustered pairs of ascogonia and antheridia. The septal pore plugs in asci are highly differentiated dome-shaped arrays of tubular elements inside the ascus (e.g. Carroll, 1967; van Brummelen, 1989). That is the "*Ascodesmis* type" of ascal septal pore plug (Kimbrough, 1994). Using primarily these characters, Kimbrough (1989) placed *Ascodesmis*, *Eleutherascus*, *Coprotus* and *Pyronema* in a new suborder of Pezizales, Pyronemineae. The genus *Pyronema* also produces small, gymnohymenial apothecia (Fig. 1a and b), with a similar developmental pattern. Septal pore plugs in asci of *Pyronema* are similar to those of Ascobolaceae and *Ascodesmis*, *Eleutherascus* and *Coprotus*, although not identical. Compared to Ascobolaceae the intercalary band of the plug is of a different electron density and compared to *Ascodesmis* the radiating tubular elements are smaller and fewer (Kimbrough, 1994). Our results show that *Pyronema* is distantly related to *Coprotus* and *Ascodesmis*; clearly Pyronemineae is paraphyletic based on molecular phylogenetics (Fig. 2). This suggests that very simple, reduced apothecial forms have evolved several times within the C lineage (see also *Monascella* in the *Otidea* lineage, Section 4.5).

Boubovia shares a gymnohymenial development of the apothecia with *Coprotus* and Ascodesmidaceae, as well as with *Pulvinula* (Brummelen and Kristiansen, 1999). On the other hand, *Lasiobolus*, which forms a sister group to Ascodesmidaceae, has a cleistohymenial development with the apothecia opening in the late mesohymenial phase (i.e. the ascogenous system and asci are enclosed in the excipulum until the ascospores are maturing). Cleistohymenial development appears to be most common within Pezizomycetes; other members of the Pezizomycetes studied thus far, except for Ascobolaceae, have cleistohymenial apothecia that develop from a single ascogonial coil (Kimbrough, 1989). van Brummelen (1994) maintained that *Coprotus* should be transferred to Thelebolaceae (Pezizales), along with taxa he considered to possess an ancestral operculum type. Later however, van Brummelen (1998) determined, using TEM, that *Coprotus lacteus* has the "*Octospora* type" ascus (with reference to the fine structure of the ascus wall and operculum), characteristic of Pyronemataceae in the broad sense. Nevertheless, *Coprotus* was excluded from Pezizales with other Thelebolaceae and placed in Leotiomycetes (in Thelebolales; e.g. Eriksson et al., 2001; Lumbsch and Huhndorf, 2010). The relationship of *Coprotus* has not been studied before using molecular data. Our results substantiate that *Coprotus* belongs to Pezizomycetes; *Coprotus ochraceus* is deeply nested within the class. *Coprotus* is a large genus with 23 recognized species and the type species, *C. sexdecimsporus*, should be sequenced to firmly place the genus. Several *Coprotus* species have multi-spored asci (16, 32, 64 or 256 spores per ascus), and no species of Pezizomycetes with more than 8-spored asci has so far been included in molecular phylogenetic analyses.

A unique septal pore plug in the asci and ascogenous hyphae has been described for *Pulvinula* and *Geopyxis*, the "pulvinuloid-type" (Kimbrough, 1994). This type is considered strikingly similar to the early stages of the ascal pore plug type found in Helvellaceae, having 'V-shaped' striations, quite different from other pore types found in Pyronemataceae so far (Kimbrough and Gibson, 1989, 1990; Kimbrough, 1994). Also, strong similarities between the septal pore plug in *Geopyxis* and *Urnula craterium* (Sarcosomataceae) have been found (Li and Kimbrough, 1995). It was suggested that pore plugs composed of a rather loose, granular matrix with more elaborate 'V-shaped' striations, seen in *Geopyxis* and members of the Sarcoscyphaceae and Sarcosomataceae, are intermediate between those of Pyronemataceae (as Otideaceae) and Helvellaceae (Kimbrough, 1994; Li and Kimbrough, 1995). *Geopyxis* and *Pulvinula* possess uninucleate spores, which clearly exclude these genera from the Helvellaceae (tetranucleate spores) and Sarcocyphaceae and Sarcosomataceae (multinucleate spores). This suggests that septal pore plugs in the asci may prove to be informative for family assignment of the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages. Still, only a limited number of taxa have been studied thus far, and no information is available for the ascus septal pore plugs in *Boubovia*, *Tarzetta* and the *Pseudombrophila* lineage.

Confirming previous results (Tanabe et al., 1999), the anamorphic species *Cephaliophora irregularis* and *C. tropica* are shown to be members of Ascodesmidaceae. Contrary to Tanabe et al. (1999) based on SSU rDNA sequences and a smaller sampling of Pezizomycetes, our analyses recovered *C. irregularis* and *C. tropica* as a monophyletic group (Fig. 2 and Suppl. Fig. 2), sister to an *Ascodesmis–Eleutherascus* clade. No teleomorphic states have been reported for these *Cephaliophora* species and no anamorphic states have been reported for *Ascodesmis* or *Eleutherascus*.

4.4.3. Glaziellaceae

Glaziella aurantiaca, the only species of Glaziellaceae, is for the first time firmly placed in molecular phylogenetic analyses. Our data suggests it belongs to the clade of Ascodesmidaceae and the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages. *Glaziella* has had a confusing taxonomic history and has even been treated in its own order (Gibson et al., 1986). It produces unusual ascomata that are more or less epigeous, large, bright yellow to orange, completely hollow with a basal opening, and with monosporic asci scattered within a rather thin, gelatinous ascoma wall. The spores are large, 300–500 μm, and are left embedded in the ascoma wall when the asci disintegrate. Paraphyses are lacking. Based on the results presented here it most likely constitutes a separate distinct lineage, giving support to the monotypic family.

4.5. Early diverging lineages within Pyronemataceae s. str

The two lineages *Pyropyxis* and *Otidea*, recovered previously (Perry et al., 2007), are now strongly supported as successive sister lineages to the rest of Pyronemataceae *s. str*. *Pyropyxis* was tentatively placed in the tribe Aleurieae *sensu* Korf (1973; subfamily Scutellinioideae) (Egger, 1984), but a close relationship with *Geopyxis* was also indicated. Egger (1984) suggested *Pyropyxis* might provide a link between *Geopyxis* and the guttulate genera of the tribe Aleurieae (e.g. *Aleuria*, *Pulvinula* and *Rhodotarzetta*). The composition of the *Pyropyxis* lineage is surprising; there are no known morphological or ecological characters to support a close relationship between *Pyropyxis* and *Smardaea*. *Smardaea* was placed in subfamily Ascodesmidioideae (as *Pulparia*) (Korf, 1972, 73), based on the presence of purple pigment in all parts of the ascomata, including the ascospore wall.

A close relationship is for the first time suggested between *Monascella* and *Warcupia* (PP, ML- and MP-BP 100%), two unusual fungi within Pezizomycetes. They form a strongly supported sister group to *Otidea* (PP and ML-BP 100%, MP-BP 98%). *Monascella* produces highly reduced ascomata that are superficial, embedded in aerial mycelium; with a simple excipulum; without paraphyses; and with a small number of clavate to saccate asci with a thin, rather persistent wall that lacks an operculum (Guarro and von Arx, 1986). In contrast, *Warcupia* produces globose, cleistothecial ascomata (230–300 μm in diam.) that open irregularly distally at maturity to expose the asci and spores; paraphyses are present and persist at maturity (Paden and Cameron, 1972). Like *Monascella*, the asci lack an opening mechanism, but are cylindrical to clavate and deliquesce slowly after the ascospores mature, and appear in small, randomly oriented clusters along the wall of the locule. The ascomatal development differs in that *Monascella* ascomata develop from ascogonia surrounded by coiled antheridia (gymnohymenial development) (Guarro and von Arx, 1986), whereas in *Warcupia* they develop from multi-celled coiled ascogonia with trichogynes that are surrounded by sterile hyphae forming the ascomatal wall (cleistohymenial development) (Paden and Cameron, 1972). The development in *Monascella* is similar to the development in *Ascodesmis*, *Eleutherascus*, *Coprotus* and *Pyronema*, whereas ascogonial coils have been observed in most other Pezizomycetes (Kimbrough, 1989). This suggests that very simple, reduced apothecial forms, developing from clustered pairs of ascogonia and antheridia, have evolved at least three times within the C lineage (in the Ascodesmidaceae-*Boubovia*, *Otidea* and *Pyronema* lineages). *Monascella* is currently classified in Onygenaceae (Onygenales, Eurotiomycetes) (Lumbsch and Huhndorf, 2010), or placed questionably in Pyronemataceae (Kirk et al., 2008). Previous placement in Pyronemataceae was based on phylogenetic analysis of ITS sequences (Stchigel et al., 2001) that resolved *Monascella* in a highly supported clade with *Lamprospora* sp. and *Pyronema domesticum* (the only two pyronemataceous taxa included in that analysis). *Warcupia* was originally placed in the cleistothecial family Eoterfeziaceae (Paden and Cameron, 1972; Benny and Kimbrough, 1980; within Pezizales), but was transferred to the tribe Theleboleae of Pyronemataceae *sensu* Korf (1972) by Jeng and Krug (1976), following the notion that closely related genera with exposed hymenia and cleistothecial genera are better accommodated in one rather than separate families.

Species of *Otidea* produce apothecia (i.e. with an exposed hymenium at maturity) and operculate asci with forcible spore discharge. Recently however, it was shown that a semi-hypogeous to hypogeous truffle-like form, without active spore discharge, has evolved within *Otidea* (Smith and Healy, 2009). This is not surprising since truffle or truffle-like forms have evolved multiple times independently within Pezizomycetes and Pyronemataceae, with only one exception known (*Paurocotylis*), always in ectomycorrhizal lineages (Læssøe and Hansen, 2007). This is also the case in *Otidea* that has been detected as ectomycorrhizal (e.g. Moser et al., 2009; Smith et al., 2007). The close relationship of *Otidea* to *Monascella* and *Warcupia* is surprising and seems only supported morphologically by the ascospores that are ellipsoid, smooth, hyaline and with two (or sometimes three in *Warcupia*) oil guttules. *Otidea* was placed in Otideeae in the subfamily Otideoideae (Korf, 1972), along with *Ascosparassis* (not available for molecular study) and *Psilopezia* (now Rhizinaceae). The family Otideaceae has had several different circumscriptions (Eckblad, 1968; Korf and Zhuang, 1991). Our analyses do not support any of these proposed relationships of *Otidea*.

4.6. Evolution of trophic strategies and habitat for fruiting

Our ASRs show that the ancestor of lineage C and Pyronemataceae *s. str*. was a soil inhabiting, saprobic fungus ($P = 0.9330/0.9963$; $P = 0.9649/0.9913$). This result implies that an ectomycorrhizal lifestyle has evolved independently from a saprobic lifestyle seven to eight times within lineage C, and six times within Pyronemataceae *s. str*. (Figs. 3, 1c–f and j). It also demonstrates that no reversals to a free-living, saprobic lifestyle have occurred from either symbiotic or parasitic Pyronemataceae. This is substantiated by a recent study using the genus *Amanita* (Basidiomycota), which demonstrated the irreversible loss of genes required for a free-living lifestyle associated with the evolution of an ectomycorrhizal symbiosis (Wolfe et al., 2012). Although comparison of genomic traits in two ectomycorrhizal fungi, *Tuber melanosporum* (the Périgord black truffle, Pezizomycetes) and *Laccaria laccata* (Basidiomycota), has shown that genetic predispositions for symbiosis – this so-called "symbiotic toolbox" evolved via different routes in ascomycetes and basidiomycetes (Martin et al., 2010). *Tuber melanosporum* lacks a large set of carbohydrate cleaving enzymes, but still has an invertase gene (not present in *L. laccata*, which is completely dependent on its host for its provision of sucrose), and therefore can access and hydrolyse plant-derived sucrose, suggesting that it may be less dependent on its host. Similarly, the ectomycorrhizal *Trichophaea brunnea* (in the *Scutellinia–Trichophaea* lineage (Fig. 2)) has been shown to be able to effectively degrade cellulose, produce phenol oxidases and hydrolyze pectin, suggesting it may be capable of living independent of its host (Danielson, 1984; Egger, 1986). Our ASR results are consistent with recent studies that inferred multiple origins of ECM within the Agaricales (Basidiomycota) with no reversals to a free-living condition (Matheny et al., 2006).

Pure culture synthesis studies (e.g. Danielson, 1984; Egger and Paden, 1986) as well as studies in which fungi isolated from mycorrhizal root tips were induced to develop apothecia in culture (Yang and Wilcox, 1984), were among the earliest to demonstrate the ability of some pyronemataceous species to form mycorrhizae. Since then, many species have been identified as mycobionts in molecular studies of ectomycorrhizal communities (reviewed in Tedersoo et al. (2010)), some including morpho-anatomical descriptions (Tedersoo et al., 2006; Wei et al., 2010), and new species or genera continue to be discovered as ectomycorrhizal. Most recently *Parascutellinia carneosanguinea*, *Aleurina imaii*, "*Pustularia patavina*", *Rhodoscypha ovilla*, *Unicava sp.* (*gen. ined.*), and an as yet unknown clade were discovered as ectomycorrhizal (Tedersoo et al., in press). This indicates that further ectomycorrhizal lineages within Pezizomycetes are still likely to be revealed. In our ASR analyses we scored the genus *Sowerbyella* as saprobic. Although this genus has been suggested to be ectomycorrhizal, it has not yet been found in molecular community studies and direct evidence is lacking.

Very few vascular plant parasites are known within Pezizomycetes and only *Pyropyxis rubra* has been recognized as such within

Pyronemataceae (Egger and Paden, 1986; Tedersoo et al., 2010). Obligate bryosymbiotic species are found in the genera *Lamprospora*, *Neottiella* and *Octospora* (Fig. 1g). In our model-based analyses these form a moderate to strongly supported monophyletic group (PP 100%, ML-BP 77%) within the *Octospora* lineage, as a sister group to a strongly supported group of the non-bryosymbiotic genera *Rhodoscypha* and *Rhodotarzetta* (PP, ML and MP-BP 100%) (Fig. 2). This differs from our previous analyses (Perry et al., 2007), which weakly resolved the non-bryosymbiotic clade within the bryosymbiotic clade. The genus *Moravecia* (not sampled here) was highly supported within *Lamprospora* in our previous analyses. This was especially interesting because *Moravecia* was erected as a non-bryosymbiotic genus (Benkert et al., 1987). Since the publication of our previous results, Benkert (2011) has reexamined the two known species of *Moravecia* and found infections on rhizoids of small bryophytes. Two additional obligate bryosymbiotic genera, *Filicupula* and *Octosporella*, are considered closely related to *Lamprospora*, *Neottiella* and *Octospora*. In a large phylogenetic study of bryosymbiotic species across all of the Ascomycota (Stenroos et al., 2009), two species of *Octosporella* and a species of *Lamprospora* sp. were included. The results showed that *Lamprospora* was nested within *Octosporella*. This not only implies *Octosporella* belongs to the bryosymbiotic clade of the *Octospora* lineage, but also questions the monophyly of the genus. *Filicupula* was introduced to accommodate a divergent *Octosporella* species (Yao and Spooner, 1996b) and likely belongs to this same clade. Our ASR indicates that a bryosymbiotic life strategy has arisen only once in the *Octospora* lineage. Since the only other lineage within Pezizomycetes containing species that can be considered as bryosymbiotic is the *Cheilymenia* lineage, with *C. sclerotiorum*, we suggest that bryosymbiotism has evolved only twice within the class. Our ASR was unable to reconstruct the ancestral node of the *Octospora* lineage, which could be either bryosymbiotic or ectomycorrhizal. The monotypic *Rhodoscypha* was recently shown to be ectomycorrhizal (Tedersoo et al., in press), but the trophic strategy for *Rhodotarzetta* is still unknown (coded with a "?" in our ASR). A bryosymbiotic lifestyle has evolved in numerous lineages across the Ascomycota (even with highly specializied niches) and preliminary results indicate that most bryosymbionts are derived from saprobic ancestors (Stenroos et al., 2009). The association between species of the *Octospora* lineage and bryophytes has been interpreted as rhizoid parasitism in nature (Benkert, 1993; Döbbeler, 1979). The apothecia of these fungi can be borne directly upon their bryophyte host(s), but often the species fruit on soil near the host and the relationship is therefore not directly apparent. Many species have restricted host ranges and are associated with a single bryophyte species or genus; others have a wide spectrum of hosts (Benkert, 1993). The majority of species are associated with acrocarpous mosses, although a small number of taxa are parasitic on pleurocarpous mosses (*Octospora* section *Wrightoideae*) and liverworts (e.g. *Neottiella ricciae*, *Filicupula*, *Octosporella*). All species studied are characterized by hyphae that attack the subterranean living rhizoids by means of elaborate infection structures consisting of appressoria connected to intracellular haustoria (Döbbeler, 1979, 2002). Apart from the Pezizomycetes, no other fruit-body-forming ascomycetes are known to infect the subterranean part of bryophytes (Döbbeler, 2002). This specific type of parasitism may explain the success of the bryosymbiotic clade (*Filicupula*, *Lamprospora*, *Neottiella*, *Octospora*, *Octosporella*), which represents a highly speciose group with an estimated 150 species (Kirk et al., 2008).

Species of Pezizomycetes have also been detected as symbionts of partly autotrophic orchids (i.e. they rely partly on fungi for carbon and mineral nutrients). For example, species of *Epipactis* associated nearly exclusively with ectomycorrhizal Tuberaceae and several Pyronemataceae, including *Wilcoxina*, *Genea*, *Geopora* and *Trichophaea woolhopeia* (Selosse et al., 2004; Tešitelova et al., 2012; Tedersoo et al., in press). Also two recently diverged lineages of South African orchids of the subtribe Coryciinae have shifted fungal symbionts to saprobic *Tricharina* and *Pezizaceae* species, respectively (Waterman et al., 2011). These relationships are highly specific; the same fungal partners were found in different geographical areas, and clades of orchids displayed conserved preference for a particular fungal partner.

Surprisingly, Pezizomycetes have been discovered to act as both foliar endophytic and as endolichenic fungi using culturing and sequence analysis (U'Ren et al., 2010, 2012). In the seasonally dry, fire-dominated montane forests of Arizona, Pyronemataceae and related Pezizomycetes comprised >50% of all isolates from lichens and photosynthetic tissue of plants, especially mosses. Using phylogenetic analysis of LSU sequences derived from Pyronemataceae ascomata and plant endophytes and endolichenic fungi, Tedersoo et al. (in press) show that these taxa are distinct from ectomycorrhizal fungi. Two possible exceptions are *Trichophaea brunnea* and *T. hemisphaerioides*; both scored as EcM in our ASR analysis (the latter suggested to be mutualistic under some conditions by Egger and Paden (1986)). The endophytic/endolichenic taxa were otherwise affiliated with species of *Anthracobia*, *Pyronema*, *Smardaea*, *Tricharina*, non-mycorrhizal *Trichophaea s. l.*, *Trichophaeopsis* (Pyronemataceae *s. str.*), *Boubovia*, *Geopyxis*, and non-mycorrhizal *Pulvinula* species (Tedersoo et al., in press). Notably, the majority of these taxa are known to be pyrophilous (producing apothecia on burnt ground). In our ASR analysis we consider these taxa as saprobic. It is nevertheless likely that these fungi obtain some nutrition from their hosts, living plant tissue or lichen, during this part of their lifecycle, although no differentiated structures for nutrient exchange have been observed. Looking at substrate utilization patterns *in vitro* in post-fire Pyronemataceae and other Pezizomycetes, it appears that the majority are opportunistic decomposers or facultative biotrophs – utilizing the major non-lignified components of the soil (Egger, 1986).

Comparing the ASRs of trophic strategies and habitat for fruiting, it is not surprising to find that substrate specialists (pyrophilous, coprophilous, nitrophilous, or wood inhabiting) and endophytic/endolichenic Pyronemataceae have evolved in mainly saprobic lineages, although a few pyrophilous species have been shown to form ectomycorrhizae. Also many saprobic Pyronemataceae produce ascomata on newly disturbed ground.

4.7. Evolution of carotenoids, hairs and ascospores guttulation

Our ASR confirms that the production of carotenoid pigments has evolved several times in distantly related taxa within Pezizomycetes (in both lineage B and C), as suggested previously (Landvik et al., 1997; Perry et al., 2007). Nevertheless, our results support that production of carotenoids (Figs. 1a, h, i and k) is a synapomorphic trait characterizing a monophyletic group of pyronemataceus members. The ancestor of Pyronemataceae *s. str.*, excluding the *Pyropyxis* and *Otidea* lineages (node 6), is reconstructed with high probability to have produced ascomata with carotenoids, indicating that carotenoid production has subsequently been lost in some clades. This is consistent with the ideas of Arpin (1969), but he did not consider that carotenoids could be subsequently lost, and Aleuriaceae *sensu* Arpin (1969) does not conform to the group delimited here. Interestingly, it appears that most of those clades lacking carotenoids contain ectomycorrhizal members, viz. the *Humaria* lineage, *Rhodoscypha* and the *Geopora–Trichophaea* clade. Carotenoids are bright yellow, orange to red organic pigments that are synthesized in all photosynthetic organisms, and many heterotrophic bacteria and fungi – but generally not in animals. To date more than 600 carotenoid derivatives are known, the majority consisting of a 40-carbon skeleton (Sandmann and Misawa, 2002).

Carotenoid synthesis is not a uniform feature of fungi although reported in most classes. Fungi synthesize either bicyclic β-carotene or monocyclic γ-carotene as the end product of the pathway, or within Ascomycota and Basidiomycota more complex derivatives (Sandmann and Misawa, 2002). Within the Ascomycota, special carotenoids have been found in Pezizomycetes and the order Sordariales. Pezizomycetes possess the potential to synthesize acyclic keto carotenoids and monocyclic structures derived from γ-carotene and torulene. Special carotenoids found in the class are for example phillipsiaxanthin (in *Phillipsia* and *Cookeina*) and plectaniaxanthin (in *Sarcoscypha coccinea* and other Sarcoscyphaceae and Pyronemataceae taxa) (Arpin and Liaaen-Jensen, 1967; Arpin, 1969); aleuriaxanthin in *Aleuria aurantia*, *Melastiza cornubiensis* (Fig. 1h), and *Scutellinia umbrarum*, in Pyronemataceae (Arpin, 1969; Schrantz and Lemoine, 1995); and a unique β.γ-carotene with a terminal methylene group in *Caloscypha fulgens*, in Caloscyphaceae (Arpin et al., 1971). Carotenoids in fungi probably accumulate in lipid globules (Fig. 1n and o) and in membranes, and may provide antioxidant protection (Johnson and Schroeder, 1996). Carotenoids have the potential to inactivate radicals, quench singlet oxygen and dissipate the energy from excited photosensitizers as heat (Britton, 1995). In heterotrophic organisms, including fungi, carotenoids have been shown to protect against oxidative stress and photoreactions [caused by light and UV radiation] (e.g. Blanc et al., 1976; Will et al., 1984). The reason for the loss or inactivation of carotenoid biosynthetic genes (and their enzyme production) in some clades of Pyronemataceae *s. str.* remains unknown. Our results suggest that the acquisition of the ectomycorrhizal lifestyle may play a role in loss or suppression of carotenoid production in this group.

Many species of Pyronemataceae produce ascomata covered with distinct, projecting hairs of differing construction (±rooting, ±pigmented) (Fig. 1e, h, k, l and m), which have been used to delimit tribes within a large Pyronemataceae (Korf, 1972), or subfamilies, in combination with the presence or absence of carotenoids (Korf and Zhuang, 1991). Our ASR suggest however, that different types of hairs do not constitute synapomorphic traits, nor does the absence or presence of hairs. Importantly, true hairs are restricted to the core group of Pyronemataceae *s. str.* (except true, superficial hairs have also evolved in *Lasiobolus* within Ascodesmidaceae and in the *Pseudombrophila* lineage), the Chorioactidaceae–Sarcoscyphaceae–Sarcosomataceae clade and some Tuberaceae. The ancestral node of Pyronemataceae *s. str.* excluding the *Pyropyxis* and *Otidea* lineages (node 6) was recovered with equivocal character states, highlighting that this group is especially prone to shifts among states. True, superficial, blunt or pointed hairs (Fig. 1e, h, m) have evolved in several clades; the epigeous species of the *Humaria* lineage share stiff, thick-walled brown, superficial, pointed hairs (in the hypogeous, truffle-like *Genea*, hairs are absent or blunt), but this type of hair is also present in *Spooneromyces* (in the *Aleuria* lineage), and in *Tricharina* and *Trichophaea* (in the *Scutellinia–Trichophaea* lineage). A transition from blunt to pointed hairs, or reversal, has happened within three clades, implying that these states are not useful for delimiting monophyletic groups or genera.

Independent origins of true rooting hairs (Fig. 1k and l) include either the common ancestors of the *Cheilymenia–Scutellinia–Trichophaea* lineages (with subsequent losses and gains) and *Paratrichophaea*; or the ancestor of *Cheilymenia*, the main group of *Scutellinia*, *Scutellinia sp*. A and *Paratrichophaea*. It should be said that several species in the genus *Cheilymenia* possess several different types of hairs, both superficial (pointed and blunt) and rooting hairs, but have been coded as rooting hairs in our ASRs (as SIMMAP does not allow for multiple states). A unique type of hair, termed stellate (superficial, thick-walled, septate hairs, having several radiating arms), is confined to some *Cheilymenia* species (e.g. *Scutellinia crucipila*, here shown to be a member of *Cheilymenia*). Our results show that several genera, partly delimited on type of excipular hairs are not monophyletic.

The evolutionary patterns of ascospores guttulation (Fig. 1p–r) indicate that this is a homoplasious character that cannot be used to delimit taxa at higher levels within Pezizomycetes. It may however, still be a character that can be used in combination with other characters to delimit genera. We coded spore guttulation as a binary state (absent or present), but the coding could be refined to include multiple states, such as one or two large, or multiple small guttules. It may be that such a coding would show a different pattern. This study confirms the difficulties of finding morphological synapomorphies within Pyronemataceae *s. l.*

5. Conclusions and future directions

At the inter-familial and -generic level within Pezizomycetes, the RPB1 region (A–C) exhibits the greatest phylogenetic signal per sequenced base pair, followed by the RPB2 (6–11) region, based on number of putative parsimony informative characters and highly supported clades. The EF-1α region appears to be less informative. Introns are rich and the positions dynamic in the EF-1α, often lineage-specific and diagnostic for many deeper nodes. Third codon positions do not appear to be markedly saturated under model-based analyses, but we suggest these should be excluded under parsimony analyses for resolving deeper level relationships.

Our four-gene data set produced a highly resolved phylogeny with many strongly supported lineages, both at shallow and deep taxonomic levels. A strongly supported C-lineage is resolved. The families Chorioactidaceae, Sarcosomataceae and Sarcoscyphaceae, for the first time using molecular data, form a strongly supported, early diverging monophyletic group within lineage C. Also the family Glaziellaceae is for the first time placed firmly (using model-bases analyses), as a sister group to the clade of Ascodesmidaceae and the *Boubovia*, *Geopyxis*, *Pseudombrophila* and *Pulvinula* lineages. We suggest these four lineages be excluded from the taxonomic circumscription of Pyronemataceae, but postpone formal descriptions of one or more new families for these lineages until more data become available and the relationships among these taxa are more fully resolved. A monophyletic Pyronemataceae is delimited and ten strongly supported lineages are identified within the family, two of which are firmly placed. With these results, analyses incorporating a more intense sampling of taxa and genes focusing on the Pyronemataceae *s. str.* and/or the Ascodesmidaceae–Glaziellaceae clade can now be undertaken. Such analyses, coupled with studies addressing ultrastructural features such as septal pore plugs in the base of the asci, will be fruitful in improving our understanding of the evolutionary history of these fungi and to finally propose a formal classification for the family. The current results also pinpoint generic boundaries in need of further study; several genera are newly shown or corroborated to be non-monophyletic (to be addressed elsewhere).

Very simple, gymnohymenial apothecial forms, with excipula that are highly reduced or lacking, are suggested to have evolved at least three times within lineage C, in the Ascodesmidaceae-*Boubovia*, *Otidea* and *Pyronema* lineages. At the same time, cleistothecial forms (ascomata that never open) evolved at least three times within lineage C, in the *Pseudombrophila*, *Otidea* and *Lasiobolidium* lineages. The ascus operculum and the ability to discharge spores actively has been lost in the cleistothecial forms, and in the gymnohymenial *Eleutherascus* and *Monascella*. The anamorphic *Cephaliophora irregularis* and *C. tropica* form a monophyletic group and are confirmed to belong to Ascodesmidaceae.

Our ASR gives new insight into the evolution of essential life history traits. Ascomata carotenoid pigment and different hair

types are shown to be homoplasious characters. Interestingly our ASR support that the ancestor of Pyronemataceae *s. str.*, excluding the *Pyropyxis* and *Otidea* lineages, produced ascomata with carotenoids and that carotenoid production was subsequently lost in some clades. This explains at large the problems this feature has caused in Pyronemataceae classifications. It appears that most of the clades lacking carotenoids contain ectomycorrhizal members and we speculate that the loss or inactivation of the production of carotenoids may be correlated with a transition to an ectomycorrhizal lifestyle. Bryosymbiotism is suggested to have evolved only twice within Pezizomycetes, in the *Octospora* lineage and in *Cheilymenia* (*C. sclerotiorum*). The association between species of the *Octospora* lineage and bryophytes has been interpreted as rhizoid parasitism. This type of parasitism, known thus far among fruit-body-forming ascomycetes only in Pezizomycetes, may explain the success of this highly speciose bryosymbiotic clade. The Rhizinaceae and Caloscyphaceae form for the first time a strongly supported monophyletic group, using model-based analyses. These families contain some of the few plant parasitic species known in Pezizomycetes (otherwise known only in Sarcosomataceae and *Pyropyxis*). Thick-walled, rooting hairs are suggested to have evolved at least four times within Pyronemataceae, whereas stellate hairs are unique to *Cheilymenia*. In this study we traced the evolution of selected characters within Pyronemataceae, but other morphological features such as excipulum structure of the ascomata (i.e. the cell structure of the tissue containing the asci and paraphyses), would be interesting to examine in future investigations. The current knowledge of trophic strategies within Pezizomycetes are fairly advanced, but as research in this field continues to progress so will our understanding of the ecology and evolution of these diverse fungi.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.01.014.

References

Altekar, G., Dwarkadas, S., Huelsenbeck, J.P., Ronquist, F., 2004. Parallel Metropolis-coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20, 407–415.

Amicucci, A., Zambonelli, A., Guidi, C., Stocchi, V., 2001. Morphological and molecular characterization of *Pulvinula constellatio* ectomycorrhizae. FEMS Microbiol. Lett. 194, 121–125.

Arpin, N., 1969. Les caroténoïdes des Discomycètes: essai chimotaxinomique. Bull. Mens. Soc. Linn. Lyon 38 (Suppl. l), 1–169.

Arpin, N., Liaaen-Jensen, S., 1967. Recherches chimiotaxinomiques sur les champignons. Fungal carotenoids: III- Nouveaux carotenoides, notamment sous forme d'esters tertiaires, isoles de *Plectania coccinea* (Scop. ex Fr.) Fuck. Phytochemistry 6, 995–1005.

Arpin, N., Fiasson, J.L., Bouchez-Dangye-Caya, M.P., Francis, G.W., Liaaen-Jensen, S., 1971. A new $C_{40}H_{56}$ carotene with a terminal methylene group. Phytochemistry 10, 1595–1601.

Benkert, D., 1993. Bryoparasitic Pezizales: ecology and systematics. In: Pegler, D.N., Boddy, L., Ing, B., Kirk, P.M. (Eds.), Fungi of Europe: Investigation, Recording and Conservation. Royal Botanic Garderns, Kew, pp. 147–156.

Benkert, D., 2011. Ergänzende beobachtungen zu *Moravecia* Benkert, Caillet & Moyne (Pezizales). Z. Mykol. 77, 157–160.

Benkert, D., Caillet, M., Moyne, G., 1987. *Moravecia*, eine neue gattung der Pyronemataceae (Pezizales). Z. Mykol. 53, 139–144.

Benny, G.L., Kimbrough, J.W., 1980. A synopsis of the orders and families of *Plectomycetes* with keys to genera. Mycotaxon 12, 1–91.

Blanc, P.L., Tuveson, R.W., Sargent, M.L., 1976. Inactivation of carotenoid-producing and albino strains of *Neurospora crassa* by visible light, blacklight, and ultraviolet radiation. J. Bacteriol., 616–625.

Bollback, J.P., 2006. SIMMAP: Stochastic character mapping of discrete traits on phylogenies. BMC Bioinform. 7, 88.

Bollback, J.P., 2009. SIMMAP 1.5 Documentation online. <http://www.simmap.com/pgs/priors.html>.

Britton, G., 1995. Structure and properties of carotenoids in relation to function. FASEB J. 9, 1551–1558.

Brummelen, J. van, 1989. Ultrastructure of the ascus and the ascospore wall in *Eleutherascus* and *Ascodesmis* (Ascomycotina). Persoonia 14, 1–17.

Carroll, G., 1967. The fine structure of the ascus septum in *Ascodesmis sphaerospora* and *Saccobolus kerverni*. Mycologia 59, 527–532.

Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. 17, 540–552.

Danielson, R.M., 1984. Ectomycorrhiza formation by the operculate discomycete *Sphaerosporella brunnea* (Pezizales). Mycologia 76, 454–461.

Davidson, R.W., 1950. *Urnula craterium* is possibly the perfect state of *Strumella coryneoidea*. Mycologia 42, 735–742.

Denton, A.L., McConaughy, B.L., Hall, B.D., 1998. Usefulness of RNA polymerase II coding sequences for estimation of green plant phylogeny. Mol. Biol. Evol. 15, 1082–1085.

Dissing, H., 2000. Pezizales. In: Hansen, L., Knudsen, H. (Eds.), Nordic Macromycetes, vol. 1. Ascomycetes, Nordsvamp, Copenhagen, pp. 55–127.

Döbbeler, P., 1979. Untersuchungen an moosparasitischen Pezizales aus der verwandtschaft von *Octospora*. Nova Hedwigia 31, 817–864.

Döbbeler, P., 2002. Microniches occupied by bryophilous ascomycetes. Nova Hedwigia 75, 275–306.

Eckblad, F.-E., 1968. The genera of operculate discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. Nytt Mag. Bot. 15, 1–191.

Egger, K.N., 1984. *Pyropyxis*, a new pyrophilous operculate discomycete with a *Dichobotrys* anamorph. Can. J. Bot. 62, 705–708.

Egger, K.N., 1986. Substrate hydrolysis patterns of post-fire ascomycetes (Pezizales). Mycologia 78, 771–780.

Egger, K.N., Hibbett, D., 2004. The evolutionary implications of exploitation in mycorrhizas. Can. J. Bot. 82, 1110–1121.

Egger, K.N., Paden, J.W., 1986. Biotrophic associations between lodgepole pine seedlings and postfire ascomycetes (Pezizales) in monoxenic culture. Can. J. Bot. 64, 2719–2725.

Eriksson, O.E., Baral, H.-O., Currah, R.S., Hansen, K., Kurtzman, C.P., Rambold, G., Læssøe, T., 2001. Outline of Ascomycota – 2001. Myconet 7, 1–88.

Gibson, J.L., Kimbrough, J.W., Benny, G.L., 1986. Ultrastructural observations on Endogonaceae (Zygomycetes). II. Glaziellales ord. nov. and Glaziellaceae fam. nov.: new taxa based upon light and electron microscopic observations of *Glaziella aurantiaca*. Mycologia 78, 941–954.

Ginns, J.H., 1968. *Rhizina undulata* pathogenic on Douglas-fir seedlings in Western North America. Plant Dis. Reporter 52, 579–580.

Guarro, J., von Arx, J.A., 1986. *Monascella*, a new genus of Ascomycota. Mycologia 78, 869–871.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hansen, K., Pfister, D.H., 2006. Systematics of the Pezizomycetes – the operculate discomycetes. Mycologia 98, 1029–1040.

Hansen, K., Læssøe, T., Pfister, D.H., 2001. Phylogenetics of the Pezizaceae, with an emphasis on *Peziza*. Mycologia 93, 958–990.

Hansen, K., LoBuglio, K.F., Pfister, D.H., 2005a. Evolutionary relationships of the cup-fungus genus *Peziza* and Pezizaceae inferred from multiple nuclear genes: RPB2, β-tubulin, and LSU rDNA. Mol. Phylogenet. Evol. 36, 1–23.

Hansen, K., Pfister, D.H., Hibbett, D.S., 1999. Phylogenetic relationships among species of *Phillipsia* inferred from molecular and morphological data. Mycologia 91, 299–314.

Hansen, K., Perry, B.A., Pfister, D.H., 2005b. Phylogenetic origins of two cleistothecial fungi, *Orbicula parietina* and *Lasiobolidium orbiculoides*, within the operculate discomycetes. Mycologia 97, 1023–1033.

Hansen, K., Weber, N.S., Landvik, S., 2008. Phylogenetic relationships and distribution of *Karstenella* (Pezizomycetes). Karstenia 48, 13–19.

Harrington, F.A., Pfister, D.H., Potter, D., Donoghue, M.J., 1999. Phylogenetic studies within the Pezizales. I. 18S rRNA sequence data and classification. Mycologia 91, 41–50.

Hofstetter, V., Miadlikowska, J., Kauff, F., Lutzoni, F., 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: a case study of the Lecanoromycetes (Ascomycota). Mol. Phyl. Evol. 44, 412–426.

James, P., Whelen, S., Hall, B.D., 1991. The RET1 gene of yeast encodes the second-largest subunit of RNA polymerase III. J. Biol. Chem. 266, 5616–5624.
Jeng, R.S., Krug, J.C., 1976. *Coprotiella*, a new cleistothecial genus of the Pyronemataceae with ascospores possessing de Bary bubbles. Mycotaxon 4, 545–550.
Johnson, E.A., Schroeder, W.A., 1996. Microbial carotenoids. Adv. Biochem. Eng. Biotechnol. 53, 119–178.
Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinform. 9, 286–298.
Katoh, K., Misawa, K., Kuma, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl. Acids Res. 30, 3059–3066.
Kimbrough, J.W., 1970. Current trends in the classification of Discomycetes. Bot. Rev. 36, 91–161.
Kimbrough, J.W., 1989. Arguments towards restricting the limits of the Pyronemataceae (Ascomycetes, Pezizales). Mem. N. Y. Bot. Gard. 49, 326–335.
Kimbrough, J.W., 1994. Septal ultrastructure and Ascomycete systematics. In: Hawksworth, D.L. (Ed.), Ascomycete Systematics: Problems and Perspectives in the Nineties. NATO ASI Series 269. Plenum Press, New York, pp. 127–141.
Kimbrough, J.W., Gibson, J.L., 1989. Ultrastructural observations on Helvellaceae (Pezizales; Ascomycetes). III. Septal structures in *Helvella*. Mycologia 81, 914–920.
Kimbrough, J.W., Gibson, J.L., 1990. Ultrastructural and cytological observations of apothecial tissues of *Geopyxis carbonaria* (Pezizales, Ascomycetes). Can. J. Bot. 68, 243–257.
Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., 2008. Ainsworth and Bisby's Dictionary of the Fungi, 10th ed. CAB International, Wallingford.
Korf, R.P., 1972. Synoptic key to the genera of the Pezizales. Mycologia 64, 937–994.
Korf, R.P., 1973. Discomycetes and Tuberales. In: Ainsworth, G.C., Sparrow, F.K., Sussman, A.S. (Eds.), The Fungi: An Advanced Treatise. Academic Press, Inc., New York, pp. 249–319.
Korf, R.P., Zhuang, W.-Y., 1984. The ellipsoid-spored species of *Pulvinula* (Pezizales). Mycotaxon 20, 607–616.
Korf, R.P., Zhuang, W.-Y., 1991. A preliminary discomycete flora of Macaronesia: part 15, Terfeziaceae, and Otideaceae, Otideoideae. Mycotaxon 40, 413–433.
Kumar, S., Skjæveland, Å., Orr, R.J.S., Enger, P., Ruden, T., Mevik, B.-H., Burki, F., Botnen, A., Shalchian-Tabrizi, K., 2009. AIR: a batch-oriented web program package for construction of supermatrices ready for phylogenomic analyses. BMC Bioinform. 10, 357.
Landvik, S., Egger, K.N., Schumacher, T., 1997. Towards a subordinal classification of the Pezizales (Ascomycota): phylogenetic analyses of SSU rDNA sequences. Nord. J. Bot. 17, 403–418.
Landvik, S., Eriksson, O.E., Berbee, M.L., 2001. *Neolecta* – a fungal dinosaur? Evidence from β-tubulin amino acid sequences. Mycologia 93, 1151–1163.
Le Gal, M., 1953. Les Discomycètes de Madagascar. Prodrome à une Flore Mycologique de Madagascar et Dépendances publié sous la Direction de Roger Heim, vol. 4, pp. 1–465.
Lehmann, J., Eisenhardt, C., Stadler, P.F., Krauss, V., 2010. Some novel intron positions in conserved *Drosophila* genes are caused by intron sliding or tandem duplication. BMC Evol. Biol. 10, 156.
Li, L.-T., Kimbrough, J.W., 1995. Septal structures in the Sarcoscyphaceae and Sarcosomataceae (Pezizales). Int. J. Plant Sci. 156, 841–848.
Liu, C.-Y., Zhuang, W.-Y., 2006. Phylogeny of some genera in Pyronemataceae (Pezizales, Ascomycetes). Mycosystema 25, 546–558.
Liu, Y.J., Whelen, S., Hall, B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol. Biol. Evol. 16, 1799–1808.
Lumbsch, H.T., Huhndorf, S.M., 2010. Myconet 14. Outline of Ascomycota – 2009, in Fieldiana. Life Earth Sci. 1, 1–42.
Læssøe, T., Hansen, K., 2007. Truffle trouble – what happened to the Tuberales? In: Hawksworth, D.L., LaGreca, S. (Eds.), New Bottles for Old Wine. Mycol. Res. 111, 1075–1099.
Maddison, D.R., Maddison, W.P., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer Ass., Sunderland, MA.
Marek, S.M., Hansen, K., Romanish, M., Thorn, R.G., 2009. Molecular systematics of the cotton root rot pathogen, *Phymatotrichopsis omnivora*. Persoonia 22, 63–74.
Martin, F., Kohler, A., Murat, C., Balestrini, R., Coutinho, P.M., Jaillon, O., Montanini, B., Morin, E., Noel, B., Percudani, R., Porcel, B., Rubini, A., Amicucci, A., Amselem, J., Anthouard, V., Arcioni, S., Artiguenave, F., Aury, J.-M., Ballario, P., Bolchi, A., Brenna, A., Brun, A., Buée, M., Cantarel, B., Chevalier, G., Couloux, A., Da Silva, C., Denoeud, F., Duplessis, S., Ghignone, S., Hilselberger, B., Lotti, M., Marçais, B., Mello, A., Miranda, M., Pacioni, G., Quesneville, H., Riccioni, C., Ruotolo, R., Splivallo, R., Stocchi, V., Tisserant, E., Viscomi, A.R., Zambonelli, A., Zampieri, E., Henrissat, B., Lebrun, M., Paolocci, F., Bonfante, P., Ottonello, S., Wincker, P., 2010. Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464, 1033–1038.
Mason-Gamer, R.J., Kellogg, E.A., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst. Biol. 45, 524–545.
Matheny, P.B., Liu, Y.L., Ammirati, J.F., Hall, B.D., 2002. Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). Am. J. Bot. 89, 688–698.
Matheny, P.B., Curtis, J.M., Hofstetter, V., Aime, M.C., Moncalvo, J.-M., Ge, Z.-W., Yang, Z.-L., Slot, J.C., Ammirati, J.F., Baroni, T.J., Bougher, N.L., Hughes, K.W., Lodge, D.J., Kerrigan, R.W., Seidl, M.T., Aanen, D.K., DeNitis, M., Daniele, G.M., Desjardin, D.E., Kropp, B.R., Norvell, L.L., Parker, A., Vellinga, E.C., Vilgalys, R., Hibbett, D.S., 2006. Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98, 982–995.
Matheny, P.B., Wang, Z., Binder, M., Curtis, J.M., Lim, Y.W., Nilsson, H., Hughes, K.W., Hofstetter, V., Ammirati, J.F., Schoch, C.L., Langer, E., Langer, G., McLaughlin, D.J., Wilson, A.W., Frøslev, T., Ge, Z.-W., Kerrigan, R.W., Slot, J.C., Yang, Z.-L., Baroni, T.J., Fischer, M., Hosaka, K., Matsuura, K., Seidl, M.T., Vauras, J., Hibbett, D., 2007. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Mol. Phyl. Evol. 43, 430–451.
Millanes, A.M., Diederich, P., Ekman, S., Wedin, M., 2011. Phylogeny and character evolution in the jelly fungi (Tremellomycetes, Basidiomycota, Fungi). Mol. Phylogenet. Evol. 61, 12–28.
Moncalvo, J.M., Lutzoni, F.M., Rehner, S.A., Johnson, J., Vilgalys, R., 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49, 278–305.
Moravec, J., 2005. A world monograph of the genus *Cheilymenia* (Discomycetes, Pezizales, Pyronemataceae). Libri Bot. 21, 1–256.
Moser, M., Frank, J.L., D'allura, J.A., Southworth, D., 2009. Ectomycorrhizal communities of *Quercus garryana* are similar on serpentine and nonserpentine soils. Plant Soil 315, 185–194.
Paden, J.W., Cameron, J.V., 1972. Morphology of *Warcupia terrestris*, a new ascomycete genus and species from soil. Can. J. Bot. 50, 999–1001.
Paden, J.W., Sutherland, J.R., Woods, T.A.D., 1978. *Caloscypha fulgens* (Ascomycetidae, Pezizales): the perfect state of the conifer seed pathogen *Geniculodendron pyriforme* (Deuteromycotina, Hyphomycetes). Can. J. Bot. 56, 2375–2379.
Perry, B.A., Hansen, K., Pfister, D.H., 2007. A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol. Res. 111, 549–571.
Petersen, P.M., 1985. The ecology of Danish soil inhabiting Pezizales with emphasis on edaphic conditions. Oper. Bot. 77, 1–38.
Pfister, D.H., 1976. A synopsis of the genus *Pulvinula*. Occas. Pap. Farlow Herb. Cryptog. Bot. 9, 1–19.
Pfister, D.H., Slater, C., Hansen, K., 2008. Chorioactidaceae: a new family in the Pezizales (Ascomycota) with four genera. Mycol. Res. 112, 513–527.
Posada, D., 2008. JModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Rambaut, A., 2002. Se–Al. Sequence Alignment Editor. Version 2.0 alpha 11. University of Oxford, Oxford. <http://tree.bio.ed.ac.uk/software/seal/>.
Rehner, S., Buckley, E., 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97, 84–98.
Rifai, M.A., 1968. The Australasian Pezizales in the Herbarium of the Royal Botanic Gardens Kew. Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen II. vol. 57, pp. 1–295.
Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Sandmann, G., Misawa, N., 2002. Fungal carotenoids. In: Osiewacz, H.D. (Ed.), The Mycota X. Industrial Applications. Springer, Berlin, pp. 247–262.
Schoch, C.L., Shoemaker, R.A., Seifert, K.A., Hambleton, S., Spatafora, J.W., Crous, P., 2006. A multigene phylogeny of the Dothideomycetes using four nuclear logi. Mycologia 98, 1041–1052.
Schoch, C.L., Sung, G.-H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., de Hoog, G.S., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.-O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Acomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. Syst. Biol. 58, 224–239.
Schrantz, J.P., Lemoine, Y., 1995. Carotenoid composition of mycelium and apothecia in the discomycete *Scutellinia umbrarum*. Phytochemistry 40, 33–35.
Schultz, T.R., Churchill, G.A., 1999. The role of subjectivity in reconstructing ancestral character states: a Bayesian approach to unknown rates, states, and transformation asymmetries. Syst. Biol. 48, 651–664.
Schumacher, T., 1990. The genus *Scutellinia* (Pyronemataceae). Oper. Bot. 101, 1–107.
Selosse, M.-A., Faccio, A., Scappaticci, G., Bonfante, P., 2004. Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal Septomycetes, including truffles. Microb. Ecol. 47, 416–426.
Smith, M.E., Healy, R.A., 2009. *Otidea subterranea* sp. nov.: *Otidea* goes below ground. Mycol. Res. 113, 858–866.
Smith, M.E., Douhan, G.W., Rizzo, D.M., 2007. Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol. 174, 847–863.
Spatafora, J.W., Sung, G.-H., Johnson, D., Hesse, C., O'Rourke, B., Serdani, M., Spotts, R., Lutzoni, F., Hofstetter, V., Miadlikowska, J., Reeb, V., Gueidan, C., Fraker, E., Lumbsch, T., Lücking, R., Schmitt, I., Hosaka, K., Aptroot, A., Roux, C., Miller, A.N., Geiser, D.M., Hafellner, J., Hestmark, G., Arnold, A.E., Büdel, B., Rauhut, A., Hewitt, D., Untereiner, W.A., Cole, M.S., Scheidegger, C., Schultz, M., Sipman, H., Schoch, C.L., 2006. A five-gene phylogeny of Pezizomycotina. Mycologia 98, 1018–1028.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stchigel, A.M., Cano, J., Mac Cormack, W., Guarro, J., 2001. *Antarctomyces psychrotrophicus* gen. et sp. nov. a new ascomycete from Antarctica. Mol. Res. 105, 377–382.

Stenroos, S., Laukka, T., Huhtinen, S., Döbbeler, P., Myllys, L., Syrjänen, K., Hyvönen, J., 2009. Multiple origins of symbioses between ascomycetes and bryophytes suggested by a five-gene phylogeny. Cladistics 25, 1–20.

Svrcek, M., 1977. New combinations and new taxa in operculate discomycetes (Pezizales). Ceská Mykol. 31, 69–71.

Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tanabe, Y., Nagahama, T., Saikawa, M., Sugiyama, J., 1999. Phylogenetic relationship of *Cephaliophora* to nematophagous hyphomycetes including taxonomic and nomenclatural emendations of the genus *Lecophagus*. Mycologia 91, 830–835.

Tedersoo, L., Hansen, K., Perry, B.A., Kjøller, R., 2006. Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytol. 170, 581–596.

Tedersoo, L., May, T.W., Smith, M.E., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217–263.

Tedersoo, L., Arnold, E., Hansen, K., in press. Novel aspects in the life cycle and biotrophic interactions in Pezizomycetes (Ascomycota, Fungi). Mol. Ecol. http://dx.doi.org/10.1111/mec.12224.

Těšitelová, T., Těšitel, J., Jersáková, J., Říhová, G., Selosse, M., 2012. Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. Am. J. Bot. 99, 1020–1032.

U'Ren, J.M., Lutzoni, F., Miadlikowska, Arnold, A.E., 2010. Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microb. Ecol. 60, 340–353.

U'Ren, J.M., Lutzoni, F., Miadlikowska, J., Laetsch, A.D., Arnold, A.E., 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. Am. J. Bot. 99, 898–914.

van Brummelen, J., 1994. Problems in the systematics of Pezizales. In: Hawksworth, D.L. (Ed.), Ascomycete Systematics: Problems and Perspectives in the Nineties. NATO ASI Series 269. Plenum Press, New York, pp. 303–314.

van Brummelen, J., 1998. Reconsideration of relationships within the Thelebolaceae based on ascus ultrastructure. Persoonia 16, 425–469.

van Brummelen, J., Kristiansen, R., 1999. A new species of *Boubovia* (Pezizales) from the Hvaler archipelago in Norway. Persoonia 17, 265–271.

Waterman, R.J., Bidartondo, M.I., Stofberg, J., Combs, J.K., Gebauer, G., Savolainen, V., Barraclough, T.G., Pauw, A., 2011. The effects of above- and belowground mutualisms on orchid speciation and coexistence. Am. Nat. 177, E54–E68.

Wei, J., Peršoh, D., Agerer, R., 2010. Four ectomycorrhizae of Pyronemataceae (*Pezizomycetes*) on Chinese Pine (*Pinus tabulaeformis*): morpho-anatomical and molecular-phylogenetic analyses. Mycol. Progress 9, 267–280.

Wiens, J.J., 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47, 568–581.

Will, O.H., Newland, N.A., Reppe, C.R., 1984. Photosensitivity of pigmented and nonpigmented strains of *Ustilago violacea*. Curr. Microbiol. 10, 295–302.

Wolfe, B.E., Tulloss, R.E., Pringle, A., 2012. The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. PLoS ONE 7, e39597.

Yang, C.S., Wilcox, H.E., 1984. An E-strain ectendomycorrhiza formed by a new species, *Tricharina mikolae*. Mycologia 76, 675–684.

Yao, Y.-J., Spooner, B.M., 1996a. Delimitation of *Boubovia* and *Pulvinula*. Mycol. Res. 100, 193–194.

Yao, Y.-J., Spooner, B.M., 1996b. Notes on British *Octosporella* with a new genus, *Filicupula* (Pezizales). Kew. Bull. 51, 193–196.

Zhang, N., Castlebury, L.A., Miller, A.N., Huhndorf, S.M., Schoch, C.L., Seifert, K.A., Rossmann, A.Y., Rogers, J.D., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Sung, G.-H., 2006. An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98, 1076–1087.