# Systematics of the Pezizomycetes—the operculate discomycetes

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**Abstract:** The Pezizomycetes (order Pezizales) is an early diverging lineage within the Pezizomycotina. A shared derived character, the operculate ascus, supports the Pezizales as monophyletic, although functional opercula have been lost in certain taxa. Phylogenetic relationships within Pezizales were studied using parsimony and Bayesian analyses of partial SSU and LSU rDNA sequences from 100 taxa representing 82 genera and 13 of the 15 families currently recognized. Three primary lineages are identified that more or less correspond to the A, B and C lineages resolved in previous analyses using SSU rDNA: (A) Ascobolaceae and Pezizaceae; (B) Discinaceae-Morchellaceae and Helvellaceae-Tuberaceae; (C) Ascodesmidaceae, Glaziellaceae, Pyronemataceae, Sarcoscyphaceae and Sarcosomataceae. In contrast the monotypic Rhizinaceae and Caloscyphaceae are resolved as two independent lineages. Bayesian analyses support a relationship among *Rhizina* and two species of *Psilopezia* (Pyronemataceae). Only lineage C is highly supported. The B and C lineages form a strongly supported monophyletic group. None of these lineages corresponds to earlier proposed suborders. The A and B lineages are supported by certain morphological features (e.g. ascus bluing reaction in iodine, cytology of spores and paraphyses, septal pore structures and excipulum structure); these characters have been subject to homoplasy. Lineage C is the largest and most heterogeneous, and no unifying morphological features support its recognition. The Pyronemataceae, in which almost half of the species in the order are found, is not monophyletic because the Ascodesmidaceae and Glaziellaceae are nested within it. The relationships among all families in the C lineage remain uncertain. The origin of various forms of ascomata, including hypogeous forms (truffles and truffle-like), epigeous cleistothecia, simple reduced apothecia and highly elaborate, stipitate forms (helvelloid and morchelloid), are discussed.

## INTRODUCTION

The Pezizales, the only order of the Pezizomycetes, is characterized by asci that generally open by rupturing to form a terminal or eccentric lid or operculum. The ascomata are apothecia or are closed structures of various forms that are derived from apothecia. Apothecia range in size from less than a millimeter to ca. 15 cm and may be sessile or stalked (FIG. 1). The order includes epigeous, semihypogeous to hypogeous (truffles) taxa. The ascospores are single-celled, bipolar symmetrical, and usually bilaterally symmetrical, ranging from globose to ellipsoidal or occasionally fusoid. Some ascospores develop surface ornamentations in the form of warts, ridges or spines. The tissues of the ascomata are fleshy and often fragile. In a few cases ascomata are highly reduced or lacking. Anamorphic states are reported from several hyphomycetous form genera. The conidia are formed blastically; no phialidic states are known in the Pezizales. The Pezizales are saprobic, mycorrhizal or plant parasitic; the biotic interactions of many taxa are not known. Species are collected on soil, wood, leaves and dung. Soil-inhabiting species often fruit in habitats with a high pH and low content of organic matter, including disturbed ground (for ecology see Petersen 1985). The greatest diversity of species occurs in temperate regions or at high elevation. Several members of the Sarcoscyphaceae and Sarcosomataceae are common in tropical regions. The order includes ca. 1125 described species (Kirk et al 2001), classified in 15 families (Eriksson 2006).

*Characters used in the classification.*—The layering of walls, structure of the apical apparatus and histochemistry of the ascus, as observed with light and transmission electron microscopy (TEM), are used in the classification of the Pezizales (summarized by Brummelen 1994). In Pezizaceae and Ascobolaceae, the periascus (a mucilaginous extra-ascal layer) becomes blue in iodine solutions. In the Sarcoscyphaceae, Sarcosomataceae and the *Chorioactis* clade the lateral walls of the asci are thick and have particular layering as seen in TEM. In the Sarcoscyphaceae the operculum is eccentric and thickened. The interpretation of the structures in such asci has been the subject of debate (Eckblad 1968, Samuelson 1975). Asci in most hypogeous and cleistothecial
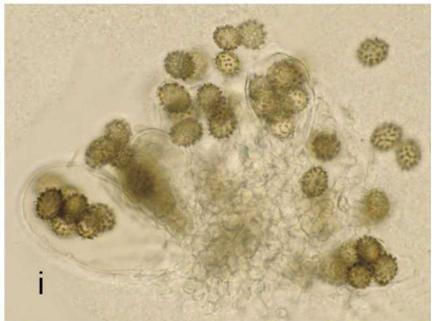
forms lack an operculum and spores are discharged passively. In some taxa (e.g. *Carbomyces* and *Orbicula*) asci disintegrate and the spores form a powdery mass. Operculate asci are known outside Pezizales (Brummelen 1998, Kohlmeyer and Kohlmeyer 1979, Verkley 1995) but these are structurally different in wall layering, and their similarity to the pezizalean operculum seems to be a case of convergence.

Additional features employed in classification are arrangement of hyphae in the sterile tissues of the apothecium, presence of hairs and/or tomentum, spore shape, ornamentation, presence or absence of oil guttules and hymenial pigmentation (Boudier 1907, Nannfeldt 1932, 1949, Eckblad 1968, Korf 1972, 1973). The number of nuclei (uni- or multinucleate) in ascospores and paraphyses (Berthet 1964) has been used to assign taxa to families. Presence, absence and types of carotenoid pigments were studied by Arpin (1969) and used as a basis of a classification he presented. Ultrastructural characters of septal pore plugs and Woronin bodies correlate to some degree with families and lineages in the order (Kimbrough 1994).

*Variation in apothecial form.*—Although formation of an apothecial ascomata is normally the rule in members of the Pezizales, in some cases the ascomata develop cleistothecium- and perithecium-like morphologies (Corner 1931, Döbbeler 2004, Hansen et al 2005b). In other cases there is a reduction or loss of sterile tissues entirely. Brummelen (1967) described the developmental patterns in the genera *Ascobolus* and *Saccobolus*, and this system has been adopted widely across the order. Development may be cleistohymenial or gymnohymenial. Cleistohymenial ascomata never open or may open at different stages in development of the hymenial layers. Thus, hymenia of cleistohymenial types may be exposed early in development of the ascoma, before asci are formed or mature, or opening of the ascoma may occur only at the final stages when asci are fully mature. Cleistohymenial types that remain closed might be confused with cleistothecia of other unrelated groups (e.g. Eurotiales).

*The circumscription of the order.*—In early treatments both operculate and inoperculate taxa were included in the Pezizales (Bessey 1907, EA Bessey 1950, Seaver 1928). The use of the Pezizales in the restricted sense of the operculate discomycetes can be traced to Nannfeldt (1932). Eckblad (1968), Rifai (1968), Kimbrough (1970), Korf (1972), Brummelen (1994), Landvik et al (1997) and Pfister and Kimbrough (2001) reviewed the order and its evolving classification. While not always recovered as a monophyletic group, the five-gene molecular phylogenetic study of the Pezizomycotina in this volume (Spatafora et al) resolves the Pezizales as monophyletic, although only with weak support.

The circumscription of the Pezizales has been shaped by inclusion and exclusion of taxa. On the one hand, the Pezizales was expanded considerably when (Trappe 1979) abandoned the order Tuberales and transferred most hypogeous genera to families of the Pezizales. Since then the hypothesis that the ascomycete truffles evolved independently within different evolutionary lineages of Pezizales has been confirmed and refined by ascus septal ultrastructure, number of nuclei in spores and molecular phylogenetics (summarized by Læssøe and Hansen in review). On the other hand, *Thelebolus* and related genera (Thelebolaceae) were excluded from the Pezizales based on ascoma development and ascus structure (e.g. Samuelson and Kimbrough 1978) and molecular phylogenetic studies (Landvik et al 1998). These taxa now are considered as allied with the Leotiomycetes. Nevertheless, Thelebolaceae show a wide range of ascus dehiscence mechanisms (operculate, bilabiate or inoperculate; Brummelen 1998) and some taxa still might belong to the Pezizales.

*Subordinal classifications.*—Chadefaud (1946) and Le Gal (1946a, b) introduced the idea that there are two types of operculate asci: thin-walled asci with terminal

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FIG. 1. Examples of ascoma types and habits in the Pezizales. a–b. Pezizaceae and Ascobolaceae (lineage A). a. *Peziza succosa* apothecia exuding yellow milk where cut, on disturbed soil (KH-98-34, C). b. *Saccobolus* sp. apothecia with protruding asci, dark colored by purplish spores, on dung (JHP-96.006). c–d. Discinaceae-Morchellaceae (lineage B). c. *Gyromitra californica* (KH-97-26, FH). d. *Morchella esculenta.* e. Rhizinaceae: *Rhizina undulata* (TL-5357, C). f. *Psilopezia juruensis* (T. Læssøe, AAU 59604, C). g. Caloscyphaceae: *Caloscypha fulgens,* insert shows the green color of the apothecia when brushed (KH-97-17, FH). h.–o. Pyronemataceae-Ascodesmidaceae-Sarcosomataceae-Sarcoscyphaceae (lineage C). h. *Pyronema domesticum* on burnt ground (JHP-03.136, C). i. *Ascodesmis nigricans* simple eugymnohymenial apothecium in LM (CBS 389.68). j. *Lasiobolus diversisporus* apothecium with protruding asci and stiff pointed hairs, on dung (JHP-98.020, C). k. *Octospora* cf. *axillaris* parasitic on moss (KS-94–194, C). l. *Scutellinia scutellata* (KS-94-46, C). m) *Otidea onotica* (JHP-04.107, C). n. *Plectania nannfeldtii* stipitate apothecia emerging from melting snow (KH-97-16, FH). o. *Phillipsia domingensis* (TL-6014, C). Photos: b, d, h, j, m ©Jens H. Petersen/MycoKey; a, c, g, i, k, l, n K. Hansen; e, f, o T. Læssøe.

opercula and thick-walled asci with thickened, often eccentric opercula, the so-called suboperculate asci. Le Gal proposed the family Sarcoscyphaceae for these, but for nomenclatural reasons it first was published validly by Eckblad (1968). Within the Sarcoscyphaceae two tribes were recognized (Le Gal 1953): one brown-black, the Urnuleae, and one bright-colored, the Sarcoscypheae. These tribes later were recognized as families, the Sarcoscyphaceae and the Sarcosomataceae (Korf 1970). Earlier Rifai (1968) had divided the Pezizales into two suborders, the Sarcoscyphineae (for those with suboperculate asci) and the Pezizineae. Kimbrough (1989) created a new suborder, Pyronemineae, for *Pyronema*, *Ascodesmis* and additional genera with eugymnohymenial or paragymnohymenial apothecia. Thus, three suborders have been recognized. Molecular phylogenetic studies, however, do not support these subordinal distinctions (Landvik et al 1997, Harrington et al 1999). Landvik et al (1997) detected three major lineages within the Pezizales using SSU rDNA: (A) Pezizaceae and Ascobolaceae, (B) Helvellaceae, Morchellaceae, Tuberaceae, and *Caloscypha* and (C) Sarcoscyphaceae, Sarcosomataceae, Ascodesmidaceae, Glaziellaceae and Pyronemataceae. The relationships among the three lineages were not resolved. Several families and/or lineages have been the focus of molecular phylogenetic studies that have refined family boundaries and provided insight into relationships among families. In the present study characters from an additional region of the rDNA, the LSU, and increased taxon sampling are used to further investigate the evolution of pezizalean fungi.

## MATERIAL AND METHODS

*Specimens.*—A data matrix containing 99 species of Pezizales and *Neolecta vitellina* was constructed with sequences from LSU and SSU rDNA (SUPPLEMENTARY TABLE I). Sequences were selected to represent all sublineages within Pezizales based primarily on O'Donnell et al (1997), Harrington et al (1999), Hansen et al (2001, 2005b) and Perry et al (in preparation), including 82 genera and 13 families (out of ca. 164 genera and 15 families). The monotypic families Karstenellaceae and Carbomycetaceae are not included. *Neolecta vitellina* was used as outgroup.

*Molecular methods and analyses.*—Laboratory techniques followed procedures outlined in Hansen et al (2002, 2005a). Twenty-eight new SSU sequences, included in this study, are deposited in GenBank (SUPPLEMENTARY TABLE I). The combined SSU and LSU alignment is available from TreeBASE as accession M2779. Individual and combined analyses of the LSU and SSU rDNA were performed using the portable version of PAUP 4.0b10 for Unix (Swofford 2002) and MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001) on G5 Macintosh computers. Search strategies were

reported earlier by Hansen et al (2005a), except for Bayesian analyses that consisted of two parallel searches, each run for 5 000 000 generations. The trees sampled before the chains reached a split deviation frequency of 0.05 were discarded as burn-in, while the remaining trees were used to calculate the posterior probabilities (PP) of the clades. The GTR+I+G model of nucleotide substitution was found to fit each of the individual datasets best, using a hierarchical likelihood ratio test as implemented in the program MrModeltest 2.2 (Nylander 2004). Maximum parsimony (MP) analyses of the SSU rDNA used a two-step search (due to an exceedingly large number of trees generated), while analyses of the LSU used a single step search.

## RESULTS AND DISCUSSION

*Phylogenetic analyses.*—The LSU region is highly variable across all the Pezizales and exclusion of various regions was explored in analyses. Weakly supported branches were affected by the exclusion of characters, but this process also resulted in lower resolution toward the tips of the tree. Therefore in the final analyses all characters were included. No supported conflict (PB [parsimony bootstrap] $\geq 75\%$, PP $\geq 95\%$) was detected between the individual LSU and SSU gene trees. The combined dataset consisted of 2726 characters of which 746 were parsimony informative. Parsimony analyses resulted in 62 most parsimonious trees (MPTs). The strict consensus tree of all MPTs is highly resolved, but many of the deeper branches have only low support. Bayesian analyses reached an average standard deviation of split frequencies below 0.05 after approximately 420 000 generations, and the first 4200 trees were excluded as the burn-in. Bayesian posterior probabilities support many of the terminal relationships in the phylogeny with confidence but also fail to support some of the deeper nodes.

*Subordinal relationships.* Three lineages are identified (FIG. 2) that overall correspond to the A, B and C lineages resolved by Landvik et al (1997). Of these lineages only C is well supported; lineages A and B are without support and not resolved in parsimony bootstrap analyses. Lineage A is resolved as a sister group to the rest of the Pezizales, which form a strongly supported monophyletic group. In contrast to previous results, which placed the monotypic genera *Rhizina* (O'Donnell et al 1997) and *Caloscypha* (Landvik et al 1997) in the B lineage, our analyses resolve these taxa (now recognized as Rhizinaceae and Caloscyphaceae) as two independent lineages (FIG. 2). Two species of the genus *Psilopezia* (currently classified in Pyronemataceae) are suggested as closely related to *Rhizina*, although supported only by Bayesian PP (99%). *Caloscypha*, *Rhizina* and *Psilopezia* appear to be

highly divergent and their placement is uncertain. In multigene analyses by Spatafora et al (this volume) the A, B and C lineages are strongly supported with *Rhizina* and *Caloscypha* as successive sister taxa to a strongly supported Morchellaceae-Discinaceae clade as lineage B. Their analyses, however, include only 4–5 taxa from each of the A, B and C lineages, which might affect the groupings and support.
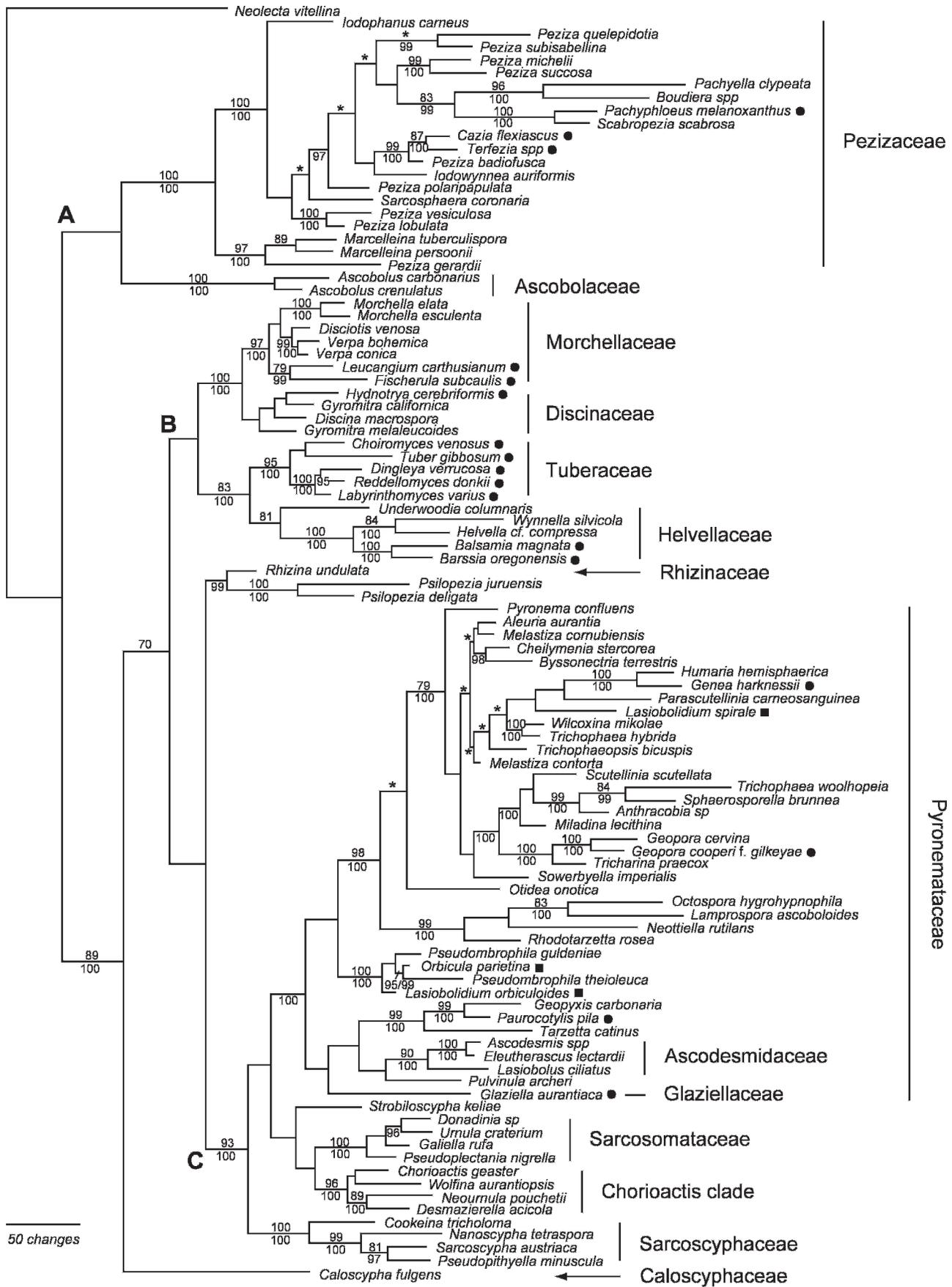
*Pezizaceae-Ascobolaceae* (Fig. 1a–b). Several features support a common ancestry of the Pezizaceae and Ascobolaceae, such as the amyloid reaction of the asci that is absent from other pezizalean families, the presence of an internal circular indentation delimiting the operculum and some similarities in the type of ascus septum pore plugging (e.g. Brummelen 1978, Kimbrough 1994). The amyloid reaction of the asci has been lost in some taxa within lineage A (e.g. in *Marcelleina*), several hypogeous taxa (Hansen et al 2001) and some species of *Ascobolus* sec. *Ascobolus* (Brummelen 1967). The ascospores in both families are uninucleate. The excipulum structure is generally composed of thin-walled globose cells often with interwoven hyphae. Each family is highly supported as monophyletic. The Ascobolaceae, as we recognize it, includes three genera, *Ascobolus*, *Saccobolus* and *Thecotheus*, all of which have been included in molecular studies (Landvik et al 1997, 1998). The distinction between Ascobolaceae and Pezizaceae is not completely clear-cut, and a few genera (e.g. *Boudiera*, *Iodophanus* and *Thecotheus*) have been alternatively classified in either family (e.g. Eckblad 1968, Kimbrough and Korf 1967, Korf 1972). *Ascobolus* and *Saccobolus* have a unique violet-pigmented epispore of the ascospores (Fig. 1b), a feature absent in *Thecotheus*. All species of Ascobolaceae have diffusely amyloid asci, but this type of amyloid reaction also is found in some lineages of the Pezizaceae. The ascus septal pore plug, although with similarities, is of a distinct type in each of the families (Kimbrough 1994).

Molecular phylogenetic analyses of the Pezizaceae using partial sequences from LSU, RPB2 and β-tubulin, identified 14 fine-scale lineages within the family (Hansen et al 2005a; representatives of 12 of these fine-scale lineages are included here, Fig. 2). Species of *Peziza* occur in eight of the lineages, spread among other genera of the family, confirming the nonmonophyly of the genus (Norman and Egger 1999, Hansen et al 2001). The analyses support several scenarios for a revised classification of *Peziza* and Pezizaceae that is under way (Hansen and Pfister in progress). The truffle genus *Terfezia* is nested within the Pezizaceae (Fig. 2) thus relegating Terfeziaceae to synonymy (see Hansen and Trappe 2001). Hypogeous taxa have evolved multiple times within

the Pezizaceae; 13 of 25 genera currently placed in the family are hypogeous. No truffles are known in the Ascobolaceae. In the Pezizaceae there are saprobic and mycorrhizal taxa that are either epigeous (e.g. *Peziza succosa* Fig. 1a) or truffle-like (Tedersoo et al 2006). The Ascobolaceae all are presumed saprobic; the majority are coprophilous, some occur on other organic substrates or on soil.

*Morchellaceae-Discinaceae-Helvellaceae-Tuberaceae* (Fig. 1c–d). A Morchellaceae-Discinaceae clade is identified as a strongly supported sister group to a Helvellaceae-Tuberaceae clade (Fig. 2), confirming molecular results by O'Donnell et al (1997). The families as emended by O'Donnell et al (1997) are resolved as monophyletic, but the Discinaceae with only weak support. Helvellaceae was restricted by O'Donnell et al (1997) to tribus Helvelleae Dissing (1966) of Helvellaceae and the hypogeous *Barssia* and *Balsamia* (Balsamiaceae). Discinaceae and a monotypic Rhizinaceae were resurrected for the remaining helvellaceous genera of Dissing (1966). One of the primary characters previously used to distinguish Helvellaceae, tetranucleate spores, was suggested to be a plesiomorphic character rather than uniquely derived within Helvellaceae; other families recognized with tetranucleate spores include Discinaceae and some taxa in Tuberaceae. A strictly hypogeous Tuberaceae was maintained and emended by O'Donnell et al (1997) to include *Tuber*, *Choriomyces*, *Dingleya*, *Labyrinthomyces* and *Reddellomyces*; all these genera, except *Tuber*, were previously classified in other families.

Ascus septal pore plugs, which are dome-, cone- or dumbbell-shaped structures with V-shaped striations, support lineage B (Kimbrough 1994). The spores are tetra- or multinucleate (multinucleate in Morchellaceae), with a variable number of nuclei and spores per ascus in the Tuberaceae. The families contain many of the largest members of the order, with some of the most elaborate apothecia: sessile to stipitate, cup-shaped to expanded, cerebriform, saddle-shaped to campanulate, including forms with strongly folded, gyrose to sponge- or honeycomb-like hymenia (Fig. 1c–d). They include many of the commercially important taxa in the order such as *Morchella* and *Tuber*. Members of the Morchellaceae and Discinaceae fruit on soil, sometimes in connection with rotten wood, or directly on wood, woodchips or sawdust, and are possibly saprobic, ectomycorrhizal (e.g. *Hydnotrya*, Tedersoo et al 2006) or facultative ectomycorrhizal or mycorrhizal-like (e.g. *Morchella*, Dahlstrom et al 2000). The Helvellaceae and Tuberaceae include taxa that fruit exclusively on/in soil, and all will likely prove to be ectomycorrhizal (for evidence so far see Tedersoo et al 2006).

*Neolecta vitellina*

*Iodophanus carneus*

Pezizaceae
- Peziza quelepidotia
- Peziza subisabellina
- Peziza michelii
- Peziza succosa
- Pachyella clypeata
- Boudiera spp
- Pachyphloeus melanoxanthus ●
- Scabropezia scabrosa
- Cazia flexiascus ●
- Terfezia spp ●
- Peziza badiofusca
- Iodowynnea auriformis
- Peziza polaripapulata
- Sarcosphaera coronaria
- Peziza vesiculosa
- Peziza lobulata
- Marcelleina tuberculispora
- Marcelleina persoonii
- Peziza gerardii

Ascobolaceae
- Ascobolus carbonarius
- Ascobolus crenulatus

Morchellaceae
- Morchella elata
- Morchella esculenta
- Disciotis venosa
- Verpa bohemica
- Verpa conica
- Leucangium carthusianum ●
- Fischerula subcaulis ●

Discinaceae
- Hydnotrya cerebriformis ●
- Gyromitra californica
- Discina macrospora
- Gyromitra melaleucoides

Tuberaceae
- Choiromyces venosus ●
- Tuber gibbosum ●
- Dingleya verrucosa ●
- Reddellomyces donkii ●
- Labyrinthomyces varius ●

Helvellaceae
- Underwoodia columnaris
- Wynnella silvicola
- Helvella cf. compressa
- Balsamia magnata ●

Rhizinaceae
- Barssia oregonensis ●

- Rhizina undulata
- Psilopezia juruensis
- Psilopezia deligata

Pyronemataceae
- Pyronema confluens
- Aleuria aurantia
- Melastiza cornubiensis
- Cheilymenia stercorea
- Byssonectria terrestris
- Humaria hemisphaerica
- Genea harknessii ●
- Parascutellinia carneosanguinea
- Lasiobolidium spirale ■
- Wilcoxina mikolae
- Trichophaea hybrida
- Trichophaeopsis bicuspis
- Melastiza contorta
- Scutellinia scutellata
- Trichophaea woolhopeia
- Sphaerosporella brunnea
- Anthracobia sp
- Miladina lecithina
- Geopora cervina
- Geopora cooperi f. gilkeyae ●
- Tricharina praecox
- Sowerbyella imperialis
- Otidea onotica
- Octospora hygrohypnophila
- Lamprospora ascoboloides
- Neottiella rutilans
- Rhodotarzetta rosea
- Pseudombrophila guldeniae
- Orbicula parietina ■
- Pseudombrophila theioleuca
- Lasiobolidium orbiculoides ■
- Geopyxis carbonaria
- Paurocotylis pila ●
- Tarzetta catinus

Ascodesmidaceae
- Ascodesmis spp
- Eleutherascus lectardii
- Lasiobolus ciliatus
- Pulvinula archeri

Glaziellaceae
- Glaziella aurantiaca ●

- Strobiloscypha keliae

Sarcosomataceae
- Donadinia sp
- Urnula craterium
- Galiella rufa
- Pseudoplectania nigrella

Chorioactis clade
- Chorioactis geaster
- Wolfina aurantiopsis
- Neournula pouchetii
- Desmazierella acicola

Sarcoscyphaceae
- Cookeina tricholoma
- Nanoscypha tetraspora
- Sarcoscypha austriaca
- Pseudopithyella minuscula

Caloscyphaceae
- Caloscypha fulgens

50 changes

A  B  C

*Rhizinaceae and Psilopezia* (FIG. 1e–f). The monotypic genus *Rhizina* has always been considered closely related to members of the Helvellaceae and Discinaceae, mainly because of the one-layered excipulum of *textura intricata* and spore ornamentation similar to species of *Discina* (e.g. Dissing 1966, Abbott and Currah 1997). It has been treated separately in a tribe Rhizineae of Pezizaceae (Korf 1954) and as a monotypic family Rhizinaceae (Benedix 1961). Korf (1958) and Benedix (1961) considered Rhizinaceae to share a common ancestor with Discinaceae. Molecular phylogenetic analyses support *Rhizina undulata* as a separate lineage, and it therefore was removed from the Helvellaceae by O'Donnell et al (1997), but sister group relationships have not been resolved. In our analyses *Rhizina* (FIG. 1e) forms a monophyletic group with *Pilopezia juruensis* (FIG. 1f) and *P. deligata* as a sister group to the C lineage (FIG. 2). Several distinct characters support the separation of *Rhizina*: thick-walled setae in the hymenium originating from cells in the excipulum; indeterminate growth at the margin of the apothecia; early coalescence of multiple fruit bodies; and rhizoids attaching the apothecia to the substrate (Abbott and Currah 1997). It is pathogenic on conifers, causing mortality, and is especially damaging to seedlings planted on newly burned sites, and it usually fruits on burnt ground (e.g. Jalaluddin 1967, Ginns 1968).

The "psilopezioid fungi," *Psilopezia* (and *Pachyella*, Pezizaceae), were recognized for a group of operculate discomycetes with fruiting bodies broadly attached to the substrate, on wet or submerged wood or other decaying plant parts. Pfister (1973a, b) restricted *Psilopezia* to taxa with nonamyloid asci, ascospores with an outer spore wall that loosens in Cotton Blue in lactic acid and/or iodine solutions, an outer excipulum of parallel hyphae perpendicular to the outside and gel, if present, located in the inner layer of the outer excipulum. In this sense, *Psilopezia* was treated in the tribe Otideeae of Pyronemataceae (Korf 1972, Pfister 1973b) based on the lack of carotenoids, nonamyloid asci, uninucleate spores and anatomical similarities to *Otidea*. We have not been able to extract DNA from specimens of the type species, *P. nummularia*, but assuming that the genus is monophyletic the placement within Pyronemataceae is not supported by the molecular data.

*Rhizina* and *Psilopezia* both produce brownish, pulvinate to gibbose apothecia that are broadly attached to the substrate (FIG. 1e–f) but otherwise are sufficiently distinct in morphology and ecology to question a close relationship.

*Caloscyphaceae* (FIG. 1g). The family Caloscyphaceae was erected for the monotypic genus *Caloscypha* (Harmaja 2002) based partly on phylogenetic analyses of SSU rDNA (Landvik et al 1997), which placed *Caloscypha fulgens* within a *Helvella/Tuber* clade. Nevertheless, in analyses forcing *Caloscypha* to group with lineage C could not be rejected. In our MP analysis *Caloscypha* is weakly resolved as a sister group to the clade of lineages B and C (FIG. 2). *Caloscypha* has been pointed out to be rather isolated in the Pezizales or Pyronemataceae (Eckblad 1968, Nannfeldt in Korf 1972). An unidentified carotenoid was found in the apothecia of *Caloscypha* and *Sowerbyella*, which also share β-carotene as a major pigment (Arpin 1969). This led Le Gal (1969), followed by Korf (1972, 1973), to place *C. fulgens* in the Sowerbyelleae of Otideaceae/Pyronemataceae. This relationship is not supported by molecular phylogenetic analyses; *Sowerbyella* is deeply nested within Pyronemataceae (FIG. 2). *Caloscypha fulgens* produces bright orange-yellow apothecia that are unique within the Pezizales by turning green or bluish with age or when touched or broken (FIG. 1g). Spore cytology and presence of carotenoids suggest that *C. fulgens* belongs to the C lineage, whereas the ascospore wall type ("*Morchella*" type, Harmaja 1974) suggests affinities with the B lineage. The anamorphic state, *Geniculodendron pyriforme*, is a pathogen of conifers seeds (Paden et al 1978). *Caloscypha fulgens* occurs in temperate and alpine areas, fruits in early spring or after snowmelt.

*Pyronemataceae-Ascodesmidaceae-Glaziellaceae-Sarcosomataceae-Chorioactis clade-Sarcoscyphaceae* (FIG. 1h–o). Lineage C is the largest and most heterogeneous assemblage. The MP analysis indicates that Pyronemataceae is not monophyletic, because Ascodesmidaceae and Glaziellaceae are nested within it (FIG. 2). The relationships among these families however, are not supported, and in Bayesian analyses Glaziellaceae and Ascodesmidaceae are resolved as successive sister lineages of Pyronemataceae. In fact no interfamilial relationships in the C lineage have support. The majority of the Pyronemataceae sampled form a highly

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FIG. 2.  Phylogenetic relationships within the Pezizomycetes inferred from combined analysis of LSU and SSU rDNA. One of 62 MP trees. Terminal taxa represent individual specimens (SUPPLEMENTARY TABLE I). Branches with asterisks collapse in the strict consensus of all MP trees. Numbers above branches are posterior probabilities (PP ≥ 95%) obtained from 50% majority rule consensus tree of the 45 800 trees sampled from a Bayesian MCMC analysis; numbers below branches are MP bootstrap proportions (PB ≥ 70%). ● = hypogeous ascomata; ■ = cleistothecia. Three primary lineages identified are labeled A, B and C for discussion.

supported monophyletic group. This is in agreement with recent results by Perry et al (in preparation) based on LSU rDNA and a much larger pyronemataceous taxon sampling. The family is the largest in Pezizales, including 78 genera, representing about half of the known species within the order (ca. 500 spp.). It has been a default family for pezizalean taxa with uninucleate spores and iodine negative asci that lack distinguishing anatomic characters by which they could be segregated into natural families. The concept of the Pyronemataceae 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 for the remaining taxa. Korf (1972) proposed five subfamilies and 11 tribes in his broadly inclusive Pyronemataceae. The divisions into families or subfamilies (Arpin 1969, Korf 1972, 1973, Kimbrough 1970) were based mainly on the pigmentation of the apothecia, spore pigmentation and guttulation, and the presence or absence of a subiculum. The division into tribes (Korf 1972) was based further on characters such as presence or absence, origin and pigmentation of apothecial hairs. Kimbrough (1989) argued for a restricted concept of the Pyronemataceae to include only *Pyronema* (FIG. 1h) and *Coprotus*, distinguished by gymnohymenial apothecia that arise from clusters of paired ascogonia and antheridia, opercula resulting from changes in the outer ascus wall, and complex, striated septal pore plugs in the base of asci. Based on the presence or absence of carotenoids and hairs of the apothecia, Korf and Zhuang (1991) placed numerous other taxa once placed in the family (not including Ascodesmidaceae and Thelebolaceae) in two subfamilies of the Otideaceae, Otideoideae and Scutellinieae (for *Otidea* and *Scutellinia* see FIG. 1l–m). Molecular phylogenetic analyses do not support a segregation of *Pyronema* from the Otideaceae (Landvik et al 1997). Our analyses confirm this position, with *Pyronema* nested within a moderately supported clade including species from each of the subfamilies (FIG. 2). This clade furthermore is nested within a strongly supported clade including *Otidea* and a clade of *Lamprospora*, *Octospora* (FIG. 1k), *Neottiella* and *Rhodotarzetta*. Nevertheless the rDNA sequences of *Pyronema* appear to be somewhat divergent, as do sequences of *Otidea* (FIG. 1m) and *Sowerbyella*, and their sister group relationships are still unresolved (FIG. 2). Perry et al (in preparation) identified 12 larger clades within Pyronemataceae, nine of which are represented in our analyses (FIG. 2). None of these

clades corresponds to any of the restricted families, subfamilies or tribes that have been employed for the pyronemataceous taxa. Molecular analyses imply that there has been extensive homoplasy in most of the morphological characters, such as carotenoid pigments and hairs of the apothecia, which have been used to delimit suprageneric taxa within this group. The relationships among the clades were not resolved using rDNA sequences (FIG. 2, Perry et al in preparation) and analyses with additional genes are needed to provide a robust phylogenetic hypothesis for this group (Hansen et al in progress).

Species of Ascodesmidaceae are among the most highly reduced apothecial forms in lineage C with simple eugymnohymenial apothecia (without excipula) that consist of a bundle of asci surrounded by paraphyses on a small base (FIG. 1i) or of solitary asci (Brummelen 1989). Brummelen (1981) reintroduced Ascodesmidaceae as a monotypic family. Based on the complex ultrastructure of the asci and spores (Brummelen 1978, Merkus 1976), Brummelen (1981) considered *Ascodesmis* a specialized fungus with a strongly reduced ascoma. Despite the absence of an opening mechanism for the ascus, *Eleutherascus* was considered closely related to *Ascodesmis*, based on identical development of the spore ornamentation (Brummelen 1989), and was added to the Ascodesmidaceae. Molecular phylogenetic analyses show that *Ascodesmis* and *Eleutherascus* are closely related to *Lasiobolus* and group with members of Pyronemataceae (FIG. 2; Landvik et al 1998, Perry et al in preparation). The molecular results do not resolve with confidence the question of whether Ascodesmidaceae should be retained as a separate family. Kimbrough (1989) accepted the Ascodesmidaceae *sensu* Brummelen in his suborder Pyronemineae and added the genus *Amauroascus* (now Onygenales). Kimbrough (1989, 1994) considered the septal pore plug in the base of the asci to be unique and similar in Ascodesmidaceae and Pyronemataceae (*Pyronema* and *Coprotus*). The genus *Pyronema* also produces simple, gymnohymenial apothecia, with an excipulum of few to several layers of cells (FIG. 1h), developing from clusters of paired ascogonia and antheridia (e.g. Moore and Korf 1963). Clearly the suborder Pyronemineae is paraphyletic in molecular phylogenetic analyses (FIG. 2), which suggests that very simple, reduced apothecial forms have evolved at least twice in the Pezizales.

*Glaziella aurantiaca*, the only species of Glaziellaceae, has had a confusing taxonomic history and once even its own order (Gibson et al 1986). The ascomata are unusual; they are more or less epigeous, and large, bright yellow to orange, completely hollow with a basal opening, with monosporic asci scattered

within a rather thin, gelatinous ascoma wall and large spores, 300–500 μm. The spores are left embedded in the ascoma wall when the asci disintegrate. Paraphyses are lacking. Molecular phylogenetic analyses have shown that *Glaziella* belongs to Pezizales (Landvik and Eriksson 1994) and that it is related to genera of the Pyronemataceae (FIG. 2, Landvik et al 1997) or Sarcoscyphaceae (Harrington et al 1999), but neither relationship is supported.

The restricted families Sarcoscyphaceae and Sarcosomataceae and the *Chorioactis* clade are each highly supported (FIG. 2). The clade of *Chorioactis, Desmazierella* (Sarcoscyphaceae in Korf 1970, 1972, Eriksson 2006), *Neournula* and *Wolfina* (Sarcosomataceae in Korf 1970, 1972, Eriksson 2006), here referred to as the *Chorioactis* clade, was first identified by Harrington et al (1999) although without bootstrap support (PB 30%). The circumscription of this clade is being explored further using RPB2, LSU and SSU rDNA sequences and morphology (Pfister et al in preparation). The Sarcoscyphaceae and Sarcosomataceae as delimited here are supported as distinct from each other and from the rest of the C lineage by morphology and ecology. The Sarcoscyphaceae includes species with brightly colored apothecia having carotenoids (FIG. 1o), and hairs, if present, are light colored. The species of Sarcosomataceae have dark apothecia (FIG. 1n) and dark hairs. The apothecia in both families are mostly leathery or corky, or sometimes tough-gelatinous, due to an excipulum composed of *textura intricata*. The spores are as far as known multinucleate, and in Sarcoscyphaceae the paraphyses cells are multinucleate whereas they are uninucleate in Sarcosomataceae (Berthet 1964).

Ascus septal pore plugs are highly variable among taxa in the C lineage, with five different types described in Pyronemataceae, distinct from those in the Sarcoscyphaceae, Sarcosomataceae and Ascodesmidaceae (as summarized by Kimbrough 1994). None of the five types found in Pyronemataceae so far correspond to any of the groupings found by molecular phylogenetic analyses. A few characters are unique to the lineage, although not all taxa have them; apothecial hairs and carotenoid pigments are found only in the C lineage (except carotenoids are also present in apothecia of *Caloscypha* and *Iodophanus*).

Pyronemataceae occur on various types of soil, wood, other plant debris and dung. The majority of species have been considered saprobic, but their trophic strategies are not well studied and remain unknown. One group (here represented by the genera *Octospora, Lamprospora* and *Neottiella*) is parasitic on the rhizoids of bryophytes (Döbbeler 1979, FIG. 1k) and an increasing number of species are being identified as ectomycorrhizal (Tedersoo et al 2006).

The Ascodesmidaceae are considered saprobic; all known species are coprophilous. Species of Sarcoscyphaceae, Sarcosomataceae and the *Chorioactis* clade are likely saprobic and occur on wood, other plant parts or soil. One species, *Urnula craterium*, is considered to cause a plant disease, *Strumella* canker on oak (Davidson 1950, anamorphic state *Conoplea globosa*), and there might well be others. Pyronemataceae reach their highest diversity in temperate zones and arctic alpine areas, whereas many taxa of Sarcoscyphaceae and Sarcosomataceae are tropical and if temperate typically fruit in the spring (FIG. 1n).

*Ascomata form and habit.*—Several types of ascomatal forms exist within the Pezizales. Epigeous apothecia of various shapes with forcible spore discharge are the most common form and occur in each of the A, B and C lineages. This form is presumably the ancestral state. Hypogeous or semihypogeous, closed ascomata with a correlated loss of active spore dispersal have evolved independently at least 15 times in six families (Læssøe and Hansen in review, FIG. 2). The truffle or truffle-like forms show various degrees of folding and compression of the hymenium from hollow to more or less solid ascomata to ascomata without hymenia or with external hymenia. Of interest, all ascomycetous truffles and truffle-like forms, with the exception of *Elaphomyces* (Eurotiales, Eurotiomycetes), belong to the Pezizales. Epigeous cleistothecial forms, which likewise have lost the ability to discharge spores actively, have evolved at least three times within the Pyronemataceae (Hansen et al 2005b, Perry et al in preparation). Highly reduced apothecia have evolved within the Pezizales at least twice, the most extreme being *Eleutherascus* (Ascodesmidaceae) with species producing indehiscent asci singly or sparsely aggregated without any excipulum cells. Our data support the view that the reduced apothecia of e.g. *Eleutherascus, Ascodesmis* and *Pyronema* are evolutionarily derived structures within the Pezizales rather than representing an ancestral state. The large and complex fruit bodies of lineage B have inspired several evolutionary hypotheses. Most authors (e.g. Nannfeldt 1937, Dissing 1966) regarded these as highly derived forms, based in part on their tetra- and multinucleate spores. This view was also supported by Kimbrough (1994), who considered the septal pore plug at the base of the asci in taxa of Helvellaceae and Morchellaceae to be the most elaborate within the Pezizales. Molecular data suggest that the turbinate, pulvinate to cupulate forms of lineage A are a sister group to the rest of the Pezizales. Pileate, helvelloid and morchelloid, cupulate and hypogeous forms occur in lineage B, where minute or reduced ascomata are unknown so far. Because of the lack of support for lineages A and B, and for the

relationships among the B and C lineages, the origin and evolution of these most complex ascomata types remains in question.

*Concluding remarks.*—The combined LSU and SSU phylogeny of an expanded taxon sampling support previous results regarding relationships within Pezizomycetes but suggests that more than the three primary lineages (A, B and C) might exist; the Caloscyphaceae and Rhizinaceae might be independent lineages. Although lineage C is the most heterogeneous, it is the only lineage with strong support. In addition to the broader sampling of taxa in this study a multigene approach is needed to resolve the interfamilial/subordinal relationships within the Pezizomycetes. In the past 10 y several molecular phylogenetic studies have focused on the Pezizales and provided significant insight into the evolution of these fungi. The circumscription of the order and the delimitation of families have been clarified or are being clarified. At the generic and species level many problems remain to be solved.

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