Phylogenetics of the Pezizaceae, with an emphasis on *Peziza*

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Abstract: Phylogenetic relationships among members of the Pezizaceae were studied using 90 partial LSU rDNA sequences from 51 species of *Peziza* and 20 species from 8 additional epigeous genera of the Pezizaceae, viz. *Boudiera*, *Iodophanus*, *Iodowynnea*, *Kimbropezia*, *Pachyella*, *Plicaria*, *Sarcosphaera* and *Scabropezia*, and 5 hypogeous genera, viz. *Amylascus*, *Cazia*, *Hydnophymous*, *Ruhlandiella* and *Tirmania*. To test the monophyly of the Pezizaceae and the relationships to the genera *Marcelleina* and *Pfistera* (*Pyronemataceae*), 6 species from the families *Ascobolaceae*, *Morchellaceae* and *Pyronemataceae* were included. Maximum parsimony and maximum likelihood analyses of these sequences suggest that the Pezizaceae is paraphyletic, because the non-amyloid *Marcelleina* is nested within it. If *Marcelleina* were transferred to the Pezizaceae, then the family would be monophyletic. Although the Pezizaceae is traditionally characterized by amyloid asci, our results indicate that the amyloid reaction is a symplesiomorphy, which has been lost in some lineages, e.g., in those including *Marcelleina* and *Cazia*. Nodes deep in the tree could not be resolved, but 7 groups of species (I–VII) are generally well supported or present in all trees. *Peziza* species, which constitute the core of the family, are present in all groups except group III, confirming the non-monophyly of the genus. The analyses indicate that the other included genera of the Pezizaceae are all nested within *Peziza*, the placement of *Iodophanus* being unresolved. The morphologically distinct *Peziza gerardii*, which forms a clade with *Marcelleina*, appears to be the sister group to the rest of the Pezizaceae. Morphological features were studied and evaluated in the context of the phylogeny. Distinct types of ascus amyloid reactions were found to support different rDNA lineages, e.g., a distinct amyloid ring zone at the apex is a synapomorphy for group IV, an intense and unrestricted amyloid reaction of the apex is mostly found in group VI, and asci that are weakly or diffusely amyloid in the entire length are present in group II. Other morphological features, such as spore surface relief, guttulation, excipulum structure and pigments, while not free from homoplasy, do support the groupings. Anamorphs likewise provide clues to higher-order relationships within the Pezizaceae. Several macro- and micromorphological features, however, appear to have evolved several times independently, including ascomatal form and habit (epigeous, semi-hypogeous or hypogeous), spore discharge mechanisms, and spore shape. Parsimony-based optimization of character states on our phylogenetic trees suggested that transitions to truffle and truffle-like forms evolved at least three times within the Pezizaceae (in group III, V and VI). The 9 hypogeous species included are nested in lineages with epigeous pezizaceous taxa. Species with apothecia of various shapes and with forcible spore discharge are spread among all groups and the apothecium is suggested to be symplesiomorphic in the Pezizaceae. The results indicate that the apothecia forming Pezizaceae have given rise to at least 3 different forms of hypogeous ascomata without forcible spore discharge: ptychothecia, sterothecia and exothecia.

Key Words: ascus amyloid reactions, epigeous-hypogeous evolution, molecular phylogeny, nLSU rDNA, Pezizales, systematics

INTRODUCTION

The Pezizaceae (Pezizales) displays great variation in ascomatal forms. It includes taxa that produce epigeous, sessile or stipitate, cupulate, discoid, turbinate, pulvinate or sparassoid ascomata, or semi-hypogeous to hypogeous, closed, folded to solid ascomata (FIGS. 1–21). The ascomata range in size from a few mm to more than 10 cm in diam, and are often fleshy, soft and brittle. Anatomical and biochemical diversity is found in characters such as spore discharge mechanism; amyloid reaction of the ascus; spore shape, color, ornamentation, and guttulation; pigmentation of the paraphyses; and excipulum structure (TABLE I).
Ecologically, the family covers a broad range of niches, fruiting on all types of soil, sand, clay, limestone, burnt ground, dung and wood. Many species are known to prefer soil with a high pH and in some cases a low content of organic matter (Petersen 1967, 1985). The family is primarily restricted to temperate zones and arctic-alpine areas, although a few strictly tropical taxa are known. The majority of species are considered to be saprotrophs, although most hypogeous and a few epigeous species are claimed to be ectomycorrhizal (Maia et al. 1996 and references therein), but in general their trophic statuses are not
### Table I. Characters used in the delimitation of genera in the Pezizaceae (based on published and original observations)

<table>
<thead>
<tr>
<th>Genus</th>
<th>Ascoma: position; forcible spore discharge</th>
<th>Ascoma: type; shape when epigeous</th>
<th>Ascus: +/- amyloid; type of amyloid reaction</th>
<th>Spore: shape; guttulation</th>
<th>Spore: color; +/- ornamentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amylaceus</strong></td>
<td>hy, se; -</td>
<td>Ptychothecium to stereothecium</td>
<td>+; weakly over the entire length</td>
<td>Globose; 1 or several</td>
<td>Hyaline to pale brown; + spines, rods or cones</td>
</tr>
<tr>
<td><strong>Boudiera</strong></td>
<td>ep; +</td>
<td>Apothecium, subglobose, turbinate to pulvinate</td>
<td>+; weakly over the entire length</td>
<td>Globose; 1 or several</td>
<td>Pale brown; + echinulate to reticulate</td>
</tr>
<tr>
<td><strong>Cazia</strong></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>-</td>
<td>Ellipsoid; 1</td>
<td>Hyaline; + minutely warty-reticulate</td>
</tr>
<tr>
<td><strong>Hapsidomyces</strong></td>
<td>ep; +</td>
<td>Apothecium, turbinate</td>
<td>+; weakly over the entire length</td>
<td>Globose; 0</td>
<td>Hyaline to pale yellow; + reticulate and spiny</td>
</tr>
<tr>
<td><strong>Hydnobolites</strong></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>+/-; weakly (2% KOH pretreatment)</td>
<td>Globose; 0</td>
<td>Hyaline to pale brown; + reticulate</td>
</tr>
<tr>
<td><strong>Hydnotryopsis</strong></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>+; diffusely over the entire length</td>
<td>Ellipsoid; ?</td>
<td>Hyaline to brownish yellow; + warty to pitted to ridged</td>
</tr>
<tr>
<td><strong>Iodophanus</strong></td>
<td>ep; +</td>
<td>Apothecium, pulvinate</td>
<td>+; weakly over the entire length</td>
<td>Ellipsoid to broadly ellipsoid; 0</td>
<td>Hyaline; + warty or episporium wrinkled</td>
</tr>
<tr>
<td><strong>Iodowynnea</strong></td>
<td>ep; +</td>
<td>Apothecium, convoluted to highly folded, arising from a hypogeous stipe</td>
<td>+; intensely over the entire length</td>
<td>Ellipsoid; 1 or 2, rarely several</td>
<td>Hyaline; + longitudinally ridged and warty</td>
</tr>
<tr>
<td><strong>Kimbropezia</strong></td>
<td>ep; +</td>
<td>Apothecium, discoid</td>
<td>+; intensely over the apex</td>
<td>Ellipsoid; 0</td>
<td>Hyaline; -</td>
</tr>
<tr>
<td><strong>Mycoclelandia</strong></td>
<td>hy, se; -</td>
<td>Ptychothecium</td>
<td>+; diffusely, more intense near the apex</td>
<td>Broadly ellipsoid to subglobose; 1 or 2</td>
<td>Hyaline; -</td>
</tr>
<tr>
<td><strong>Pachyella</strong></td>
<td>ep; +</td>
<td>Apothecium, discoid, flat to pulvinate, broadly attached to the substrate</td>
<td>+; diffusely over the entire length</td>
<td>Ellipsoid; 2</td>
<td>Hyaline; -/+ warty, ridged</td>
</tr>
<tr>
<td><strong>Pachyphloeus</strong></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>±; weakly over the entire length</td>
<td>Globose; 0 ?</td>
<td>Hyaline to pale brown; + spinose to verrucose; +/- embedded in mucilage</td>
</tr>
<tr>
<td><strong>Peziza</strong></td>
<td>ep, rarely se, hy; + rarely -</td>
<td>Apothecium, discoid to cupulate, rarely sporassoid, or rarely psychothecium</td>
<td>+; ring zone at the apex, intensely unrestricted over the apex or evenly over the entire length (diffusely, weakly or intensely)</td>
<td>Ellipsoid or fusiform; 0, 2-or multi-guttulate, rarely 1</td>
<td>Hyaline or pale brown; -/+ warty, ridged to reticulate, spiny, apiculate</td>
</tr>
<tr>
<td>Paraphyses: pigments; gelatinous matrix; epithecium</td>
<td>Excipulum: outer</td>
<td>Excipulum: medullary</td>
<td>Trophic status; substrate</td>
<td>Anamorphs</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
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<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>Outermost textura globulosa to angularis, giving rise to surface tomentum. Inner layer of broad hyphae, mixed with inflated cells</td>
<td>Textura intricata, +/− large cells</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Hyaline or brownish walls</td>
<td>Textura globulosa to angularis</td>
<td>Dense textura intricata</td>
<td>sa ?, ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Paraphyses present: pigments ?</td>
<td>Textura intricata, at septa, the cells often inflated</td>
<td>Textura intricata</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Yellowish contents</td>
<td>Textura globulosa to angularis</td>
<td>Textura intricata</td>
<td>sa; co</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>Textura globulosa (± isodiametric cells) with brownish, amorphous material in the intercellular spaces</td>
<td>Textura globulosa (± isodiametric cells), or textura intricata</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Paraphyses present: pigments ?</td>
<td>Textura globulosa (± isodiametric cells), sometimes with interwove hyphae</td>
<td>Textura intricata</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Usually containing carotenoid pigments</td>
<td>Textura globulosa to angularis, sometimes with interwoven hyphae</td>
<td>Textura globulosa intermixed with hyphae</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Hyaline</td>
<td>Textura globulosa intermixed with hyphae, outermost cells arranged in short chains</td>
<td>Textura globulosa intermixed with hyphae</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Hyaline</td>
<td>Textura intricata, with very short hyphae, extending towards outside</td>
<td>A layer of textura intricata, between two layers of textura angularis to globulosa</td>
<td>sa ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Hyaline</td>
<td>Textura intricata or textura globulosa</td>
<td>Textura globulosa, of large, thin-walled cells</td>
<td>ec ?; te</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Dark granules in the apical cells</td>
<td>Textura globulosa to angularis, outer cells terminating in flexuous hairs embedded in a gelatinous matrix</td>
<td>Textura intricata +/− gelatinous matrix</td>
<td>sa ?; li</td>
<td>Oedocephalum-like</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>Subangular to globose isodiametric cells, on outside scabrous to verrucose</td>
<td>Isodiametric cells</td>
<td>ec ?; te</td>
<td>Glischroderma ?</td>
<td></td>
</tr>
<tr>
<td>+/- pigmented granules; +/- embedded in granular matrix</td>
<td>Textura globulosa or textura angularis, of large, thin-walled cells +/− interwoven hyphae, or smaller globose cells elongating to a hyphoid layer towards the outside</td>
<td>One, two or more layers, of textura globulosa or textura angularis, of large, thin walled cells +/− interwoven hyphae, or of parallel hyphae</td>
<td>sa, ec; te, co, py, li</td>
<td>Oedocephalum, Chromelosporium, Chromelosporium-like, or hyaline to colored, 0–3 septate resting spores</td>
<td></td>
</tr>
</tbody>
</table>
TABLE I. Continued

<table>
<thead>
<tr>
<th>Genus</th>
<th>Ascoma: position (^b); forcible spore discharge</th>
<th>Ascoma: type; shape when epigeous</th>
<th>Ascus: +/- amyloid; type of amyloid reaction</th>
<th>Spore: shape; guttulation</th>
<th>Spore: color; +/- ornamentation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plicaria</em></td>
<td>ep; +</td>
<td>Apothecium, discoid to cupulate</td>
<td>+/- weakly over the entire length</td>
<td>Globose; few to multi-guttulate</td>
<td>Pale brown; +/- warty, spiny, reticulate</td>
</tr>
<tr>
<td><em>Rhodopesiza</em></td>
<td>ep; +</td>
<td>Apothecium, cupulate</td>
<td>+/- over the entire length</td>
<td>Broadly ellipsoid; 1 small evanescent guttule</td>
<td>Pale yellowish; + tuberculate</td>
</tr>
<tr>
<td><em>Ruhlandiella</em></td>
<td>se; -</td>
<td>Exothecium</td>
<td>+/- weakly over the entire length</td>
<td>Globose; 1</td>
<td>Pale brown; + reticulate-areolate</td>
</tr>
<tr>
<td><em>Sarcosphaera</em></td>
<td>ep, se; +</td>
<td>Apothecium, hollow, initially hypogeous, becoming exposed and split into acute rays</td>
<td>+/- weakly over the entire length (a little stronger at the apex)</td>
<td>Ellipsoid; 2</td>
<td>Hyaline; + low warty</td>
</tr>
<tr>
<td><em>Scabropezia</em></td>
<td>ep; +</td>
<td>Apothecium, cupulate</td>
<td>+/- over the entire length</td>
<td>Globose; 1</td>
<td>Pale brown; + truncate or delicate warts</td>
</tr>
<tr>
<td><em>Sphaerozone</em></td>
<td>hy, se; -</td>
<td>Exothecium</td>
<td>+/- weakly over the entire length</td>
<td>Globose; 0</td>
<td>Reddish brown; + irregular warty</td>
</tr>
<tr>
<td><em>Terfezia</em></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>-</td>
<td>Globose; 0</td>
<td>Hyaline to pale brown; + spiny, papillate, reticulate, +/- mucilage-embedded</td>
</tr>
<tr>
<td><em>Tirmania</em></td>
<td>hy; -</td>
<td>Stereothecium</td>
<td>+; diffusely, +/- weakly</td>
<td>Globose to broadly ellipsoid; 1</td>
<td>Hyaline; +/- minutely roughened</td>
</tr>
</tbody>
</table>

\(^a\) Monotypic genus.

\(^b\) Position of ascoma; ep = epigeous, hy = hypogeous, se = semihypogeous.

\(^c\) Trophic status: sa = saprobic, ec = ectomycorrhizal. Substrate: te = terrestrial, co = coprophilous, li = lignicolous, py = pyrophilous.

well studied. A study by Warcup (1990), with single spore isolation and incubation experiments did, however, show several species, both epigeous and hypogeous, to be ectomycorrhizal.

Eriksson (2000) lists 19 genera in the Pezizaceae, with a circumscription based mainly on Rifai (1968), Kimbrough (1970) and Trappe (1979). Recent molecular phylogenetic studies have led to the inclusion of *Pachyphloeus* and *Terfezia* in the family (Norman and Egger 1999, Percudani et al 1999) (TABLE I). The Pezizaceae is chiefly characterized by amyloid asci, a feature shared only with the Ascomycota within the Pezizales. The spores are uninucleate, usually thin-walled, globose, ellipsoid or fusiform, hyaline or pale brown, smooth or with cyanophilic ornamentations. The excipulum consists, at least partly, of large isodiametric cells. In addition, ultrastructural and refined cytochemical studies have led to further delimiting characters such as the presence of electron-opaque convex and biconvex bands in septal pores of the ascospores (so far recorded in *Peziza*, *Plicaria*, *Iodophanus*, *Sarcosphaera*, *Pachyphloeus* and *Hydnobolites*) (Curry and Kimbrough 1983, Kimbrough and Curry 1985, Kimbrough et al 1991, Kimbrough 1994).

Molecular phylogenetic studies of the Pezizales to date have included relatively few taxa from the groups with amyloid asci. With regard to the Pezizaceae, the work of Norman and Egger (1996, 1999) deserves special note, because it provides broader
Table I. Extended Continued

<table>
<thead>
<tr>
<th>Paraphyses: pigments; gelatinous matrix; epithecium</th>
<th>Excipulum: outer</th>
<th>Excipulum: medullary</th>
<th>Trophic status; substrate</th>
<th>Anamorphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embedded in dark granular matrix</td>
<td>Textura globulosa to angularis, and intermixed broad hyphae</td>
<td>Textura globulosa and intermixed broad hyphae</td>
<td>sa ?, ec; py</td>
<td>Chromelosporium</td>
</tr>
<tr>
<td>Orange granules (carotenoid granules)</td>
<td>Textura globulosa to angularis, of subglobose to polygonal cells</td>
<td>Small textura globulosa angularis, occasionally intermixed with hyphae</td>
<td>sa ?; te</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gelatinous sheath; exceeding the asci Brownish contents</td>
<td>Textura globulosa to angularis, thick-walled cells</td>
<td>Textura globulosa to angularis</td>
<td>ec; te</td>
<td>Chromelosporium-like</td>
</tr>
<tr>
<td>Small brownish guttules; embedded in a coloured, granular matrix</td>
<td>Textura globulosa, outermost cells thick-walled, forming conical pustules</td>
<td>Textura intricata</td>
<td>sa ?; ec ?; te, li</td>
<td>Glischooderma ?</td>
</tr>
<tr>
<td>Exceeding the asci</td>
<td>Textura globulosa to angularis, cells thick-walled</td>
<td>Textura globulosa to angularis</td>
<td>ec ?; te</td>
<td>Unknown</td>
</tr>
<tr>
<td>Absent</td>
<td>Textura intricata, of broad hyphae with inflated cells</td>
<td>Textura intricata, of broad hyphae with inflated cells</td>
<td>ec ?; te</td>
<td>Unknown</td>
</tr>
<tr>
<td>Absent</td>
<td>Textura intricata, of broad, thin-walled hyphae and many inflated cells</td>
<td>Textura intricata, of broad, thin-walled hyphae and many inflated cells</td>
<td>ec ?; te</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

The family: delimitation and important morphological characters.—For nomenclatural purposes the Peziza- ceae can be traced to Dumortier (1829) (Hawksworth and David 1989), as a family of the order Fungariceae (fungi with a distinct external hymenium), to include genera with the spores born on the upper surface of a cup-shaped receptacle. Dumortier’s circumscription was essentially the same as used by Fries (1822, as “Pezizeae”), but Fries did not use a consistent method of ranking in his system, and therefore Fries is not accepted as author of the Pezizaceae contrary to the use by, e.g., Eckblad (1968), Kimbrough (1970) and Korf (1972). Dumortier (1829) listed Peziza and Ascomobolus, along with other genera of the discomycetes, Stictis, Bulgaria, Tympanis, Cenangium, Patellaria and Helotium, and two genera of basidio- mycetes, Solenia and Ditiola. This system was based solely on macroscopic features and has long been regarded as artificial.

Boudier (1885) was the first to delimit a group, “the Aleuriés,” which more or less circumscribes the...
core of the Pezizaceae as we recognize it today. The group was placed in the "division Operculés," distinguished by asci that open with an operculum. Besides the presence or absence of an operculum, Boudier introduced and used further important microscopic characters, such as the amyloid reaction of asci, spore shape and number of oil guttules in the spores in his classification of the "fleshy discomycetes." The "Aleuriés" was distinguished by amyloid asci and in general having cup-shaped, sessile ascomata without hairs on the receptacle. The genera *Lepidotia* Boud., *Aleuria* (Fr.) Gill. and *Galactinia* (Cooke) Boud. (=*Peziza*), *Sarcosphaera*, *Sphaerosoma* Klotzsch, and *Plicaria* were included. Later Boudier (1907) treated the group at tribus level ("Aleuriés") and added his new genus *Pachyella*, but *Sphaerosoma* was excluded. *Pachyella* was segregated from *Peziza* and *Psilotpezia* Berk. on the basis of the pulvinate to convex apothecia and lack of or only weakly amyloid asci. The absence of an amyloid reaction for taxa in the tribe was contrary to the definition of "Aleuriés," but Boudier also knowingly included species in *Plicaria* that had non-amyloid asci.

Boudier's (1907) circumscription of the Aleurieae was generally followed by Le Gal (1947, as tribus Aleurieae), Rifai (1968), Eckblad (1968) and Korf (1972, 1973b) (as Pezizaceae), and Dennis (1981, as tribus Pezizeae). Additional characters, such as spore ornamentation of cyanophilic material (Le Gal 1947), uninculeate spores (Berthet 1964a), absence of carotenoid pigments (Arpin 1969) and the anatomy of the ascomata, consisting of mostly large isodiametric cells, were discovered and used to refine the limits of the family. In culture, many species of the Pezizaceae were found to produce *Oedocephalum* or *Chromelosporium* conidial states (Rifai 1968).

Trappe (1979) emended the family to include hypogeous taxa with chambered or marbled glebae and cylindrical to saccate asci which lacked functional opercula. He recognized the artificial nature of the Tuberales, abandoned the order and assigned most taxa to families within the Pezizales. Hypogeous species of *Peziza*, and the genera *Mycoceleadna*, *Hydnophytopsis*, *Amylascus* and *Tirmania* were accepted in the Pezizaceae. Trappe considered the hypogeous taxa to be derived from various epigeous taxa within the family. This hypothesis was based primarily on similarities in the anatomy of the excipulum and on the presence of amyloid asci. Currently 10 genera, almost half of the genera accepted in the Pezizaceae, have semi-hypogeous ascomata (Eriksson 2000, Table 1), while six hypogeous species are placed in *Peziza* (e.g., Figs. 17–18) (Trappe 1979).

The amyloid reaction. Amyloid asci were described by Nylander (1868), Karsten (1869) and Boudier (1879) in a number of species of *Peziza* and *Ascobolus* Pers. Korf (1972) attached some importance to ascus amyloid reactions. He recognized the Pezizaceae for taxa with asci amyloid reactions: i) in an apical ring, ii) over the apex, or iii) rarely in their whole length. Taxa in the Ascobolaceae tribus *Iodophaneae* were described as having diffusely amyloid asci (tribus *Ascobolaceae* showed non-amyloid or diffusely amyloid asci). Nevertheless, Korf included *Pachyella*, characterized by diffusely amyloid asci, in the Pezizaceae. Except for the genera *Pachyella*, *Iodophanus* and *Boudiera*, the location and extent of the amyloid reaction have not been used as characters to delimit taxa within the Pezizaceae. Within the Ascobolaceae, differences in ascus reactions were noticed for some sections in the genus *Ascobolus*: intensely amyloid in sect. *Dasyobolus* (Sacc.) Brumm. (in all but one species), faintly amyloid or none at all in several mainly terrestrial and lignicolous species of sect. *Ascobolus*, and non-amyloid in sect. *Pseudoascoledesmis* Brumm. (van Brummelen 1967). All species in the remaining four sections of *Ascobolus* showed a "general" amyloid reaction of the ascus wall. Van Brummelen (1967) found the intensity of the reaction to be constant for each species although significant differences existed between related species. A diffuse amyloid reaction was described by Pfister (1973) in *Pachyella* as "not restricted to the apex of the ascus, nor is it in the form of a J+ ring at the apex of the ascus," and that it "is present either as an external layer on the ascus wall (which may separate from the ascus wall proper), or occurs in the gel which surrounds the asci and paraphyses and is not restricted to the wall."

Morphological and cytochemical studies of amyloid asci were performed on five species of the Pezizaceae and Ascobolaceae, using light microscopy and transmission electron microscopy (TEM) (Samuelson 1978). The amyloid site in *Peziza succosa*, *Saccobolus depauperatus* (Berk. and Broome) E.C. Hansen and *Theotheus pelletieri* (Crouan) Boud. appeared to be an exogenous mucilaginous coat, whereas in *Iodophanus granulipolaris* Kimbrough it appeared to be the ascal wall proper. However, Samuelson stated that the amyloid reaction may be the result of more than one site of activity. *Peziza succosa* was strongly but unrestrictedly amyloid over the apex ("10–14 μm down the sides of the ascus wall"). *Peziza* species with a distinct amyloid ring zone at the apex were not studied. According to van Brummelen (1978) the site of the amyloid reaction is strictly confined to an extra-ascal layer, the periascus (a mucilaginous substance), which varies in thickness. In *Boudiera* and *Iodophanus* it is more or less uniform, but in *Peziza ammophila* and *P. succosella* it develops into a ring-shaped thickening at the apex. In *P. ammophila* a thick ring with
an indented central portion forms, whereas in *P. suc-osella* the periascus is considerably thickened but uniformly so over the ascus apex (van Brummelen 1978: figs. 7, 8). Such ultrastructural studies help to explain the variation in the amyloid reaction of asci, which is observed in the Pezizaceae.

The genera: delimitation and relationships.—Epigeous genera. *Peziza* Fr. (1822) is the largest genus within the Pezizales, with approximately 100 currently accepted species (Hohmeyer 1986, Korf 1972). It is a complex and heterogeneous assemblage, that constitutes the core of the Pezizaceae. Although many keys (Dissing 2000, Hohmeyer 1986, Häffner 1995, Le Gal 1941, Maas Geesteranus 1967, Moser 1963, Romagnesi 1978) and descriptions (e.g., Donadini 1978, 1979a, b, 1980a, Moravec 1985) of European species exist, *Peziza* has never been monographed and the infrageneric relationships are poorly understood. A number of subgenera and sections have been proposed, but only few attempts to provide a coherent system have been made (Donadini 1977, 1978). Furthermore, controversy has surrounded the generic delimitation of *Peziza*. Recent molecular studies suggest that the genus is not monophyletic, but rather is composed of at least two distinct clades (Norman and Egger 1996, 1999).

The nomenclature of *Peziza* is complicated on several counts. It is a pre-starting point name originating from Dillenius (1719). It has been applied to a number of cup-shaped fungi including both basidiomycetes and ascomycetes. Linnaeus (1753) adopted the name *Peziza* in Species Plantarum, the starting point work, for a group of fungi now referred to *Cyathus* Willd. (Basidiomycota) (Dennis 1983). Post-linnaean authors, particularly Bulliard (1791) and Persoon (1801), included primarily discomycetes, both operculate and inoperculate, in *Peziza*, thus making important contributions to the modern concept of the genus. Since Fries (1822) adopted the concept of these authors (not Linnaeus's concept) and placed what became the lectotype of *Peziza* L. (= *Peziza lentifera* L. = *Cyathus olla* Pers.) in *Nidularia* Fr. (Dennis 1983), Fries can be considered as having described *Peziza* as a new genus with a sanctioned status (Yao et al 1995). *Peziza* in the sense of Fries has been questioned. Eckblad (1968) did not recognize *Sarcosphaera* as distinct from *Peziza* has been questionable. Eckblad (1968) did not recognize *Sarcosphaera* as a distinct genus, and treated the species in *Peziza*. However, most contemporary taxonomists accept *Sarcosphaera* (Pouzar 1972, Korf 1973b, Pfister 1979a, Dissing 2000).

*Plicaria*Fuckel (1870) was introduced to accommodate species mostly treated by modern authors as *Peziza* and *Plicaria*, with both ellipsoid and globose spores, united by a few characters, e.g., thick-walled, brownish, ornamented spores. *Peziza vesculosa* and others were placed in *Pustularia* Fuckel, while *Peziza* in Fuckel’s classification included small stipitate inoperculate discomycetes. Fuckel erected *Aleuria* Fuckel for the non-amyloid, *P. aurantia* Pers. (the modern accepted concept of *Aleuria*). Boudier (1885) restricted *Plicaria* to the globose-spored species and was followed by most contemporary and later workers. Rehm (1894) used *Plicaria*, for only ellipsoid-spored species with amyloid asci, and erected *Plicariella* (Sacc.) Rehm for the globose-spored species; he rejected the name *Peziza*. Some authors have maintained *Plicaria* emend. Boud. as a separate genus (Eckblad 1968, Rifai 1968, Dennis 1981, Dissing and Pfister 1981, Hirsch 1985, Norman and Egger (corresponding to *Aleuria* and *Galactinia* sensu Boud. respectively). Le Gal abandoned *Peziza* as a “nomen confusum.” The most recent classifications (Dennis 1981, Dissing and Pfister 1981, Dissing 2000, Eckblad 1968, Rifai 1968) follow Le Gal’s (1953a) circumscriptio of the genus, but under the name *Peziza*. As such, *Peziza* is defined as having epigean, discoid to cupulate apothecia, a few mm to ca 15 cm in diam, sessile or with a short stipe (Figs. 2, 4, 9–12, 15, 20–21), amyloid asci, ellipsoid or rarely fusiform spores and an excipulum most often stratified in two or more layers of globose thin-walled cells intermixed with hyphae or a hyphal layer; lactiferous hyphae may be present. We have adopted this circumscription as the starting point for taxonomic comparisons in this study. Nevertheless, a few species are placed in the genus which produce more unusual ascomatal forms, i.e., sparassoid (Boudier 1899, Korf 1973a) or hypogeous, closed, hollow, often folded ascomata (Figs. 17–18) (Burdall 1968, Trappe 1979). The hypogeous forms have persistent hymenia and amyloid asci, but lack forcible spore discharge (TABLE 1).

*Sarcosphaera* Auerw. (1869) was segregated from *Peziza* on the basis of its macro-morphology and initial hypogeous development. It produces large, enclosed, hollow apothecia, initially underground, which as they mature, usually become exposed and split into irregular acute rays (FIG. 13). The asci mature before the apothecia open, but are in organized hymenia, and the spores are forcibly discharged. Whether to recognize *Sarcosphaera* as distinct from *Peziza* has been questionable. Eckblad (1968) did not recognize *Sarcosphaera* as a distinct genus, and treated the species in *Peziza*. However, most contemporary taxonomists accept *Sarcosphaera* (Pouzar 1972, Korf 1973b, Pfister 1979a, Dissing 2000).

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1996, Dissing 2000), while others have regarded it as a part of Peziza in the sense adopted here (e.g., following Korf 1960, 1972, Le Gal 1953b, 1962). Whether the shape of the spores is a reliable character at the generic level has been a persistent controversy. Other characters have been proposed to support the recognition of Plicaria. These are pale brownish spores, paraphyses embedded in a granular matrix, dark-colored apothecial pigments (brown pigment on the walls of the asci), Chromelosporium anamorphs and a carbonicolous habitat (Fig. 16). Egger (1987) found that Plicaria trachycarpa, P. endocarpoides and some species of Peziza (P. atrovinosa, P. vacinii, P. retiderma Cooke, P. rifaii J. Moravec & Spooner and P. ostracoderma) and stated that if Plicaria is to be recognized "the inclusion in it of species with non-globose spores appears unavoidable." Phylogenetic analyses by Norman and Egger (1996) including most of these taxa, indicated that the globose-spored Plicaria is monophyletic, but nested within Peziza. They accepted the genus ad interim even though it implied a paraphyletic Peziza. In this study we are using the name Plicaria as emended by Boudier.

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Boudiera Cooke (1877) has been placed in the Ascobolaceae (Boudier 1907, Korf 1972, 1973b) due to the turbinate to pulvinate ascomata (Fig. 6), protruding asci and pale brownish spores, but its relationship

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has been much disputed. Eckblad (1968) stated that *Boudiera* did not seem to be closely related to any other genus with protruding asci, but he, nevertheless, referred it to the Ascobolaceae. *Boudiera* was redivised (Dissing and Schumacher 1979) and placed in the Pezizaceae by Dissing and Korf (1980) and Hirsch (1980) along with other genera with globose spores and amyloid asci. Dissing and Korf emphasised characters of the anatomy and morphology of the ascomata and suggested that *Boudiera* is in a line with *Plicaria* on the one hand, and *Ruhlandiella* and *Sphaerozone* on the other. Hirsch (1980) considered *Boudiera* closely related to *Plicaria*, as did Rifai (1968). Hirsch also pointed out similarities in the excipulum structure, besides spore color, amyloid reactions of asci (which blue evenly over the entire length in both *Plicaria* and *Boudiera*) (Table 1), agglutination of paraphyses and often a complex spore ornamentation. He did not find protruding asci, a consistent generic character in *Boudiera*. Molecular phylogenetic analyses (Landvik et al 1997, Norman and Egger 1999) support the placement of *Boudiera* in the Pezizaceae.

*Pachyella* Boud. (1907) (Fig. 5) was synonymised with *Peziza* (Donadini 1980b, Eckblad 1968, Seaver 1928), but was accepted by Le Gal (1947, 1953a), Korf (1972) and Pfister (1973) based on the presence of a well-developed cortical zone of globose cells which on the outside terminate in hyaline, hyphoid hairs, embedded in a gel and asci usually diffusely amyloid. Donadini (1980b) gave *Pachyella* subgeneric rank under *Peziza*. Most taxonomists have followed Pfister (1973). Hirsch (1984) even regarded *Pachyella* as so isolated within the Pezizaceae that he proposed a tribus Pachyelleae for this taxon alone, opposed to tribus Pezizaceae including all other genera of the family.

*Iodophanus* Korf (Kimbrough and Korf 1967) was segregated from *Ascophanus* Boud. (tribus Pseudoascoboleae, Ascobolaceae) and was, along with *Theothecus* Boud., assigned to the tribus “Pezizaceae” (Pezizaceae) rather than the tribus Theloboleae (Pezizaceae), because they possess amyloid asci, spores with cyanophilic spore markings and an *Oedoeophalum* coindial state (Gamundi and Ranalli 1964). Korf (1972, 1973b) later treated *Iodophanus* in the Ascobolaceae (tribe Iodophaneae), emphasizing the thick-walled spores in early stages of development, the carotenoid pigments, and asci that protrude prominently at maturity (Fig. 1). Furthermore, Korf stated that the tribe Iodophaneae (*Iodophanus, Boudiera, Thecotheus* and *Sphaerosoma*) seems surely to represent the link between Pezizaceae and Ascobolaceae. On the contrary, *Iodophanus* has been regarded as a “primitive type” within the Pyronemataceae by Eckblad (1968), based on the paraphyses’ containing carotenoid pigments, the excipulum structure, and the diffusely amyloid type of ascus. A close relationship between *Iodophanus, Peziza* and *Plicaria* has been indicated by septal ultrastructure of asci and ascogenous hyphae (Kimbrough and Curry 1985). Two ascal septum types were found; the pezizoid and the ascoboloid. The genus *Thecotheus* shared the ascoboloid type with *Ascobolus* and *Saccobolus* Boud. Molecular phylogenetic studies support the relationship of *Iodophanus* to the Pezizaceae and of *Thecotheus* to Ascobolaceae (Landvik et al 1997, 1998).

*Scabropezia* Dissing & Pfister (1981) was segregated from *Plicaria* and *Peziza*, based on the anatomy of the apothecia. In this genus the outer surface of the ascomata is pustulate (Fig. 7), the pustules being composed of aggregates of globose to angular cells clearly separated from the medullary excipulum of textura intricata. Only two species are known.

*Hapsidomyces* J.C. Krug & Jeng (1984) was erected for *H. venezuelensis* Krug & Jeng and assigned to the Pezizaceae based on the amyloid asci and the thin-walled, pale-colored spores. Krug and Jeng considered it to be closely related to *Boudiera* (see Table 1), but sufficiently distinct by its early cleistohymenial development; a distinct apothecial margin; short, narrow asci with a short stipe; a rather narrow operculum; filiform paraphyses with yellowish content; small, eguttulate spores, and a coprophilous habitat. As in *Boudiera*, the asci protrude at maturity.

*Kimbropezia* Korf & Zhuang (1991) was described on the basis of a cyanophilic and Congo Red-staining lens-shaped thickening (“opercular lens”) within the ascus operculum. The asci were further noted to be unusually brittle and with rather abruptly narrowed and often twisted bases. The genus was assigned to the Pezizaceae, differing from *Peziza* only by the very peculiar asci (see Table 1). Van Brummelen (1998) studied the ascus apical structure by TEM and found it to be unique. He considered *Kimbropezia* best placed as a rather aberrant genus in the Pezizaceae. On the contrary, molecular analysis by Norman and Egger (1999) placed *Kimbropezia* within *Peziza*.

*Rhodopeziza* Hohmeyer & Moravec (Moravec 1994) was described for *R. tuberculata* (Gamundi). Moravec & Hohmeyer (= *Aleuria tuberculata* Gamundi). It differs from *Aleuria* in having amyloid asci and tuberculate spores which are not regularly biguttulate. According to Moravec (1994) it has a special position in the Pezizales, with its carotenoid granules in the paraphyses and asci being amyloid over the entire length. It shares characters with *Iodophanus* in particular, especially the presence of carotenoids, ornamented spores and diffusely amyloid asci. It was

Iodotyphnya Medel, Guzmán & Chacón (Medel et al 1996) was segregated from *Peziza* based on its large, convoluted to highly folded apothecia, arising from a buried stipe, and longitudinally verrucose-striate spores. One of the authors, Korf, reserved judgement on whether the new taxon should be considered a subgenus of *Peziza* or merit generic rank. Le Gal (1953a) and Pfister (1974) treated the species in *Galaciniia* and *Peziza* respectively.

Garrelta Brumm., Korf & Rifai (van Brummelen 1967) is included in this study to test the hypothesis of a close relationship to *Peziza* gerardii.

**Hypogeous genera**. The truffle or truffle-like genera presented below, except for *Mycocelendia* and *Caizia*, were originally described in the order Tuberales (Tuberaceae or Terfeziaceae) due to their hypogeous habit and lack of forcible spore discharge.

Hydnobolites Tul. & C. Tul. (1843) was described for *H. cerebriformis* Tul. & C. Tul. It was referred to Tuberales by Gilkey (1954b) and Korf (1973b), but placed in the Terfeziaceae by Hawker (1954) and Trappe (1971, 1979) because the spores are globose and there is no organized hymenium with paraphyses at any stage of development. This position has been accepted by most subsequent authors. The genus was distinguished from others in the Terfeziaceae by having an interior layer of solid undifferentiated tissue in which the asci are randomly distributed. Meandering veins deeply penetrate the interior of the ascomata from the outer surface. These are lined with outer excipulum tissue. The asci are non-amyloid without pretreatment in KOH, therefore *Hydnobolites* was not placed in the Pezizaceae. Trappe (1979) regarded *Hydnobolites* to be close to both *Pachyphloeus* and *Terfezia*. The recent discovery of several cytological and ultrastructural features by Kimbrough et al (1991) made them suggest that *Hydnobolites* belongs in *Pachyphloeus*. These features are the electron-dense, bi-convex bands in septal pores of the asci, dextrinoid and weakly amyloid asci (only after pretreatment with 2% KOH) and a type of spore wall deposition similar to members of *Pachyphloeus*.

Pachyphloeus Tul & C. Tul. (1844) was erected for *P. melanoxanthus* (Tul.) Tul & C. Tul. It was, like *Hydnobolites*, referred to Tuberales by Gilkey (1954b) and Korf (1973b), and placed in the Terfeziaceae by Trappe (1979). Hawker (1954) placed the genus in the Tuberales E. Fisch. It lacks a hymenium and asci irregularly line veins or are randomly produced in the medullary excipulum. An apical depression or pore leading into a few open veins is characteristic of the ascomata. The verrucose outer excipulum of isodiametric cells separates *Pachyphloeus* and *Hydnobolites* from other genera of the Terfeziaceae (Trappe 1979). However, amyloid asci occur in some species of *Pachyphloeus* (e.g., in the type species), and based on this and anatomical features, the genus has been placed in the Pezizaceae by Dissing and Korf (1980) and Dissing (2000). Molecular phylogenetic analyses has recently confirmed this placement (Norman and Egger 1999, Percudani et al 1999).

Terfezia (Tul. & C. Tul.) Tul. & C. Tul. (1851) was elevated from *Choeromyces* section *Terfezia* Tul. & C. Tul. to generic rank. It was distinguished by having pockets of fertile tissue separated by sterile, but otherwise undifferentiated veins, highly ornamented spores and non-amyloid asci (Trappe 1971, 1979). It is the type genus of Terfeziaceae, and the most heterogeneous genus of the family. Recent molecular phylogenetic analyses suggest that *Terfezia* is not monophyletic, and that it is nested within *Pezizaceae* (Norman and Egger 1999, Percudani et al 1999).

Sphaerozone Zobel (Corda 1854) was described for *S. tubulosei Zobel (= Sphaerosoma ostiolatum Tul. & C. Tul.)*, which has reddish brown spores. Dissing and Korf (1980) studied the type species and discovered that the asci are amyloid, contrary to previous reports by Korf (1972, 1973b). Under the impression that asci were non-amyloid Korf treated the genus in the Terfeziaceae, rather than a genus in *Pezizaceae* as was subsequently done by Dissing and Korf (1980). It differs from *Ruhlandiella* by having paraphyses without gelatinous sheaths and persistent asci.

Tirmania Chatin (1891) was described for *T. africana* Chatin (= *Tuber niveum* Desf.). It was placed in the Terfeziaceae by Trappe (1971) and Korf (1973b). It resembles *Terfezia*, but differs by having smooth or at most minutely roughened spores and amyloid asci. Based on the amyloid reaction and its anatomy, Trappe (1979) transferred the genus to the *Pezizaceae*.

Ruhlandiella Henne (1903) was redescribed and emended by Dissing and Korf (1980), who selected a neotype for the type species *R. berolinensis*. They found the asci to be amyloid and the paraphyses to have characteristic gelatinous sheaths (features not mentioned in Hennings’ original description). The ascomata are relatively small, hypogeous or semi-hypogeous, solid, pulvinate to globose ascomata and with the hymenium covering the outer surface and permanently exposed (Fig. 19) (Dissing and Korf 1980, Galán and Moreno 1998, Warcup and Talbot 1989 as *Muciturbo* Talbot). Asci are evanescent at maturity. Dissing and Korf (1980) referred *Ruhlandiella* to the *Pezizaceae*, with other globose-spored, amyloid genera. A close relationship to *Sphaerozone*, in a lineage with *Boudiera* and *Plicaria*, was suggested.

Hydnortrypss Gilkey (1916) was erected for *H. setchellii*, but later synonymized with the globose-
spored *Choiromyces* Vittad. (Tuberaeaceae) (Gilkey 1954b). Trappe (1975a), however, showed that it has amyloid asci and, based on this and its ellipsoid spores, the genus was reinstated. Fischer (1938) and Trappe (1979) placed the genus in the Pezizaceae. The ascomata are solid, but the asci and paraphyses are arranged in hymenia.

*Amylascus* Trappe (1971) (FIG. 8) was segregated from *Hydnobolites* on the basis of its thick-walled, amyloid asci, echinulate spores and the verrucose outer excipulum of large inflated cells which gives rise to a tomentose outer surface (Trappe 1971, 1975b, Beaton and Weste 1982). It was described in the Terfeziaceae for a single species, *H. herbertianus* Cribb. Later, Trappe (1975b) considered the genus to be closely related to the Geneaceae in both form and anatomy, and the similarities between *Hydnobolites* and *Amylascus* to be the result of parallel evolution. When restricting Terfeziaceae to include only genera with non-amyloid asci, however, Trappe (1979) transferred *Amylascus* to Pezizaceae.

*Mycoelelandia* Trappe & Beaton (1984) was proposed to replaced *Clelandia* Trappe (1979), a homonym of *Clelandia* J.M. Black (Violaceae). It was placed in the Pezizaceae, and noted to resemble *Peziza* s.l. in several anatomical features, but differing in its “cleistothecial” ascomata and indehiscent asci (Trappe 1979). The asci are diffusely amyloid with the reaction strongest near the apex.

*Cazia* Trappe (1989) was assigned initially to the Helvellaceae due to the non-amyloid asci and large isodiametric cells in the excipulum. It differed from other similar hypogeous genera by its hyaline, minutely ornamented spores, crooked asci and a yellow KOH reaction of the fresh medullary excipulum. The genus was identified as a member of the Pezizaceae, using sequences from the nLSU and nSSU rDNA (O’Donnell et al 1997).

**MATERIALS AND METHODS**

**Material studied.**—Forty eight collections of *Peziza* representing 39 species were selected to cover the range of macro- and micromorphological characters, and habitats and substrates recognized within the genus. To critically assess the monophyly of *Peziza* and define its sistergroup relationships, representatives of the genera *Amylascus*, *Boudiera*, *Hydnotryopsis*, *Iodowynnea*, *Pachyella*, *Ruhlandiella* and *Tirmania* were included (Tables I, II). For a few selected species more than one collection was sequenced to verify the LSU rDNA sequences and estimate the intraspecific variation. LSU rDNA sequences from 12 additional species of *Peziza* and representatives of the genera *Cazia*, *Iodophanus*, *Kimbropezia*, *Plicaria*, *Sarosphaera* and *Scabropezia* were obtained from GenBank (TABLE II). To assess the monophyly of the Pezizaceae and the relationship to the genera *Marcelleina* and *Pistera* Korf & W.Y. Zhuang (currently placed in the Pyronemataceae), species belonging to the Ascobolaceae (*Ascobolus*), Morchellaceae (*Verpa* Fr.) and Pyronemataceae (*Aleuria* Masseae, *Otidea* (Pers.) Bonord., *Smarada* Srček) were included. Generic type species were used as far as possible (TABLE II). *Neolecta vitellina* was used to root the tree because phylogenetic analyses at more inclusive levels places *Neolecta* Speci. basal to the rest of the fruit-body producing ascomycetes and the budding yeasts (Landvik et al 1993, Landvik 1996). To test the influence of the outgroup on the analyses, *Neolecta* was replaced with *Orbilia* Fr., which has been suggested as the sister group to the Pezizales (Harrington et al 1999, Platt and Spatafora 2000).

Several collections of most included *Peziza* species have been studied morphologically, and existing types or other original material have been examined. The amyloid reactions of asci have been examined in additional specimens of other included genera. A list of the specimens is available from KH. The published voucher specimen for *Pachyella clypeata* (ALTA 9029, GenBank SSU: AF133144, LSU: U40619) (Norman and Egger 1999) was examined by KH and DHP and was redetermined as *Peziza subsabellina*. The voucher specimens for the other sequences obtained from GenBank have not been studied morphologically by us.

**Morphological methods.**—The voucher material was studied using light microscopy, and was identified or identifications were verified by KH. All species of *Pachyella* were further confirmed by DHP. Special notice was paid to the intensity and location of the amyloid reaction in ascii by using Melzer’s reagent (1.5 g iodine, 5.0 g potassium iodide, 100 g chloral hydrate, 100 mL distilled water), applied with or without KOH pretreatment (2% or 10% KOH). Other chemicals used were Cotton Blue in lactic acid (1% in 875 mL lactic acid, 63 mL glycerol, 62 mL water) and Congo Red in ammonia (0.3% in commercial ammonia cleaner). For critical species the spore surface was viewed by an AMR1000 scanning electron microscope (SEM). The anatomical structures of the excipulum were studied using hand sections (for fresh material) or sections made on a freezing microtome. Microanatomical terminology follows Korf (1973b). Photographs of the asci amyloid reactions were made with an Olympus BH 2 microscope and an Olympus C35AD-4 camera, and processed in Photoshop (Adobe Photoshop 4.0).

**Molecular methods.**—DNA was isolated from dried or fresh ascomata and was extracted as outlined in Hansen et al (1999). Serial dilutions of DNA (1:10, 1:100, 1:1000) were used as template for the polymerase chain reaction (PCR). The 5’ end of the nuclear large subunit rDNA (nLSU-rDNA), spanning domains D1 and D2, was amplified using the primers LROR and LR5 (for a few taxa LR5 was replaced with LR3 or LR7) (Moncalvo et al 2000). PCR products were purified either directly or following agarose gel electrophoresis and band excision using QIAquick spin columns (Qiagen 1997). For critical species the spore surface was viewed by an AMR1000 scanning electron microscope (SEM). The anatomical structures of the excipulum were studied using hand sections (for fresh material) or sections made on a freezing microtome. Microanatomical terminology follows Korf (1973b). Photographs of the asci amyloid reactions were made with an Olympus BH 2 microscope and an Olympus C35AD-4 camera, and processed in Photoshop (Adobe Photoshop 4.0).
were included, treated as missing data (gap = missing), or were equally weighted and unordered. Gapped positions were repeated by varying the transition:transversion ratio of substitution. The proportion of invariable sites was set to zero and an equal parameter values were obtained using the Rogers-Swofford two-parameter model for unequal base frequencies. The proportion of invariable sites was set to zero and an equal rate of substitution was assumed for all sites. The starting point was set to 0.01 (the optimal value from the previous analyses). This method does not optimize the tree topologies and bootstrap values, with only slightly fewer resolved nodes.

No size variability due to introns was observed. LSU rDNA sequences from the same morphological species showed either no (e.g., *P. depressa*, *P. echinospora*) or several substitutions or deletions (e.g., *P. gerardii*, *P. subisabellina*, *Pachyella babingtonii*). Intraspecific genetic variation was in some cases larger than interspecific variation, e.g., the nucleotide diversity within *P. gerardii* was 3.1% and within *Pachyella babingtonii* 1.7%, whereas the average nucleotide diversity between the Peziza species within group IVa was only 1.3%. Identical sequences of the same morphological species were left out of the analyses.

**Phylogenetic analyses.**—Under gap = missing coding there were 319 parsimony informative characters. With gapped positions omitted this number decreased to 249. Maximum parsimony analysis produced over 15 000 equally most parsimonious trees
TABLE II. List of species used in the molecular phylogenetic study, with voucher specimen information and GenBank accession numbers. Information on new sequences are listed first, followed by information on sequences obtained from GenBank. Numbers in parentheses are used to indicate multiple collections of a single taxon.

<table>
<thead>
<tr>
<th>Species</th>
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<th>Year and collector</th>
<th>GenBank</th>
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<td>Aleurina imaii (Korf) W.Y. Zhuang &amp; Korf</td>
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<td>Hydnotryopsis setchellii Gilkeya</td>
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<td>Hydnotryopsis sp.</td>
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<td>Iodowynnea auriformis (Le Gal) Medel, Guzmán &amp; Chacón (1)</td>
<td>CUP-ME566 (CUP). Mexico</td>
<td>1986, Ramón-Farias</td>
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<td>1981, R. Kaushal</td>
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<td>Marcelleina tuberculispora K. Hansen &amp; Sandal</td>
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<td>Pachyella violaceonigra (Rehm) Pfister</td>
<td>s.n. (FH). Switzerland</td>
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<td>Peziza ammophila Durieu &amp; Mont.</td>
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<td><em>Peziza subisabella</em> (2)</td>
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<td><em>Peziza sp. 5</em></td>
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<td>1983, H. Dissing</td>
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<td><em>Smardaea amethystina</em> (W. Phillips) Svec*</td>
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<td><em>Tirmania nivea</em> (Desf.: Fr) Trappe*</td>
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<td><em>Ascobolus lineolatus</em> Brumm.</td>
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<td><em>Cazia flexiascus</em> Trappe*</td>
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<td><em>Glischroderma</em> sp.</td>
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<td><em>Iodophanus carneus</em> (Pers.) Korf*</td>
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### TABLE II. Continued

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<tr>
<th>Species</th>
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<th>Year and collector</th>
<th>GenBank</th>
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<tr>
<td><em>Kimbropezia campestris</em> Korf &amp; W.Y. Zhuang&lt;sup&gt;a&lt;/sup&gt;</td>
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<td><em>Neolecta vitellina</em> (Bres.) Korf &amp; J.K. Rogers</td>
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<td><em>Peziza alaskana</em> Cash</td>
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<td>Canada</td>
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<td><em>Peziza atrovirensa</em> Cooke</td>
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<td><em>Peziza badia</em> (2)</td>
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<td><em>Peziza fimeti</em> (Fuckel) E.C. Hansen&lt;sup&gt;e&lt;/sup&gt;</td>
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<td><em>Peziza subsipellina</em> (3)&lt;sup&gt;b&lt;/sup&gt;</td>
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<td><em>Sarcosphaera coronaria</em> (Jacq.) Boud.&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> Generic type species.

<sup>b</sup> Published as *Pachyella chryphaeta* (Schw.) Le Gal (Norman & Egger 1996, 1999).

<sup>c</sup> Published as *Peziza vesiculosa* Bull. (Norman & Egger 1999).

(MPT) of 1832 steps under gap = missing coding (consistency index [CI] = 0.362, retention index [RI] = 0.650), and 1366 steps with gapped positions omitted ([CI] = 0.354, [RI] = 0.658). Although the degree of resolution varied in parts of the trees, no conflict between the strict consensus trees of the two sets of analyses was observed (Fig. 23, trees with gapped positions excluded are not shown). Trees obtained in analyses with *Orbilia* as the outgroup (data not shown) were identical to trees obtained with *Neolecta* as the outgroup. The large number of equally parsimonious trees reflects the inability of the data to resolve the higher order relationships, and relationships between closely related species (Fig. 23). However, 7 clades were identified (groups I–VII) that were generally well supported (as measured by bootstrapping) or present in all trees (Figs. 22–23). The Pezizaceae was suggested to be paraphyletic (bootstrap MPT 98%, ML 94%), because *Marcelleina* was nested within it (Figs. 22–24). Ascobolaceae (represented by *Ascobolus* under this sampling) was confirmed to be its sister group. The included, currently accepted genera in the Pezizaceae (Table I) were all nested within *Peziza*, with *Iodophanus* (Fig. 1) in a basal position, either as the sister group to the rest of the Pezizaceae or nested within the Pezizaceae. *Peziza gerardii*, which forms a clade with *Marcelleina* (Figs. 2–3) (bootstrap 96%), is the sister group to
Pezizaceae

Epigeous, with forcible spore discharge

Hypogeous, without forcible spore discharge

Equivocal state

20 steps

Peziza natriphiila (1,2)
Peziza quelepidotia

Peziza polaripapulata
Peziza sp. 4
Peziza lutulolofflava

Peziza otracodermia

Peziza whitei

Peziza ellipospora

Peziza subototia

Peziza subisabellina (1)
Peziza subisabellina (2)
Peziza subisabellina (3)

Peziza centrinata (2)
Peziza centralinata (1)
Peziza centralinata (3)

Peziza subisabellina (1)
Peziza subisabellina (2)
Peziza subisabellina (3)

Peziza centralinata (1)
Peziza centralinata (2)
Peziza centralinata (3)

Peziza subisabellina (1)
Peziza subisabellina (2)
Peziza subisabellina (3)

Peziza gerardi (1,2)
Peziza gerardi (3)

Marcelleina tuberculipora
Marcelleina personii

Pachyella babingtonii (1)
Pachyella babingtonii (2)
Pachyella punctipora

Boudiera dennisii

Pachyella violaeornigra

Pachyella babingtonii (3)

Pachyella violaeornigra

Pachyella violaeornigra

Pachyella violaeornigra

Pachyella violaeornigra

Pachyella violaeornigra

Pachyella violaeornigra

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the rest of the Pezizaceae in the MLT (Fig. 24). In
the strict consensus of the MPTs, the basal node of
the Pezizaceae is unresolved, with Iodophanus, the
clade of P. gerardii and Marcelleina, and the rest form-
ing a trichotomy (Fig. 23). Species of Peziza occurred
in all groups except group III, often along with other
genera of the Pezizaceae. Several taxa constitute sep-
arate lineages of uncertain placement: P. apiculata,
P. retrocurvata (Fig. 21), and the clade of P. natro-
phila and P. quelepidotis (Figs. 22, 23).

The optimal MLT’s found under the two sets of
analyses using different starting trees do not have sig-
nificantly different likelihood scores (Kishino-Has-
gawa test, P < 0.05). The optimal MLT was found
with a log likelihood score of −11308.898, under a
transition:transversion ratio of 2.0:1. The ML analyses recovered the
groupings found by the MP analyses, with some mi-

Amyloid reaction types.—Distinct types of ascus amy-
loid reactions were found to correspond to different
rDNA lineages (Fig. 24). The location and intensity
of the amyloid reaction are constant in a given spe-
cies, but varies between species. Four types of reac-
tions were identified: (i) faintly amyloid over the en-
tire length (sometimes more strongly in upper half),
but intensely and unrestrictedly amyloid over the
apex (most taxa in group VI, P. succosa and P. mich-
elii); (ii) faintly amyloid over the entire length, ex-
tremely amyloid in a distinct ring zone at the
 apex, restricted to the area of dehiscence of the oper-
culum (a synapomorphy for group IV); (iii) evenly
amyloid over the entire length, either: (iii-a) weakly,
perhaps slightly stronger at the apex, or diffusely am-
yloid [periascus and hymenial gel amyloid (Pfister
1973)], or rarely non-amyloid (Iodophanus, P. gerar-
dii, group II, Pachyella, Boudiera, Amylascus, Hydro-
tryopsis, Plicaria, Tirmania, Ruhlandiella, P. natrophi-
la, P. polaripapulata and Peziza sp. 4); (iii-b) strongly
amyloid (Iodowynnea, P. ellipsoida, P. luteoloflavida,
P. retrocurvata, P. whitei and Sarcosphaera); (iv) Non-
amyloid (Marcelleina and Casia). The amyloid reac-
tion is a fairly stable character, but rarely may be ab-
sent due to change of humidity (e.g., long periods of
rain) or an overripe state of the hymenium, e.g., pre-
sumed in Pfistera (van Brummelen 1998) and other
Peziza species. The periascus (an extra-ascal mucila-
genous coat, as defined by van Brummelen 1978) can
disappear after heating (Donadini 1985) or be
removed in microscopic preparation by applying
pressure on the cover slip and sliding it back and
forth (Samuelsen 1978).

DISCUSSION

Overview.—The Pezizaceae appear to be paraphyletic
as currently circumscribed (Eriksson 2000) because
Marcelleina is nested within it. If Marcelleina is trans-
ferred to the Pezizaceae, then the family would be a
strongly supported monophyletic group (Figs. 22–
24). Our data fail to resolve the higher level relation-
ships within the Pezizaceae (Fig. 23) or result in res-
olutions of relationships that are not well-supported
by bootstrap analyses (Fig. 24). The weakness in our
estimate of the phylogeny may be due to having too
few informative characters, or to missing key taxa in
our sampling (especially species of Peziza with apic-
ulate spores). Addition of more characters from oth-
er molecules (such as the protein-coding nuclear
genes Beta-tubulin or RPB2), and expansion of the
set of taxa to include more aberrant species of Peziza,
may improve resolution deep in the tree and “break
up” long branches (Graybeal 1998). Nevertheless,
seven groups of species are identified that may even-
tually be formally recognized. Confirming recent mo-
lecular studies, Peziza is not supported as monophy-
letic (Norman and Egger 1996, 1999). Peziza species
are spread among all groups, except group III, and
it is evident from this that Peziza will have to be sub-
divided into several genera, or a very wide concept.

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Fig. 22. Phylogeny of the Pezizaceae inferred from nLSU-rDNA sequences. One of more than 15 000 equally parsimonious
trees (1832 steps), generated under gaps = missing coding. Terminal taxa represent individual specimens (TABLE II). Branch-
es with asterisks collapse in the strict consensus of all most parsimonious trees. Numbers by branches are bootstrap frequencies
(values greater than 50%). Branch lengths are proportional to the number of steps (character changes) along the branch. Branch colors and dotted branches represent character state optimizations (using MacClade). Species of Peziza are in bold-
face. Taxa in grey are hypogeous, without forcible spore discharge. Bracketed groups I–VII are discussed in the text. Diamond
indicates suggested position of P. vesiculosa from subsequent analysis (see note added after acceptance of the manuscript).
Fig. 23. Strict consensus of 15,000 equally parsimonious trees generated under gaps = missing coding. Numbers by branches are bootstrap frequencies (values greater than 50%). Species of Peziza are in boldface. Taxa in grey are hypogeous, without forcible spore discharge. Bracketed groups I–VII correspond to groups I–VII in Fig. 22. Diamond indicates suggested position of P. vesiculosa from subsequent analysis (see note added after acceptance of the manuscript).
would have to be accepted, to better reflect phylogeny.

Ascomata forms and habit.—Four different forms of ascomata exist within the Pezizaceae. Epigeous apothecia of various shapes (Table I) with forcible spore discharge, are the most common and occur in groups I–VII. This form is likely to be symplesiomorphic, implying that the apothecia-forming members of the Pezizaceae have given rise to at least three different forms of hypogeous ascomata without forcible spore discharge (sensu Weber et al. 1997): (i) ptychothecia: enclosed, hollow, subglobose to globose, often convolute ascomata, which may have one or a few open chambers, and a persistent, recognizable hymenium of asci and paraphyses typically lining the hollow chambers (Figs. 17, 18) (in group VI); (ii) sterothecia: more or less solid, fleshy ascomata, with the asci either solitary, scattered relatively evenly throughout the medullary excipulum or grouped in dispersed pockets or meandering veins (Figs. 8, 14) (in group III, V, VI); (iii) exothecia: solid, pulvinate to globose ascomata, in which the hymenium covers the outer surface (leaving no discernible margin) and permanently exposed, with the asci distinctly shorter than the paraphyses (Fig. 19) (in group VI).

At least three independent origins of hypogeous forms within the Pezizaceae were supported by the LSU rDNA gene trees (in group III, V and VI) (Figs. 22, 24). The most parsimonious interpretations of the molecular phylogenies suggest that forcible spore discharge has been lost once in group III; lost twice, or lost once and gained once in group V; and either lost twice and gained once, or lost once and gained twice in group VI (Figs. 22, 24). However, separate constraint analyses forcing the hypogeous taxa within either group V or VI into monophyletic groups, could not be rejected using the Kishino-Hasegawa test (Table III). Forced monophyly of the hypogeous taxa within group VI, did not yield trees that were significantly worse ($P < 0.05$) than the unconstrained trees (Table III). Likewise, forced monophyly of the two species of Hydnophryopsis in group V could not be rejected ($P < 0.05$). Trees with constrained monophyly of the hypogeous taxa within groups V and VI, respectively, but analyzed together, were not significantly longer than the unconstrained trees (Table III). Likewise, forced monophyly of the two species of Hydnophryopsis in group V could not be rejected ($P < 0.05$). However, under this constraint the MLT was significantly worse than the unconstrained optimal MLT ($P < 0.05$) (Table III). The most conservative conclusion is thus, that forcible spore discharge has been lost only once within each of the groups V and VI.

Phylogeny within the Pezizaceae.—Group I. This is a highly supported clade (bootstrap 96%) of the morphologically distinct *Peziza gerardii* and two species of *Marcelleina*, *M. persoonii* and *M. tuberculosphora*. It is a likely sister group to the rest of the Pezizaceae (Fig. 24). Contrary to the current classification, which accepts *Marcelleina* in the Pyronemataceae (Eriksson 2000) or Otideaceae (Hawksworth et al. 1995), our analyses place the non-amyloid *Marcelleina* in the Pezizaceae. The genus is restricted to species with small purplish apothecia, 2–10 mm in diam (Fig. 3), with light brown pigments in the walls of the hyphae in the outer excipulum and hyaline spores (Moravec 1987). The purplish (brownish) pigments are found in the paraphyses and subhymenium. *Smardaea* and *Aleurina*, presumed to be closely related to *Marcelleina* (Zhuang and Korf 1986, Moravec 1987, Hansen et al. 1998), are supported in the Pyronemataceae (grouping with *Otidea* and an undescribed genus (TL 6236)) (Figs. 22, 23). *Smardaea* and *Greletia* Donadini (the latter not included in this study) are distinguished by purplish pigments in all parts of the apothecia, including the spore ornamentation, and by a clearly differentiated medullary excipulum (Sréczek 1969, Donadini 1979c, Moravec 1987). Korf (1972) placed *Marcelleina* (as *Pulpinia* P. Kars.) in the subfamily Ascodesmidoideae (Pyronemataceae). To him, the genus seemed to provide a link between *Sphaerosezone* and *Aleurina* (as *Jafneadelphus* Rfai). Moravec (1987) revised and placed *Marcelleina* in a new subfamily, Aleurinoideae (Pyronemataceae), along with *Aleurina*, *Eoaleurina* Korf & W.Y. Zhuang, *Greletia*, *Smardaea* and *Sphaerosoma*.

Although the Pezizaceae has generally been characterized by amyloid asci, these results indicate that the amyloid reaction might be lost (type iv) in some lineages, such as in the branch leading to *Marcelleina* (Figs. 22, 24). Boudier (1907) placed species of *Marcelleina* (as *Plicaria*), including the type species *M. persoonii*, in the “Aleurieaes” (= Pezizaceae), presumably based on the globose spores and the excipulum structure. He fully realized that the asci were non-amyloid (Boudier 1905–1910, plate 308). Eckblad (1968) also regarded *Marcelleina* to be very close to *Plicaria*, the non-amyloid asci being one of only a few differentiating characters. He placed it in the Pezizaceae, despite the non-amyloid asci, and concluded that it would be “... attaching too much importance to a single, widely distributed character to keep *Marcelleina* out of its otherwise close affinity to *Plicaria* and *Peziza*.” Other contemporary taxonomists have considered *Marcelleina* as logically placed among the non-amyloid genera. However, morphological similarities between *P. gerardii* and *Marcelleina* (Figs. 2, 3), *Smardaea* and *Greletia* were noted by Schumacher and Jenssen (1992).

Macroscopically, and in habitat requirements (en-
TABLE III. Evaluation of different constrained tree topologies in MP and ML analyses, compared to the MPTs and the optimal MLT, respectively, using the Kishino-Hasegawa test (p < 0.05)

<table>
<thead>
<tr>
<th>Tree</th>
<th>MP Tree lengtha</th>
<th>Significantly worse?</th>
<th>Ln likelihood</th>
<th>Difference in LnL</th>
<th>Standard deviation</th>
<th>Significantly worse?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unconstrained optimal MLT</td>
<td>-11308.898</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Unconstrained MPT</td>
<td>1832</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypogeous taxa within group VI monophyletic</td>
<td>1839 (+7)</td>
<td>no</td>
<td>-11348.976</td>
<td>-40.078</td>
<td>27.051</td>
<td>no</td>
</tr>
<tr>
<td>Hypogeous taxa within group V monophyletic</td>
<td>1835 (+3)</td>
<td>no</td>
<td>-11926.140</td>
<td>-17.243</td>
<td>25.651</td>
<td>no</td>
</tr>
<tr>
<td>Hypogeous taxa within group VI and V both monophyletic</td>
<td>1842 (+10)</td>
<td>no</td>
<td>-11532.500</td>
<td>-43.602</td>
<td>16.248</td>
<td>yes</td>
</tr>
</tbody>
</table>

* Difference in length between MPTs and constrained trees in parentheses.

riched, often calcareous soil), species of Marcelleina (Fig. 3) are very similar to P. gerardii (Fig. 2). Peziza gerardii shares anatomical characters with Marcelleina, although the fusiform spores are very different. Within the Pezizaceae, P. gerardii has a unique spore shape and ornamentation (observed by SEM): large, slightly inequilateral, narrowly fusiform spores, with an ornamentation of low, longitudinal, cyanophobic ribs (Berthet 1970), and 1 to 3 large and several small guttules. Spores in Marcelleina are globose, smooth or ornamented, with one large gultule. The purplish (-brownish) pigments in P. gerardii are located in the paraphyses, subhymenium and excipulum. Peziza gerardii and species of Marcelleina have an excipulum composed entirely of globose to angular cells, which, toward the outer surface, become smaller (outer excipulum hardly discernible), and from which scattered short rows of cells project (appressed, hyphoid, septate hairs). The weakly amyloid, general staining of the asci (type iiia) in P. gerardii is also a distinct and rare feature within the genus Peziza.

Group II. This strongly supported (bootstrap 99%) group includes two unusual species of Peziza, P. subisabellina (Fig. 4) and P. bananicola. Both species have asci that are diffusely amyloid (type iiia) (Fig. 24). Presumably based on this feature, P. subisabellina has recently been combined in Pachyella (Fig. 5) (Trimbach 1999), a placement in contradiction with our molecular results (Figs. 22, 24) as well as the morphological characters. Diffusely amyloid asci are typical for the genus Pachyella, but P. subisabellina does not possess other pachyelloid characters (see Background Information). Spore shape and guttulation in Pachyella is uniform, broadly ellipsoid containing two large guttules, and differs from P. subisabellina which has almost fusiform spores with many small guttules. Our analyses support Pachyella in group III.

Peziza sect. Purpureodiscus G. Hirsch (Hirsch 1992) was proposed for P. subisabellina and P. kreiseli G. Hirsch, based on the diffusely amyloid asci, reddish pigments in the apothecia (Fig. 4), smooth spores without guttules and clavate paraphyses. A segregation of P. subisabellina from the "core group of Peziza species" is also supported by our analyses. Sequences from three different collections of P. subisabellina are included in this study, one occurring on wood (Winterhoff 8844) and two on soil (RK96.54, ALTA9029). Several differences in the LSU have accumulated between these individuals, with the two specimens on soil grouping together, although all three form a well-supported monophyletic group (bootstrap 99%) (Fig. 22).

Peziza bananicola is one of the few strictly tropical Peziza species. It has two unique features: an extensive subiculum that covers and binds debris from ba-
nana plants, and a peculiar spore ornamentation of sparse, irregularly spaced, very thin, acutely pointed spines (Pfister 1991). The spores are narrowly ellipsoid and eguttulate. *Peziza subisabellina* and *P. bananicola* share certain excipular features, being very thick and dense. However, the medullary excipulum is stratified in three layers in *P. bananicola*, but is composed of a single uniform layer in *P. subisabellina*. Other less known tropical species, e.g., *P. luteorosella* (Le Gal) Pfister and *Galactinia tapesoides* Le Gal (Le Gal 1960), are morphologically similar to *P. bananicola* (Pfister 1991) and may belong to group II. *Peziza luteorosella* has spore ornamentation much like *P. bananicola*, but has an unstratified excipulum like *P. subisabellina*. Hymenial colors in the three tropical species and *P. subisabellina* range from white, yellowish, rose, reddish to purplish brown.

**Group III.** Four genera and one form-genus are represented in this clade (bootstrap MPT 56%, supported by all MPT, Fig. 23). *Boudiera dennissii* (Fig. 6) forms a monophyletic group with three species of *Pachyella*, *P. violaceonigra*, *P. punctispora* (Fig. 5) and *P. babingtonii* (bootstrap MPT 91%, MLT 84%). This is in contradiction with the ML analysis by Norman and Egger (1999) based on nSSU rDNA sequences. In their analysis *Boudiera* (*B. acanthosphora* Dissing & Schumacher) clusters with *Iodophanus* (Fig. 1), basically in the Pezizaceae (<50% bootstrap), which supports the placement of these genera in a tribe, Iodophaneae (Korf 1972, 1973b). However, *Pachyella* is not represented in the study by Norman and Egger (1999), since the included *Pachyella* is *Peziza subisabella* (see Materials and Methods). A close relationship between *Boudiera* and *Picearia*, as has been suggested (see Background Information), is also not supported by our analyses. Species of *Boudiera* lack gelatinous excipular tissues and hyphoid hairs embedded in a gel, typical in the genus *Pachyella*. Nevertheless, the lineage is united by asci evenly amyloid, and the lineages of *P. babingtonii* are each 42 and 26 steps. The branch uniting the lineages of *Boudiera* and *Pachyella* is 39 steps. Long internode lengths could most probably be obviated in this clade by including more species of *Boudiera* and *Pachyella*.

*Scabropezia* and the mitosporic *Glischroderma* Fuckel form a highly supported clade with the truffle *Amylascus tasmanicus* (bootstrap 100%). Another truffle, *Pachyphloeus melanoxanthus* (type of *Pachyphloeus*), is suggested as closely related to *Scabropezia*, based on nSSU rDNA sequences (Norman and Egger 1999) and morphology (Dissing and Pfister 1981). Both truffles produce ascomata with a tough-fleshy medullary excipulum and asci randomly distributed throughout the tissue (= stereothecia) (Fig. 8). The asci are weakly amyloid over the entire length (type iia), but some other species of *Pachyphloeus* are described with non-amyloid ascus (Dissing and Korf 1980, Dissing 2000). Dissing and Pfister (1981) noted the similarities in the structure of the outer excipular layers in *Pachyphloeus* and *Scabropezia*, which is highly pustulate or verrucose (Fig. 7), of thick-walled, large, globose cells, a feature also shared with *Amylascus* (Trappe 1971, 1975b, Beaton and Weste 1982). *Scabropezia scabrosa*, *A. tasmanicus* and *P. melanoxanthus* have globose spores, with an ornamentation of prominent conical or truncate warts, which in *P. melanoxanthus* and *A. tasmanicus* are embedded in mucilage. The spores in *Scabropezia* and *P. melanoxanthus* are brownish at maturity. *Scabropezia* often occurs on calcareous soil, or rarely on decaying wood, and attempts to germinate spores of *Scabropezia* failed (Dissing and Pfister 1981). Based on these observations, and the fact that most hypogeous fungi are thought to be ectomycorrhizal (Trappe and Masé 1977), we believe this lineage could be mycorrhizal.

**Group IV.** This group is weakly supported by bootstrap analyses (<50%), but is present in all MPTs (Fig. 23) and in the MLT (Fig. 24). The presence of a distinct amyloid ring zone at the apex of the asci (type ii, Fig. 24) is a synapomorphy for group IV. Such a reaction is present in all species, except in *Kimbropezia*, in which asci are strongly amyloid apically (Korf and Zhuang 1991), and in *P. emileia*, *P. howsei* and *P. ampelina*, which show a less restricted amyloid reaction, although *P. howsei* (KH-97-98) showed a distinctly amyloid ring zone in fresh material. Asci with a strongly amyloid ring were noted previously by Strček (1970) in several of the species in clade IVa. Group IV is also united by the type of anamorph produced. All species with known anamorphs produce an *Oedoecephalum* state. An exception is *Peziza aff. merdae* which produces a *Chromelosporium* state (Norman and Egger 1999). *Oedoecephalum* anamorphs are reported for species in both clade...
IVa (for *P. ammophila*, *P. ampliata*, *P. cerea*, *P. echinispora*, *P. micropus*, *P. repanda*, *P. sylvestris*, *P. varia* and *P. vesiculosa*) and in IVb [for *P. subsulcata* (as *P. praeterrisa*), *P. petersii* and *P. loblata* (as *Galactinia violacea* (Pers.) Le Gal)] (e.g., Berthet 1964a, b, Padren 1972, 1973, Webster et al 1964). Additionally, spores with a unilateral gelatinous coating have only been found in group IV. The gelatinous spore coating was observed in *P. ampliata*, *P. granulosa*, *P. subcitrina* and *P. vesiculosa*. This character has rarely been reported, probably because it is difficult to observe in dried material.

Group IV includes one strongly supported clade, IVa, with bootstrap MPT 91%, MLT 55% (equivalent to Boudier’s genus *Aleuria* pro parte 1907). This clade contains common *Peziza* taxa, characterized by mostly large, yellowish brown disc- to cup-shaped apothecia (Fig. 9), finely warted or smooth, eguttulate spores (*P. domiciliana* has two small guttules in the spores), and simple, hyaline or pale yellowish paraphyses. They occur on rich soil mixed with litter or woody debris or mortar, on strongly decayed trunks, manured substrate or dung. A subset of these taxa was also highly supported as monophyletic in ML analysis by Norman and Egger (1999). Since the type species of *Peziza*, *P. vesiculosa*, is included, this clade will have to serve as a core group for a future circumscription of the genus.

Morphological, ecological, and molecular divergence (nLSU rDNA) based on the branch lengths (Figs. 22, 24) and nucleotide diversity are low within group IVa, which may suggest a recent radiation. Additionally, species limits are controversial and the number of species recognized varies. The group is taxonomically problematic due to lack of diagnostic morphological characters. *Peziza* species with ellipsoid, smooth, small (ca 15 µm) and eguttulate spores were referred to as the “*Peziza repanda-varia-micropus-cerea* complex” by Rifai (1968). He stated that this complex needs a prolonged field and laboratory study to establish species limits. The most detailed study of this complex is by Svrcek (1972, 1973) who also included species with finely warted spores. A detailed study of group IVa using morphology and more rapidly evolving sequences is in progress.

*Peziza ammophila* differs in its habitat and development. It fruits partly buried in sand (Fig. 10), among *Ammophila* (Poaceae). The (pseudo-)stipitate ascomata are deeply cup-shaped and the margins split into irregular rays, becoming flattened on the sand surface. Based on this characteristic development, *P. ammophila* has been placed in *Sarcosphaera* (Fig. 13) (e.g., Kotodab and Pouz 1963, Seaver 1928). Pouzar (1972) considered *P. ammophila* and another species, *P. austrogaster* (Rodway) Rifai, to be a “unique” entity within *Peziza*. He proposed a new subgenus of *Peziza*, *Asterosphaera* Pouz., with *P. ammophila* as the type species. Most authors have considered the similarities in macromorphological development between *Sarcosphaera* and *P. ammophila* to be a result of convergent or parallel evolution. These hypotheses is congruent with the molecular results. Furthermore, *P. ammophila* shares all other morphological characters of clade IVa. *Peziza echinispora* differs from other members of this clade in its spore ornamentation of isolated, regular spines and its occurrence on burnt ground. Other characters such as eguttulate spores, an *Oedocephalum* anamorph, and the distinctly amyloid ascus ring zone, justify its placement in this group. Apart from the guttulate spores, *P. domiciliana* is also aberrant in this clade by having a pinkish (violet) tinge in the hymenium otherwise characteristic of clade IVb.

The monotypic genera *Kimbropezia* and *Pfistera*, described on the basis of peculiar ascus apices (Korf and Zhuang 1991) are nested within clade IVa. *Kimbropezia campestris* was assigned to the Pezizaceae, while *Pfistera* was excluded due to the supposedly non-amyloid asci. Asci in *Pfistera pyrophila* were described as having a very thin, arched apical dome at maturity, which may split vertically, horizontally or irregularly. Van Brummelen (1998) studied the ascus apical structures in these species, and in species he supposed might be related [*P. michelii*, *P. emileia* and *Geopyxis majalis* (Fr.) Sacc.] using TEM. Our results support van Brummelen’s conclusion, that *Pfistera campestris* belongs in *Peziza*. It was found to display two different types of ascus dehiscence, the second type perhaps developing from the first type, resulting from less than optimum conditions during development. The apical structure of the “typical” ascus was of the *Peziza* type. In addition, *Pfistera pyrophila* shows several characters that are typical for clade IVa and microscopic examination (by DHP and KH) revealed a few small patches of faintly amyloid material in the hymenium, around some ascal tips (pretreated with 2% KOH). Van Brummelen observed “remains of a moderately reactive periascus, too thin to be observed by light microscopy.” Contrary to the phylogenetic analysis by Norman and Egger (1999) and to our results, van Brummelen considered *Kimbropezia* to be distinct from *Peziza*. He found that asci of *Kimbropezia*, in addition to having the unique “opercular lens,” was more strongly amyloid than those of *Peziza*, and the annular indentation or weakened zone characteristic of *Peziza* was completely absent. Dehiscence was found to be by a circumscissile, rather irregularly delimited rupture in the thin part of the ascus wall, around the opercular lens. We suggest that this apical structure and the mechanism of dehis-
cence are derived within clade IVa from a typical Peziza-type and that the “opercular lens” is an autapomorphy. The excipulum structure in Kimbropezia is, as also pointed out by Korf and Zhuang (1991), typical for species in clade IVa; the spores are ellipsoid, smooth and eguttulate (Table 1). Therefore, Kimbropezia should be included in Peziza. The operculate dehiscence has been lost completely several times within Peziza (e.g., see Group VI) and the Pezizales (e.g., Trappe 1979, O’Donnell et al 1997, Percudani et al 1999). It should be noted that in ML analysis by Norman and Egger (1999) the truffle Terfezia terfezioides (Matt.) Trappe is supported within, whereas species in clade IVb were treated in both with low, short branched ridges and warts. Four of these “outlines” appear as aggregations of many small guttules, after pretreatment in 10% KOH, either dark and intense or at least of yellow or golden color. Sarcosphaera has yellow juice when cut or bruised. Peziza species with apothecia that yield a colored juice are also present in the ascomata (Fig. 12) and eguttulate spores. Peziza lobulata has eguttulate spores, while P. proteana and P. petersonii have spores with low, short branched ridges and warts. Four of the species are pyrophilous, P. petersonii, P. proteana, P. subviolacea and P. lobulata. Peziza exogelatinosa occurs in forest, e.g., Peziza species in group VI, and these two types may not be homologous. Guttules may, however, always be present to a greater or lesser degree, but the character may not be expressed in some species. If such is the case, lack of guttules is not phylogenetically informative. The “outlines” of two guttules have been observed (by KH in fresh material) in the spores of several specimens of group IVa. These “outlines” appear as aggregations of many smaller oil drops, after pretreatment in 10% KOH, staining with Sudan black in alcohol (a lipid stain) and clearing with 50% alcohol.

Group V. In these analyses, Sarcosphaera and two species of the hypogeous Hydnotryopsis, H. setchellii and H. sp. form a highly supported clade within group V (bootstrap 100%) (Figs. 22-24). The ascomata of Hydnotryopsis have hymenia located in labyrinthiform chambers or meandering veins, but these are folded and compressed, making them solid (= stereothecia) (Fig. 14) (Trappe 1979). Hypogeous fruiting of Sarcosphaera may occur (Trappe 1975a, 1979). In such cases the apothecia occasionally become folded and form chambers, thus resembling psychothecia, but the asci retain functional opercula. Sarcosphaera coronaria fruits in spring to early summer and its development is likely associated with selection for reduction in water loss, as it is thought to be in truffles (e.g., Thiers 1984). The excipulum is thick and tough, probably also correlated with microclimatic aridity and initial development underground. It is composed of a distinct outer layer (ca 750 μm) of textura epiendomoida to textura prismatica on the outermost region, a medullary layer (ca 450 μm) of large globose cells and a subhymenium of smaller globose cells. A close relationship between Sarcosphaera and Hydnotryopsis has not previously been suggested. Trappe (1979) suggested that, “in its evolution to a solid gleba, Hydnotryopsis is related to Peziza a step farther away than Mycoelelandia.” Sarcosphaera differs anatomically from Peziza in the outer excipular layer. Hydnotryopsis and Sarcosphaera both have asci evenly amyloid over the entire length (type iii), and ellipsoid, amorphous ornamented spores (in SEM spores of Sarcosphaera have an ornamentation of low, rounded warts, covered by a thin amorphous layer). Sarcosphaera is only found associated with mycorrhiza-forming trees, and is often locally abundant where it occurs, year after year in the same spot, suggesting that it is ectomycorrhizal. Basal to these taxa is an as yet unidentified species of Peziza sp. 3, collected on experimental plots treated with urea (supported by all MPT’s and the MLT, but bootstrap <50%). It shares features with species of Peziza in group IV (a whitish, cup-shaped apothecium, smooth, biguttulate spores, and hyaline or pale yellowish paraphyses, slightly enlarged and curved towards the apices), but has asci strongly amyloid over the apex.

The Hydnotryopsis/Sarcosphaera clade appears as a sister group to common epigeous species of Peziza sharing features with those in group VI. However, Peziza succosa, P. succosella and P. michelii possess lactiferous hyphae in the excipulum, which yield a yellow juice when cut or bruised. Peziza species with apothecia that yield a colored juice are also present in

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group VI (for example *P. saniosa* (Fig. 15) and *P. badiofusca*), but in these hyphae yield a bluish or watery juice. This character however, needs further study, since lactiferous hyphae are likely to be present in many species of *Peziza*. Thus, Le Gal (1937, 1953a) described and illustrated lactiferous hyphae also in species without or seemingly without staining flesh or juice. *Peziza succosa*, *P. succosella* and *P. micheli* have spores with irregular, elongated warts and two large guttules, asci strongly amyloid over the apex (type i), and brownish colors in the hymenium (yellowish brown, olivaceous yellow to greyish brown, or first violet, then brownish). They occur on rich disturbed soil in deciduous forests, always in association with ectomycorrhizal fungi. *Peziza aff. brunneoatra* devides by having dark brown apothecia, lacking a yellow juice and in having finely warted spores packed with numerous small guttules (sensu Dennis (1978) in Norman and Egger (1999)).

**Group VI.** Although group VI has low bootstrap support (< 50%), it is present in all equally parsimonious trees (Fig. 23) and in the MLT (Fig. 24). It includes one weakly supported clade (bootstrap MPT 53%, MLT 51%) of 11 epigeous species of *Peziza* (equivalent to Boudier’s genus *Galactinia* pro parte 1907) and several truffles and truffle-like taxa. The epigeous *Peziza* species are united morphologically by mostly dark brownish, disc- to cupshaped apothecia, often with an olivaceous or purple tinge; highly warted, ridged to reticulate spores, with two, rarely one, large guttules; dark pigmented paraphyses; and asci intensely and unrestrictedly amyloid over the apex (type i). The hypogeous taxa have asci that are evenly amyloid over the entire length, either weakly or strongly (type iii), or are non-amyloid (type iv).

The relationship of several of the epigeous ellipsoid-spored species has been studied, and discussed in relation to the globose-spored *Plicaria* (Pfister 1979b, Egger 1987, Moravec and Spooner 1988, Norman and Egger 1996, 1999) (see Background Information for previous hypotheses). Our analyses confirm a close relationship of *Plicaria* (Fig. 16) to the more inclusive *Peziza* clade within group VI (Fig. 15). *Plicaria* is supported as monophyletic in the MLT (bootstrap 67%) (Fig. 24), but the placement of the smooth-spored *P. endocarpoides* (the only smooth-spored species in group VI) is unresolved in the MPTs (Figs. 22, 23). The three *Plicaria* species with ornamented spores are supported as monophyletic with a 65% bootstrap value. Globose, ellipsoid to fusiform-spored taxa group together in several lineages within the *Pezizaceae* (in group I, III, VI) (TABLE I), indicating that spore shape is not phylogenetically informative at these higher taxonomic levels. As shown by Norman and Egger (1996, 1999) to maintain *Plicaria* as a monophyletic group will leave *Peziza* (or the *Peziza* species in group VI) as a paraphyletic group. Some important characters of *Plicaria* are shared with some of the *Peziza* species, e.g., pale brownish spores at maturity and paraphyses encrusted with amorphous matter. Such characters seem to be synapomorphic to the *Peziza* species in group VI. Pale brownish spores are found in *P. atrovirinosa*, *P. vaccinii* and *P. alaskana*. Paraphyses encrusted with amorphous matter are present in *P. alaskana*, *P. badiofusca*, *Peziza sp. 1*, *P. vaccinii*, *P. depressa* and *P. phyllogena*. Other *Peziza* species reported with pale brownish spores, e.g., *P. retiderma* and *P. rifaii* (Moravec and Spooner 1988), and paraphyses encrusted with amorphous matter, e.g., *P. badiofuscoide* Donadini (Donadini 1979b), are likely members of group VI, but have not been sequenced by us. In contrast to the rest of the epigeous taxa in group VI, the asci in *Plicaria* are weakly amyloid over their entire length (type iiiia).

The 11 epigeous *Peziza* species in the more inclusive clade of group VI constitute a homogeneous group, both in macro- and microscopical features, and levels of divergence in the nLSU rDNA sequences are low based on the branch lengths (Figs. 22, 24). The clade of *P. badiofusca*, *Peziza sp. 1*, *P. badia*, *P. limnaea* and *P. alaskana* (Figs. 22–24) represents a complex of species centering around *P. badia* (see Le Gal 1937, Donadini 1979b, Pfister 1979b), that possesses spores with ornamentations of warts elongated into short ridges, which may anastomose to form an incomplete to almost complete, irregular reticulum. Less known unsampled species that may belong to this clade are *P. phlebospora* (Le Gal) Donadini, *P. badiofuscoide* and *P. badioide Donadini*. A closer study of these species, mainly distinguished by small differences in spore size and ornamentation and in habitat, is needed. *Peziza depressa* differs from members of this group by having spores ornamented with distinct, regular, high warts, and a warm reddish brown hymenium. *Peziza saniosa*, *P. badiofuscoide* and *P. badioide* Donadini. A closer study of these species, mainly distinguished by small differences in spore size and ornamentation and in habitat, is needed. *Peziza depressa* differs from members of this group by having spores ornamented with distinct, regular, high warts, and a warm reddish brown hymenium. *Peziza saniosa*, *P. badiofuscoide* and *P. badioide* Donadini. A closer study of these species, mainly distinguished by small differences in spore size and ornamentation and in habitat, is needed. *Peziza depressa* differs from members of this group by having spores ornamented with distinct, regular, high warts, and a warm reddish brown hymenium. *Peziza saniosa*, *P. badiofuscoide* and *P. badioide* Donadini. A closer study of these species, mainly distinguished by small differences in spore size and ornamentation and in habitat, is needed.
which become fused towards the poles, often forming apical caps; and its fruiting habit, on decaying wood or woody debris in wet habitats (Wells and Kempton 1967, Elliott and Kaufert 1974, Ginns 1980, Harmaja 1986). Its spores do not possess two large guttules as is typical for the group VI Peziza species, but rather have many small guttules that aggregate towards the poles. The truffles and truffle-like taxa nested within group VI show diverse ascomatal forms. Two hypogeous species of Peziza, *P. whitei* (FIG. 18) and *P. ellipsospora* (FIG. 17), produce pycnocystidia (TABLE I) (Gilkey 1916, Trappe 1975a, 1979, Beaton and Weste 1982). Both species have hyaline, broadly ellipsoid spores, which are minutely papillose in *P. ellipsospora* and reticulate in *P. whitei*. Gilkey (1916) described *P. ellipsospora* in *Hydnotrya* Berk. & Broome, based on the macro-morphology, but erected a new genus, *Hydnopicilia* Gilkey for *P. whitei* (Gilkey 1954a). Peziza *whitei* has been placed in *Hydnocystis* Tul. and *Hydnoplicata* Gilkey (see Trappe 1975a). Burdsall and Korf (Burdsall 1968) and Trappe (1975, 1979) transferred the two species to *Peziza*, based on the amyloid reaction of the asci, spore ornamentation, ascocarp structure, and supposedly operculate asci. Later Trappe (pers comm) found that these species lack an operculum and the spores are not forcibly discharged. Our molecular results support the placement of the two species among the core group of epigeous *Peziza* species in group VI (FIGS. 22–24). *Peziza ellipsospora* is a sister species to *Cazia*. Other phylogenetic analyses (Norman and Egger 1999, Percudani et al. 1999) have further shown *Cazia* to be closely related to the truffle *Terfezia arenaria* (Morris) Trappe. *Cazia* and *Terfezia* produce spongy peridium and both have non-amyloid asci (type iv) (TABLE I). As in the branches leading to *Marcellinea* (in group I) and *Terfezia terfezioides* (possibly in group IV based on Norman and Egger 1999), the amyloid reaction might be lost along a branch leading to *Cazia flexiascus* and *Terfezia arenaria*. This suggests that the amyloid reaction has been lost at least three times within the Pezizaceae. *Cazia flexiascus* has ellipsoid, hyaline, incompletely warty-reticulate spores, with one guttule (Trappe 1989), much like the spores of *P. whitei* and *P. ellipsospora*. Two species of *Terfezia*, *T. nivea* and *T. pinoyi*, also producing spongy peridium (Trappe 1971, Moreno et al. 2000), are nested within group VI. They group strongly together (bootstrap MPT 99%, MLT 93%) as a sister group to most of the epigeous and the hypogeous *Peziza* species and *Cazia* (FIGS. 23–24). The similarity in excipulum structure between *Cazia*, *Terfezia* and *Terfinaria* adds support to the close relationship between these taxa (TABLE I).

*Ruhlandiella berolinensis* produces exothecia (FIG. 19) (Dissing and Korf 1980, Warcup and Talbot 1989 as *Muciturbo*, Galán and Moreno 1998). The excipulum is composed entirely of globose to angular cells. Asci are evanescent at maturity. The paraphyses in both *R. berolinensis* and *P. whitei* exceed the asci and are embedded in a gelatinous sheath. Spores in *R. berolinensis* are probably dispersed when the hymenium and upper part of the ascoma break down to form a mucilaginous mass. *Ruhlandiella berolinensis* groups with *P. ostracoderma* in all trees (FIGS. 22–24), but with low bootstrap support.

Few anamorphs have been reported for the group VI taxa, but where reported they are referable to *Chromelosporium* (*Peziza ostracoderma*, *Plicaria endocarpoides*, *P. trachycarpa*), *Chromelosporium*-like states (*Ruhlandiella*), or are unnamed states, producing hyaline to colored, 0–3 septate resting spores [*P. saniosa*, *P. phyllogena* as *Galactinia badioconfusa* (Korf) Srřček] (Berthet 1964a, b, Paden 1972, Hennebert 1973, Hennebert and Korf 1975, Warcup and Talbot 1989). Most of the epigeous *Peziza* species occur on bare, often disturbed soil, under ectomycorrhiza-forming trees. *Plicaria* species and *Peziza vaccinii* are pyrophilous, while *P. ostracoderma* also occurs on other types of sterilized soil and *P. griseorosea* on very degraded wood or soil mixed with wood.

**Group VII.** Two highly supported clades, VIIa and VIIb (bootstrap 96% to 100%) are united in group VII, but with only weak support in the MLT and the MPT (bootstrap <50%) (FIG. 22, 24). Clade VIIa includes three species of *Peziza, P. polaripapulata, P. sp.* 4 and *P. luteoloflavida*. The apothecial colors of these species are in the range of pale yellow, ochre-yellow, warm yellow, brownish yellow to golden brown, dark brown with an olivaceous tinge, or olive yellow to olive (FIG. 20) (Kristiansen and Schumacher 1993, Hansen et al. 1998). They are united by asci being evenly amyloid over the entire length (type iii). The spores contain two small guttules or are eguttulate. *Peziza* sp. 4 has smooth spores (in SEM), whereas *P. luteoloflavida* has spores with a fine, irregular, undulating reticulum, and *P. polaripapulata* has spores with isolated, rounded to elongated warts, becoming more or less fused at the poles to a cap-like thickening. Based on the spore ornamentation *P. polaripapulata* has been placed with the “pseudoapiculate *Peziza* species” in subgenus *Phaeopezia* (Sacc.) J. M. Mavorvec (Häffner 1995), along with *P. phyllogena* and the apiculate-spored *P. apiculata*. A close relationship of these taxa is not supported in our analyses, which places *P. phyllogena* in group VI and *P. apiculata* either as a sister lineage to group II or III. However, to test the monophyly of taxa with pseudoapiculate and apiculate spores, more sampling is necessary. *Peziza*
polaripapulata (Fig. 20) occurs on very degraded hardwood, sawdust or soil in calcareous forests and P. luteoloflavida on sand on river banks. Peziza sp. 4 was collected among mosses, on a forest road surfaced with cinders.

Clade VIIa consists of two collections of the strictly subtropical or tropical genus, Iodowynnea. Our results support the segregation of Iodowynnea from the main groups of Peziza, but a close relationship to the Peziza species in clade VIIa is possible. The asci in Iodowynnea auriformis are evenly and strongly amyloid over the entire length (type iiib), and the apothecial colors have red tones. It fruits on soil or vegetable debris in forests with ectomycorrhizal trees (Medel et al. 1996).

Taxa not supported in group I–VII. Peziza natrophila and P. quelepidotia form a highly supported lineage (bootstrap 100%, Figs. 22–24), the placement of which is unresolved. But, in our analyses this group never falls within the main groups of Peziza. This observation is supported by the ascus amyloid reaction in P. natrophila, which is weak over the entire length (type iii). Peziza quelepidotia was placed in its own genus Lepidotia, recognized by ellipsoid, eguttulate spores and distinctly stipitate or obconical apothecia. The apothecial colors have red tones. It fruits on soil or vegetable debris in forests with ectomycorrhizal trees (Medel et al. 1996).

Taxonomic conclusions.—The 14 genera sampled by us, and currently accepted in the Pezizaceae (Eriksen 2000), are confirmed to be members of the family. The analyses, however, suggest that the Pezizaceae should be emended to include the non-amyloid genus Marcellina. The Ascobolaceae is confirmed to be the sister group to the Pezizaceae, which supports the general view that the amyloid reaction of the ascus is phylogenetically informative in the Pezizales. Peziza and most other genera of the Pezizaceae, as they are currently delimited, cannot be maintained in a monophyletic classification system. Peziza is composed of at least 6 major lineages, most of which include other genera of the Pezizaceae, and 4 species of Peziza occur in separate lineages of yet uncertain placement. Peziza should be disassembled into groups that more closely reflect the phylogeny, and several genera now recognized in the family require emendation or change in status. Group IV includes the type species of Peziza, P. vesiculosa, and should serve as a core group for a future circumscription of Peziza. The genera Kimbropezia and Pfistera should be included in Peziza. A close relationship between Sarcosphaera and the truffle Hydnorotrys is suggested for the first time. Likewise, a close relationship between Scabropezia and the truffle Amylascus is strongly indicated.

Overall, these LSU analyses imply that there has been extensive homoplasy in some of the morphological characters that previously have been used to delimit taxa. Hypogeous ascomata with concomitant loss of active spore discharge have evolved independently several times, suggesting that their derivation from apothecial Pezizaceae may involve relatively simple genetic changes. For example, stereothecia have been derived within group III, V and VI. In addition, ptychothecia and exothecia have evolved within group VI. Derivation of ptychothecia and stereothecia from apothecia involves the evolution of enclosed hymenia or asci, while the derivation of exothecia involves the evolution of extremely convex hymenia. Spore shape has been emphasized in the delimitation of genera, but our results indicate that species with divergent spore form may be closely related. Globose-spored species occur in group I, III and VI, along with ellipsoid- or fusiform-spored species. The amyloid reaction of the ascus appears to be a variable character that has been lost in some lineages. On the other hand, different types of ascus amyloid reactions correspond to different lineages and seem to provide clues to relationships within the Pezizaceae. Other characters, such as apothecial colors, spore surface relief, guttulation, excipulum structure and anamorph morphologies, seem to be phylogenetically informative, although considerable variation within
and between groups occur. For example, apothecial colors are purplish in group I, in the range of yellowish, rose, reddish to purplish (brown) in group III, paler yellowish-brown in group IVa, violaceous to vinaceous in group IVb, and dark brownish in group VI. Smooth or finely verrucose spores, without or with two small guttules, are mostly present in group fairly uniform within most groups. Oedocephalum anamorphs are produced by taxa in group III and IV, and by species of Iodophanus, while Chromelosporium anamorphs are produced mainly by group VI taxa.

It is still premature to propose a phylogenetic classification of the Pezizaceae. For example, the monophyly of some of the genera, e.g., Boudiera and Scabropezia, has not been tested and six genera (TABLE I) are not represented in this study. Most importantly, an additional dataset is needed to provide a more robust estimate of the phylogeny. Further studies of morphological characters, especially of pigment composition and excipulum structure, and of anamorph type and trophic status in the context of a robust molecular phylogeny, that will facilitate refining hypotheses of homology, may be useful in understanding relationships within the Pezizaceae.

Note on P. vesiculosa added after acceptance of the manuscript.

In a detailed and large scale study now underway of the group IVa Peziza species using ITS rDNA sequences it was discovered that the published voucher specimen for P. vesiculosa (ALTA 9066, GenBank LSU: AF133170, SSU: AF133155, ITS: AF133182) (Norman and Egger 1999) was misidentified. The specimen (ALTA 9066) has been examined morphologically by K. Hansen and has been determined to be Peziza fimeti. A comparison of the ITS1 sequence of ALTA 9066 with the larger ITS data set confirms this finding. In this paper ALTA 9066 has been renamed P. fimeti in FIGS. 22–24, Steen A. Elborne, David S. Hibbett, Roy Kristiansen, and Jens H. Petersen for supplying photographs, J. H. Petersen for scanning the color photographs, and two anonymous reviewers for their insightful comments on the paper. We thank Michael Castellano, Daniel Luoma and the US Forest Service, Oregon State University for permission to reproduce the photographs in FIGS. 14 AND 17–19, and Sara Landvik, Novozymes A/S, for doing the PCR and sequencing of the LSU of P. vesiculosa added after acceptance of the paper. Funding was provided by an NSF grant (DEB-9521944) to DHP and by a grant from the Faculty of Science, University of Copenhagen to KH.

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