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Phylogenetic studies within the Pezizales. I. 18S rRNA sequence data and classification

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Abstract: The order Pezizales has been divided into two suborders. One suborder, the Sarcoscyphineae, was originally described to include members whose asci were characterized by an unusual apical structure, the suboperculum. Disagreements as to how this structure should be defined, and indeed, whether or not it exists at all, have rendered the status of the suborder controversial. The two families within this suborder are the Sarcoscyphaceae and the Sarcosomataceae. Recent ultrastructural work demonstrates that there is an apical thickening which is restricted to the Sarcoscyphaceae. In order to test the monophyly of the suborders of the Pezizales and examine the relationships within the Sarcoscyphineae, phylogenetic analyses were carried out using DNA sequence data from the 18S rRNA gene. The strict consensus tree based upon these data shows both the Sarcoscyphineae and the Pezizineae as paraphyletic. These data suggest that the subordinal taxa currently recognized within the Pezizales should be abandoned and the taxonomy revised to reflect phylogenetic relationships. Strongly supported clades (i.e., greater than 95% bootstrap value, 1500 replicates) include: the Pezizaceae, the Morchellaceae, the Sarcoscyphaceae, the Helvellaceae, and a clade that includes the Sarcosomataceae (which is paraphyletic), and the Otidiaceae (represented only by 2 taxa). The genus *Pindara*, formerly placed in the Sarcoscyphaceae, is nested within the Helvellaceae, and *Wynnea*, assigned to the Sarcosomataceae by some authors, is positioned in the Sarcoscyphaceae.

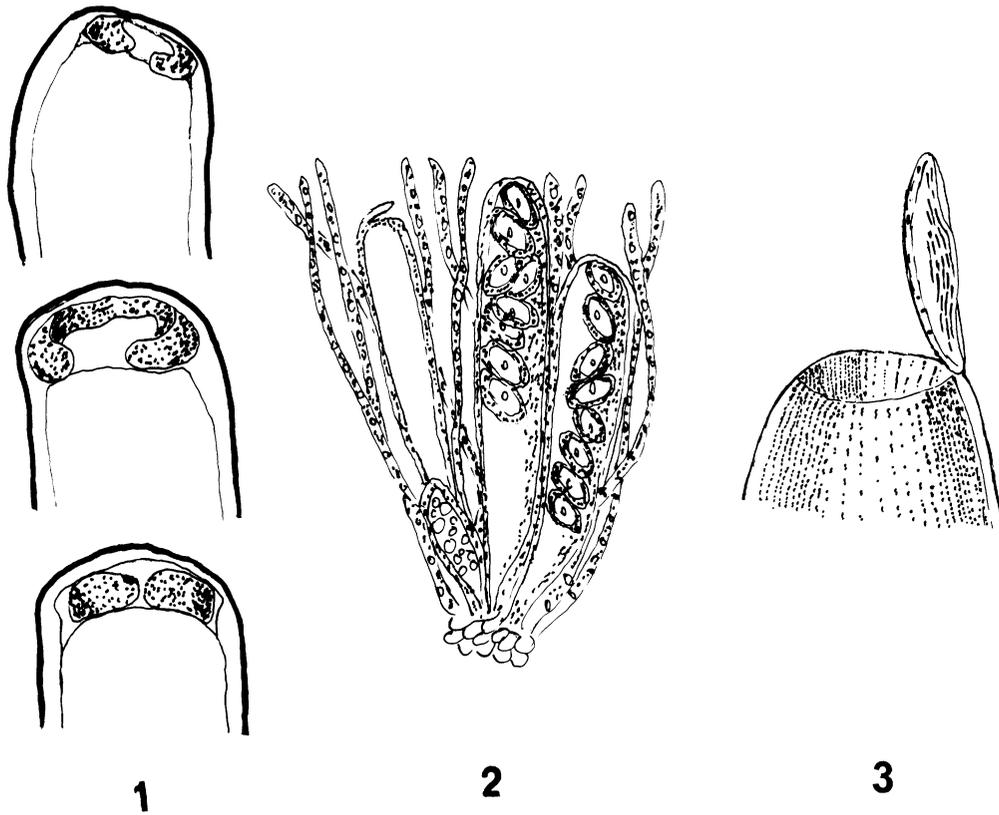
Key Words: cladistics, Pezizineae, Sarcoscyphineae

INTRODUCTION

Traditional classification schemes (e.g., Schroeter 1893, Boudier 1907, Atkinson 1915, Nannfeldt 1932, Bessey 1964) classified those fungi forming apothecial ascomata into a major group, the discomycetes, (Ascomycetes). Phylogenetic analyses using gene sequence data, however, have confirmed that morphological characters such as apothecial ascomata, ascus and ascospore shape are not necessarily reliable characters for inferring phylogenetic relationships (Berbee 1996, Berbee and Taylor 1992a, b, 1995, Momol et al 1996, Spatafora and Blackwell 1994, Saenz et al 1995). Phylogenetic analyses of 18S rRNA gene sequence data (Gargas and Taylor 1995, Spatafora 1995, Landvik 1996, Landvik et al 1997) have shown the discomycetes to be a paraphyletic assemblage and have confirmed earlier suppositions that the two principle orders, the Pezizales and the Helotiales, are not closely related (Nannfeldt 1932, van Brummelen 1978, Eckblad 1968, 1972). The order Pezizales, however, has been considered a monophyletic group, though not strongly supported (Spatafora 1995, Landvik 1996, Landvik et al 1997).

Because the ascus has been the single most-studied anatomical character within the Pezizales, this study focuses on the relevance of the ascus apex on the classification of this group. Members of the Pezizales have a dehiscence mechanism that typically involves an apical circumscissile rupture of the ascus wall to produce a lid, the operculum. Some pezizalean species, however, have asci in which the operculum has been lost or reduced (Trappe 1979), or in which the ascus ruptures by a slit. Asci with opercula are also found in taxa outside the Pezizales, such as *Orcadia* (Kohlmeyer and Kohlmeyer 1979), but these fungi have never been classified within Pezizales and their relationship to other Ascomycota is uncertain.

The significance of the operculum (FIGS. 2, 3) for discomycete classification was first recognized by Boudier (1879, 1885, 1907). He divided the discomycetes by the mode of dehiscence. Two series were recognized, those with an operculum and those without. Many of Boudier's peers (e.g., Cooke 1892, Karsten 1885, Rehm 1895, Saccardo 1889, Schroeter 1893) however, did not agree with this division. Seaver's classic treatments (1927, 1928, 1942, 1951) divide the discomycetes along Boudier's lines. Two French



FIGS. 1–3. Ascal apices according to Boudier and LeGal. 1. Apical chamber seen from different perspectives in *Sarcosoma* $\times 1500$. The stippled structure is LeGal's coussinet or suboperculum (redrawn from LeGal 1946b). 2. Boudier's interpretation of *Ascobolus* asci and paraphyses with an empty ascus on the left showing an operculum $\times 450$ (redrawn from Schröeter, J. 1893). 3. Boudier's interpretation of the lid on the ascus of *Ascobolus* $\times 1200$ (redrawn from Schröeter, J. 1893).

workers (Chadefaud 1942, 1943, 1946, Le Gal 1946a, b, 1953) focused their observations on the mechanisms of ascal dehiscence in the Pezizales (operculate discomycetes). Using light microscopic observations on the ascal apex morphology, Le Gal distinguished an ascal apex that was characterized by having a thickened sub-apical pad (“coussinet”) in the apical chamber—the suboperculum (FIG. 1). In 1953, she presented the view that this was a link between those taxa she considered the Pezizales, and the evolutionarily primitive inoperculate Helotiales. She described the family Sarcoscyphaceae as the “natural” intermediary group for these species with subopercula. Le Gal (1947) proposed two tribes, the Sarcoscyphaeae for species with brightly-colored apothecia and the Urnulae for those with brown to black apothecia.

Many classification schemes (Nannfeldt 1949, Korf 1957, 1958, 1970, 1972, 1973, Dennis 1953, 1960, 1978, Denison 1963, 1965, 1972) followed Le Gal's separation of the suboperculate from the operculate taxa. Eckblad (1968) was the first to raise issue with the definition and the structure of the suboperculate ascus. He noted (1968, 1972) that some taxa included in the Sarcoscyphaceae did indeed have the thick-

ened eccentric opercula but other taxa, traditionally placed in the family, had no such structures. The suboperculate ascus became the locus for ultrastructural studies; details of its structure interpreted from light microscopy were questioned by investigators using TEM (van Brummelen 1975, 1978, 1994, Samuelson 1975, Samuelson and Kimbrough 1978). Structurally some of the taxa show two thickened apical sublayers (Bellemère 1994, Bellemère et al 1990). This interpretation differed from Le Gal's original concept (1946b) and Samuelson's (1975) revised version, but is similar to that of Samuelson et al (1980). Numerous researchers (Das and Pant 1984, Denison 1963, 1965, 1967, 1969, 1972, Gamundi 1957, 1959, 1971, Korf 1957, 1963, 1970, 1972, Korf and Waraitch 1971, Korf and Zhuang 1991, Paden and Tylutki 1968, 1969, Paden 1983, Pant and Tewari 1970, Rifai 1968, Wang and Zhuang 1997, Zhuang 1991, 1993) however, have used the term suboperculate without describing it or fully accounting for the range of variability seen with the light microscope. When Rifai (1968) proposed a suborder for the operculate taxa (Pezizineae) and one for the suboperculates (the Sarcoscyphineae), he was codifying the commonly held

view that ascus morphology was fundamentally different between the two groups and that it was an important character in the classification of Pezizales. At the time Korf (1970) reviewed the classification of the suborder he noted: (i) the family Sarcoscyphaceae as proposed by Le Gal (1946b, 1947) was a *nomen nudum*; (ii) Eckblad (1968) provided a Latin diagnosis for the family; and (iii) both Eckblad (1968) and Le Gal (1969) had overlooked the fact that Kobayasi (1937) had properly described a family as Sarcosomataceae, which included one of the genera treated in the Sarcoscyphaceae. Thus, the Sarcoscyphaceae was superfluous if *Sarcosoma* was included. Korf (1970) provided a choice: either maintain the currently accepted taxonomic arrangement of one family (Sarcosomataceae) with two tribes (Sarcosomateae and Sarcoscyphaeae) or recognize two emended families, each with two tribes: the Sarcoscyphaceae (Sarcoscyphaeae Korf, Boedijnopezizeae Korf) and the Sarcosomataceae (Sarcosomateae Korf, Galielleae Korf). Later tribes added to each family included Pithyeae (Sarcoscyphaceae, Denison 1972) and Pseudoplectaniae, a *nomen nudum* (Sarcosomataceae, Bellemère et al 1990). The family distinctions were supported by cytological investigations of Berthet (1964). Members of both families have ascospores that are plurinucleate while the Sarcoscyphaceae has plurinucleate paraphyses, and the Sarcosomataceae has uninucleate paraphyses, though complete cytological data for some taxa are absent. The Sarcoscyphaceae was not universally accepted as a valid family name until 1991 (Eriksson and Hawksworth).

Several recent phylogenetic studies using the 18S rRNA gene sequence data have shown that the Pezizales are a monophyletic group within the discomycetes (Gargas and Taylor 1995, Spatafora 1995, Landvik 1996, Landvik et al 1997). Thus, for this study, we tested the hypothesis that the suborder Sarcoscyphineae is monophyletic and analyzed the phylogenetic relationships among the genera and families.

MATERIALS AND METHODS

Sampling.—The fungi used in this study (TABLE I) were from herbarium or personal collections. Due to scarcity and/or poor condition of collections, only 20 of the 24 genera of the Sarcoscyphineae were used in this analysis. DNA was isolated from lyophilized mycelia or from herbarium specimens. When possible, mycelia for DNA extraction was obtained from ascospore-germinated cultures, either from fresh or herbarium specimens less than five yr then lyophilized. DNA was extracted from fresh or herbarium material as described in O'Donnell et al (1997) using the phenol-based method. Approximately 1–5 ng of extracted DNA was used as template for polymerase chain reaction (PCR) amplification in 50 μ L

reactions. The 18S rRNA gene was either amplified as a single fragment with primer pairs PNS1 and NS8, or as two overlapping fragments with the primer pairs PNS1/NS41 and NS 51/ITS 2 (<http://www.botany.duke.edu/fungi/mycolab>). PCR protocol/amplification and thermal cycling parameters followed Harrington (1998). The PCR products were gel purified then cleaned with QIAquick PCR purification kit (Qiagen Inc, Chatsworth, California) following the manufacturer's instructions and were used directly for the sequencing procedures. The DNA mass was estimated using ethidium bromide staining with GibcoBRL's Low DNA Mass Ladder (Life Technologies, Gaithersburg, Maryland). For DNA sequencing reactions, approximately 10–30 ng/1 μ L of DNA added to a 10 μ L reaction with the ABI PRISM dye terminator cycle sequencing reaction kit (Perkin-Elmer Corp., Foster City, California). Nucleotide sequences were determined in both directions. Sequences were analyzed in an Applied Biosystems 373A DNA sequencer using 5% acrylamide gels (Long Ranger, AT Biochem, Malvern, Pennsylvania; or Sequagel-6, National Diagnostics, Atlanta, Georgia), and were edited with Sequencher (ver. 3.0, Gene Codes Corp., Inc., 1995).

Orbilbia was chosen as the outgroup taxon to test the potential monophyly of the Sarcoscyphineae and root the cladograms. Preliminary cladistic analyses of the 18S rRNA gene sequence data of various discomycete taxa (Harrington unpubl) placed *Orbilbia* as the sister group to the Pezizales.

Sequence alignment and phylogeny reconstruction.—Sequences were aligned with MALIGN (Wheeler and Gladstein 1993) using the pair and build options, an internal gap cost of 6 and a substitution cost of 4, and further adjusted by eye. The complete alignment is available from Treebase (<http://www.herbaria.harvard.edu/treebase>). All sites from the alignment were included in the phylogenetic analyses. Cladistic analyses using unweighted parsimony were performed on microcomputers. The heuristic option in NONA (Goloboff 1993) was used to search for trees using random taxon addition and branch swapping. The tree searching command was mult100, which for 100 replications randomizes the taxon addition order, creates a Wagner tree, and then submits it to tree bisection-reconnection branch swapping (TBR). Phylogenetic bootstrapping (1500 replications, Felsenstein 1985) was implemented in PAUP (Swofford 1993) with the following options, simple taxon addition sequence, tree bisection-reconnection branch swapping, and mulpars (retains all shortest trees) options not in effect.

RESULTS

We found no introns in any of the 18S rRNA gene sequences. The sequences ranged from 1655–1765 bp. In order to expedite alignment, all were edited to begin and end with the same highly conserved sequences. The final alignment of complete 18S rRNA sequences included 1710 sites. Of these, 369 were variable and 199 were potentially phylogenetically informative. Phylogenetic analysis of the 18S

TABLE I. Fungi included in this study

Genus	Species	Family	Locality	GenBank
<i>Balsamia</i> Vittad.	<i>magnata</i>	Helvellaceae	CA, USA	U42656
<i>Byssonectria</i> P. Karst.	<i>aggregata</i>	Otideaceae	Sweden	Z30241
<i>Chorioactis</i> Eckblad	<i>geaster</i>	Sarcosomataceae	TX, USA	AF104340
<i>Cookeina</i> Kuntze	<i>tricholoma</i>	Sarcoscyphaceae	Puerto Rico	AF006311
<i>Desmazierella</i> Lib.	<i>acicola</i>	Sarcosomataceae	Norway	AF104341
<i>Discina</i> (Fr.) Fr.	<i>macrospora</i>	Discinaceae	MI, USA	U42651
<i>Donadinia</i> Bellemère & Melendez-Howell	sp.	Sarcosomataceae	NY, USA	AF104342
<i>Galiella</i> Nannf. & Korf	<i>rufa</i>	Sarcosomataceae	GA, USA	AF004948
<i>Glaziella</i> Berk.	<i>aurantiaca</i>	Glaziellaceae	Sweden	Z49753
<i>Helvella</i> L.	<i>lacunosa</i>	Helvellaceae	OR, USA	U42654
<i>Komposocypha</i> Pfister	<i>chudei</i>	Sarcoscyphaceae	Uganda	AF006316
<i>Microstoma</i> Bernstein	<i>flocosum</i>	Sarcoscyphaceae	PA, USA	AF006313
<i>Morchella</i> Pers.	<i>elata</i>	Morchellaceae	MI, USA	U42641
<i>Nanosocypha</i> Denison	<i>tetraspora</i>	Sarcoscyphaceae	Puerto Rico	AF006314
<i>Neournula</i> Paden & Tylutki	<i>pouchetii</i>	Sarcosomataceae	OR, USA	AF104666
<i>Orbilina</i> Fr.	<i>fimicola</i>	Orbiliaceae	MA, USA	AF006307
<i>Otidea</i> (Pers.) Bonord.	<i>onotica</i>	Otideaceae	MA, USA	AF006308
<i>Peziza</i> Fr.	<i>sylvestris</i>	Pezizaceae	MA, USA	AF006309
<i>Phillipsia</i> Berk.	<i>domingensis</i>	Orbiliaceae	Puerto Rico	AF006315
<i>Pindara</i> Velen.	<i>terrestris</i>	Sarcoscyphaceae	Norway	AF006306
<i>Pithya</i> Fuckel	<i>cupressina</i>	Sarcoscyphaceae	OR, USA	AF023613
<i>Plectania</i> Fuckel	sp.	Sarcosomataceae	Puerto Rico	AF104344
<i>Pseudopithyella</i> Seaver	<i>minuscula</i>	Sarcoscyphaceae	CA, USA	AF006317
<i>Pseudoplectania</i> Fuckel	<i>nigrella</i>	Sarcosomataceae	Japan	AF104345
<i>Sarcoscypha</i> (Fr.) Boud.	<i>austriaca</i>	Sarcoscyphaceae	Norway	AF006318
<i>Sarcosoma</i> Casp.	<i>mexicana</i>	Sarcosomataceae	OR, USA	AF104346
<i>Strobiloscypha</i> Weber & Denison	<i>keliae</i>	Pezizaceae?	OR, USA	AF006310
<i>Tuber</i> Bull.: Fr	aff. <i>gibosum</i>	Tuberaceae	OR, USA	AF023609
<i>Urnula</i> Fr.	<i>craterium</i>	Sarcosomataceae	NH, USA	AF104347
<i>Wolfina</i> Eckblad	<i>aurantiopsis</i>	Sarcosomataceae	OH, USA	AF104664
<i>Wynnea</i> Berk. & M. A. Curtis	sp.	Sarcoscyphaceae	Japan	AF006319
<i>Wynella</i> Boud.	<i>silvicola</i>	Helvellaceae	ID, USA	U42655

rRNA gene sequences of 31 ingroup taxa with *Orbilina* as the outgroup resulted in ten trees of 817 steps with a consistency index (CI) of 0.54 and a retention index (RI) of 0.63. Bootstrap values are given above the corresponding nodes in the strict consensus tree (FIG. 4). The bootstrap majority rule tree (not shown) is the same with the exception of the position of the genus *Glaziella*. In the strict consensus tree *Glaziella* is sister to the Sarcoscyphaceae, whereas in the bootstrap tree *Glaziella* is sister to a clade of four sarcosomateous genera currently placed in the Sarcosomataceae. Four major clades are resolved in the consensus tree and the phylogram of branch lengths (FIG. 5): the Tuberaceae/ Helvellaceae clade, the Morchellaceae/Discinaceae clade, a restricted though unresolved clade including *Sarcosoma* with five other genera, and a superclade composed of elements of the following: Otidiaceae, Sarcoscyphaceae, *Glaziella*, and four genera (*Wolfina*, *Desmazierella*, *Chorioactis* and *Neournula*) at present assigned

to the Sarcosomataceae. The first and last of these were strongly supported (>95% bootstrap value). Other strongly supported clades include those corresponding to the families Sarcoscyphaceae and Helvellaceae; *Pindara*, which was formerly classified in the Sarcoscyphineae (Korf 1970, 1972, 1973), is nested within the Helvellaceae. *Strobiloscypha* originally placed in the Sarcosomataceae (Weber and Denison 1995) is sister to a clade, which contains *Glaziella*, Otidiaceae, Sarcoscyphaceae (all taxa) and six sarcosomateous genera (*Sarcosoma*, *Urnula*, *Plectania*, *Pseudoplectania*, *Galiella* and *Donadinia*).

DISCUSSION

The molecular data presented here support Landvik (1996) and Landvik et al (1997) conclusions that two of the Pezizalean suborders, Sarcoscyphineae and Pezizineae, are paraphyletic. As in Landvik's tree (Landvik 1996, Fig. 1), *Peziza* is sister to all other taxa in

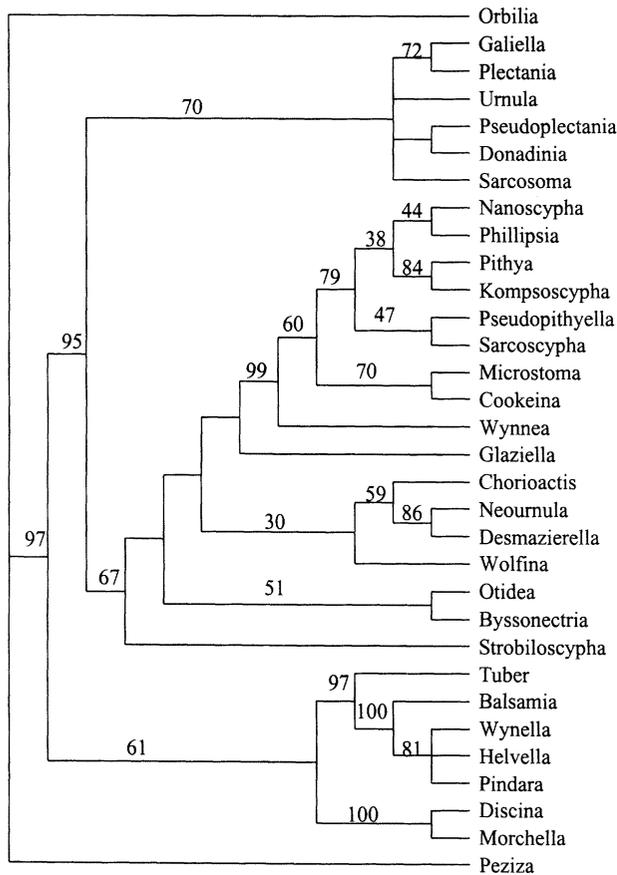


FIG. 4. Strict consensus of 10 most parsimonious trees (length = 817; CI = 0.54, RI = 63; resulting from analyses using NONA and PAUP 3.1.1). Numbers above the branches represent bootstrap support values for 1500 replicates.

the order which were included in this study (FIG. 2). In addition, *Pindara*, though currently classified in the Sarcoscyphineae, is nested within a clade corresponding to the family Helvellaceae (Pezizinae). Finally, the remaining included taxa of Pezizineae (*Otiidea* and *Byssonectria*) form a clade within a larger clade whose other members are all classified in Sarcoscyphineae. The paraphyly of the suborder Sarcoscyphineae is not surprising since the suboperculum has been variously interpreted in the literature. Based upon ultrastructural studies (Bellemère 1994, Bellemère et al 1990), the two families placed in the suborder (Sarcosomataceae, Sarcoscyphaceae) differ in ascal apex morphology. All the Sarcoscyphaceae have a thickened apical wall distally (or at the apex). Such thickenings are lacking in members of the Sarcosomataceae.

Velenovsky's (1934) initial placement of *Pindara* was close to *Helvella*. This position was later confirmed by Svřček (1947). Based on examinations of the original illustrations, Eckblad (1968) concluded that Velenovsky's drawing of the asci indicated *Pin-*

dara was inoperculate rather than operculate. On the other hand, Korf (1970, 1972, 1973), placed *Pindara* within the Sarcoscyphineae, stating that the asci were "suboperculate." Kristiansen (1984) called for more evidence to determine whether or not *Pindara* really did possess suboperculate asci. Our molecular data support Cabello's (1988) morphological analysis, which places this rarely collected genus in Helvellaceae. We have confirmed (scanning electron microphotographs, FIG. 6c, d) that *Pindara* does not have a thickened or eccentric ascal apex (cfr. *Sarcoscypha coccinea* FIG. 6b), but is more like those found in the Pezizineae (e.g., *Caloscypha* FIG. 6a). Furthermore, in *Pindara*, the ectal excipulum structure agrees with that found in members of the Helvellaceae. Specifically, in *Pindara* the cells of the outer excipulum form a palisade of longitudinally elongated cells.

Of the two families that comprise the Sarcoscyphineae, the Sarcoscyphaceae are monophyletic, while the Sarcosomataceae are paraphyletic. The relationships among the genera in the Sarcoscyphaceae s.s. do not agree completely with current tribal classifications; two tribes Sarcoscyphaceae which included: *Sarcoscypha*, *Phillipsia*, *Nanoscypha*, *Komposocypha*, *Geodina* and Pithyae (*Desmazierella*, *Thindia*, *Pseudopithyella*, *Pithya*) are paraphyletic, but the remaining tribe, Boedijnopezizeae (*Cookeina*, *Microstoma*) is monophyletic. The last tribe is united by simultaneous ascus/ascospore development. The placement of genera in the Sarcoscyphaceae have rarely been disputed with the exception of *Phaedropezia* (= *Acervus*), *Wynnea*, *Nannfeldtiella* and *Desmazierella*. *Komposocypha* has been included with the Sarcosomataceae by Eriksson and Hawksworth (1996) and in Hawksworth, (1995), but it should be treated in the Sarcoscyphaceae as initially intended (Pfister 1989). Based upon anatomical and developmental studies, *Phaedropezia* as *Acervus* (Pfister 1972, 1975, 1985) and *Nannfeldtiella* (= *Pseudombrophila*, Harmaja 1979, van Brummelen 1995) have been excluded from these families. At different times both *Wynnea* and *Desmazierella* have been placed with either the darkly colored members of the Sarcoscyphaceae, tribe Sarcosomataceae or with the brightly-colored genera, tribe Sarcoscyphaceae. The brown shades (i.e., assuming absence of carotenoids) of the mature ascomata of *Wynnea* have led some authors (Le Gal 1947, Eckblad 1968, Rifai 1968, Denison 1969, Cabello 1988) to classify it with dark colored members. Harrington (unpubl), using HPLC with fresh specimens at different stages of maturity, indicated that carotenoids are present. Korf (1972) has also stated that the paraphysis cells in *Wynnea* are plurinucleate, a condition of the Sarcoscyphaceae and not the Sar-

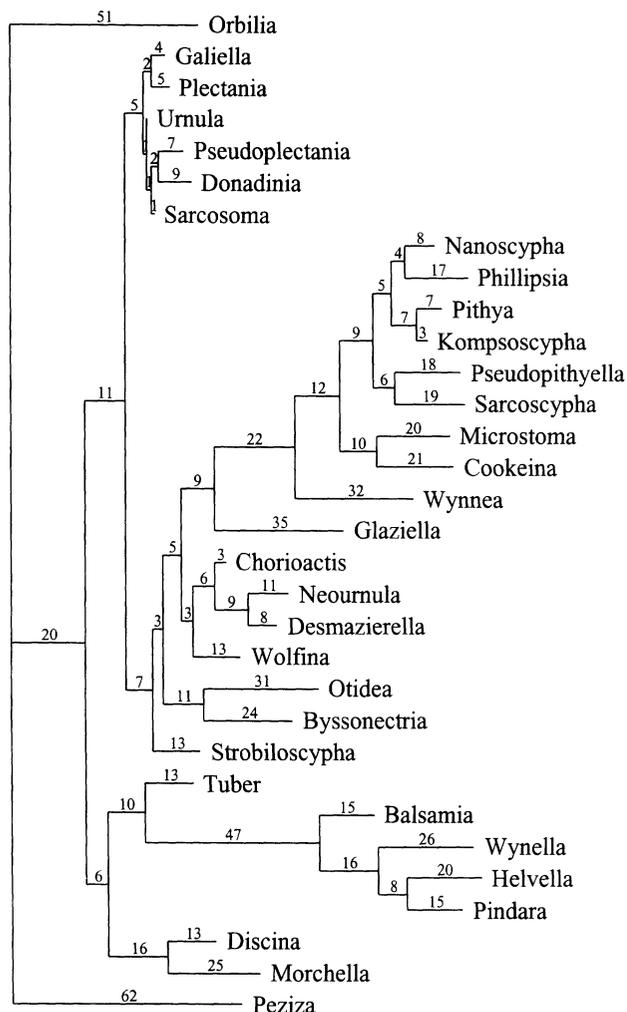


FIG. 5. Phylogram representing one of the ten most equally parsimonious trees derived from nucleotide sequences from the 18S rRNA. Numbers indicated above branches are branch lengths.

cosomataceae. The 18S rRNA data support the assignment of *Wynnea* to the Sarcoscyphaceae.

Nannfeldt (1949) moved *Desmazierella* from the Pezizaceae to the Sarcoscyphaceae, tribe Urnuleae, a position that was followed by Berthet (1964), Eckblad (1957, 1968) and Korf (as the Sarcosomataceae, 1972). Huhtinen and Mäkinen (1984) agreed however, with Denison's (1972) decision to include it as a member of the tribe Pithyeae (Sarcoscyphaceae) while Dennis (1978) thought its affinities were with the Humariaceae. Our analyses agree with recent sequence data (Landvik 1994, 1996, Landvik et al 1997) that this genus is not closely related to either the Sarcosomataceae s.s. or the Sarcoscyphaceae. In addition, morphological characters in *Desmazierella*, such as bristle-like hymenial hairs, support its placement outside of these two families.

The odd genus *Glaziella*, was classified in the Zy-

gomycota because its large zygosporolike structures are similar to one-celled spores (chlamydospores) (Höhnel 1913, Thaxter 1922, Boedijn 1933) found in the Endogonales. This was understandable because the single-spored ascus deliquesces early in spore development, leaving the ascospores embedded in the sporocarp (Gibson 1985) where they resemble chlamydospores. Ultrastructural and light microscopic studies of the ascospores, septa and asci, provided evidence for the transfer of *Glaziella* to the Ascomycota. An order and family were created for it since the authors (Gibson 1985, Gibson et al 1986) could not accommodate it within existing orders. Recently *Glaziella* has been shown to have affinities with members of the families Otidaceae and Pyronemataceae (Landvik 1994, 1996, Landvik et al 1997). In the present study, the bright orange-colored *Glaziella*, is sister to the Sarcoscyphaceae. The exact position of the genus within the Pezizales, however, is not strongly supported in either Landvik's studies (1996, 1997) or this one (Bootstrap value of 36%), and requires further investigation.

Six of the ten genera of the Sarcosomataceae are grouped in one major clade, but that clade lacks internal resolution. Although formerly proposed tribes Galielleae Korf (*Galiella*, *Neournula*, *Wolfina*) and Sarcosomataceae sensu Korf (*Chorioactis*, *Pseudoplectania*, *Plectania*, *Urnula*, *Sarcosoma*, *Desmazierella*), have no support, there is, however, support for the restricted tribe Pseudoplectanieae (*Donadinia* and *Pseudoplectania*).

The morphologically similar genera *Plectania* and *Pseudoplectania* differ most markedly in spore shape (ellipsoidal vs. globose in *Pseudoplectania*), but some authors (e.g., Paden 1983, Korf and Zhuang 1991) have suggested that they should be united. The 18S rRNA gene sequences for *Plectania* and *Pseudoplectania* differed at 19 positions and these 2 sequences are not sister to one another in any of the 10 most parsimonious trees. *Plectania* is sister to *Galiella* while *Pseudoplectania* is with *Donadinia* (FIG. 5). Li and Kimbrough (1995) have also provided evidence that *Plectania* and *Pseudoplectania* differ in spore wall ontogeny. These results suggest that *Plectania* and *Pseudoplectania* are distinct, but an analysis, including data from more species of these four genera, as well as those of *Sarcosoma* and *Urnula*, is needed in order to determine whether or not changes in the generic level taxonomy of this group are warranted. If either *Plectania* or *Pseudoplectania* is shown to be paraphyletic with respect to the other, they should be united or additional taxa recognized. If both are monophyletic, then the decision becomes one of ranking. Though studies suggest excluding *Galiella* from the Sarcoscyphineae (Bellemère et al 1990, van Brum-

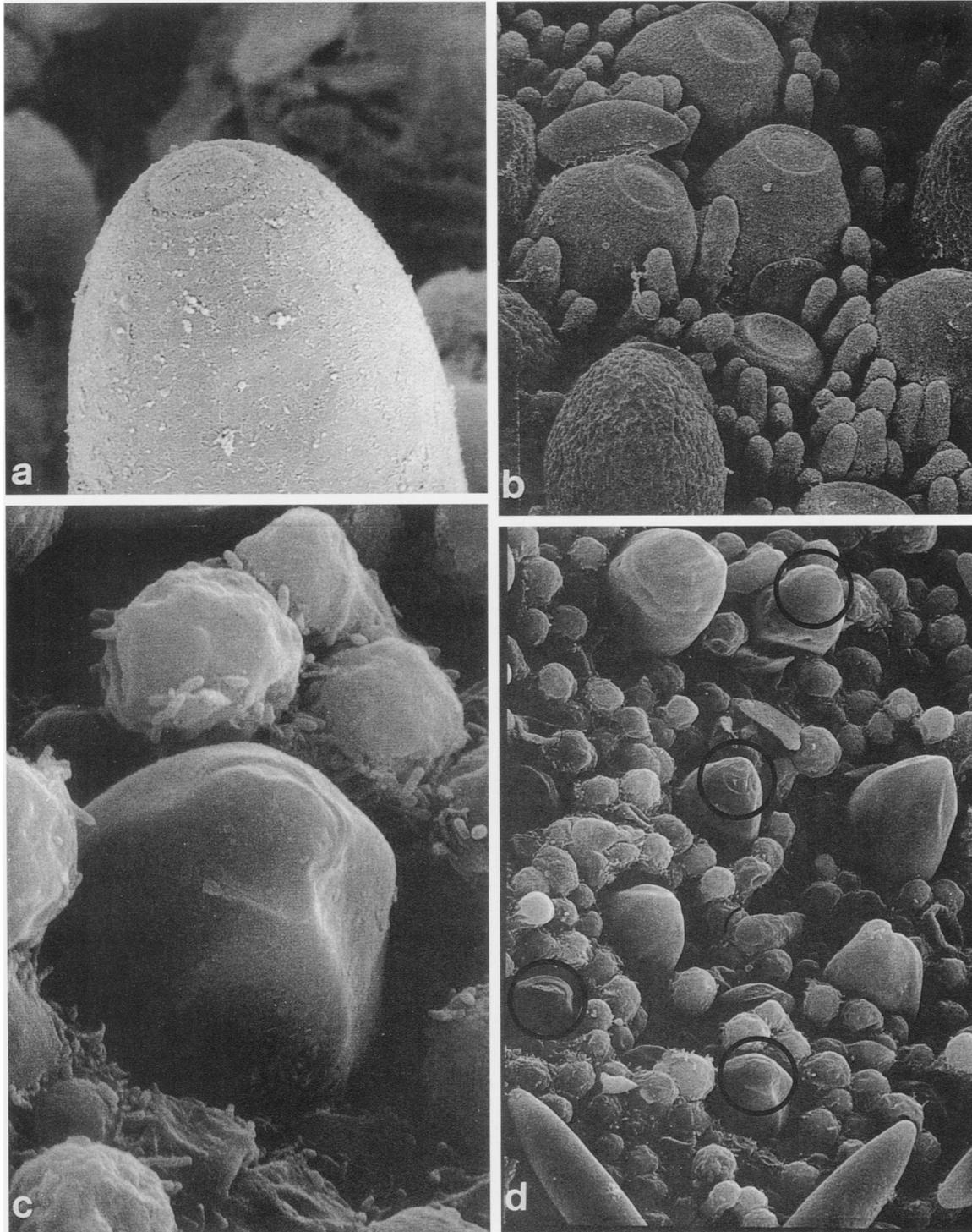


FIG. 6. SEM photomicrographs of pezizalean ascus apices. A. *Caloscypha fulgens*, $\times 8000$. B. *Sarcoscypha coccinea* $\times 9000$. *Pindara terrestris* $\times 5200$. Several ascus apices with black circles of *Pindara terrestris* $\times 1000$.

melen 1994) our study indicates a sister relationship (bootstrap value 70%) between *Galiella* and *Plectanina*. Li and Kimbrough's (1996) ultrastructural study of spore development also suggest retaining *Galiella*

as a member of the Sarcosmataceae. With the exception of *Strobiloscypha*, the remaining genera of the family (*Chorioactis*, *Wolfina*, *Neournula*, and *Desmazierella*) are sister to the clade including the Sarcos-

cyphaceae and *Glaziella*. The only sarcosomataceous taxon not included in this study was *Korfiella* for which suitable material was not available.

When *Strobiloscypha keliae* was described (Weber and Denison 1995), its placement in the family Sarcosomataceae was tentative. In this study, this taxon is sister to a larger clade including members of the Otideaceae, the Sarcoscyphaceae, and the Sarcosomataceae, as well as *Glaziella*. The inclusion of more genera, especially of Otideaceae, is needed to help resolve the placement of this species.

Genera of the Sarcoscyphaceae not included in this analysis but listed by Systema Ascomycetum (Eriksson and Hawksworth 1996) are *Rickiella*, *Aurophora*, *Geodina* and *Thindia*. *Aurophora* is of questionable status and is currently under study. It is held as distinct from *Phillipsia* based on the presence of a gelatinous excipular layer. *Rickiella* is considered a synonym of *Phillipsia* (Korf 1973), but has lacunose excipular tissues which is unlike any found in *Phillipsia*; further studies are needed on whether or not *Rickiella* should be merged with *Phillipsia* (Pfister 1987). Adequate material was not available of *Geodina* and *Thindia*.

Taxonomic changes supported by this study include transfer of *Pindara* to the Helvellaceae and emendation of the family Sarcosomataceae s.s. to include only *Galiella*, *Plectania*, *Urnula*, *Pseudoplectania*, *Donadinia*, and *Sarcosoma*. The results presented here also suggest that the subordinal ranks within Pezizales should be abandoned, and tribal ranks within the Sarcoscyphaceae and Sarcosomataceae revised. It is premature; however, to make formal taxonomic modifications based solely on the inferences from a single gene. Certain clades appear here that have not been previously detected (e.g., the clade of *Chorioactis*, *Neourrnula*, *Wolfina* and *Desmazierella*). The monophyly of these groups should be tested with additional data. Currently we are completing a study that combines morphological and molecular characters for most of the taxa used in this study to further elucidate relationships in this order.

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