

## ***Papulosa amerospora* accommodated in a new family (Papulosaceae, Sordariomycetes, Ascomycota) inferred from morphological and molecular data**

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To investigate the systematic position of the unitunicate pyrenomycete *Papulosa amerospora*, we performed phylogenetic analyses of SSU rDNA sequences from 37 ascomycetes. Among these sequences were some new ones from taxa that might be related to *Papulosa*: Hyponectriaceae (*Hyponectria buxi*, *Monographella nivalis*), Phyllachorales (*Phyllachora graminis*), and Xylariales (*Barrmaelia melanotes*, *Poronia punctata*). Our results showed 100% bootstrap support for a clade of all unitunicate pyrenomycetes, the class Sordariomycetes. We also found strong support for recognizing the subclasses Hypocreomycetidae and Xylariomycetidae. The remaining taxa, belonging to subclass Sordariomycetidae, appeared as a polyphyletic group in one analysis, but was monophyletic when shorter SSU sequences were used. *Barrmaelia melanotes*, *Poronia punctata*, *Hyponectria buxi*, and *Monographella nivalis* are members of Xylariomycetidae, but we could not determine whether *Monographella* should be included in Hyponectriaceae. The new family Papulosaceae is erected for *Papulosa* on molecular and morphological bases, but the exact systematic position of *Papulosa* within subclass Sordariomycetidae is still uncertain, since the genus did not cluster consistently with any of the included taxa. Phyllachorales are not closely related to Diaporthales, as previously suggested.

Key Words—Ascomycota; evolution; pyrenomycetes; systematics.

*Juncus roemerianus* Scheele is an important halophyte in salt marshes along the mid Atlantic coast of the U.S.A. Kohlmeyer and Volkmann-Kohlmeyer discovered that culms of this species were inhabited by many undescribed marine and terrestrial ascomycetes (e.g., Kohlmeyer and Volkmann-Kohlmeyer, 1993; Kohlmeyer et al., 1995; 1997). Several species deviated from other ascomycetes in important morphological traits and were difficult to place in the ascomycete system. One such species was *Papulosa amerospora* Kohlm. & Volkm.-Kohlm. (Kohlmeyer and Volkmann-Kohlmeyer, 1993), a unitunicate pyrenomycete with perithecia immersed in lower parts of senescent culms and with asci having an apical amyloid ring. Lateral paraphyses line the inner surface of the locule, and the peridium consists of an outer layer of cells with small lumina and an inner layer of cells with larger lumina, forming a *textura angularis*. Kohlmeyer and Volkmann-Kohlmeyer could not place this new genus and species with certainty into any order or family. They found that a key by Müller and von Arx (1973) lead to Diaporthaceae (i.e. Valsaceae) and one by Barr (1990) to Diaporthales. However, the important features of that order, as circumscribed by Barr in 1978 (e.g. pseudoparenchymatous centrum, peridium of *textura epidermoidea*, non-amyloid ring in the asci, absence of paraphyses) deviated very much from *Papulosa amerospora*, and the genus could not be included in that order even if it was conceived wider (sensu Cannon, 1988) to include the Phyllachorales. The authors, there-

fore, placed the genus in the group “Unitunicate ascomycetes *incertae sedis*” (Eriksson and Hawksworth, 1991).

We have tried to find molecular evidence for the relationships of *Papulosa* Kohlm. & Volkm.-Kohlm. by performing phylogenetic analyses of SSU rDNA data from that genus and representatives of four classes of ascomycetes (sensu Eriksson and Winka, 1997), viz. (1) Sordariomycetes (perithecia, unitunicate ascus), (2) Dothideomycetes (ascostromata, bitunicate ascus), (3) Eurotiomycetes (cleistothecia, pseudoprototunicate ascus), and (4) Pezizomycetes (apothecia, operculate ascus). All orders of these classes were represented. We focused on the class Sordariomycetes, since this group consists of fungi with perithecia and unitunicate asci. Within the class, the main criterium we used to select candidate families for *Papulosa* was presence of amyloid ring in the ascus, but we also included representatives from Diaporthales and Phyllachorales since keys had lead Kohlmeyer and Volkmann-Kohlmeyer (1993) to these taxa. Most of the included SSU sequences were retrieved from GenBank, but five species were sequenced by us, viz. *Phyllachora graminis* (Pers.) Nitschke (Phyllachoraceae, Phyllachorales), *Barrmaelia oxyacanthae* (Mont.) Rappaz and *Poronia punctata* (L.) Fr. (Xylariaceae, Xylariales), *Hyponectria buxi* (DC.) Sacc. and *Monographella nivalis* (Schaffnit) E. Müll. (Hyponectriaceae). The latter is a family of uncertain affinities, but with amyloid asci. *Barrmaelia oxyacanthae* is a

member of the Xylariales but it lacks the characteristic amyloid ring in the ascus. *Phyllachora graminis* and *Hyponectria buxi* were unavailable as cultures and, therefore, DNA was extracted from herbarium specimens.

## Materials and Methods

### Specimens studied:

*Barrmaelia oxyacanthae* (syn. *Anthostoma melanotes* Berk. & Broome). Sweden, Uppland, Dalby par., Viggeby, on *Populus tremula*, iii. 1987, leg. K. and L. Holm, isol. O. Constantinescu (UPSC 2132).

*Hyponectria buxi*. Denmark, Copenhagen, Western Cemetery, on *Buxus sempervirens*, 13. vii. 1998, leg. Thomas Læssøe (UME 31430).

*Monographella nivalis* (anam. *Microdochium nivale* (Fr.) Samuels & I. C. Hallett). Sweden, Östergötland, Norsholm, on wheat, vii. 1990, leg. and isol. G. Rydström (UPSC 3273).

*Papulosa amerospora*. USA, North Carolina, Carteret County, Broad Creek, 34°43'N, 76°55'77"W, on *Juncus roemerianus*, 8. viii. 1993, leg. J. Kohlmeyer (Herb. J. K. 5517).

*Phyllachora graminis*. Sweden, Ångermanland, Hörnefors par., Norrbyskär, Stengrundet, on *Elymus caninus*, 20. ix. 1997, leg. Ove E. Eriksson (UME 31349).

*Poronia punctata*. Australia, on horse dung, vi. 1987 (UPSC 2189; duplicate of CBS 628.78).

DNA from these specimens, except *Hyponectria buxi*, was extracted with the Nucleon PhytoPure Plant DNA extraction kit (Scotlab Bioscience), or according to the Whiting et al. (1997) protocol. The fresh material of *Hyponectria buxi* was used directly for PCR, without DNA extraction, as described by Wolinski et al. (1997). The SSU rRNA gene was PCR amplified with the primers SL1 5'-TGG TTG ATC CTG CCA GTA-3' (Landvik et al., 1996) and NS8 (White et al., 1990), or as two overlapping fragments: SL1 (Landvik et al., 1996) and KW65 5'-GCA GAC AAA TCA CTC CAC-3' (Winka et al., 1998), and KW34 5'-TCA GAG GTG AAA TTC TTG GA-3' (Winka et al., 1998) and NS8 (White et al., 1990). If no PCR product was visible on a 1% SeaKem agarose gel stained with EtBr, a reamplification with one or two internal primers was done; NS1 (White et al., 1990), and SL887 5'-ACC TTG TTA CGA CTT TTA-3' (Landvik, 1996).

For the PCR amplifications, Ready-To-Go PCR Beads (Amersham Pharmacia Biotech), or standard PCR protocols with AmpliTaq Gold (Perkin Elmer=PE) was used. The SSU rRNA gene was automatically sequenced with the ThermoSequenase Dye Terminator cycle sequencing pre-mix kit (Amersham Pharmacia Biotech, Sweden) or the AmpliTaq DNA Polymerase FS Dye Terminator Cycle Sequencing Ready Reaction kit (PE). Different sets of sequencing primers were used to obtain almost complete SSU rDNA sequences from the five species. The sequencing primers were NS1, NS6 and NS7 (White et al., 1990); SL43 and SL56 (Landvik et al., 1993); SL87 (Landvik and Eriksson, 1994); SL122, SL344 and SL887 (Landvik et al., 1996); KW3, KW34, KW65 and KW7

(Winka et al., 1998). The sequencing products were run on an ABI 377A automatic sequencer (PE), and the resulting sequences were corrected and aligned with AutoAssembler™ 1.4.0 (PE). To verify the sequences from the herbarium specimens, about two thirds of the SSU gene were sequenced from a second specimen and compared with the corresponding sequence from the first specimen. The sequences from the two separate collections supported each other.

Our new SSU rDNA sequences were compared with 31 homologous sequences, retrieved from GenBank (Table 1). The sequences were aligned manually in SeqApp 1.9a169 (Gilbert, 1993). A model of the secondary structure of SSU rRNA (Hendriks et al., 1992) was used to improve the alignment, which can be obtained from the authors on request. The phylogenetic analyses were performed with PAUP\* 4.0b1 (Swofford, 1999). Three representatives from the discomycete order Pezizales were used as outgroup. Two different datasets were analysed. The first analysis included 31 taxa with (almost) complete SSU rDNA sequences. The length of the alignment was 1728 bp. In the second analysis, 6 further taxa, from which we had only shorter SSU rDNA sequences, were included. Six of the almost complete sequences were excluded, as they were not expected to influence the topology of the trees. The total length of this alignment was reduced to 998 bp for all sequences. Both parsimony (heuristic search with 10 random sequence additions) and distance (neighbor joining; (Saitou and Nei, 1987), Jukes and Cantor distance algorithm (Jukes and Cantor, 1969) analyses were performed. Gaps were treated as a fifth character and were given equal weight as the other characters, or treated as missing data. Support for the branches was tested with 500 bootstrap replicates. The log likelihood of each of the trees was calculated and the differences evaluated with the Kishino-Hasegawa test (Kishino and Hasegawa, 1989) in PAUP\*.

## Results

The parsimony analysis of the 344 phylogenetically informative characters in the 1728 bp dataset resulted in five trees (length 998, CI=0.59, RI=0.74). One of these is shown in Fig. 1. In a strict consensus tree the branches indicated with an asterisk (\*) collapsed. Four trees were obtained if gaps were treated as missing data (332 phylogenetically informative characters remained), and the tree length decreased to 950. The strict consensus tree of these four trees was identical to the consensus tree obtained with gaps included, but the support for *Papulosa amerospora*+Ophiostomatales was reduced from 91% to 80%. A neighbor-joining analysis of the same dataset yielded a tree where the Eurotiales was basal to the unitunicate pyrenomycetes, and *P. amerospora*+Ophiostomatales was the sister group of Diaporthales (65% bootstrap support, tree not shown). In a Kishino-Hasegawa test in PAUP\*, none of the trees differed significantly from the others.

The second dataset was analysed in the same way.

Table 1. Fungal species and GenBank accession numbers for SSU rDNA sequences.

Species <sup>a</sup>	Accession no.	Family <sup>b</sup>	Order <sup>b</sup>	Class/Subclass <sup>c</sup>
<i>Alternaria brassicicola</i>	U05197	Pleosporaceae	— <sup>d</sup>	Dothideomycetes
<i>Aspergillus fumigatus</i>	M60300	Trichocomaceae	— <sup>d</sup>	Eurotiomycetes
<i>Barrmaelia oxyacanthae</i>	AF064048	Xylariaceae	Xylariales	Sordariomycetes/Xylariomycetidae
<i>Camarops microspora</i>	Z49783	Boliniaceae	Sordariales	Sordariomycetes/Sordariomycetidae
<i>Ceriosporopsis halima</i>	U47843	Halosphaeriaceae	Halosphaeriales	Sordariomycetes/Hypocreomycetidae
<i>Chaetomium elatum</i>	M83257	Chaetomiaceae	Sordariales	Sordariomycetes/Sordariomycetidae
<i>Cryphonectria cubensis</i>	L42439	Valsaceae	Diaporthales	Sordariomycetes/Sordariomycetidae
<i>Diaporthe phaseolorum</i>	L36985	Valsaceae	Diaporthales	Sordariomycetes/Sordariomycetidae
<i>Diatrype disciformis</i>	U32403	Diatrypaceae	Diatrypales	Sordariomycetes/Xylariomycetidae
<i>Glaziella aurantiaca</i>	Z49753	Glaziellaceae	Pezizales	Pezizomycetes
<i>Glomerella cingulate</i> <sup>e</sup>	M55640	<i>Incertae sedis</i>	Sordariales	Sordariomycetes/Hypocreomycetidae
<i>Gyromitra esculenta</i>	Z30238	Discinaceae	Pezizales	Pezizomycetes
<i>Halosphaeria appendiculata</i>	U46872	Halosphaeriaceae	Halosphaeriales	Sordariomycetes/Hypocreomycetidae
<i>Hypocrea lutea</i>	D14407	Hypocreaceae	Hypocreales	Sordariomycetes/Hypocreomycetidae
<i>Hypomyces chrysospermus</i>	M89993	Hypocreaceae	Hypocreales	Sordariomycetes/Hypocreomycetidae
<i>Hyponectria buxi</i>	AF130976	Hyponectriaceae	<i>Incertae sedis</i>	Sordariomycetes/Xylariomycetidae
<i>Magnaporthe salvinii</i>	U78546	Magnaporthaceae	<i>Incertae sedis</i>	Sordariomycetes/Sordariomycetidae
<i>Monographella nivalis</i> <sup>f</sup>	AF064049	?Hyponectriaceae	<i>Incertae sedis</i>	Sordariomycetes/Xylariomycetidae
<i>Neurospora crassa</i>	X04971	Sordariaceae	Sordariales	Sordariomycetes/Sordariomycetidae
<i>Obolarina dryophila</i>	Z49784	Xylariaceae	Xylariales	Sordariomycetes/Xylariomycetidae
<i>Ophiostoma stenoceras</i>	M85054	Ophiostomataceae	Ophiostomatales	Sordariomycetes/Sordariomycetidae
<i>Papulosa amerospora</i>	AF064050	<i>Incertae sedis</i>	<i>Incertae sedis</i>	Sordariomycetes/Sordariomycetidae
<i>Petriella setifer</i>	U43908	Microascaceae	Microascales	Sordariomycetes/Hypocreomycetidae
<i>Phyllachora graminis</i>	AF064051	Phyllachoraceae	Phyllachorales	Sordariomycetes/Sordariomycetidae
<i>Phyllachora sp.</i>	U78542	Phyllachoraceae	Phyllachorales	Sordariomycetes/Sordariomycetidae
<i>Pleospora herbarum</i>	U05201	Pleosporaceae	Dothideales	Dothideomycetes
<i>Poronia punctata</i>	AF064052	Xylariaceae	Xylariales	Sordariomycetes/Xylariomycetidae
<i>Pseudallescheria boydii</i>	U43913	Microascaceae	Microascales	Sordariomycetes/Hypocreomycetidae
<i>Scedosporium prolificans</i> <sup>g</sup>	U43910	Microascaceae	— <sup>d</sup>	Sordariomycetes/Hypocreomycetidae
<i>Setosphaeria rostrata</i>	U42487	Pleosporaceae	Dothideales	Dothideomycetes
<i>Sordaria fimicola</i>	X69851	Sordariaceae	Sordariales	Sordariomycetes/Sordariomycetidae
<i>Sporothrix schenckii</i> <sup>h</sup>	M85053	Ophiostomataceae	— <sup>d</sup>	Sordariomycetes/Sordariomycetidae
<i>Thermoascus crustaceus</i>	M83263	Trichocomaceae	Eurotiales	Eurotiomycetes
<i>Trichophyton rubrum</i>	X58570	Arthrodermataceae	— <sup>d</sup>	Eurotiomycetes
<i>Urnula hiemalis</i>	Z49754	Sarcosomataceae	Pezizales	Pezizomycetes
<i>Valsa leucostoma</i> <sup>i</sup>	M83259	Valsaceae	Diaporthales	Sordariomycetes/Sordariomycetidae
<i>Xylaria carpophila</i>	Z49785	Xylariaceae	Xylariales	Sordariomycetes/Xylariomycetidae

a Author names are found in Eriksson and Winka (1999).

b Familial and ordinal names according to Eriksson and Hawksworth (1998). (Note: Diatrypaceae in Xylariales, Pleosporaceae in Pleosporales, and Papulosaceae *incertae sedis* in this paper).

c Classification according to Eriksson and Winka (1997). (Note: *Glomerella cingulata* in Hypocreomycetidae and *Papulosa amerospora* in Sordariomycetidae in this paper).

d Anamorphs not included in these classifications.

e As *Colletotrichum gloeosporioides* in GenBank.

f As *Microdochium nivale* var. *nivale* in UPSC.

g As *Lomentospora prolificans* in GenBank.

h As *Ophiostoma schenkii* in GenBank.

i As *Leucostoma persoonii* in GenBank.

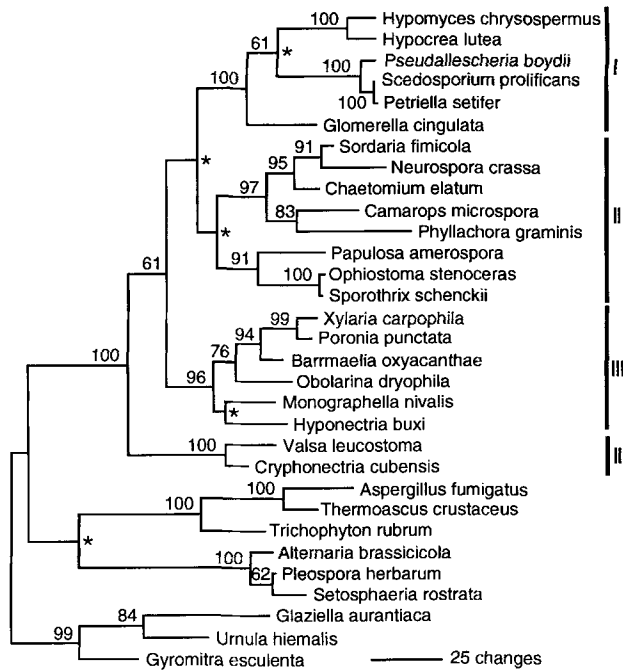


Fig. 1. One of the five most parsimonious trees from a heuristic search in PAUP\*, performed on the 1728 bp dataset. Length 998 steps, CI=0.59, RI=0.74. Bootstrap values from 500 replicates are included. Branch lengths are proportional to number of substitutions. Subclasses according to Eriksson and Winka (1997) are indicated by the roman numerals I, II, and III. Nodes marked with \* collapse in a strict consensus of the five most parsimonious trees.

When gaps were coded as a fifth character, six trees of length 568 (CI=0.60, RI=0.71) were found, based on 185 phylogenetically informative characters. The strict consensus tree is included as Fig. 2. Treating gaps as missing data also yielded six trees of equal length (552), 178 phylogenetically informative characters remained. The strict consensus tree of these six trees was identical to the tree in Fig. 2. The NJ tree from the shorter dataset strongly resembles the tree in Fig. 1, but differs in having Diaporthales as sister group to *P. amerospora*/Ophiostomatales/Sordariales/Phyllachorales, and in this clade being the sister group to Xylariales + *Monographella nivalis*, *Hyponectria buxi* and *Diatrype disciformis* (Hoffm.) Fr. (tree not shown). In a Kishino-Hasegawa test in PAUP\*, the parsimony trees obtained from the analysis with gaps treated as fifth character state had likelihood values significantly lower than the trees obtained with gaps treated as missing data. The trees were identical in topology, so the difference in likelihood was a result of differences in branch lengths.

## Discussion

The unitunicate pyrenomycetes constitute a monophyletic group supported by high bootstrap values in phylogenetic studies of the ascomycetes (Berbee and Taylor, 1992; Eriksson and Winka, 1998; Spatafora and Black-

well, 1994). The group was treated as a separate class, Sordariomycetes, by Eriksson and Winka (1997), and three subclasses were recognized: Hypocreomycetidae, Sordariomycetidae and Xylariomycetidae. In the present paper the class received 100% bootstrap support and *Papulosa amerospora* was found to be a member of this class. That was expected from morphological studies, but the position of the genus within the class was uncertain. Among its morphological features, the amyloid ring in the ascus top indicated that it probably belonged in subclass Xylariomycetidae, in which a majority of the members have such ascus tops.

Subclass Hypocreomycetidae (I) was represented by six taxa in our phylogenetic analysis of the data set of longer sequences (Fig. 1) and appeared as a clade with 100% bootstrap support. The six taxa belong in Hypocreaceae [*Hypocrea lutea* (Tode) Petch, *Hypomyces chrysospermus* Tul.], Microasceae [*Petriella setifer* (Alf. Schmidt) Curzi, *Pseudallescheria boydii* (Shear) McGinnis, *Scedosporium prolificans* (Hennebert & B. G. Desai) E. Guého & de Hoog], and Sordariales *inc. sed.* [*Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk.]. In the analysis of the data set of shorter sequences (Fig. 2) *Petriella setifer* was substituted by two members of the Halosphaeriaceae (*Ceriosporopsis halima* Linder, *Halosphaeria appendiculata* Linder). The support for the subclass was in this case 99%. Thus, SSU rDNA sequences do not indicate that *Papulosa* belongs in this subclass. Also the morphology is different. Dark coloured, immersed perithecia characterize many genera in the Halosphaeriaceae also, but the asci lack amyloid ring in the ascus and the hamathecium is different. The same refers to *Glomerella* Spauld. & H. Schenk. which has been suggested to be related to Sordariales because of a *Sordaria* type centrum, and of similar development (Uecker, 1994). *Glomerella* has also been included in Phyllachorales (Barr, 1983; Eriksson and Hawksworth, 1993), or in Xylariales (Barr, 1990). Spatafora and Blackwell (1994) found that *Glomerella cingulata* occurred in the same clade as Microascales and Hypocreales/Clavicipitales, and so do we (Fig. 2). In a taxonomic review of the Phyllachorales (Silva-Hanlin and Hanlin, 1998) the authors indicate that *Glomerella* is not a true member of Phyllachorales.

Subclass Xylariomycetidae (III) received 96% support in the analysis of the long sequences, 81% in that of the shorter sequences. This was the clade we assumed might be the right position of *Papulosa*, and most probably then the family Hyponectriaceae. Our SSU rDNA sequences from *Hyponectria buxi* and *Monographella nivalis* confirm that Hyponectriaceae should be included in this subclass. A close relationship with the Xylariales was assumed by Barr (1990) on the basis of the morphology (esp. the amyloid ring in the ascus top). However, more sequences are required to show whether *Monographella* Petr. should be referred to the Hyponectriaceae. Although both appeared in the same cluster as Xylariaceae (*Barrmaelia oxyacanthae*, *Obolarina dryophila*, *Poronia punctata*, *Xylaria carpophila*) with 96% bootstrap support, the two genera did not form a

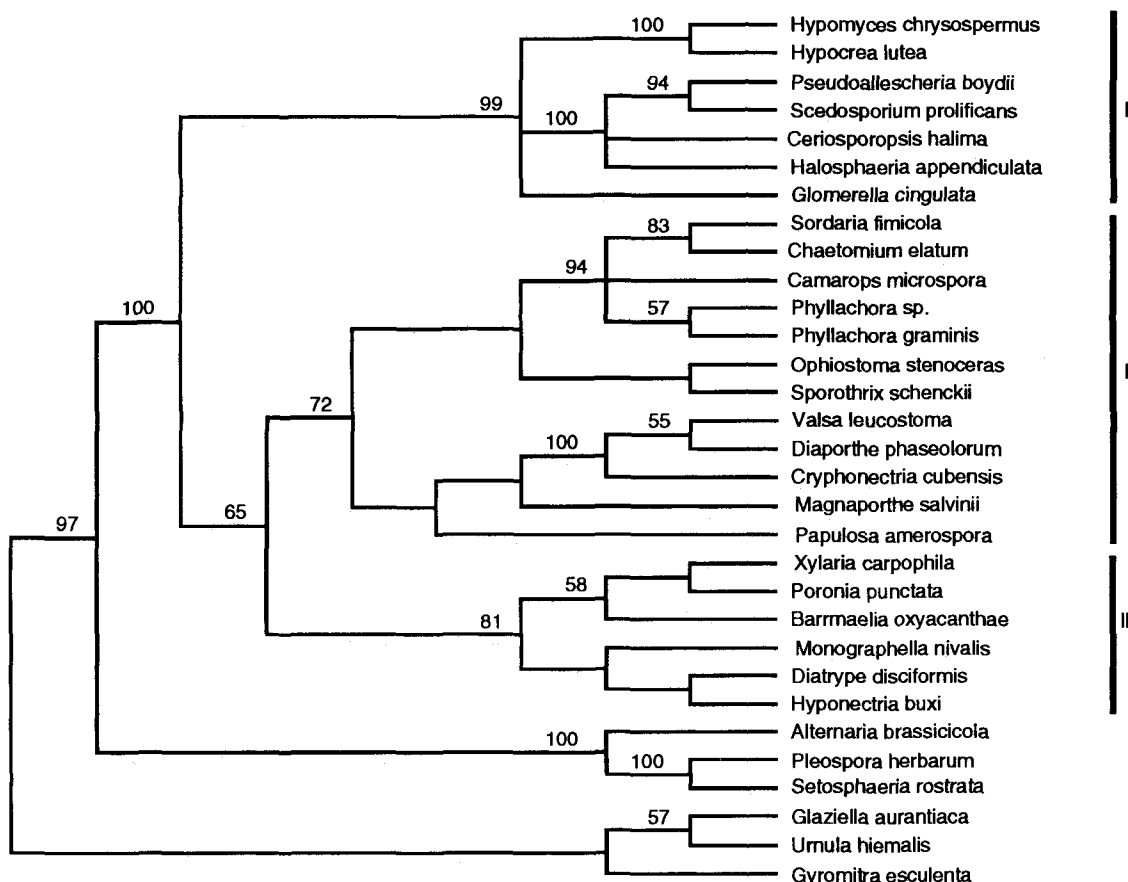


Fig. 2. Strict consensus tree of six equally parsimonious trees from a heuristic search in PAUP\*, performed on the 998 bp dataset. Length 568 steps, CI=0.60, RI=0.71. Bootstrap values from 500 replicates are included. Subclasses according to Eriksson and Winka (1997) are indicated by the roman numerals I, II, and III.

monophyletic clade in all trees. In the analysis of the shorter sequences, *Hyponectria buxi* formed a cluster with *Diatrype disciformis* (not included in the first analysis), although with low bootstrap support. The Diatrypaceae has been accommodated in a separate order (Chadefaud, 1960; Hawksworth and Eriksson, 1986) but the possibility of affinities to Xylariaceae has been considered and discussed by Rogers (1994) and Barr (1990), and the two orders Diatrypales and Xylariales were merged by Eriksson and Winka (1997). *Papulosa* did not nest in this subclade and an amyloid ring in the ascus is, therefore, not a character exclusive for this subclass. It is probably a plesiomorphic character in the Xylariomycetidae, but has been lost in some members, e.g. *Barrmaelia oxyacanthae*. The morphology of the ascumata in that species is very similar to *Anthostomella* Sacc., with black immersed perithecia provided with a black clypeus, but that genus has a typical xylariaceous amyloid ring in the ascus and ascospores with a germ slit, another character lacking in *Barrmaelia* Rappaz. However, on the basis of our molecular study there is no doubt that *Barrmaelia* with non-amyloid ascus is a member of the Xylariaceae, whereas *Papulosa* with amyloid ring not even is a member of the class Xylariomycetidae.

Subclass Sordariomycetidae (II) formed a clade with

72% support in the analysis of the short sequences and *Papulosa* appeared basal to *Magnaporthe* R. A. Krause & R. K. Webster and Diaporthales (Fig. 2). In the analysis of the longer sequences, the taxa in the suborder formed a polyphyletic clade, since the Diaporthales appeared basal to the other pyrenomycetes. In the core group, one of the subclades consisted of members of the Sordariales [*Camarops microspora* (P. Karst.) Shear, *Chaetomium elatum* Kunze, *Neurospora crassa* Shear & B. O. Dodge, *Sordaria fimicola* (Rob.) Ces. & De Not.] and the Phyllachorales (*Phyllachora graminis*), and was supported by 97%. Cannon (1988) merged Phyllachorales with Diaporthales, because he thought it was untenable to continue to recognize them on the basis of differences in centrum structure. The other branch, consisting of *Ophiostoma stenoceras* (Robak) Nannf. (anamorph *Sporothrix schenckii* Hektoen & C. F. Perkins) and *Papulosa*, was supported by 91%. A close relationship is very surprising. The morphology is very different. *Ophiostoma* species have small superficial ascumata with long beaks, no hamathecium, small unicellular ascospores, etc. However, *Ophiostoma* differs from other members of the subclass also. The position of *Papulosa* is most probably among the taxa that are currently included in subclass Sordariomycetidae, but no really

close relative of the genus has as yet been sequenced (SSU rDNA). None of the three orders (Diaporthales, Ophiostomatales and Sordariales) and 15 families in the present subclass Sordariomycetidae (sensu Eriksson and Winka, 1998) can accommodate *Papulosa*.

However, Magnaporthaceae, one of the families that is currently listed among Ascomycota *incertae sedis*, has to be discussed. It was described by Cannon (1994) for a group of fungi centered on *Magnaporthe* and showing many similarities, "most notably in teleomorph form and pathogenic effects". Our studies indicate that this family belongs in subclass Sordariomycetidae. *Magnaporthe salvinii* (Catt.) R. A. Krause & R. K. Webster came close to *Papulosa amerospora* in our analysis of shorter sequences, but with weak bootstrap support (< 50%). Although some putative representatives of the family have amyloid asci, *Magnaporthe* has non-amyloid, thick-walled asci, that even have been interpreted as being bitunicate (von Arx and Müller, 1975; von Arx, 1979). *Papulosa* has very different asci and it does not seem to produce any anamorph, in contrast to the members of the Magnaporthaceae, most of which are necrotrophic parasites on root systems and stem bases. The latter also have a mycelium with appressoria, which have not been seen in *Papulosa*. There are other morphological differences too (e.g. ascospore shape, septation, colour). Longer sequences of *Magnaporthe* and the other members of the family are required to demonstrate their relationships and to show whether the family is monophyletic. However, *Papulosa* can not be a member of that family or any other family currently recognized in the subclass. Cannon (1994) pointed out *Monographella* Petr., with amyloid ring in the ascus, as a possible member of Magnaporthaceae, and did not agree with Eriksson and Hawksworth (1998) who referred the genus to Hyponectriaceae (with a "?"). However, in our analyses, both *Monographella* and *Hyponectria* cluster with the Xylariales (Fig. 2).

None of the other families listed among Ascomycota *incertae sedis* is likely to be closely related to *Papulosa*. Most of the families in that group have non-amyloid asci, and those with amyloid asci differ in other respects. That is true, for instance, for the Protothelenellaceae, which are pyrenomyces with dark-coloured perithecia seated in algal crusts on soil, peat, etc. The apical apparatus in the ascus top consists of double rings. The ascospores are muriform and smooth.

The relationships of the Halosphaerales have recently been examined by Spatafora et al. (1998). In their analyses the genera *Lulworthia* Sutherl. and *Lindra* I. M. Wilson did not cluster with the rest of the Halosphaerales but appeared basal to all unitunicate pyrenomyces. To investigate the possible relationship between these genera and *Papulosa*, we included two SSU rDNA sequences from *Lulworthia* and *Lindra* in our second analysis (tree not shown). They appeared basal to the rest of the Sordariomycetes and thus not closely related to *Papulosa*.

**Conclusions** The exact phylogenetic position of *Papulosa* can not be established from our results, there are too

few SSU rDNA sequences available from the unitunicate pyrenomyces. *Papulosa* seems to be closest related to Ophiostomatales, Diaporthales, Sordariales and Phyllochorales, we therefore accommodate the genus in subclass Sordariomycetidae. The genus does not fit into any family in that subclass. Therefore, we describe the new family Papulosaceae, to accommodate the single genus *Papulosa*.

**Papulosaceae Winka & O. E. Erikss., fam. nov.** *Perithecia* solitaria, immersa, ostiolata, cum collo, coriacea, atrobrunnea. *Peridium* texturam angularem formans. *Hamathecium*: Periphyses et paraphyses. *Asci* cylindrici, unitunicati, persistentes, annulo apicali amyloidei. *Ascosporae* uniseriales, ellipsoideae, unicellulares, hyalinae, verruculosae.

Typus: *Papulosa* Kohlm. & Volkm.-Kohlm. (Syst. Ascomycetum 11: 96, 1993).

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