

Archiascomycetes: detection of a major new lineage within the Ascomycota

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For phylogenetic analysis of the higher fungi, we sequenced the nuclear small subunit rRNA (18S rRNA) gene from *Taphrina populina*, the type species of the genus *Taphrina*, and *Protomyces lactucae-debilis*. The molecular phylogeny inferred from these 2 sequences and 75 sequences from the DNA data bank divided the Ascomycota into three major lineages: the hemiascomycetes, the euascomycetes, and the archiascomycetes, newly described herein. The former two lineages are monophyletic, whereas the archiascomycetes, which originated first and are comprised of *Taphrina*, *Protomyces*, *Saitoella*, *Schizosaccharomyces*, and *Pneumocystis*, may not be monophyletic. Among the archiascomycetes, the *Taphrina/Protomyces* branch is monophyletic. Confirmation of the archiascomycetes as a monophyletic taxonomic class will require comparison of additional genetically defined characters.

Key Words—archiascomycetes; fungal evolution; molecular phylogeny; 18S rRNA gene sequence; Taphrinalean fungi.

Introduction

Taphrina and *Protomyces* have no ascoma, and according to Ainsworth's classification (1973), these fungi are accommodated in the class Hemiascomycetes. The anamorphic species *Saitoella complicata* Goto et al., which is placed in the Blastomycetes, shares characteristics with both ascomycetes and basidiomycetes (Sugiyama et al., 1993). In many respects, the fission yeast *Schizosaccharomyces* is quite divergent from the budding ascomycetous yeasts (Taylor et al., 1993). Fungal molecular systematics (e.g., Bruns et al., 1992; Berbee and Taylor, 1993; Nishida et al., 1993) have considered *Pneumocystis carinii* to be a member of fungi since Edman et al. (1988) made its placement within a phylogenetic tree derived from comparison of 18S rRNA sequences. Based on 18S rRNA sequence comparisons, the phylogenetic position of the ascomycetes is examined and taxonomic placements are shown.

Materials and Methods

The strains which we sequenced in this study are *Taphrina populina* Fr. CBS 337.55 (ex *Betula nana*, A. J. Mix) and *Protomyces lactucae-debilis* Sawada IFO 6899 (Tubaki, 1957). DNAs amplified by the polymerase chain reaction were purified by agarose gel electrophoresis.

Sequences were obtained directly from the amplified DNAs with Sequenase version 2.0 (U.S. Biochemical). Seventy-seven higher fungal 18S rRNA sequences were aligned using CLUSTAL V (Higgins et al., 1992). The species names and the accession numbers in the DNA database are shown in Table 1. A total of 1,418 positions was considered excluding positions with gaps. The bootstrap procedure (Felsenstein, 1985) was used to sample the data 1,000 times.

Results and Discussion

Our molecular phylogeny (Fig. 1) clearly indicates the existence of the two divisions, Ascomycota and Basidiomycota, among the higher fungi. Their topologies are well supported by bootstrapping. Both divisions appear to be monophyletic as already suggested by molecular phylogenetic analyses (e.g., Bruns et al., 1992; Berbee and Taylor, 1993; Nishida and Sugiyama, 1993). The Ascomycota showed three major lineages (Fig. 1). The first major lineage contains *Taphrina deformans*, *T. populina*, *T. wiesneri*, *Protomyces lactucae-debilis*, *P. inouyei*, *Saitoella complicata*, *Schizosaccharomyces pombe*, and *Pneumocystis carinii*. The lineage diverged first among the ascomycetes, whereas the hemiascomycetes (ascomycetous yeasts) and euascomycetes (filamentous ascomycetes) evolved more recently (cf., Berbee and Taylor, 1993; Nishida and Sugiyama, 1993; Nishida et al., 1993). In the archiascomycete lineage, *Taphrina populina*, the type species of the genus *Taphrina*, formed a cluster with *T. deformans* and *T. wiesneri* (99.8% support in bootstrap procedure). *Protomyces lactucae-debilis* is most closely related to *P. in-*

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Table 1. Organisms and Sequences.

| Organisms | Accession numbers and References |
|-------------------------------------|-----------------------------------|
| <i>Protomyces lactucae-debilis</i> | D14164 This study |
| <i>Taphrina populina</i> | D14165 This study |
| <i>Protomyces inouyei</i> | D11377 Nishida et al., 1993 |
| <i>Taphrina wiesneri</i> | D12531 Nishida and Sugiyama, 1993 |
| <i>Saitoella complicata</i> | D12530 Nishida and Sugiyama, 1993 |
| <i>Cystofilobasidium capitatum</i> | D12801 Suh and Sugiyama, 1993 |
| <i>Mrakia frigida</i> | D12802 Suh and Sugiyama, 1993 |
| <i>Erythrobasidium hasegawianum</i> | D12803 Suh and Sugiyama, 1993 |
| <i>Rhodosporidium toruloides</i> | D12806 Suh and Sugiyama, 1993 |
| <i>Filobasidium floriforme</i> | D13460 Suh and Sugiyama, 1993 |
| <i>Byssochlamys nivea</i> | M83256 Berbee and Taylor, 1992a |
| <i>Chaetomium elatum</i> | M83257 Berbee and Taylor, 1992a |
| <i>Eremascus albus</i> | M83258 Berbee and Taylor, 1992a |
| <i>Leucostoma persoonii</i> | M83259 Berbee and Taylor, 1992a |
| <i>Monascus purpureus</i> | M83260 Berbee and Taylor, 1992a |
| <i>Ophiostoma ulmi</i> | M83261 Berbee and Taylor, 1992a |
| <i>Talaromyces flavus</i> | M83262 Berbee and Taylor, 1992a |
| <i>Thermoascus crustaceus</i> | M83263 Berbee and Taylor, 1992a |
| <i>Ascosphaera apis</i> | M83264 Berbee and Taylor, 1992a |
| <i>Pseudallescheria boydii</i> | M89782 Berbee and Taylor, 1992b |
| <i>Hypomyces chrysospermus</i> | M89993 Berbee and Taylor, 1992b |
| <i>Microascus cirrosus</i> | M89994 Berbee and Taylor, 1992b |
| <i>Dipodascopsis uninucleata</i> | U00969 Berbee and Taylor, 1993 |
| <i>Eurotium rubrum</i> | U00970 Berbee and Taylor, 1993 |
| <i>Taphrina deformans</i> | U00971 Berbee and Taylor, 1993 |
| <i>Tilletia caries</i> | U00972 Berbee and Taylor, 1993 |
| <i>Ustilago hordei</i> | U00973 Berbee and Taylor, 1993 |
| <i>Endomyces geotrichum</i> | U00974 Berbee and Taylor, 1993 |
| <i>Pleospora rudis</i> | U00975 Berbee and Taylor, 1993 |
| <i>Tremella globospora</i> | U00976 Berbee and Taylor, 1993 |
| <i>Tremella moriformis</i> | U00977 Berbee and Taylor, 1993 |
| <i>Auricularia auricula</i> | L22254 Swann and Taylor, 1993 |
| <i>Auricularia polytricha</i> | L22255 Swann and Taylor, 1993 |
| <i>Calocera cornea</i> | L22256 Swann and Taylor, 1993 |
| <i>Dacrymyces chrysospermus</i> | L22257 Swann and Taylor, 1993 |
| <i>Dacrymyces stillatus</i> | L22258 Swann and Taylor, 1993 |
| <i>Heterotextus alpinus</i> | L22259 Swann and Taylor, 1993 |
| <i>Pseudohydnum gelatinosum</i> | L22260 Swann and Taylor, 1993 |
| <i>Sporidiobolus johnsonii</i> | L22261 Swann and Taylor, 1993 |
| <i>Tremella foliacea</i> | L22262 Swann and Taylor, 1993 |
| <i>Torulasporea delbrueckii</i> | X53496 Hendriks et al., 1990 |
| <i>Candida albicans</i> | X53497 Hendriks et al., 1989 |
| <i>Leucosporidium scottii</i> | X53499 Hendriks et al., 1991a |
| <i>Clavispora lusitaniae</i> | X55526 Hendriks et al., 1991b |
| <i>Candida tropicalis</i> | M55527 Hendriks et al., 1991b |
| <i>Issatchenkia orientalis</i> | M55528 Hendriks et al., 1991b |
| <i>Dekkera bruxellensis</i> | X58052 Hendriks et al., 1992 |
| <i>Pichia anomala</i> | X58054 Hendriks et al., 1992 |
| <i>Pichia membranaefaciens</i> | X58055 Hendriks et al., 1992 |
| <i>Zygosaccharomyces rouxii</i> | X58057 Hendriks et al., 1992 |
| <i>Bullera alba</i> | X60179 Van de Peer et al., 1992 |
| <i>Sporobolomyces roseus</i> | X60181 Van de Peer et al., 1992 |

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|---------------------------------------|--|
| <i>Trichosporon cutaneum</i> | X60182 Van de Peer et al., 1992 |
| <i>Filobasidiella neoformans</i> | X60183 Van de Peer et al., 1992 |
| <i>Athelia bombacina</i> | M55638 Illingworth et al., 1991 |
| <i>Aureobasidium pullulans</i> | M55639 Illingworth et al., 1991 |
| <i>Colletotrichum gloeosporioides</i> | M55640 Illingworth et al., 1991 |
| <i>Candida glabrata</i> | X51831 Wong and Clark-Walker, 1990 |
| <i>Kluyveromyces lactis</i> | X51830 Maleszka and Clark-Walker, 1990 |
| <i>Thanatephorus praticola</i> | M92990 Bruns et al., 1992 |
| <i>Coprinus cinereus</i> | M92991 Bruns et al., 1992 |
| <i>Boletus satanas</i> | M94337 Bruns et al., 1992 |
| <i>Cronartium ribicola</i> | M94338 Bruns et al., 1992 |
| <i>Peridermium harknessii</i> | M94339 Bruns et al., 1992 |
| <i>Xerocomus chrysenteron</i> | M94340 Bruns et al., 1992 |
| <i>Schizophyllum commune</i> | X54865 Bruns et al., 1992 |
| <i>Blastomyces dermatitidis</i> | M55624 Bruns et al., 1992 |
| <i>Coccidioides immitis</i> | M55627 Bruns et al., 1992 |
| <i>Penicillium notatum</i> | M55628 Bruns et al., 1992 |
| <i>Pneumocystis carinii</i> | X12708 Edman et al., 1988 |
| <i>Neurospora crassa</i> | X04971 Sogin et al., 1986 |
| <i>Saccharomyces cerevisiae</i> | M27607 Mankin et al., 1986 |
| <i>Spongipellis unicolor</i> | M59760 Bowman et al., 1992 |
| <i>Ustilago maydis</i> | X62396 De Wachter et al., 1992 |
| <i>Schizosaccharomyces pombe</i> | X54866 Sogin's data, 1990 |
| <i>Aspergillus fumigatus</i> | C55626 Sogin's data, 1991 |
| <i>Podospira anserina</i> | X54864 Sogin's data, 1991 |

ouyei (98.7% support). The phylogenetic tree (Fig. 1) shows that *Taphrina*, *Protomyces*, *Saitoella*, *Schizosaccharomyces*, and *Pneumocystis* diverged before the hemiascomycetes and euascomycetes. We call this early lineage the archiascomycetes.

5S rRNA analysis by Watanabe et al. (1989) showed that *Pneumocystis carinii* is linked with the chytridiomycete *Phlyctochytrium irregulare* and the zygomycete *Basidiobolus magnus*. In contrast to phylogenetic analysis of 18S rRNA genes, however, the weakness of comparison of nucleotide sequences of 5S rRNA has been discussed by Bruns et al. (1991). Wakefield et al. (1992, 1993) showed that *Pneumocystis carinii* was closely related to the ustomycetous red yeasts based on the mitochondrial large subunit rRNA gene analysis. Recently, Taylor and Bowman (1993), and Taylor et al. (1994) have pointed out that phylogenetic analysis of 18S rRNA genes put *Pneumocystis carinii* near the divergence of Ascomycota and Basidiomycota and that its meiospore characters seem ascomycetous. Actually the sexual and asexual life cycle of *Pneumocystis carinii* elucidated by TEM studies (Yoshida, 1989) is similar to that to the fission yeast *Schizosaccharomyces octosporus*. Our phylogenetic tree shows that *Pneumocystis carinii* is a member of the Ascomycota (100% support in bootstrap). The dikaryotic mycelia of *Taphrina*, which forms asci and ascospores, may show a transitional position between the Ascomycota and Basidiomycota. Consequently, the phylogenetic position of *Taphrina*, based on 18S rRNA sequence data, appears correct. The species of *Taphrina* and *Protomyces* form a lineage clearly different from the hemiascomycetes. The bootstrap analysis indicated 100% support for the monophyly of this lineage. At this time, it is uncertain whether the archiascomycete lineage is monophyletic.

Common characters of the eight species which define archiascomycetes are limited. At present, the archiascomycetes, a major new lineage within the Ascomycota, is characterized as follows. Its assimilative state is hyphal or yeast-like. Its reproductive state is sexually ascogenous (but lacking ascogenous hyphae), and asexually it shows budding or fission. Neither ascomata nor conidiomata are formed.

In the sequence alignment, only four positions defined each major ascomycete lineage (Table 2). Our data are restricted to 44 taxa in the Ascomycota. More sampling is needed to confirm whether the four specific positions can characterize the archiascomycetes.

The higher fungi have two divisions: Ascomycota and Basidiomycota. The Ascomycota contains three major lineages: the hemiascomycetes, the euascomycetes, and the archiascomycetes. This is a new concept for classification of the ascomycetes. The nomenclatural proposal of archiascomycetes as a higher taxon needs verification from other types of data.

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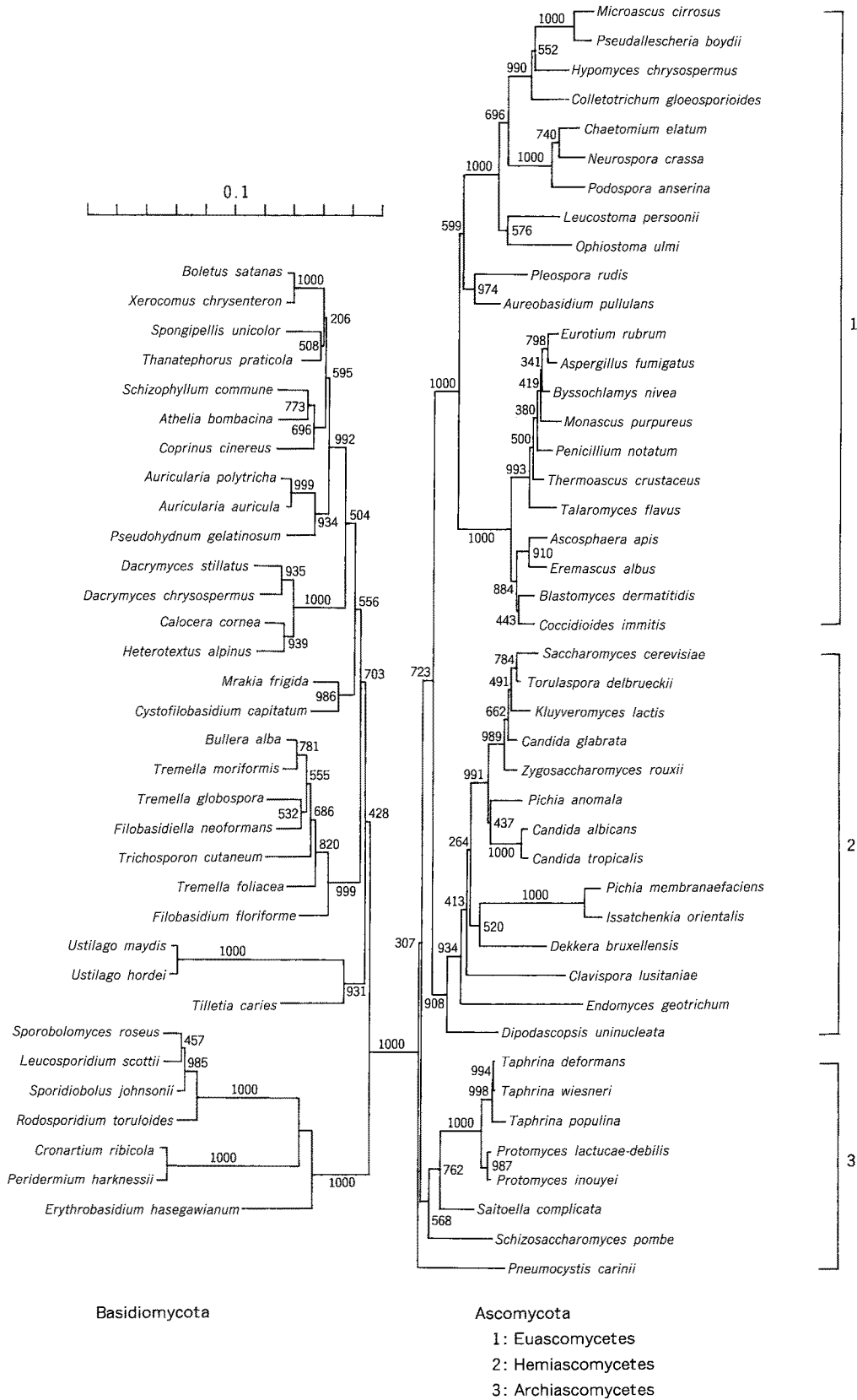


Fig. 1. Phylogenetic tree based on the nuclear 18S rRNA sequences. This unrooted tree was constructed from the evolutionary distance (Kimura, 1980) by using the neighbor-joining method (Saitou and Nei, 1987). The bar indicates the distance corresponding to ten changes per 100 nucleotide positions.

Table 2. Taxon specific positions in 18S rRNA sequences among the three major ascomycete lineages.

| Positions [#] | Archiascomycetes (8 species) | Hemiascomycetes (22 species) | Euascomycetes (14 species) |
|------------------------|---------------------------------|---------------------------------|-------------------------------|
| 478 | A | A | C |
| 479 | C | C | U |
| 883 | U | C | C |
| 970 | A | A | G |

[#]Position of the corresponding residue in the *Saccharomyces cerevisiae* 18S rRNA sequences.

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