Mycoscience 39: 487–511, 1998

Review

Relatedness, phylogeny, and evolution of the fungi

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"Traditional studies of evolution have amply demonstrated that evolution at the phenotypic level is characterized by adaptation and opportunism, irregularity in pace, and inequality of rates among lineages. In contrast, studies of molecular evolution have revealed quite different features characterized by changes that are conservative in nature, random in pattern (independent of phenotypic characters), and quite regular in pace with equal rates among diverge [*sic*] lineages for a given protein". (Kimura, M. 1983. The neutral theory of molecular evolution, pp. 308–309, Cambridge University Press, Cambridge.)

Accepted for publication 2 November 1998

Recent advances in fungal systematics are reviewed in relation to our previous studies. The usefulness of the integrated analysis of genotypic (especially 18S rRNA gene sequence comparisons) and phenotypic (especially ultrastructural and chemotaxonomic data) characters has been emphasized for the major groups and selected taxa of the fungi, and the impact to fungal systematics and evolution is discussed. Our noteworthy studies and findings are: 1) polyphyly of the chytridiomycetes and zygomycetes, 2) phylogenetic origin of the entomophthoralean fungi including *Basidiobolus*, 3) detection of a major new lineage "Archiascomycetes," comprising *Taphrina*, *Protomyces* and *Saitoella*, *Schizosaccharomyces*, and *Pneumocystis*, within the Ascomycota, and its phylogenetic and evolutionary significance, 4) polyphyletic origins of species in the anamorphic genus *Geosmithia*, and 5) phylogenetic placement of *Mixia osmundae*, species correctly and incorrectly assigned to the genus *Taphrina*, and basidiomyceotus yeasts. The newest 18S rDNA sequence-based neighbor-joining trees of the Ascomycota are demonstrated.

Key Words——18S rRNA gene sequence; archiascomycetes; fungal diversity; molecular evolution; molecular phylogenetics.

As D. L. Hawksworth (1991) has stated, "Biodiversity, the extent of biological variation on Earth, has come to the fore as a key issue in science and politics for the 1990s." This subject is also situated in the 21th century in the light of global gene resources and environmental problems such as global climate change caused by the increase of CO_2, loss of biodiversity, loss of tropical rain forests, acid rain, desertification, ozone depletion caused by freon gas, and chemical pollution (Domoto and Iwatsuki, 1997; Ishi, 1988). The leading discipline (Systematics Agenda 2000, 1994) is "systematics" among biodiversity studies, and its importance continues to increase toward the next century.

A recent new estimate provides 1.5 million species for the full extent of the fungal world, and among these only 5% have been inventoried as known and described species (Hawksworth, 1991). The fungi are of great consequence agronomically, bio-industrially, medically and biologically. In spite of such importance, our knowledge is poor about phylogeny and evolution within the fungi and between fungi and other organisms, as well as the taxonomic inventory of species diversity. As pointed out by Bruns et al. (1991), "their simple and frequently convergent morphology, their lack of a useful fossil record, and their diversity have been major impediments to progress in this field." In the past, phylogenetic speculation of the fungi was based mainly on comparative analyses of morphological, ontogenetical, and biochemical data (e.g., Bartnicki-Garcia, 1970, 1987; Bessey, 1942; Cain, 1972; Demoulin, 1974; Gäumann, 1952; LéJohn, 1971, 1974; Ragan and Chapman, 1978; Savile, 1968; Vogel, 1964). Kimura's neutral theory of molecular evolution impacted studies of the phylogeny and evolution (Kimura, 1968, 1980, 1983). In the 1980s, development of molecular biological techniques (particularly gene cloning, nucleic acid sequencing, and polymerase chain reaction), proliferation of high performance computers, and improvement of molecular evolutionary analysis programs have extended studies on relatedness, phylogeny, and evolution of organisms, in-

* Recipient of the 2nd Mycological Society of Japan's Excellent Achievement Award, 1998; the awarding lecture was given at the 42nd Annual Meeting of the Mycological Society of Japan, 16 May, 1998, Kyoto University, Kyoto. This review is based mainly on the publications intended for the Award.

cluding fungi, at the molecular level (Sugiyama and Nishida, 1995; Sugiyama et al., 1996a). In the early 1990s, such an approach steered fungal taxonomy toward fungal molecular systematics (Bruns et al., 1991; Hibbett, 1992; Kohn, 1992; Kurtzman, 1992; Reynolds and Taylor, 1993; Sugiyama, 1994; Sugiyama et al., 1996a). Studies on fungal phylogeny and evolution have already entered a new era.

Sexual and asexual reproductive structures have provided important phenotypic characters to measure relatedness and evolutionary affinities among fungi. If they lose these structures, accurate taxonomic assignment is quite difficult even at the phylum level. Conversely, nucleic acid characters, as genotypic characters, are ubiquitous and are not dependent on the expression of reproductive structures (Taylor, 1993). Nuclear DNA (nDNA) base composition and nDNA relatedness, as nucleic acid characters, have been used to define species of yeasts (e.g., Kurtzman, 1987; Kurtzman and Phaff, 1987; Nakase and Komagata, 1971; Price et al., 1978; Storck, 1966; Storck et al., 1969; for other references, see Kurtzman, 1998a), *Neurospora* (Dutta, 1976), and *Aspergillus* (e.g., Kurtzman et al., 1986). However, these molecular characters resolve only to the genetic sibling species level.

Ribosomal RNA (rRNA) and its template ribosomal DNA (rDNA) sequence comparisons, as a nucleic acid character, offer a means for estimating both close and distant relationships from prokaryotes to eukaryotes. Historically molecular phylogenetic studies of fungi go back to Walker and Doolittle's epoch-making work (1982) on several basidiomycetous species using 5S rRNA sequence divergence. Initially, analyses based on 5S rRNA sequence comparisons dramatically improved our understanding of fungal phylogeny and evolution (e.g., Blanz and Unseld, 1987; Hori and Osawa, 1987). Because only 120 nucleotides are available for comparison, however, resolution is limited (Bruns et al., 1991). In the latter half in the 1980's, molecular phylogenetic studies of the fungi shifted to the small (18S) and large (23S to 28S) subunit rRNAs. Phylogenetic analysis among distantly related taxa, i.e., at the class-phylum-kingdom level, using 18S rRNA gene sequence divergence, has contributed to well-resolved and statistically supported conclusions (e.g., Berbee and Taylor, 1992a, 1992b, 1993; Bowman et al., 1992; Bruns et al., 1991, 1992; Nagahama et al., 1995; Nishida and Sugiyama, 1993, 1994b; Nishida et al., 1995; Suh and Sugiyama, 1993a, 1994; Swann and Taylor, 1993). The analyses of the 18S and 26S rRNA partial sequences and their significance for yeasts and yeast-like fungi have been reviewed by Yamada (1994).

During the past eight years, we obtained 18S rRNA gene sequence data from the lower to higher fungi to investigate evolutionary relationships among their major groups and the selected taxa, and to assess the existing taxonomic systems and phylogenetic hypotheses that were based mainly on morphological characters. Results of these studies are, in part, described here.

Phylogenetic hypotheses and circumscription of the true fungi

Among earlier phylogenetic speculations concerning the whole fungi and related organisms that have been made during the past 10 yr, Cavalier-Smith's theory (1987) is noteworthy. He provided a framework for a taxonomic system and phylogeny for the fungal kingdom that was based mainly on cell wall chemistry, biosynthetic pathways for lysine, type of motile cells, cellular ultrastructure, and 5S rRNA sequence divergence. He included only the chytridiomycetes, zygomycetes, ascomycetes, and basidiomycetes in the kingdom Fungi. These four fungal groups are characterized by chitinous cell walls (Bartnicki-Garcia, 1970, 1987) and the α-aminoadipic acid (AAA) lysine biosynthetic pathway (Vogel, 1964, 1965). The oomycetes, hyphochytrids, labyrinthulids, thraustochytrids, and slime molds, which are cellulosic (Bartnicki-Garcia, l.c.) and have the α, β-diaminopimelic acid (DAP) lysine biosynthetic pathway (Vogel, l.c.), are excluded from the Fungi. The first four major groups have been accommodated in the pseudofungi (sensu Cavalier-Smith, 1987) and the slime molds in the kingdom Protozoa (Cavalier-Smith, 1993). In this scheme, he also speculated that Fungi and animals had a common ancestor, a choanociliate (choanoflagellate) protozoan. He concluded that the kingdom Fungi is monophyletic and also that all major eufungal taxa in the Endomycota, Ascomycota, and Basidiomycota, evolved from the Entomophthorales from a chytridiomycete ancestor by loss of cilia (flagella).

Evidence from 18S rDNA sequence divergence put an end to a debate on the circumscription of the kingdom Fungi, i.e., whether oomycetes, hyphochytrids, and chytrids were the "true" fungi, and confirmed the extent of "true" fungi (Fig. 1; cf., Bruns et al., 1991, 1992). As a result, the true fungi are chytridiomycetes, zygomycetes, ascomycetes, and basidiomycetes. The AAA lysine pathway and the presence of chitin in the cell walls strongly support the 18S rDNA sequence-based phylogeny. And, in general, the true fungi are hyphal, have cell walls through most or all of their life cycle and are exclusively absorptive in their nutrition. These "true" fungi form a monophyletic group, though below the 95% level based on bootstrap analysis, and are distinguished phenotypically from cellular slime molds (Acrasiomycetes) and plasmodial slime molds (Myxomycetes) and the oomycetes (Oomycota) (Bruns et al., 1992; Sugiyama, 1996b; cf., Fig. 1). In this phylogenetic tree, the two groups of slime molds diverged separately, prior to the terminal radiation of eukaryotes. This result is consistent with many differences between slime molds and fungi in form, function and life cycle. The oomycetes (*Achlya*, *Lagenidium* and *Phytophthora*), hyphochytrids (*Hyphochytridium*) and labyrinthulids (*Thraustochytrium* and *Ulkenia*) (Fig. 1) form a cluster with brown algae and diatoms. These organisms have heterokont flagella, one decorated with tripartite hairs, contain chlorophylls a and c, and are classified within the Chromista sensu Cavalier-Smith (1993), or the the king-

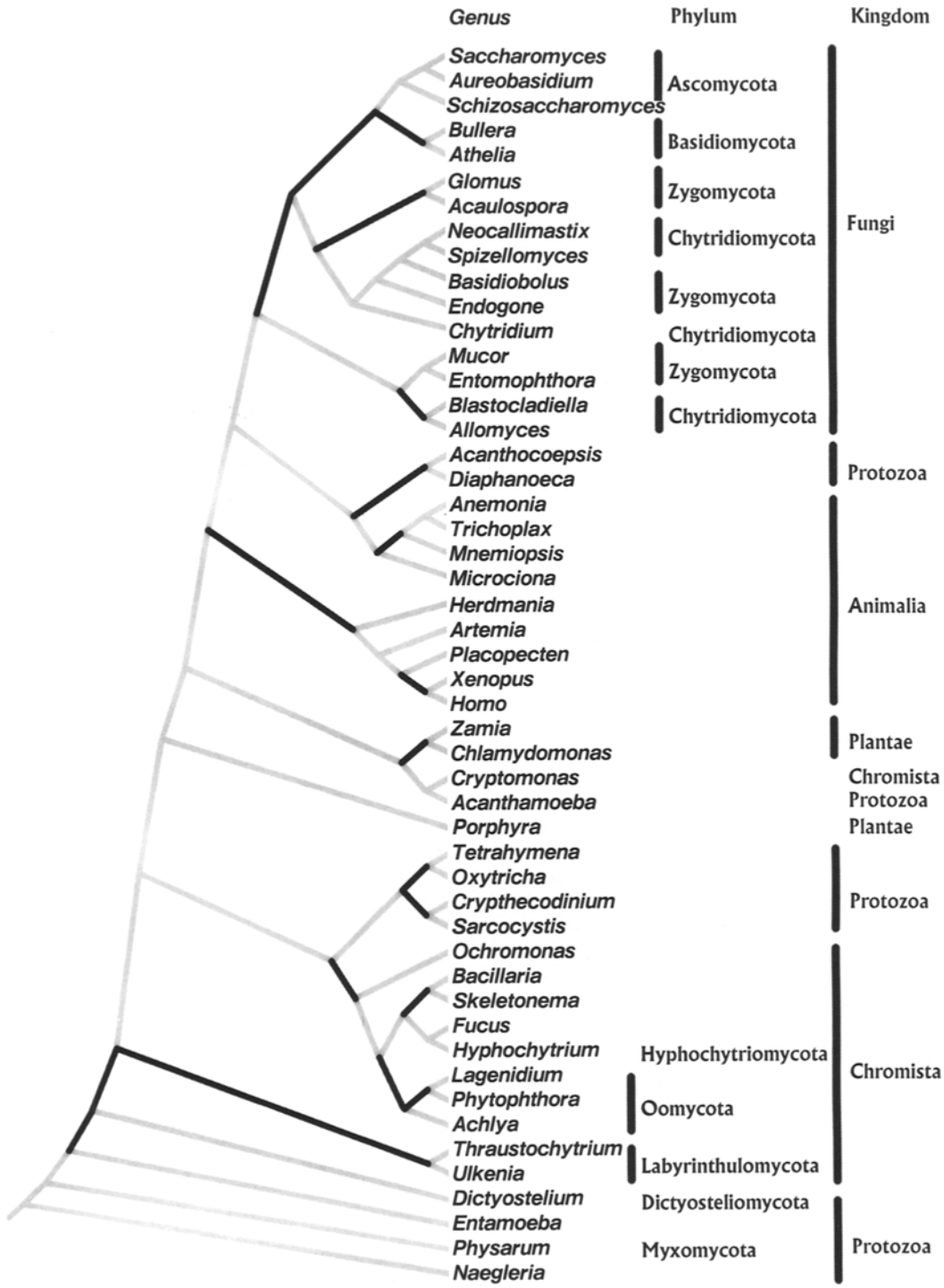


Fig. 1. A tree, inferred from 1,460 sites of 18S rRNA gene sequence from 50 eukaryotes.
Bold line indicates a branch supported by 95% or greater derived from 1,000 replications. Modified from Nagahama (1995) and Sugiyama (1996b).

dom Stramenopila (Alexopoulos et al., 1996), segregated from the Chromista. The labyrinthulids appear to be basal to other heterokont algae and oomycetes and hyphochytrids within the Chromista (van der Auwera et al., 1995; cf. Leipe et al., 1994). Maximum likelihood phylogentic analysis of the 18S rDNA suggests that *Plasmodiophora brassicae* Woronin, a severe pathogen of crucifers, may be more closely related to the alveolates than to any of the Fungi (Castlebury and Domier, 1998). Parsimony analysis of elongation factor-1α (EF: a protein

involved in the translation of messenger RNA) amino acid sequences strongly supports a monophyletic Mycetozoa, represented by the dictyostelid *Dictyostelium*, the myxogastrid *Physarum*, and the amoeboflagellate protostelid *Planoprotostelium*, being basal to the other two members. The Mycetozoa is among the multicellular eukaryotes, tentatively supported as more closely related to animals+fungi than are green plants (Baldauf and Doolittle, 1997). The use of EF-1α emphasizes the importance of developing multiple sequence data sets. As a conclusion, the phylogeny of the so-called pseudofungi and their order of divergence remains uncertain at the moment.

Evolutionary relationships between the three kingdoms of complex eukaryotes, fungi, plants and animals, are still controversial, because of lack of solid fossil evidence. However, recent 18S rDNA phylogenies and amino acid sequence of elongation factor show that the closest relatives to fungi are animals, not plants (Baldauf and Palmer, 1993; Hasegawa et al., 1985, 1993; Hendriks et al., 1991; Kumar and Rzhetsky, 1996; Sogin et al., 1989; Wainright et al., 1993). Nikoh et al. (1994) concluded that, from a phylogenetic analysis of trees inferred from 23 different protein species from the three kingdoms by three different methods, i.e., the maximum likelihood (ML) method, the neighbor-joining (NJ) method, and the maximum parsimony (MP) method, the kingdom Animalia is closely related to the kingdom Fungi and is distantly related to the kingdom Plantae. In contrast, on the basis of the ribosomal protein peptide and nucleotide sequences, Plantae and Animalia are sister clades and Fungi form a more distinct clade to them (Veuthey and Bittar, 1998). In conclusion, the full evidence from both molecules and morphology is lacking concerning the boundaries between the Fungi and other organisms. More informative molecular characters are needed to solve these evolutionary problems.

Phylogenetic relationships among the chytridiomycetes and zygomycetes

Four divisions, i.e., Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota, that now can be recognized as sharing a number of important characteristics are accommodated in the kingdom Fungi (e.g., Fig. 3 in Alexopoulos et al., 1996; Hawksworth et al., 1995; Sugiyama, 1996a) or the kingdom Eumycota (Barr, 1992). The 18S rRNA gene phylogeny supports this framework (e.g., Berbee and Taylor, 1993; Bruns et al., 1992; Gehrig et al., 1996; Nagahama et al., 1995).

In the view of many mycologists, it is believed that the chytrids are the most primitive fungi within the "true" fungi, because they are zoosporic and gave rise to the Zygomycota and other higher fungi. Molecular phylogenetic studies of the chytridiomycetes and zygomycetes are limited because economic interest is relatively low, pure cultures have not been achieved for many species, and even detection of some species is very difficult. However, such studies are indispensable to fill the gaps in knowledge of fungal evolution. rRNA sequence comparisons answered the debate as to whether flagellated fungi were "true" fungi. Early phylogenetic analysis of 5S rRNA sequences by Walker (1984) indicated that *Basidiobolus* was closely related to the chytrids. The Harpellales, an order of the Trichomycetes, was proposed to have a close relationship with the Kickxellales based on similarities in septal pore ultrastructure, cell wall structure, asexual reproductive apparatus, and serological affinity (Moss and Young, 1978), but this interpretation conflicted with Walker's 5S rRNA sequence comparison (1984). Gunderson et al. (1987) revealed that the 18S rRNA sequence-based phylogeny supported exclusion of the oomycetes. The sequence analysis of 18S rRNA genes suggested that the chytrid *Blastocladiella emersonii* Cantino & Hyatt and the higher fungi shared a common ancestor (Förster et al., 1990), and that the rumen anaerobic fungus *Neocallimastix* sp. should be assigned to the Chytridiomycetes (Bowman et al., 1992).

Our molecular phylogenetics in the Chytridiomycota and Zygomycota focused on phylogenetic divergence of the entomophthoralean fungi from nuclear 18S rRNA gene sequence comparisons (Nagahama et al., 1995) on the basis of Cavalier-Smith's hypothesis (1987). Our bootstrapped NJ and ML analyses (Fig. 2; cf. Nagahama et al., l.c.) opened a debate with existing classifications and the previously proposed hypothesis of fungal evolution. We used the choanoflagellate, *Diaphanoeca grandis*, as an outgroup, which has been suggested to be the protozoan ancestor of the true fungi (Cavalier-Smith, 1987). Between the NJ and ML trees, there were differences in the phylogenetic positions of *Blastocladiella*, *Endogone*, and the chytrids, but the statistical confidences by bootstrap analysis, supporting the branches of each species, were not high. The four species in the Entomophthorales were placed in two clusters, i.e., *Basidiobolus ranarum* Eidam in one cluster containing *Chytridium*, *Spizellomyces* and *Neocallimastix*, and the remaining three species *Conidiobolus coronatus* (Constantin) Bakto, *Entomophthora muscae* (Cohn) Fresenius, and *Zoophthora radicans* (Brefeld) Bakto in another cluster containing *Mucor*. *Glomus etunicatum* de Bary was basal to the higher fungi, i.e., ascomycetes and basidiomycetes, and *Smittium culisetae* Lichtwardt was placed close to the divergence of Entomophthorales from the chytrid-*Glomus*-ascomycete-basidiomycete clade. Neither of the trees supported a monophyletic chytridiomycetes or a monophyletic zygomycetes and instead suggested that losses of flagella occurred in several lineages during the course of fungal evolution. The concept that different chytrids lost flagella and gave rise to the major groups of filamentous fungi did not correspond with Cavalier-Smith's hypothesis (1987). The presence of zygospores, commonly observed for species in the zygomycetes, may be a convergent character and the definition of zygospores in fungal diversity should be investigated further using ultrastructural data.

Basidiobolus joined with the chytrids, *Chytridium*, *Spizellomyces*, and *Neocallimastix*, and formed a cluster with a high level (81%) of bootstrap confidence in the NJ

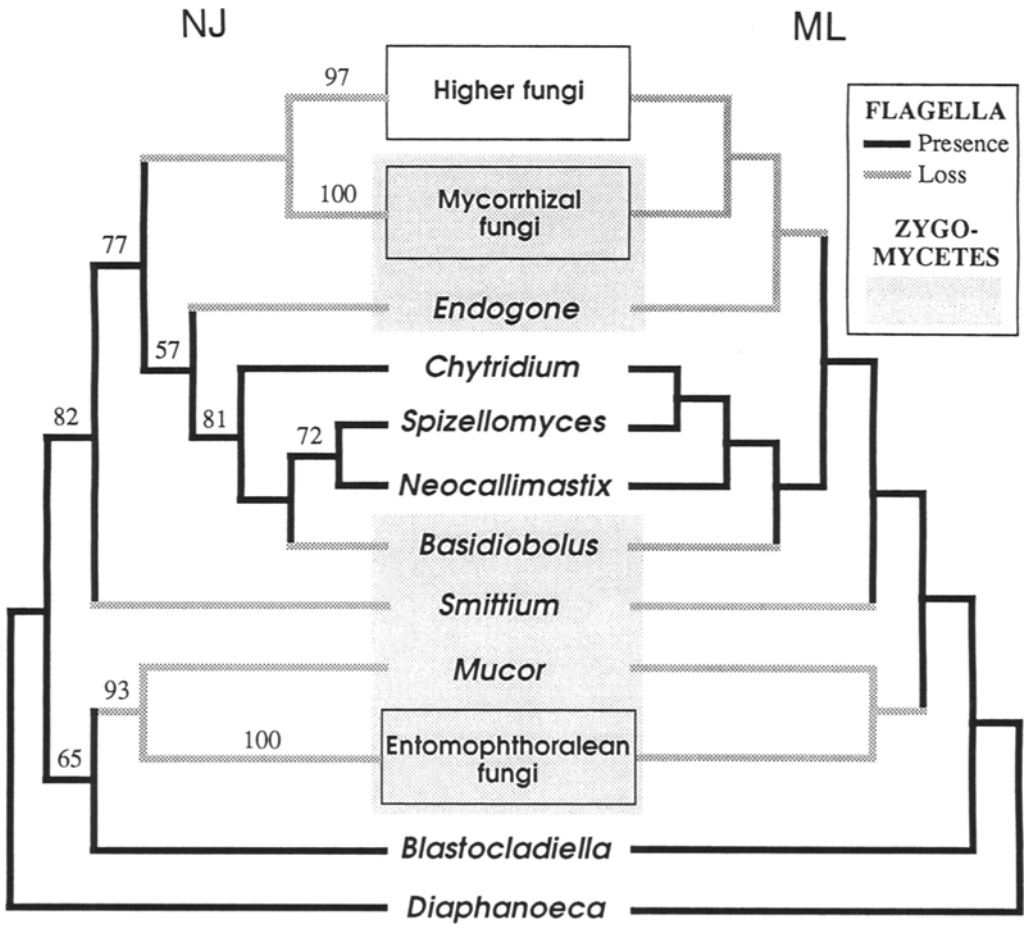


Fig. 2. Comparison of branching patterns between neighbor-joining (NJ) tree and maximum-likelihood (ML) tree, inferred from 1455 alignable sites of the 18S rRNA gene sequence (Sugiyama et al., 1996a).
Published sequences, for the four entomophthoralean fungi (*Basidiobolus ranarum* Eidam, *Conidiobolus coronatus* (Constantin) Batko, *Entomophthora muscae* (Cohn) Fresenius and *Zoophthora radicans* (Brefeld) Batko), the trichomycete *Smittium clusitae* Lichtwardt, the three higher fungi (the two ascomycetous yeasts *Saccharomyces cerevisiae* Meyen ex Hansen and *Schizosaccharomyces pombe* Lindner, and the basidiomycete *Athelia bombacina* Persoon), the four chytrids (*Blastocladiella emersonii* Cantino & Hyatt, *Chytridium confervae* (Willie) Minden, *Neocallimastix* sp. and *Spizellomyces acuminatus* (Barr) Barr), the endomycorrhizal fungus *Glomus etunicatum* de Bary, the zygomycete *Mucor racemosus* Fresenius, and the choanoflagellate *Diaphanoeca grandis* Ellis, were aligned by CLUSTAL W (Thompson et al., 1994). Topology of the left hand tree was constructed by the NJ method (Saitou and Nei, 1987), from alignment of the data sets. The percentage of bootstraps (Felsenstein, 1985) was derived from 1,000 replications, using CLUSTAL W (Thompson et al., 1994). Values below 50% are not shown. The topology of the right hand tree was constructed using the ML program DNAML from PHYLIP 3.5c (Felsenstein, 1994). The lineages where flagella were lost are drawn in gray.

tree (Fig. 2; cf. Nagahama et al., l.c.). In the ML tree, in spite of changes in branching orders among the four species, their monophyletic grouping was supported. McKerracher and Heath's finding (1985) of the presence of a postcentriole in *Basidiobolus* strongly supports the 18S rRNA gene phylogeny. Figure 2 also suggests that the entomophthoralean fungi, including *Basidiobolus*, are not monophyletic, and shows great phylogenetic divergence among the chytridiomycetes. Other discussions are fully mentioned in our original paper (Nagahama et al., 1995). Very recently phylogenetic relationships among the Harpellales and Kickxellales have been analyzed by O'Donnell et al. (1998b) using the 18S rDNA gene sequence analysis.

To establish a taxonomic system of the chytridiomycetes and zygomycetes which reflects their evolutionary relationships, further studies of their molecular phylogenetic, chemotaxonomic, and micromorphological studies (especially on ultrastructural studies) will be necessary.

Phylogenetic divergence of ascomycetes and their anamorphs

Numerous hypotheses on phylogeny and evolution of the higher fungi, i.e., the ascomycetes, basidiomycetes and their anamorphs, have been proposed (for references, see Bessey, 1942; Demoulin, 1974; Kramer, 1987;

Savile, 1955, 1968; Tehler, 1988). Among these, Savile's phylogenetic considerations of higher fungi (1955, 1968) have attracted the attention of a lot of mycologists, including me, over 40 yr as a logical hypothesis. Savile (1968) suggested that *Taphrina* was the closest survivor of the common ancestor of the higher fungi. He suspected that two major lineages evolved from "Prototaphrina" a common ancestor. One major lineage led to the present day *Taphrina* and the ascomycetes, whereas another major lines led to the

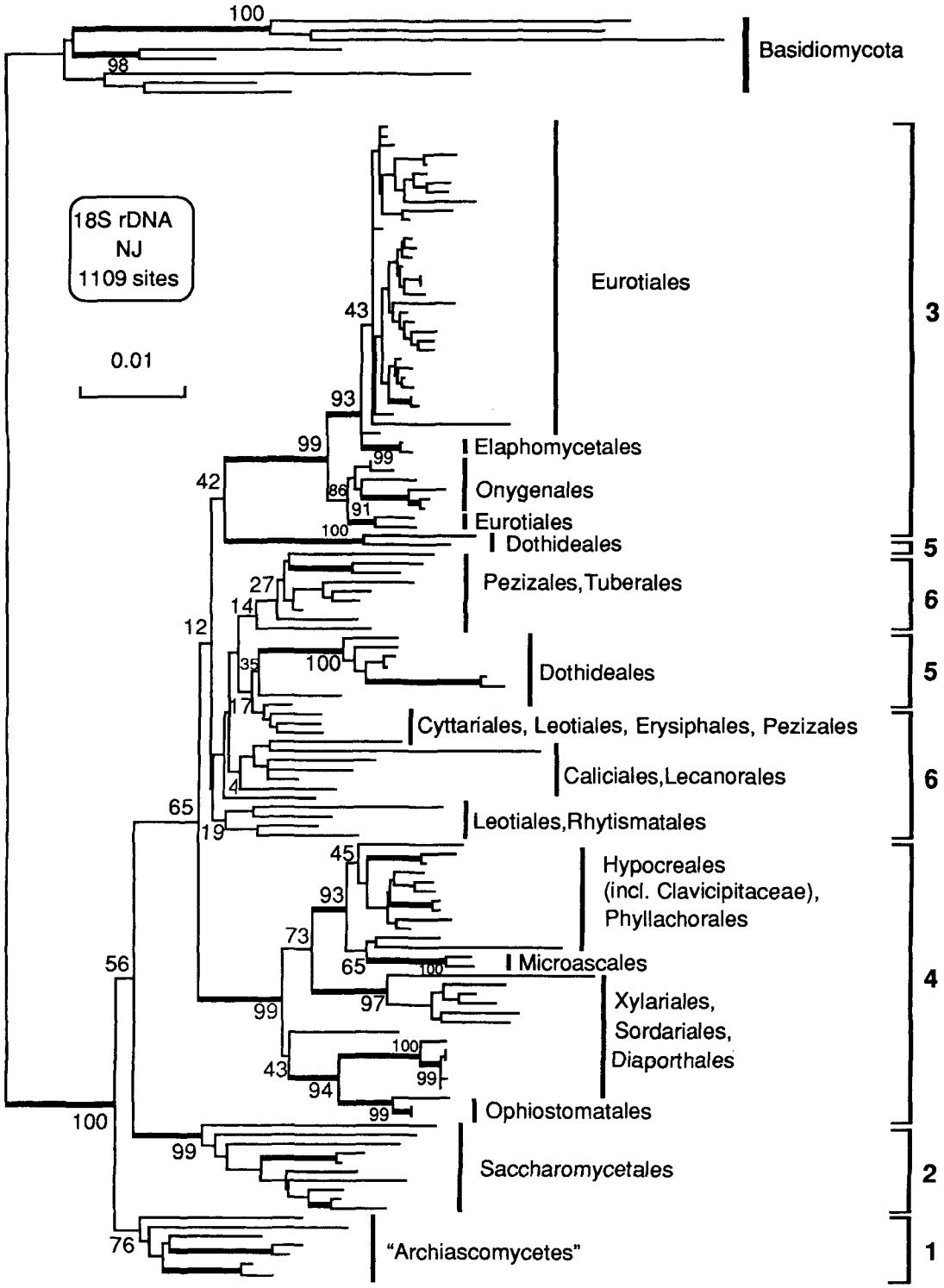


Fig. 3A.

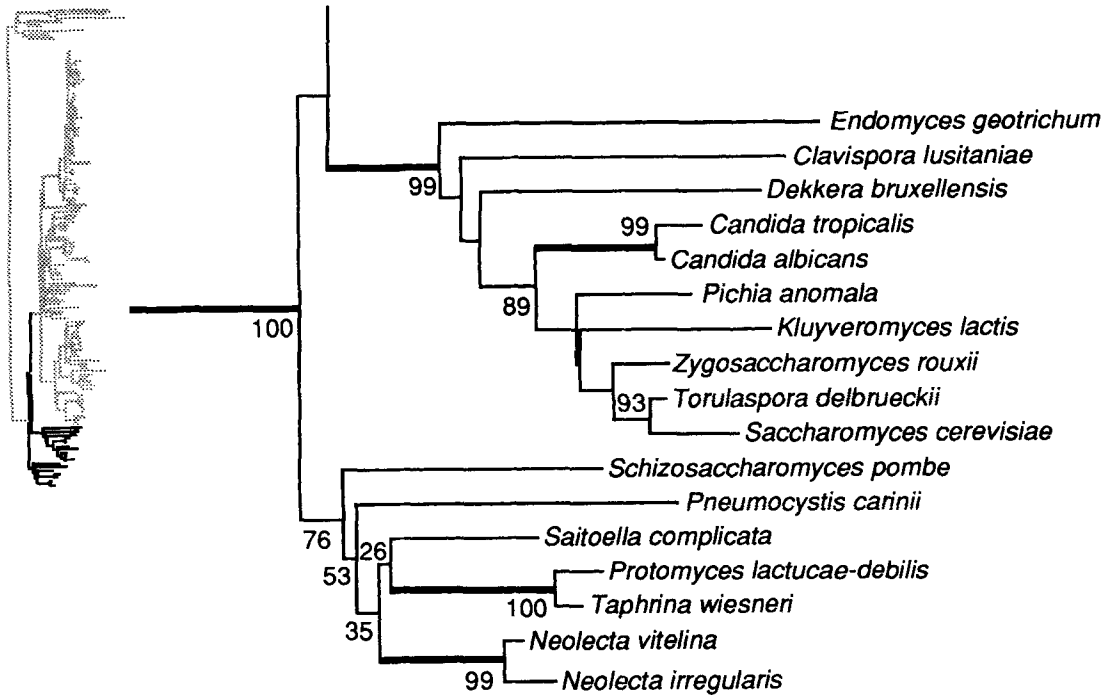
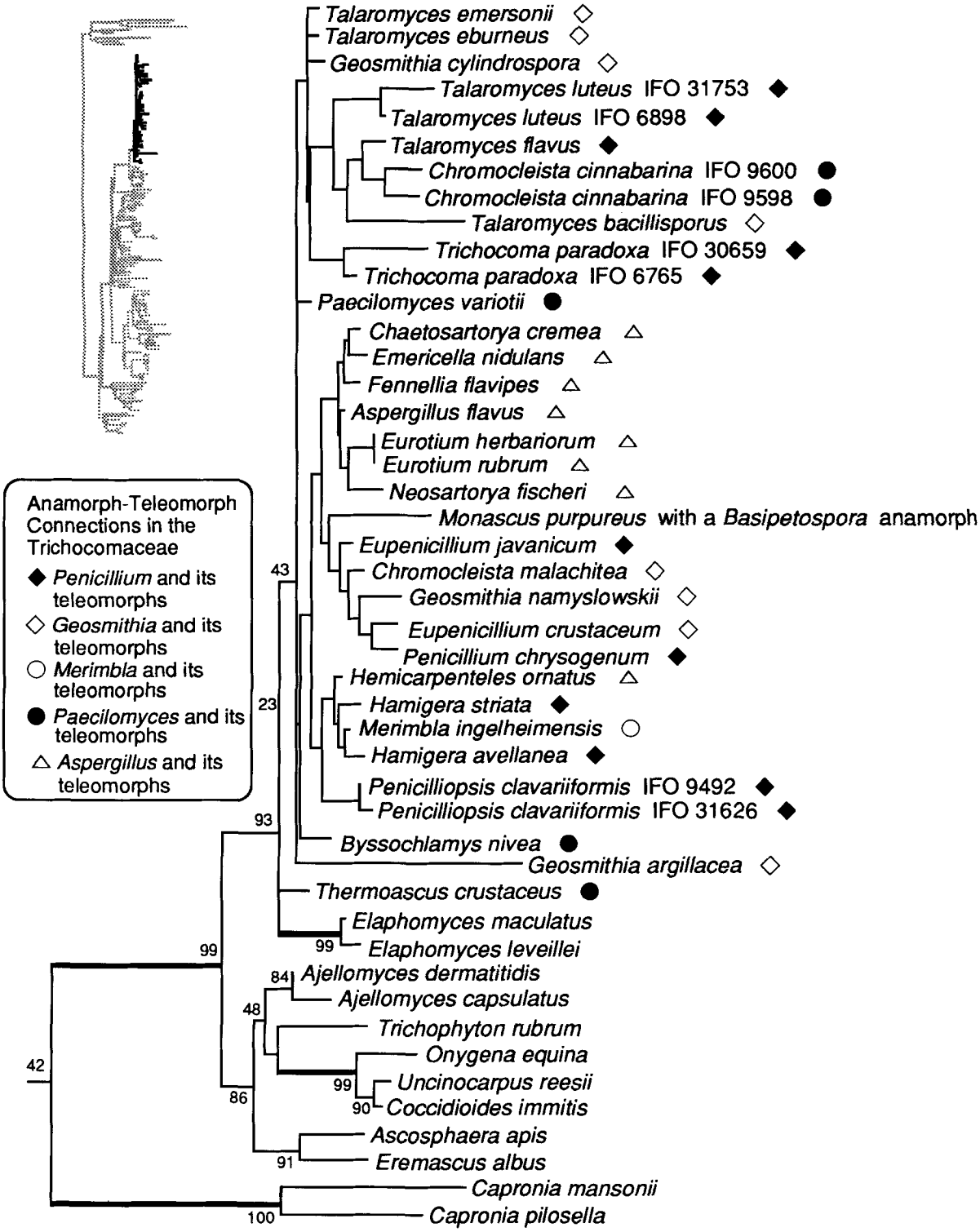


Fig. 3B.

basidiomycetes (the Uredinales line and the parasitic Auriculariaceae line) through a "Protobasidiomycete." In his phylogenetic scheme *Taphrina* was considered to be the most important fungus.

Detection of the Ascomycota and Basidiomycota Phylogenetic trees inferred from 18S rDNA sequence divergence indicate the existence of the two divisions, Ascomycota and Basidiomycota, among the higher fungi (e.g., Berbee and Taylor, 1993; Bruns et al., 1992; Gargas et al., 1995; Nishida and Sugiyama, 1993, 1994b; Nishida et al., 1993). Our NJ and ML trees (Sjamsuridzal et al., 1997), inferred from 1,363 alignable sites of 18S rRNA gene sequence from 31 selected higher fungi using *Mucor racemosus* as an outgroup, support that both divisions appear to be monophyletic, though the confidence in the branch uniting the basidiomycetes was relatively low (35% in NJ tree). The former is characterized by the ascus as a meiosporangium producing ascospores (sexual endospores), whereas the latter is characterized by the basidium as a meiosporangium producing basidiospores (sexual exospores). Both divisions appear to be monophyletic but their origin is ambiguous and controversial. In most of the higher fungi the asexual process involves the reproduction of asexual spores (e.g., conidia). Although the pleomorphy is characteristic in the higher fungi (Sugiyama, 1987), strictly anamorphic fungi appear to be phylogenetically members within the Ascomycota or Basidiomycota. The newest 18S rDNA sequence-based NJ tree (Figs. 3A–E) supports this statement. The use of the Deuteromycota or Deuteromycotina as a formal taxon is now decreased (e.g., Alexopoulos et al., 1996; Hawksworth et al., 1995; Reynolds and Taylor, 1993; Sugiyama, 1996a; Taylor, 1993, 1995). In recent years molecular data have added a new dimention to an understanding of the relationships among ascomycete orders and higher taxonomic categories, while recognizing that the ascoma characters adopted to define them are known to converge (Cain, 1972; Malloch, 1981). Hemiascomycetes, Plectomycetes, Pyrenomycetes and other traditional class level categories are no longer used formally in the fungal classification system (Alexopoulos et al., 1996; Hawksworth et

Figs. 3A, B, C, D, and E. Phylogenetic analysis of 124 ascomyceous fungi with 8 basidiomycetous fungi as outgroups. All gaps and ambiguous sites were excluded. A sequence data set of 1,162 sites of 18S rRNA gene sequence was considered. NJ tree (Saitou and Nei, 1987), based on the Kimura's two parameter model (1980; transition/transversion ratio = 2.0), was constructed using the software package Clustal W, version 1.74 (Thompson et al., 1994), on Linux (2.0.33) computer. The analysis was bootstrapped up to 1,000 replications to estimate robustness of tree structure, and numbers on the selected branches are percent bootstrap values. The scale bar indicates one base change per 100 nucleotide positions. For sequence references, see Ogawa et al. (1997) and Sjamsuridzal et al. (1997, 1999). New sequences have been determined by Ogawa and Sugiyama (unpublished). Outgroups used (top to bottom) are: *Uredinopsis intermedia* Kamei, *Rhodosporidium toruloides* Banno, *Mixia osmundae* (T. Nishida) Kramer, *Tremella globospora* Reid, *Filobasidium floriforme* L. S. Olive, *Ustilago maydis* (de Candolle) Corda, *Tilletia caries* (de Candolle) Tulasne, and *Tilletiaria anomala* Bandoni & Johri. The whole tree (A) is divided into four portions: i.e., B, archiascomycetes and hemiascomycetes; C, euascomycetes (plectomycetes); D, euascomycetes (pyrenomycetes); E, euascomycetes (other euascomycetes excluding plectomycetes and pyrenomycetes).


Talaromyces emersonii ◇
Talaromyces eburneus ◇
Geosmithia cylindrospora ◇
Talaromyces luteus IFO 31753 ◆
Talaromyces luteus IFO 6898 ◆
Talaromyces flavus ◆
Chromocleista cinnabarina IFO 9600 ●
Chromocleista cinnabarina IFO 9598 ●
Talaromyces bacillisporus ◇
Trichocoma paradoxa IFO 30659 ◆
Trichocoma paradoxa IFO 6765 ◆
Paecilomyces variotii ●
Chaetosartorya cremea △
Emericella nidulans △
Fennellia flavipes △
Aspergillus flavus △
Eurotium herbariorum △
Eurotium rubrum △
Neosartorya fischeri △
Monascus purpureus with a Basipetospora anamorph
Eupenicillium javanicum ◆
Chromocleista malachitea ◇
Geosmithia namyslowskii ◇
Eupenicillium crustaceum ◇
Penicillium chrysogenum ◆
Hemicarpenteles ornatus △
Hamigera striata ◆
Merimbla ingelheimensis ○
Hamigera avellanea ◆
Penicilliopsis clavariiformis IFO 9492 ◆
Penicilliopsis clavariiformis IFO 31626 ◆
Byssochlamys nivea ●
Geosmithia argillacea ◇
Thermoascus crustaceus ●
Elaphomyces maculatus
Elaphomyces leveillei
Ajellomyces dermatitidis
Ajellomyces capsulatus
Trichophyton rubrum
Onygena equina
Uncinocarpus reesii
Coccidioides immitis
Ascosphaera apis
Eremascus albus
Capronia mansonii
Capronia pilosella
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91
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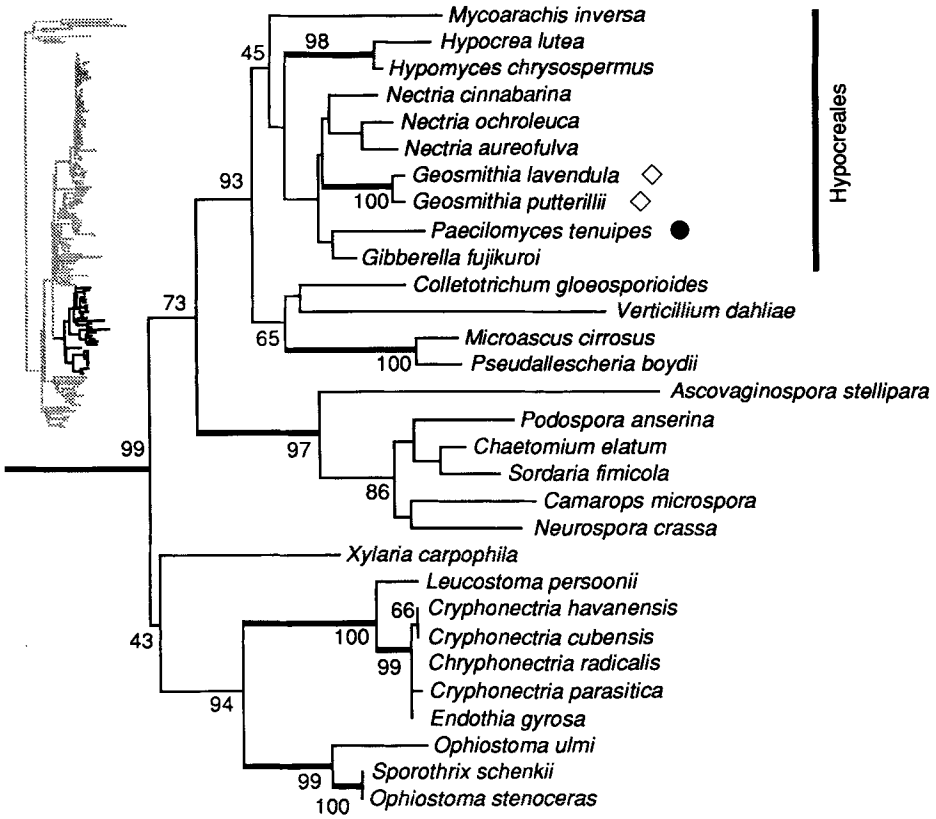
Anamorph-Teleomorph Connections in the Trichocomaceae
◆ Penicillium and its teleomorphs
◇ Geosmithia and its teleomorphs
○ Merimbla and its teleomorphs
● Paecilomyces and its teleomorphs
△ Aspergillus and its teleomorphs

Fig. 3C.


Mycoarachis inversa
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Hypomyces chrysospermus
Nectria cinnabarina
Nectria ochroleuca
Nectria aureofulva
Geosmithia lavendula ◇
Geosmithia putterillii ◇
Paecilomyces tenuipes ●
Gibberella fujikuroi
Hypocreales
Colletotrichum gloeosporioides
Verticillium dahliae
Microascus cirrosus
Pseudallescheria boydii
Ascovaginospora stellipara
Podospora anserina
Chaetomium elatum
Sordaria fimicola
Camarops microspora
Neurospora crassa
Xylaria carpophila
Leucostoma persoonii
Cryphonectria havanensis
Cryphonectria cubensis
Chryphonectria radicalis
Cryphonectria parasitica
Endothia gyrosa
Ophiostoma ulmi
Sporothrix schenkii
Ophiostoma stenoceras
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Fig. 3D.

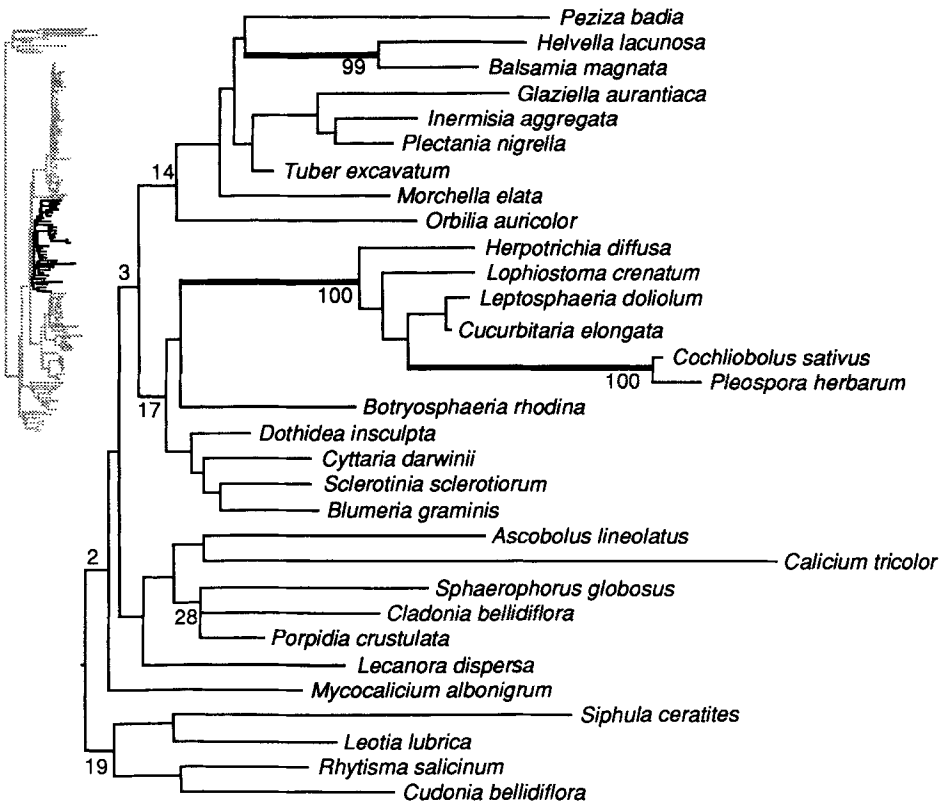
Peziza badia
Helvella lacunosa
Balsamia magnata
Glaziella aurantiaca
Inermisia aggregata
Plectania nigrella
Tuber excavatum
Morchella elata
Orbilia auricolor
Herpotrichia diffusa
Lophiostoma crenatum
Leptosphaeria doliolum
Cucurbitaria elongata
Cochliobolus sativus
Pleospora herbarum
Botryosphaeria rhodina
Dothidea insculpta
Cyttaria darwinii
Sclerotinia sclerotiorum
Blumeria graminis
Ascobolus lineolatus
Calicium tricolor
Sphaerophorus globosus
Cladonia bellidiflora
Porpidia crustulata
Lecanora dispersa
Mycocalicium albonigrum
Siphula ceratites
Leotia lubrica
Rhytisma salicinum
Cudonia bellidiflora
99
14
3
100
100
17
2
28
19

Fig. 3E.

al., 1995; Sugiyama, 1996a), and this is a reason for using hemiascomycetes, plectomycetes and pyrenomycetes as informal names until the respective groups are redefined or renamed (Ogawa et al., 1997). However, the class level names "Archiascomycetes," Hemiascomycetes, and Euascomycetes have been adopted in yeast classification by Kurtzman (1998b).

***Saitoella, Taphrina*, and detection of a major new lineage "Archiascomycetes" within the Ascomycota** The root of the anamorphic yeast *Saitoella complicata* is the two Himalayan yeast isolates identified as *Rhodotorula glutinis* (Fresenius) Harrison (Goto and Sugiyama, 1970), and its research history has been fully outlined by Sugiyama et al. (1993). This anamorphic yeast species has been characterized by molecular data (nuclear DNA base composition and nuclear DNA relatedness; Hamamoto et al., 1986a, b, 1987) as well as cultural, morphological, physiological, chemotaxonomic and ultrastructural characters (Goto and Sugiyama, 1970; Goto et al., 1987; Simmons and Ahearn, 1987; Sugiyama et al., 1985; Suh et al., 1993b; cf. Ahearn et al., 1998). Based on these characteristics, we (Goto et al., 1987) proposed a new anamorphic yeast genus *Saitoella* in the family Cryptococcaceae, with only the type species *S. complicata* Goto, Sugiyama, Hamamoto & Komagata. Species of *Taphrina, Protomyces, Rhodotorula* and its teleomorph *Rhodosporidium* (Tubaki, 1957, 1978), and *S. complicata* produce pigmented yeast colonies on artificial culture media (Goto et al., 1987; van Eijk and Roeyman, 1982). Macroscopically these fungi are very similar to each other in pure culture. Comparisons of their principal characteristics are summarized by Sugiyama and Nishida (1995) and Sugiyama et al. (1996a). *Saitoella complicata* and *Taprina wiesneri* (Ráthay) Mix, attacking Japanese cherry trees (*Cerasus yedoensis*) and causing "witches' brooms," share some characteristics with the ascomycetes and basidiomycetes. In both species, the negative diazonium blue B (DBB) reaction and negative extracellular DNase activity resemble characteristics of ascomycetous yeasts, whereas the positive urease activity and major ubiquinone system Q-10 resemble those of basidiomycetous yeasts (Goto et al., 1987; cf., Sugiyama and Nishida, 1995; Sugiyama et al., 1996a). G+C content of nuclear DNA in two species is around 50 mol%, the so-called "grey zone" (Miller et al., 1976). Ultrastructurally *S. complicata* is characterized by a two-layered cell wall that is characteristic of ascomycetous yeasts and by enteroblastic conidiogenesis, which is typical of the basidiomycetous yeasts (Goto et al., 1987; Sjamsuridzal et al., 1997; cf. Ahearn et al., 1998). Very recently we (Sjamsuridzal et al., 1997) have clarified ultrastructurally that *T. wiesneri* and the type species *T. populina* Fries have presumably the multi-layered cell wall and the enteroblastic type of conidium ontogeny, characteristic of basidiomycetous yeasts. Based on these comparisons, Goto et al. (1987) suggested a close affinity between *Saitoella* and the Taphrinales. This series of studies integrated *Saitoella*, *Taphrina*, and Savile's phylogenetic speculation into one logical string.

Nishida and Sugiyama's 18S rRNA gene phylogeny (1993) indicates the existence of the two divisions, Ascomycota and Basidiomycota, among the higher fungi, with strong bootstrap support, and both divisions appear to be monophyletic as already suggested by 18S rDNA sequence comparisons (e.g., Berbee and Taylor, 1993; Bruns et al., 1992). The molecular phylogeny (Nishida and Sugiyama, l.c.) also showed that the ascomycetes are composed of three major lineages. First is the "Archiascomycete" lineage with 86% bootstrap confidence, which contains *Taphrina wiesneri* (Ráthay) Mix, *Saitoella complicata* Goto et al., and the fission yeast *Schizosaccharomyces pombe* Lindner, and is distinguished from the ascomycetous yeasts. Second is the hemiascomycete lineage, which contains *Kluyveromyces lactis* (Dombrowski) van der Walt, *Saccharomyces cerevisiae* Meyer ex Hansen, and *Candida albicans* (Robin) Berkhout among other yeasts. Third is the filamentous ascomycetes or the euascomycete lineage, which embraces *Neurospora crassa* Shear & B. Dodge and *Podospora anserina* (Rabenhorst) Winter among other fungi. In addition, their phylogenetic tree has suggested that the ascomycetes are not ancestral to the basidiomycetes. Further analysis based on two new sequences and 75 sequences from the DNA data bank divided the Ascomycota into three major lineages (Nishida and Sugiyama, 1994b): the hemiascomycetes, the euascomycetes, and the archiascomycetes, newly described herein. The archiascomycetes are composed of *Taphrina wiesneri*, *T. populina*, *Protomyces inouyei* T. Nishida, *P. lactucae-debilis* Sawada, *Saitoella complicata*, *Schizosaccharomyces pombe*, and *Pneumocystis carinii* Delanoë & Delanoë (Delanoë and Delanoë, 1912). This major new lineage, the archiascomycetes, diverged prior to the separation of the other two major ascomycete lineages. The archiascomycetes correspond to the basal ascomycetes (Berbee and Taylor, 1993) or the early ascomycetes (Taylor et al., 1994). In another phylogenetic analysis by Sugiyama et al. (Fig. 3 in 1996a), the ascomycetous yeasts and filamentous fungi are shown to be monophyletic, whereas the archiascomycetes may not be monophyletic, although enjoying fairly high bootstrap support (ca. 81% in the NJ tree). Common characters of the eight species to define "Archiascomycetes" are limited to date (Table 1). The "Archiascomycetes" is characterized by as follows: its assimilative state is hyphal or yeastlike; its reproductive state is sexually ascogenous (but lacking ascogenous hyphae), and asexually it shows budding or fission; neither ascomata nor conidiomata are formed.

Subsequently, Landvik et al. (1993) and Landvik (1996) placed the apothecial ascomycetes *Neolecta vitellina* (Bresadola) Korf & J. K. Rogers and *N. irregularis* (Peck) Korf & J. K. Rogers in the basal ascomycete lineage defined by Berbee and Taylor (1993). Integrated analysis of genotypic (the full sequence of 18S rRNA gene from 14 *Taphrina* and 2 *Protomyces* species) and phenotypic (cell wall sugar composition, ubiquinone system, cell wall ultrastructure, and mode of conidium ontogeny) has added *Neolecta vitellina* to the "Archiascomycetes" and reinforced the archiascomycete lineage

Table 1. Characters of archiascomycetes compared to other ascomycetes.[a,b]

Character	"Archiascomycetes"						
	Taphrina	*Protomyces*	*Saitoella*	*Schizosaccharomyces*	*Pneumocystis*	Saccharomycetales	Filamentous ascomycetes
Enveloping membrane system	I	?	−	I	V	I	V
Forcibly discharged asci	+/−	+/−	−	−	−	−	+/−
Woronin bodies	?	?	−	−	−	+/−	+
Simple septal pore	+	?	−	−	−	+/−	+
Chitin	+[c]	+[c]	+[c]	+[d]	+[e]	+	+
Major ubiquinone system[f]	Q-10	Q-10	Q-10	Q-10	?	Q-6~9	Q-9, 10, 10(H_2), 10(H_4)
Ascogenous hyphae	−	−	−	−	−	−	+
Ascoma	−	−	−	−	−	−	+
Ploidy of somatic cells	n+n	2n	n	n	n/2n	n/2n	n

a,b) Modified from Alexopoulos et al. (1996), and Sugiyama and Nishida (1995).
c) Data from Nishida and Sugiyama (1994b).
d) Data from Bowen et al. (1992), and Sietsma and Wessels (1990).
e) Data from Garner et al. (1991).
f) Data from Kuraishi et al. (1991).
Abbreviations: I, associated with individual nuclei; V, enclosing all nuclei; +, present; −, absent; ?, no data.

(Sjamsuridzal et al., 1997). The newest NJ analysis (Figs. 3A, B) has placed the archiascomycete lineage within the Ascomycota with fairly high bootstrap confidence (76%). All the trees (NJ, MP, and ML) show that, within the archiascomycete lineage, 11 of the 14 *Taphrina* species and the 4 *Protomyces* species are monophyletic, and a core group of *Taphrina* and *Protomyces* is always monophyletic (Sjamsuridzal et al., 1997). Their analyses suggest that the strictly anamorphic yeast *Saitoella complicata* groups with the apothecial ascomycete *Neolecta vitellina* rather than the *Taphrina/Protomyces* branch. Asci of *N. vitellina* are occasionally filled with numerous conidia and the ascospores become conidiogenous by producing a single apical collarette from which the phialoconidia are formed (Fig. 4; Redhead, 1977). These features are similar to those of *Taphrina*, and therefore may not conflict with the proposed molecular phylogeny. It is still uncertain whether *Neolecta* forms ascogenous hyphae or if its anamorphic state is yeastlike, and further studies are needed to assign this genus within the "Archiascomycetes" and also to find the closest relatives. Very recently the class "Archiascomycetes" has been hierarchically classified by Kurtzman (1998b), and Kurtzman and Sugiyama (unpublished). The class "Archiascomycetes" accommodates the four orders Schizosaccharomycetales Prillinger et al. ex Kurtzman (incl. Schizosaccharomycetaceae), Taphrinales Gäumann & Dodge (incl. Taphrinaceae), Prorotomycetales Luttrell ex D. Hawksworth & O. E.

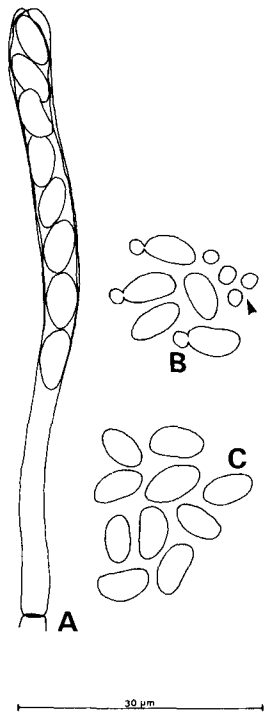

Fig. 4. *Neolecta vitellina* (Bresadola) Korf & J. K. Rogers. A, ascus with apical nasse (TRTC 10076). B, ascospores (S.A.R. 1794); arrowhead indicates ascospores producing conidia. C, ascospores (S.A.R. 1430). From Redhead, 1977; Copyright 1977 NRC Research Press; Reprinted with permission from the copyright holder and the author.

Erikkson (incl. Protomycetaceae and the anamorphic genus *Saitoella*, incertae sedis), and Pneumocystidales O. E. Eriksson (incl. Pneumocystidaceae). Following the work of Edman et al. (1988: 18S rRNA sequence) and Watanabe et al. (1989: 5S rRNA sequence), the molecular phylogeny and systematic position of *Pneumocystis carinii* (Delanoë and Delanoë, 1912) have been repeatedly discussed (Taylor and Bowman, 1993; Taylor et al., 1994; Wakefield et al., 1993). However, the closest relatives of *P. carinii* still remain uncertain.

Fungal group I introns newly found within the nuclear 18S rRNA gene (Nishida and Sugiyama, 1995; Nishida et al., 1993, 1998) in the archiascomycetous *Protomyces* species provide a good model for studies on horizontal or lateral gene transfer in the course of evolution due to interactions between the plant parasitic fungus and its host. On the basis of Berbee and Taylor's calibration (1993), *Protomyces* diverged approximately 30–40 million yr ago (mya), and the group I intron has been suggested to be transferred from the parasite to the host roughly 3–4 mya (Nishida et al., 1998).

The hemiascomycete lineage As mentioned above, the Ascomycota is composed of three major lineages, i.e., the archiascomycetes, hemiascomycetes (ascomycetous yeasts), and euascomycetes (filamentous ascomycetes). The latter two lineages appear to be monophyletic sister groups and the newest NJ tree (Fig. 3B) also indicates that the hemiascomycetes form a monophyletic group that is well supported by bootstrapping (99%). The hemiascomycetes as the formal class, based on the single order Saccharomycetales Kudryavtsev (=Endomycetales Gäumann), has now become phylogenetically circumscribed to include both the budding yeasts and yeastlike genera such as *Ascoidea* and *Cephaloascus* (Kurtzman and Fell, 1998). As a noteworthy result of our recent research (Sjamsuridzal et al., 1997), the evidence from molecular and phenotypic characters such as cell wall sugar composition, ubiquinone system, cell wall ultrastructure, and mode of conidium ontogeny, strongly suggests that *'Taphrina' farlowii* Sadebeck CBS 376.39 be excluded from the "Archiascomycetes" and placed with *Candida albicans* (C. P. Robin) Berkhout in the Saccharomycetales.

The euascomycete lineage The euascomycetes with comparatively well-developed ascomata comprise the plectomycetes, pyrenomycetes, loculoascomycetes, laboulbeniomycetes, and discomycetes based on traditional classifications. In the monophyletic euascomycete lineage (98% bootstrap support in NJ tree (Fig. 1) in Ogawa et al., 1997; Fig. 3A of this paper), two major lineages, the plectomycetes with the closed ascomata (cleistothecia) and pyrenomycetes with the flask-shaped ascomata (perithecia), appeared to be monophyletic, each receiving 99% bootstrap support. The tree topologies in Figs. 3A, C, D supported the monophyly of plectomycetes and pyrenomycetes as already detected by Berbee and Taylor (1992b) and also supported by our analyses (Nishida and Sugiyama, 1994b; Ogawa et al., 1997).

The plectomycete group The morphology-based class Plectomycetes of Fennell (1973) was defined only on the basis of the cleistothecium and diagnostic anamorphs (cf. Benny and Kimbrough, 1980) and was composed of nine families, i.e., Amorphothecaceae, Gymnoascaceae, Onygenaceae, Monascaceae, Thermoascaceae, Trichocomaceae, Eurotiaceae, Cephalothecaceae, and Pseudeurotiaceae, in the single order Eurotiales. Her concept for the the class was overturned by Malloch (1979, 1981; cf. Malloch and Cain, 1972) with emphasis on centrum structure and evolutionary simplifications and modifications. Malloch (1979, 1981) revised plectomycete taxonomy and included plectomycetous genera distributed among 19 families of Pyrenomycetes, Discomycetes, and Loculoascomycetes. Subsequently Benny and Kimbrough (1980) redefined the Plectomycetes with emphasis on centrum development and mode of discharge of the asci, and recognized six orders (Ascosphaerales, Elaphomycetales, Eurotiales, Microascales, Onygenales, and Ophiostomatales), including 12 families developing a plectomycete centrum. In recent years molecular data are adding a new dimension to understanding of the relationships between the different ascomycete orders and higher taxonomic categories (Hawksworth et al., 1995).

The plectomycete family Trichocomaceae includes cleistothecial teleomorphic genera which are associated with economically important anamorphs, such as *Penicillium*, *Geosmithia*, *Merimbla*, *Aspergillus*, *Paecilomyces*, and related genera (Malloch and Cain, 1972, 1973; Pitt, 1979a, 1995; Pitt and Samson, 1993; Samson, 1974). Phylogeny of *Penicillium* and related taxa producing a penicillus is of considerable interest because of the diversity of cleistothecial ascomata and ascospore types (Malloch and Cain, 1972; cf. Berbee et al., 1995). The teleomorphic genera associated with *Penicillium* are *Talaromyces*, *Hamigera*, *Eupenicillium*, *Trichocoma*, *Penicilliopsis* and *Chromocleista* (Malloch and Cain, 1972; Stolk and Samson, 1971, 1985; Yaguchi et al., 1993).

Our systematic research in *Aspergillus*, *Penicillium* and related teleomorphs developed abreast of chemotaxonomic work on these taxa (Kuraishi et al., 1990, 1991; Sugiyama and Yamatoya, 1990; Sugiyama et al., 1991a; Tamura et al., 1996; Yamatoya et al., 1990). Our molecular phylogenetic studies of these fungal groups go back to a partial sequence analysis of 18S rRNA from 11 selected species of *Aspergillus* and related teleomorphic genera by Chang et al. (1991). From 18S, 5.8S and ITS rDNA sequence analyses, Berbee et al. (1995) have detected that species of *Penicillium* are not monophyletic; one group diverged first within the Trichocomaceae cluster and contains *Talaromyces* spp. grouping with the *Penicilium*-producing *Talaromyces flavus* (Köcker) Stolk & Samson var. *macrosporus* Stolk & Samson and the *Geosmithia*-producing *T. bacillisporus* (Swift) C. R. Benjamin, whereas another group contains the *Penicillium*-producing *Eupenicillium javanicum* (van Beyma) Stolk & Scott, the *Aspergillus*-producing *Eurotium rubrum* König, Spieckermann & Bremer, the *Aspergillus*-producing *Neosartorya fischeri* (Whemer) Malloch

& Cain, and the *Basipetospora*-producing *Monascus purpureus* Went. Further, their phylogeny was consistent with that based on reexamination of 18S rRNA partial sequences, comprising a total of 558 nucleotides, by Chang et al. (1991; cf. Berbee et al., 1995).

Recently we (Ogawa et al., 1997) focused on molecular phylogeny of the anamorphic genus *Geosmithia* (Pitt, 1979b), which has been segregated from *Penicillium* and has the type species *G. lavendula* (Raper & Fennell) Pitt. *Geosmithia* includes species lacking a teleomorph as well as species associated with the teleomorphs *Talaromyces* and *Chromocleista*. Our NJ analysis of 1,586 sites of the 18S rRNA genes from 57 selected taxa within the Ascomycota, clearly detected that species of *Geosmithia* are polyphyletic with evolutionary affinities to at least three groups of the euascomycetes: 1) Group one is a monophyletic clade of pyrenomycetes with strong bootstrap support (100%) containing *Geosmithia lavendula*, the type of the genus, *G. putterillii* (Thom) Pitt and the hypocrealean fungi, *Gliocladium*-producing *Hypocrea lutea* (Tode) Petch and *Verticillium*/*Sepedonium*-producing *Hypomyces chrysospermus* Tulasne; 2) Group two is not as well supported (57% bootstrap) and it includes the plectomycetes *G. cylindrospora* (G. Smith) Pitt and the *Geosmithia*-producing *Taralomyces* species (i.e., *T. bacillisporus*, *T. ebruneus* Stolk, and *T. emersonii* Stolk) along with the *Penicillium*-producing *T. flavus* var. *macrosporus* of the Trichocomaceae; 3) Group three is better supported (74% bootstrap) and contains *G. namyslowskii* (Zaleski) Pitt, the *Geosmithia*-producing *Chromocleista malachitea* Yaguchi & Udagawa, the *Penicillium*-producing *Eupenicillium crustaceum* Ludwig and the *Merimbla*-producing *Talaromyces avellaneus* Thom & Turesson (≡*Hamigera avellanea* (Thom & Turesson) Stolk & Samson). The bootstrapped NJ and MP analyses, using 1,706 sites of the same gene only from plectomycetes and pyrenomycetes, demonstrate similar phylogenetic relationships. The bootstrapped NJ and MP analyses of 70 sites of 5S rDNA supported the results from the18S rDNA sequence analyses. Our 28S rDNA sequence (580 sites)-based NJ and MP analyses (Ogawa et al., l.c.) showed that, within the Hypocreales, *Geosmithia lavendula* and *G. putterillii* grouped with the hypocrealean fungi, cleistothecial, *Acremonium*-producing *Mycoarachis inversa* Malloch & Cain and *Emericellopsis terricola* van Beyma, and the strictly anamorph species of *Acremonium* in 66% or greater bootstrap support. The three cleistothecial genera *Roumegueriella* (with a *Gliocladium* anamorph), *Mycoarachis* (with an *Acremonium* anamorph), and *Emericellopsis* (with an *Acremonium* anamorph), which are characterized by the plectomycete centrum and produce phialoconidia, should be placed within the pyrenomycete family Hypocreaceae (Ogawa et al., 1997; Rehner and Samuels, 1994, 1995; cf., Spatafora and Blackwell, 1993). Another cleistothecial species, *Pseudallescheria boydii* (Shear) McGinnis, has already been placed solidly within the pyrenomycete group (Berbee and Taylor, 1992a).

Our 18S rRNA gene phylogeny (Fig. 1 in Ogawa et al., 1997) supports that species of *Penicillium*, *Geosmithia*, *Aspergillus*, *Paecilomyces*, *Basipetospora* and related teleomorphic genera, which are assignable to the Trichocomaceae sensu Malloch and Cain (1972, 1973), appeared to be monophyletic with 100% bootstrap support. However, the confidence level is low (23%) in Fig. 3C. The tree topologies in Fig. 3C, on the whole, do not conflict with earlier analyses by Berbee et al. (1995) and Ogawa et al. (1997) or with groupings based on the type of cleistothecial ascomata (Malloch and Cain, 1972; Pitt, 1979a) and the major ubiquinone systems (Kuraishi et al., 1985, 1991). The branch comprising two hypogeous fungi *Elaphomyces maculans* Vittad. and *E. laveillei* Tulasne in addition to the trichocomaceous taxa is highly supported (93% in Fig. 3C). It suggests that trichocomaceous fungi and *Elaphomyces* spp. share a common ancestor (Landvik et al., 1996; LoBuglio et al., 1996). In the NJ tree (Figs. 3A, C), two *Capronia* members of the loculoascomycete family Herpotrichiellaceae, *C. mansonii* (Schol-Schwarz) E. Müller and *C. pilosella* (Karsten) E. Müller are basal to the plectomycetes (Berbee, 1996; Untereiner et al., 1995). Berbee's parsimony analysis (1996) based on 1,509 sites 18S rRNA gene sequence indicated that *C. pilosella* clustered with the plectomycetes at the 99% confidence level. She states: "This implies that convergence in ascus morphology must have occurred." Cladistic analysis of 1,050 sites 18S rRNA gene sequence supported that a clade of dematiaceous hyphomycetes represented by *Exophiala mansonii* (Castell.) de Hoog (≡*Rhinocladiella mansonii* (Castell.) Schol-Schwarz) and *Wangiella dermatitidis* (Kano) McGinnis (≡*Exophiala dermatitidis* (Kano) de Hoog) was a sister group to a clade comprising members of two orders of cleistothecial ascomycetes, Eurotiales and Onygenales (Spathafora et al., 1995).

Our recent molecular phylogenetic analyses have extended to the *Penicillium*-producing *Trichocoma paradoxa* Junghuhn and the *Sarophorum*-producing *Penicilliopsis clavariiformis* Solms-Laubach (Ogawa and Sugiyama, 1997), and the respective type species from 18 sections in *Aspergillus* and related teleomorphs (Tamura et al., 1997), including the elucidation of the identity of the xerophilic species *Aspergillus penicillioides* Spegazzini. And, our analyses of molecular characters from 18S rDNA in *Paecilomyces, Sagenomella*, and related teleomorphs are now in progress.

The pyrenomycete group Members of Pyrenomycetes usually produce perithecial ascomata, i.e., ostiolate fruitbodies characterized by having paraphyses and inoperculate evanescent asci with an apical pore or slit (Ainsworth, 1973). This group, represented by *Neurospora crassa* Shear & B. Dodge, *Chaetomium elatum* Kunze & Schmidt: Fries, *Colletotrichum gloeosporioides* Penzig, *Hypomyces chrysospermus* Tulasne, *Microascus cirrosus* Curzi, *Ophiostoma ulmi* (Buisman) Nannfeldt, and *Leucostoma persoonii* (Nitschke) Höhnel, appeared to be monophyletic (Figs. 3A, D). The previous sequence analyses based on 18S rRNA genes (e.g., Berbee and Taylor, 1992a, 1992b; Berbee et al., 1995; Nishida and Sugiyama, 1994b; Ogawa et al., 1997) and 5S rRNA

(Hori and Osawa, 1987) supported the monophyly of pyrenomycetes.

Other euascomycete groups Analyses of 18S rRNA gene sequences support that both the loculoascomycetes (fissitunicate ascomycetes) and the discomycetes (apothecial ascomycetes) are not monophyletic (e.g., Berbee, 1996; Berbee et al., 1994; Gargas and Taylor, 1995; Ogawa et al., 1997). Within the former group, however, the loculoascomycete order Pleosporales appears as a monophyletic group including the families Pleosporaceae and Lophiostomataceae, and the loculoascomycete order Dothideales may also constitute a monophyletic group without statistical support (Berbee, 1996). As pointed by Taylor et al. (1994), among the filamentous ascomycetes with ascomata, there is an early radiation comprising the apothecial ascomycetes represented by *Morchella*, *Gyromitra* and *Inermisia* and the loculoascomycetes represented by the Pleosporales and Dothideales radiated earlier than cleistothecial and perithecial ascomycetes (Berbee, 1996; Gargas and Taylor, 1995). The newest NJ analysis of 18S rRNA gene (Figs. 3A, E) supports these evolutionary relationships but without statistical support.

According to a recent model proposed based on partial sequences of 18S rDNA for the evolution of the Laboulbeniales, the *Pyxidiophora-Rickia* lineage of the laboulbeniomycetes (obligate parasites of arthropods) lies outside the other perithecial ascomycetes among loculoascomycetes and discomycetes where taxon sampling is still incomplete (Blackwell, 1994). At the moment, therefore, previous hypotheses including *Pyxidiophora* in the Hypocreales, the Ophiostomatales, and the yeasts, including *Cephaloascus* and *Ambrosiozyma*, are not supported (Blackwell, l.c.).

Phylogenetic divergence of the basidiomycetes and their anamorphs

To date basidiomycetous yeasts represented by *Rhodosporidium*, *Leucosporidium*, *Sporidiobolus*, *Filobasidium* and *Filobasidiella* have been well characterized phenotypically and genotypically (for references, see Boekhout et al., 1993; Kurtzman and Fell, 1998; cf. Figs. 5, 6). We (Sugiyama and Nishida, 1995; Sugiyama and Suh, 1993; Sugiyama et al., 1993, 1996a, 1997; Suh and Sugiyama, 1993a, b, 1994) have focused on the phylogeny and evolutionary relationships of the basidiomycetous yeasts and relatives from integrated analysis of both genotypic and phenotypic characters including chemotaxonomic and ultrastructural data (e.g., Hamamoto et al., 1986a, b, 1987; Sugiyama et al., 1985, 1991b, 1997; Suh et al., 1993a, b). Recent 18S rDNA sequence analyses have shed light on the phylogenetic framework of basidiomycetes including basidiomycetous yeasts (Nishida and Sugiyama, 1994a, b; Nishida et al., 1995; Sjamsuridzal et al., 1997, 1999; Suh and Sugiyama, 1993a, b, 1994; Swann and Taylor, 1993; 1995a, b). The molecular phylogeny divides the Basidiomycota into three major lineages, each of which receives a relatively high bootstrap confidence level (82–100% in the NJ tree in Sjamsuridzal et al., 1997; cf., Fig. 1 in the parsimony tree in Swann and Taylor, 1995b). These are the ustilaginomycetes (smut fungi), urediniomycetes (rust fungi and teliospore-forming yeasts), and hymenomycetes (mushrooms). From the analysis of the 18S rRNA gene as a primary taxonomic character, these major lineages were newly re-classified by Swann and Taylor (1995a, b) as the classes Ustilaginomycetes, Urediniomycetes and Hymenomycetes, respectively. The newest bootstrapped NJ analysis of aligned 1,613 sites of the 18S rRNA gene (Fig. 6) supports those demonstrated previously. However, the diverging order at a class level still remains uncertain because of the lack of statistical support.

The ustilaginomycete lineage The first major lineage (Ustilaginomycetes sensu Swann and Taylor, 1995a, b; A in Figs. 5, 6) is composed of the smut fungi (Ustilaginales), represented by *Ustilago maydis* (de Candolle) Corda, *U. hordei* (Persoon) Lagerheim and *Tilletia caries* (de Candolle) Tulasne (Tilletiales), including the strictly anamorphic yeast *Sympodiomycopsis paphiopedili* Sugiyama, Tokuoka & Komagata (Sugiyama and Suh, 1998; Sugiyama et al., 1991b). The type species *Graphiola phoenicis* (Mougeot) Poiteau (Cole, 1983; Oberwinkler, 1993; Oberwinkler et al., 1982; Tubaki and Yokoyama, 1971); and *G. cylindrica* (Kobayasi, 1952) of the Graphiolaes, both parasitizing palm leaves in the tropical and subtropical region, group together with the smut fungi (Sugiyama et al., 1997). The small taxon samples of ustilaginomycetes seem to form a monophyletic group, which may be basal to other basidiomycetes.

The urediniomycete lineage The second major lineage (Urediniomycetes sensu Swann and Taylor, 1995a, b; B in Figs. 5, 6) includes the smut-like, teliospore-forming yeast species *Leucosporidium scottii* Fell, Statzell, Hunter & Phaff, *Rhodosporidium toruloides* Banno, *Sporidiobolus johnsonii* Nyland, *Erythrobasidium hasegawianum* Hamamoto, Sugiyama & Komagata (Hamamoto et al., 1988, 1991; Sugiyama and Hamamoto, 1998; Sugiyama and Suh, 1993) and *Kondoa malvinella* (Fell & I. L. Hunter) Yamada, Nagahama & Banno (≡*Rhodosporidium malvinellum* Fell & I. L. Hunter), which are characterized by a simple septal pore, and no xylose in the cell wall (cf. Table 5). *Mixia osmundae* (T. Nishida) Kramer, a *Taphrina*-like parasitic fungus previously accommodated in the Ascomycota, and the rust fungi *Cronartium ribicola* J. C. Fischer and *Peridermium harknessii* J. P. Moore are included in this lineage (Nishida et al., 1995). Among these fungi *Mixia* is phylogenetically unique. The research history about *Mixia* and *M. osmundae* has been reviewed by Sugiyama and Nishida (1995), and Sugiyama et al. (1996a). Therefore, a brief note is given herein.

The *Mixia*-basidiomycete connection Savile (1968) wrote, "Kramer (1958) later reexamined the two species of *Taphrina* attacking *Osmunda* (the most ancient ferns). He has assigned them to a more primitive genus, *Mixia*, which almost closes the gap between Taphrinales and the borderline phycomycetes." Soon after we sequenced *Taphrina wiesneri* (Ráthay) Mix and *Saitoella complicata*

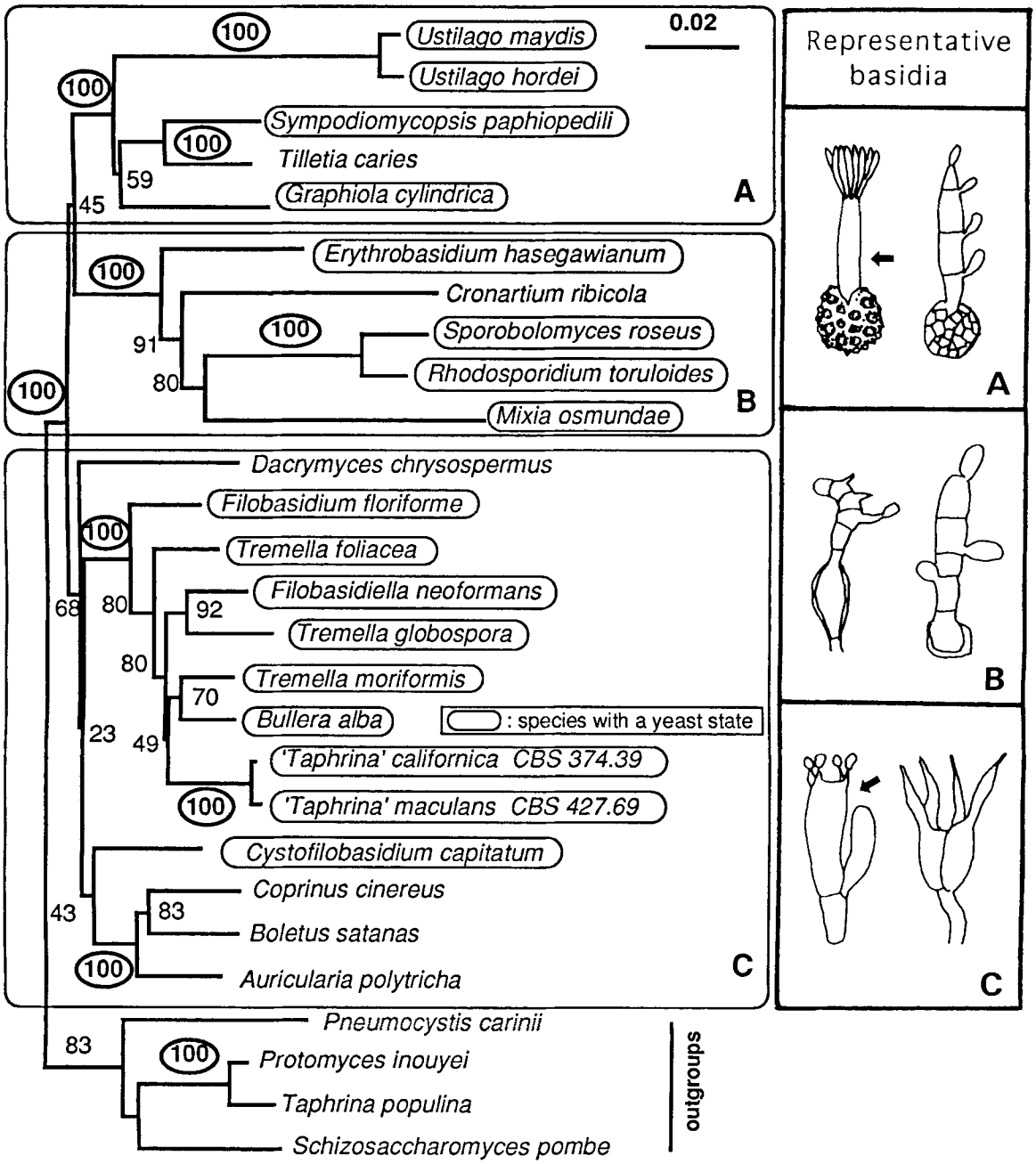

Fig. 5. Bootstrapped NJ (Saitou and Nei, 1987) tree, inferred from 1,613 alignable sites of the 18S rRNA gene sequence data set and using the selected four archiascomycetes as outgroups, shows phylogenetic relationships of basidiomycetous yeasts to other basidiomycetous fungi.

Representative basidia of three major lineages are shown at the right side. All gaps were excluded. The multiple alignment was performed using Clustal W ver. 1.7 (Thompson et al., 1994), and the distance matrix for the aligned sequences was calculated using the two-parameter method by Kimura (1980). Bootstrap values derived from 1,000 replications are shown as percentages. The scale bar indicates two base changes per 100 nucleotide positions. Arrow indicates the holobasidium, and others the phragmobasidium. In B and C (representative basidia), thick-walled, resting teliospores germinate and produce the promycelium bearing sporidia (corresponding to basidiospores). For sequence data, see Sjamsuridzal et al. (1997) and Sugiyama et al. (1997).

Goto et al., we chose *Mixia osmundae* as a new target in 18S rDNA sequencing to determine the phylogenetic placement of the major groups of the higher fungi. Strain IFO 32408, listed as *Taphrina osmunda* T. Nishida (1911) (the correct name is now *Mixia osmundae* (T. Nishida) Kramer in the Mixiaceae Kramer, 1987) in the IFO List of Cultures, 9th Edition, p. 426, 1992. We sequenced 1,780 nucleotides from this isolate in 1992 and

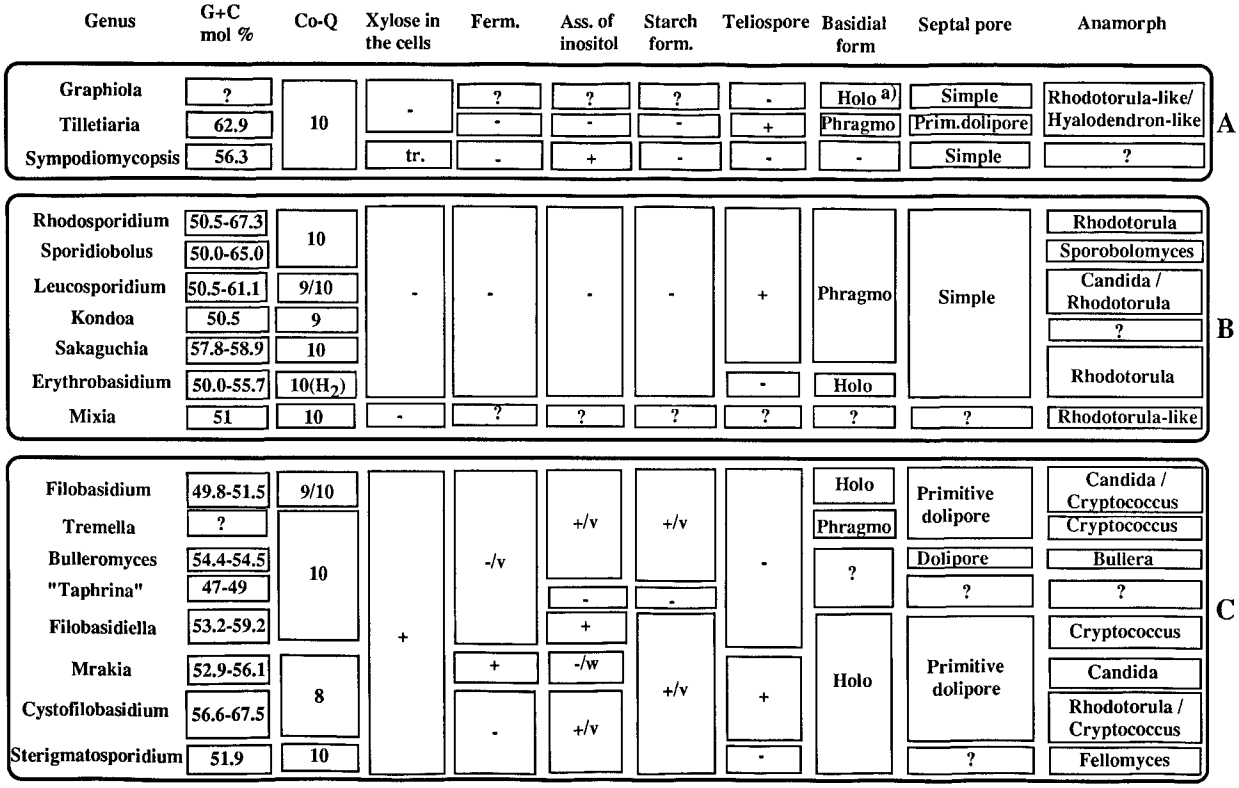

Fig. 6. Characteristics of the selected genera and species of basidiomycetous yeasts.
Abbreviations: +, positive reaction or presence; −, negative reaction or absence; v, variable; ? no data or unknown. For references, see Kurtzman and Fell (1998), Suh and Sugiyama (1993a, 1993b), and Sugiyama et al. (1991b, 1997) in addition to Sugiyama and Suh (1993).

added the sequence to our molecular phylogeny, in which *T. osmundae* was indicated as strain X (Nishida and Sugiyama, 1994a) or IFO 32408 (Sugiyama et al., 1996a). Surprisingly this isolate was clustered together with *Rhodosporidium toruloides*, and *Leucosporidium scottii*. The topology was well supported by both bootstrapped NJ and MP analyses. In May 1993 we were successful in collecting fresh material of *Mixia osmundae* growing on *Osmunda japonica*. Evidence from both molecular and morphological characters, resulting from the collaborative work of five people (Nishida et al., 1995; Sugiyama and Nishida, 1994), suggested that *M. osmundae* is not a member of the ascomycetes and not related to either the Taphrinales or Protomycetales. *Mixia osmundae* is a member of the basidiomycetes and is placed among the two rusts *Cronartium ribicola* and *Peridermium harknessii* and *Erythrobasidium hasegawianum* within the simple septate basidiomycete lineage (=the urediniomycete lineage). In this research we could not specify the unique sporangium of *M. osmundae*. If its exogenously produced spores are meiospores, the meiosporangium is the holobasidium which is quite unique in the Basidiomycota. Very recently the identity of *M. osmundae*'s sporangium has been considerably elucidated in a collaborative work with Prof. F. Oberwinkler and Dr. R. Bauer of Universität Tübingen, Germany. The results will be published elsewhere in the near future.

Molecular phylogenetics in ballistoconidium-forming yeasts and relatives have been developed by Nakase's group of Japan Collection of Microorganisms, RIKEN (e.g., Nakase et al., 1991; Sugita and Nakase, 1998; Suh and Nakase, 1995; Suh et al., 1996a, b; Takashima and Nakase, 1996; Takashima et al., 1995).

The hymenomycete lineage The third major lineage (Hymenomycetes sensu Swann and Taylor, 1995a, b) is supported with fairly high statistical support (C in Figs. 5, 6). It is formed by the filobasidiaceous yeast species, *Cystofilobasidium capitatum* (Fell, Hunter & Tallman) Oberwinkler & Bandoni, *Mrakia frigida* (Fell, Stazell, I. L. Hunter & Phaff) Yamada & Komagata, *Filobasidium floriforme* L. S. Olive, and *Filobasidiella neoformans* (Sanfelice) Vuillemin (anamorph: *Cryptococcus neoformans* (Sanfelice) Vuillemin), and the ballistospore-forming yeast species *Bulleromyces albus* Boekhout & Fonseca (anamorph: *Bullera alba* (Hanna) Derx), and the arthroconidium-forming yeast species *Trichosporon cutaneum* (de Beurmann, Gougerot & Vaucher) Ota. Members of the heterobasidiomycetes *Tremella*, *Dacrymyces* and *Auricularia*, and selected hymenomycetous taxa represented by *Athelia bombacina* Persoon, *Coprinus cinereus* (Schaeffer: Fries) S. F. Gray, *Boletus satanas* Lenz, and *Spongipellis unicolor* (Schweinitz) Murrill are also included in this lineage. In addition to these,

'Taphrina' californica Mix CBS 4374.39 and *'T.' maculans* Butler CBS 427.69 are related to the Tremellales (Sjamsuridzal et al., 1997; Figs. 5, 6 in this paper). Although the stalk conidium-forming teleomorphic yeast *Sterigmatosporidium polymorphum* Kraeplin & Schulze is not shown in Fig. 5, this taxon indicates a close relationship with *Kockovaella thailandica* Nakase, Banno & Y. Yamada and *Fellomyces polyborus* (D. B. Scott & van der Walt) Y. Yamada & Banno, which group with the tremellaceous fungi (Fig. 1 in Suh et al., 1996a; cf. Fig. 6 of this paper and Statzell-Tallman, 1998).

Traditionally the mode of basidium ontogeny and basidium morphology have been adopted as the primary source of characters to classify the basidiomycetes (Oberwinkler, 1982). There are two types of basidia, the holobasidium and the phragmobasidium. However, recent molecular phylogenetic data demand re-evaluation of the basidial morphology as a taxonomic character (e.g., Sugiyama and Suh, 1993; Suh and Sugiyama, 1994; Swann and Taylor, 1993, 1995a, b). Our targets for molecular basidiomycete phylogenetics are now extended to the whole urediniomycetes (Sjamsuridzal et al., 1999).

Pleomorphy, nomenclature, and molecular phylogeny

The pleomorphy is a marked characteristic of the higher fungi, i.e., ascomycetes, basidiomycetes, and deuteromycetes (Sugiyama, 1987; cf., 1998). Article 59 of the current International Code of Botanical Nomenclature (ICBN; Greuter et al., 1994; cf., Sugiyama, 1998) permits the use of dual nomenclature for the pleomorphic fungi, i.e., two names, one for the sexual (meiotic) reproductive state (teleomorph) and an optional name for the asexual (mitotic) reproductive state (anamorph). "Under the dual nomenclatural system, fungi exhibiting the structures associated with sexual reproduction are classified in the kingdom Fungi, but those that lack evidence of sexual reproduction are classified in the formal taxon (Deuteromycota or) Deuteromycotina" (Reynolds and Taylor, 1993, in Foreword; cf., Taylor, 1995). However, the molecular characters (both 5S rRNA and 18S rRNA sequences) do not support the separate placement of the deuteromycetes as a higher taxon (e.g., Figs. 3A–E). As mentioned above, deuteromycetes are phylogenetically assigned to either the Ascomycota or Basidiomycota (cf. Fig. 3E). It may suggest that "Asexual organisms do not contribute to the major events of evolution." (LoBuglio et al., 1993; Reynolds and Taylor, l.c.) More discussion is addressed below.

The tree topologies constructed by Geiser et al. (1996; cf. Geiser et al., 1998) using the mitochondrial small ribosomal subunit, the nuclear ribosomal ITS, and the nuclear 5.8 rRNA gene revealed that "asexual Aspergilli [sic] are often recently derived from meiotic lineages, and do not give any strong support to the existence of ancient asexual lineages." From sequence analyses of the same or different molecules (genes), such patterns of evolution have been seen in *Aspergillus* and related teleomorphs (Peterson, 1993, 1997a; Tamura et al., 1997), *Penicillium*, *Geosmithia* and their related *Talaromyces* and *Eupenicillium* teleomorphs (LoBuglio et al., 1993; Ogawa and Sugiyama, 1997; Ogawa et al., 1997; Peterson, 1995, 1997b), plectomycete dermatophytes, and *Oidiodendron* and related *Myxotrichum* teleomorphs (Bowman and Taylor, 1993; Hambleton et al., 1998), *Fusarium* and related *Gibberella* and *Nectria* teleomorphs (Guadet et al., 1989; O'Donnell, 1993; O'Donnell et al., 1998a), *Gilocladium*, *Trichoderma* and their *Nectria* and *Hypocrea* teleomorphs (Rehner and Samuels, 1994, 1995), *Acremonium* and its related hypocrealean teleomorphs (Glenn et al., 1996), rust fungi (Vogler and Bruns, 1993), and ascomycetous and basidiomycetous yeasts (for references, see Kurtzman and Fell, 1998). These data suggest that meiotic sex is lost frequently in the plectomycete family Trichocomaceae and order Onygenales, and the pyrenomycete order Hypocreales. Recent advances in fungal molecular systematics have clarified that "molecular characters offer the potential for combining the dual classification into one natural classification." (Reynolds and Taylor, 1993, in Foreword; cf. Taylor 1995.) Group Discussion entitled "What are the consequences of abandoning the deuteromycetes ?" at the Fungal Holomorph Conference in Newport, Oregon, August 1992 commented, "Mitotic (deuteromycete) names should be retained. Retention at the generic level is feasible. Mitotic genera could be placed in meiotic families." (Reynolds and Taylor, l.c.) I agreed with this statement at the Conference. I think that anamorphic genus and species names of the deuteromycetes are necessary for purposes of identification and practice (cf., Gams, 1995). Retention of the dual nomenclature for pleomorphic fungi (ICBN, Art. 59) at the genus-species level is essential for economically important deuteromycetes.

A problem concerning DNA as the type specimen has been considered by Reynolds and Taylor (1991a, b, 1992), and Haines and Cooper (1993). An example (the anamorphic yeast *Saitoella complicata*) of the use of rDNA sequence data in fungal diagnoses and descriptions has been provided by Sugiyama et al. (1993).

Phylogenetic divergence of lichen-forming fungi

The lichen lifestyle is the symbiotic association of fungi with photosynthetic members such as the green algae *Trebouxia*, *Pseudotrebouxia*, and *Trentepohlia*, and the cyanobacterium *Nostoc* (Raven et al., 1992). It is found in various representatives of the higher fungi, both the Ascomycota and Basidiomycota. However, there are no lichenized hemiascomycetes, plectomycetes, or laboulbeniomycetes. Lichen-forming fungi constitute 17,000 or even 20,000 species, suggesting that only 50–70% of the world's species are currently known (Galloway, 1992; cf., Hawksworth, 1994). Lichens occur in a variety of habitats from the Arctic and the Antarctic and all regions, e.g., on soil and bare rock, on tree trunks, on frozen substrata in the polar regions, and on plant leaves particularly in the tropics. They constitute a significant fraction of known fungal diversity.

A recent phylogenetic analysis, based on 18S rDNA sequences, suggests multiple origins of lichen symbioses in fungi. A highly resolved parsimony analysis of 1927 nucleotides of 18S rDNA sequences from 75 representative fungi suggests at least five independent origins of the lichen habit in disparate groups of the Ascomycota and Basidiomycota. Within the Basidiomycota, the phylogenetic hypothesis supports three independent origins of the lichen habit indicated by *Multiclavula*, *Omphalina* and *Dictyonema*, each corresponding to groups supported by morphological characters (Gargas et al., 1995). Within the Ascomycota, it supports at least two independent origins of the lichen habit represented by members of the order Lecanorales, and *Arthonia radiata* (Persoon) Acharius and allied species of the order Arthoniales. This molecular phylogenetic analysis concludes neither mutualism nor parasitism should be construed as endpoints in symbiont evolution because lichen associations arose from parasitic, mycorrhizal, or free-living saprobic fungi. The concept of lichen is ecologically meaningful but not phylogenetically meaningful (Gargas et al., l.c.).

Fungus radiations and estimation of geological time of origins

From the molecular and fungal fossil evidence, Berbee and Taylor (1993) estimated the absolute timing of the origin of fungal groups. They used the percentage of nucleotide substitution that lineages have accumulated since divergence from a common ancestor to estimate the relative timing of origin of fungal lineages. They also used calibration points from the fossil record such as the appearance of fossilized fungal clamp connections. Clamp connections in fungal filaments first appear in the fossil record in woody tissue from a 290 million yr old Carboniferous fern.

From their estimates, the chytridiomycetes split from a lineage of terrestrial fungi that gave rise to the zygomycetes, ascomycetes, and basidiomycetes ca. 550 million yr ago (mya). After plants invaded the land ca. 440 mya, the ascomycetes split from the basidiomycetes. The three major lineages in the Ascomycota established perhaps during the coal age, from 330 to 310 mya. Mushrooms, many ascomycetous yeasts represented by the true yeast *Kluyveromyces lactis* (Dombrowski) van der Walt and baker's yeast *Saccharomyces cerevisiae* Meyen ex Hansen, and common molds such as the economically important species of *Aspergillus*-producing *Eurotium* and *Penicillium*-producing *Talaromyces* may have evolved after the origin of angiosperm plants and in the last 200 mya. The holobasidiomycetes, including mushrooms, radiated in the Cretaceous (ca. 130 mya). The remains of two gilled mushrooms (*Archaeomarasmius leggetti* Hibbett et al., gen. et sp. nov., resembles the extant genera *Marasmius* and *Marasmiella*) were recently discovered in mid-Cretaceous (90–94 mya) amber from central New Jersey, USA. The New Jersey fossils provide another calibration point for basidiomycete molecular clocks (Hibbett et al., 1995, 1997). Hibbett et al. (l.c.) state that this date is consistent with Berbee and Taylor's conclusions (1993).

Future perspectives in fungal systematics

The news about finding of mushroom fossils by Hibbett et al. (1995, 1997) surprised our nation (The Asahi Shinbun, evening ed., 12 October 1995). The impact from the fungal fossil record is great to fungal phylogenetics and evolution but the complete connections are rare. On the other hand, DNA is the macromolecule that harbors evolutionary information as well as genetic information (Hillis et al., 1996; Miyata, 1998). "Evolution, at the molecular level, is observable as nucleotide (or base) changes in the DNA and amino acid changes in proteins" (Ridley, 1996). Examples discussed in this review indicate that the 18S rRNA gene divergence has been an especially useful molecular character to elucidate the identity of fungal taxa and their evolutionary relationships. If there are the conflicts between the molecular and morphological characters, both molecular and morphological data should be re-examined, but often the morphological data have been misinterpreted. The anamorphic yeast *Saitoella complicata* and the phytopathogen *Mixia osmundae* provide examples. As stated above, recent 18S rDNA sequence analyses indicate the existence of two divisions within in the higher fungi, the sister groups Ascomycota and Basidiomycota. This relationship suggests that the ascus and the basidium, as meiosporangia, are phylogenetically meaningful. However, the evolutionary processes forming these meiosporangia are unclear. The molecular phylogenies do not support the existence of the deuteromycetes as a distinct higher taxon. Instead they integrate both anamorphs and teleomorphs into a molecular phylogenetic tree. Recently the fungal genome project has started. The genome of *Saccharomyces cerevisiae* has been sequenced (see Saccharomyces Genome Database, http://genome-www.stanford.edu/Saccharomyces/). "Fungal sequence of mitochondrial (chytrids, zygomycetes, dikaryomycetes, Oomycota; Paquin et al., 1997) and nuclear genomes (*Saccharomyces*, *Candida*, *Aspergillus*, *Magnaporthe*, *Neurospora* (see http://fungus.genetics.uga.edu:5080/main.html)) are providing new nucleic acid targets for phylogenetics and population genetics" (Taylor, 1998). I agree with his statement. Undoubtedly, molecular information from the fungal genome projects unite molecular variation with morphology and function. How to the use for data from the fungal genome project will sway the future of fungal systematics and phylogenetics. In the light of developments in molecular biological techniques, fungal herbarium specimens are now targets in molecular phylogenetics (Bruns et al., 1990). Finally, I sincerely hope that the future of fungal molecular systematics will be illuminated with the expectations of more dramatic findings.

Acknowledgements——Dr. Stan Hughes, my post-doctoral supervisor at the Plant Research Institute (now ECORC, Agricul-

ture & Agri-Food Canada), Central Experimetal Farm, Ottawa, introduced me the world of pleomorphic fungi through hyphomycetes and sooty moulds, and taught me the importance of collecting, microscopic observation and clear thinking as well as the mycologist's spirit. The late Dr. Hiroshi Hara, Emeritus Professor of the University of Tokyo and my M. Sc. and Ph. D. research supervisor, gave me guidance and encouragement. Dr. Shoichi Kawano, one of my colleages at the Botany Department of the University of Tokyo, introduced me to the extent of biosystematics and taught me the logic of scientific writing. Dr. Kazuo Komagata, Emeritus Professor of the University of Tokyo, and Dr. Hiroshi Kuraishi, the formerly Professor of the Tokyo Univesity of Agriculture and Technology, introduced me the world of microbial chemotaxonomy and provided counsel. The late Dr. Yosio Kobayasi, Dr. Keisuke Tubaki, Emeritus Professor of the University of Tsukuba, Dr. Masami Soneda, Dr. Hiroshi Fukushima, formerly Professor of the Yokohama City University, the late Dr. Hiroshi Iizuka, formerly Professor of the University of Tokyo, and Dr. Shoji Goto, Emeritus Professor of Yamanashi University, all introduced me the world of filamentous fungi and yeasts. A number of graduate students and research students (or research fellows) have contributed the work that has been summarized herein: Dr. Norihide Amano, Mr. J.-M. Chang, Dr. S.-W. Chiu, Mr. Masao Fukagawa, Mr. Takashi Inamura, Dr. W. H. Joo, Dr. Hiromi Nishida, Dr. Hiroyuki Ogawa, Dr. Takahiko Nagahama, Mr. Katsusuke Nagai, Dr. Endang S. Rahayu, Ms. Wellyzar Sjamsuridzal, Dr. S.-O. Suh, Mr. Yoshito Tajiri, Dr. Miki Tamura, Ms. Kaori Yamatoya-Osada, and Ms. Atsuko Yoshimura-Kawada. Especially Dr. Katsuhiko Ando, Dr. Yasuo Ando, Dr. Makiko Hamamoto, Dr. Aiko Hirata, Prof. Makoto Kakishima, Prof. Tsuneyoshi Kuroiwa, Dr. Hiroki Sato, Dr. Gen Okada, Assoc. Prof. Naruya Saitou, and Assoc. Prof. Y. Akira Yokota are acknowledged as collaborators. The late Dr. Alma Barksdale, Prof. Bob Bandoni, Dr. Mary Berbee, Prof. Meredith Blackwell, Prof. Paul Blanz, Dr. Stan Hughes, Dr. Clete Kurtzman, Dr. Takashi Nakase, Prof. Franz Oberwinkler, Dr. Steve Peterson, Dr. John Pitt, Prof. John Taylor, and Prof. Keisuke Tubaki have made critical reviews of the past manuscripts and provided constructive comments. The curators of ATCC, CBS, FRR, IFO, IMI, JCM, and NRRL kindly provided fungal cultures. Dr. Hiromi Nishida (Fig. 1), Dr. Hiroyuki Ogawa and Mr. Minoru Endo (Fig. 3), and Ms. Wellyzar Sjamsuridzal (Figs. 5, 6) are acknowledged for assistance in preparing the figures of this paper. Financial supports from the Ministry of Education, Science, Sports and Culture of Japan, Mitsubishi Chemical Corp., and Suntory Limited, are also acknowledged.

Finally I am grateful to Prof. John Taylor of the University of California, Berkeley, who kindly read through the manuscript and its proof sheets and corrected the English, and also provided valuable comments on the manuscript. Dr. Scott A. Redhead of ECORC, Agriculture and Agri-Food Canada has allowed me to use his illustrations (Fig. 4 of this paper) of *Neolecta vitellina*. My thanks are also due to Dr. Naruya Saitou for kindly providing a transcription of the Kimura reference.

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