Mycologia, 98(6), 2006, pp. 996–1005.

Early diverging Ascomycota: phylogenetic divergence and related evolutionary enigmas

Junta Sugiyama[1]

Tokyo Office, TechnoSuruga Co. Ltd., Ogawamachi Kita Building 4F, Kanda Ogawamachi 1-8-3, Chiyoda-ku, Tokyo 101-0052, Japan

Kentaro Hosaka

Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois 60605-2496

Sung-Oui Suh

Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803

Abstract: The early diverging Ascomycota lineage, detected primarily from nSSU rDNA sequence-based phylogenetic analyses, includes enigmatic key taxa important to an understanding of the phylogeny and evolution of higher fungi. At the moment six representative genera of early diverging ascomycetes (i.e. *Taphrina*, *Protomyces*, *Saitoella*, *Schizosaccharomyces*, *Pneumocystis* and *Neolecta*) have been assigned to ''Archiascomycetes'' *sensu* Nishida and Sugiyama (1994) or the subphylum ''Taphrinomycotina'' *sensu* Eriksson and Winka (1997). The group includes fungi that are ecologically and morphologically diverse, and it is difficult therefore to define the group based on common phenotypic characters. Bayesian analyses of nSSU rDNA or combined nSSU and nLSU rDNA sequences supported previously published Ascomycota frameworks that consist of three major lineages (i.e. a group of early diverging Ascomycota [Taphrinomycotina], Saccharomycotina and Pezizomycotina); Taphrinomycotina is the sister group of Saccharomycotina and Pezizomycotina. The 50% majority rule consensus of 18 000 Bayesian MCMCMC-generated trees from multilocus gene sequences of nSSU rDNA, nLSU rDNA (D1/D2), RPB2 and β-tubulin also showed the monophyly of the three subphyla and the basal position of Taphrinomycotina in Ascomycota with significantly higher statistical support. However to answer controversial questions on the origin, monophyly and evolution of the Taphrinomycotina, additional integrated phylogenetic analyses might be necessary using sequences of more genes with broader taxon sampling from the early diverging Ascomycota.

Accepted for publication 19 September 2006.

[1] Corresponding author. E-mail: jsugiyam@tecsrg.co.jp

Key words: basal Ascomycota, classification, evolution, molecular phylogeny

INTRODUCTION

The ascomycete subphylum Taphrinomycotina *sensu* Eriksson and Winka (1997) was based on the provisional class ''Archiascomycetes'' proposed by Nishida and Sugiyama (1993, 1994b). The taxon was based on nuclear small subunit (nSSU) rDNA sequence analyses, and it is an assemblage of the diverse early diverging Ascomycota. The group includes taxa that have been central to evolutionary theories concerning the origin of the Ascomycota and Basidiomycota. Among numerous phylogenetic hypotheses one by Savile (1955, 1968) on the phylogeny of higher fungi has attracted the attention of mycologists for more than 50 y; it is a logical hypothesis based on phenotypic characters, particularly those of comparative morphology (Kramer 1987, Sugiyama and Nishida 1995, Sugiyama 1998, Kurtzman and Sugiyama 2001). Savile (1955, 1968) proposed that *Taphrina* Fr. represented an early divergence within the higher Fungi (i.e. the present-day *Taphrina* was the closest survivor of ''*Prototaphrina*,'' a hypothetical genus and common ancestor of the Ascomycota and Basidiomycota). One major lineage led to the present day *Taphrina* and the Ascomycota, whereas another line led to the Basidiomycota (i.e. the Uredinales line and the parasitic Auriculariaceae via a ''Protobasidiomycete''). Cavalier-Smith (1987) proposed another hypothesis on the basis of ultrastructural and molecular (5S ribosomal RNA sequences) data; he suggested that the Taphrinomycetes (including Taphrinales, Schizosaccharomycetales and Protomycetales) evolved independently from a Saccharomycetes-Mucorales-Entomophthorales group by the total loss of chitin, and he placed the Taphrinomycetes and Saccharomycetes in the phylum Endomycota. Cavalier-Smith (2001) subsequently accommodated the Taphrinomycetes and Saccharomycetes as classes within the Hemiascomycotina. The existence of chitinous walls in species of *Taphrina* (Nishida and Sugiyama 1994a, Nishida 2005) and *Schizosaccharomyces* (e.g. Bowen et al 1992) had been shown with molecular techniques and refuted a hypothesis based on loss of chitin. Additional historical background on the phylogenetic and evolutionary theories related to *Taphrina* and the

TABLE I. Characteristics of the early diverging ascomycetes compared to other ascomycetes [a, b]

Character	Taphrinomycotina							
	Taphrina	*Protomyces*	*Saitoella*	*Schizosaccharomyces*	*Pneumocystis*	*Neolecta*	Saccharomycotina	Pezizomycotina
Lifestyle	PP	PP	S	S	PA	S[c]	S	PP/S
Culturable	YES	YES	YES	YES	NO	NO	YES	YES
Ascoma	−	−	−	−	−	+	−	+
Ascogenous hyphae	−	−	−	−	−	+	−	+
Enveloping membrane system	I	?	−	I	V	V	I	V
Forcible ascospore discharge	+/−	+/−	−	−	−	+	−	+/−
Anamorph	Y	Y	Y	Y	?	?	Y	H
Woronin bodies	?	?	−	−	−	+	+/−	+
Simple septal pore	+	?	−	−	−	+	+/−	+
Ploidy of somatic cells	n+n	2n	n	n	n/2n	n	n/2n	n
Major ubiquinone system	Q-10	Q-10	Q-10	Q-10	?	?	Q-6∼9	Q-9, 10, 10(H_2), 10(H_4)

[a] Compiled from Alexopoulos et al (1996), Sugiyama (1998) and Landvik et al (2003).

[b] Exceptional data are omitted.

[c] *N. vitellina* is possibly a weak parasite on the host conifer, the ecology of three species is little known (Landvik et al 2001).

Abbreviations = PP: phytopathogenic; PA: pathogenic for animals; S: saprobic; +: present; −: lacking; ?: unknown or no data; I: associated with individual nuclei; V: enclosing all nuclei; Y: yeast/yeastlike; H: hyphal.

definition and circumscription of the Taphrinomycotina were reviewed by Sugiyama and Nishida (1995), Alexopoulos et al (1996), Sugiyama (1998) and Kurtzman and Sugiyama (2001).

As stated above Nishida and Sugiyama (1993, 1994b) initially treated their provisional class ''Archiascomycetes'' as monophyletic, accommodating five genera *Taphrina*, *Protomyces*, *Saitoella*, *Schizosaccharomyces* and *Pneumocystis*. The common characters of the five genera are: (i) A sexually reproductive state (not present in *Saitoella*) is ascogenous but ascogenous hyphae are not formed; (ii) asexual reproduction is by budding or fission; (iii) neither ascomata nor conidiomata are formed; and (iv) the vegetative state is hyphal or yeast-like (TABLE I). On the basis of nSSU rDNA sequence analyses Landvik et al (1993) and Landvik (1996) subsequently included the apothecial ascomycete genus *Neolecta* in the early diverging ascomycete lineage defined by Berbee and Taylor (1993, 1995), and Sjamsuridzal et al (1997) confirmed the addition of *Neolecta* into the group based on the same gene, thus causing an evolutionary enigma. Characteristics of all the representative genera and other ascomycete yeasts and filamentous ascomycetes are compared (TABLE I); these phenotypic characters used to circumscribe the Taphrinomycotina are unclear with the inclusion of *Neolecta* in the group.

The discovery of *Neolecta* as a member of the already diverse early diverging ascomycetes has attracted great interest from mycologists, centering on the major question whether the early diverging ascomycetes comprise a monophyletic or a paraphyletic group. Eriksson (1999) first suggested that the subphylum might be paraphyletic, and subsequent analyses have not resolved the question. A phylogeny based on the gene sequences encoding the second largest subunit RNA polymerase II (RPB2, Liu et al 1999) supported the view that the Ascomycota is composed of monophyletic groups of Taphrinomycotina (=Archiascomycetes), Saccharomycotina (=Saccharomycetes) and Pezizomycotina (=Euascomycetes); however only two early diverging species, *Schizosaccharomyces pombe* and *Neolecta vitellina*, were included in their study. On the other hand a phylogeny from β-tubulin genes (Landvik et al 2001) showed that the early diverging ascomycetes are not monophyletic but supported the hypotheses of an early divergence of *Neolecta* from superficially similar filamentous ascomycetes (= euascomycetes). The respective RPB2 and β-tubulin genes did not give a clear answer for the monophyly vs. paraphyly of the Taphrinomycotina. Taxonomically de Hoog et al (2000) and Sugiyama (2005) treated the group as the class Archiascomycetes in the phylum Ascomycota, in which the remaining phyla were Hemiascomycetes (ascomycetous yeasts) and Euascomycetes (filamentous ascomycetes), corresponding to the subphyla Taphrinomycotina, Saccharomycotina and

Pezizomycotina, respectively (Eriksson and Winka 1997, Eriksson 2005). Kirk et al (2001) reclassified the early diverging ascomycetes into four classes, the Taphrinomycetes, Schizosaccharomycetes, Pneumocystidomycetes and Neolectomycetes. Eriksson (2005) treated these as ''classes of uncertain positions in the subphylum Taphrinomycotina'' because of poor support for Taphrinomycotina as a monophyletic group, corresponding to the classification used in this volume (Blackwell et al 2006).

Here we briefly describe the respective biological profiles of six genera of early diverging ascomycetes (i.e. *Taphrina* Fr., *Protomyces* Unger, *Saitoella* Goto et al, *Schizosaccharomyces* Lindner, *Pneumocystis* P. Delanoë & Delanoë and *Neolecta* Speg.).

Taphrina.—This genus usually has been placed in the monotypic family Taphrinaceae in the Taphrinales (e.g. Kramer 1973, 1987; Kurtzman and Sugiyama 2001; Kirk et al 2001). Species of *Taphrina* are pathogenic primarily on ferns and higher plants (particularly the Rosales and Fagales). They are dimorphic with a saprobic haploid uninucleate yeast state (von Arx et al 1982), which is assignable to the anamorph genus *Lalaria* R.T. Moore (1990). This genus accommodates 95 species (Kirk et al 2001). Two representative species in the genus are well known: *T. wiesneri* Ráthay (=*T. cerasi* [Fuckel] Sadeb.), attacking Japanese cherry (''Sakura'') tree and causing witches' brooms, and *T. deformans*, causing peach leaf curl. The life cycle of the latter species is well studied cytologically (Alexopoulos et al 1996). The yeast (budding) states of species of genera *Taphrina*, *Protomyces* and *Saitoella* are morphologically, biochemically and chemotaxonomically similar (Sugiyama and Nishida 1995, Kurtzman and Sugiyama 2001, Lopandic et al 2005). Most published nSSU rDNA (Sjamsuridzal et al 1997, Bacigálová et al 2003) and nLSU rDNA (D1/D2) (Rodrigues and Fonseca 2003, Inácio et al 2004) phylogenies confirmed the monophyly of the genus with the probable exception of *T. vestergrenii* Giesenhagen to be mentioned later.

Protomyces.—This genus usually is placed in the Protomycetaceae in the order Protomycetales with four other genera (Reddy and Kramer 1975). The phylogenetic hypothesis proposed for the Protomycetales (Reddy and Kramer 1975) has not been tested, and only the genus *Protomyces* is included in our study. The remaining taxa, including *Burenia*, *Protomycopsis*, *Taphridium* and *Volkartia*, remain to be sampled. Species of *Protomyces* are also parasitic on higher plants, mainly the Apiaceae (=Umbelliferae) and Asteraceae (=Compositae), and the species can be cultured on artificial media like *Taphrina* spp. Biological and cultural properties of *Protomyces inouyei*, *P. lactucae-debilis* Sawada and *P. pachydermus* von Thümen have been characterized by Tubaki (1957). nSSU rDNA sequence phylogeny demonstrated that these three *Protomyces* spp. and *P. macrosporus* Unger form a monophyletic group (Sjamsuridzal et al 1997). In addition a core group comprising *Taphrina* (including *T. vestergrenii*) and *Protomyces* spp. always appeared to be monophyletic with strong bootstrap support (100%) (Sjamsuridzal et al 1997, Bacigálová et al 2003). As Kurtzman (1993) pointed out, the Protomycetales might be synonymous with the Taphrinales. Kirk et al (2001) and Eriksson (2005) have accommodated the two families, Taphrinomycetaceae and Protomycetaceae, into the order Taphrinales.

Saitoella.—This genus usually has been treated as incertae sedis among the early diverging ascomycetes (Eriksson 2005, Sugiyama 2005). Kurtzman and Sugiyama (2001) accommodated it within the Protomycetaceae based on nSSU rDNA sequence-based phylogeny and the primary phenotypic characteristics (e.g. Sjamsuridzal et al 1997, Sugiyama 1998). The genus comprises only the type species *S. complicata*, which is a saprobic soil-living yeast the lacks true hyphae and is superficially similar to *Rhodotorula glutinis* (Fres.) F.C. Harrison, a basidiomycetous asexual yeast. It lacks a meiotic cycle. *Saitoella* shares some characteristics typical of both ascomycetes and basidiomycetes (Goto et al 1987). The history of studies on *Saitoella* was fully described by Sugiyama et al (1993). Although previous phylogenetic studies using nSSU rDNA sequences elucidated the identity of *S. complicata* as an ascomycete (Nishida and Sugiyama 1993, 1994b; Nishida et al 1993) its evolutionary relationship to other members in the Taphrinomycotina remains unresolved.

Schizosaccharomyces.—Kurtzman (1993) and Eriksson et al (1993) placed the genus *Schizosaccharomyces* in the monotypic family Schizosaccharomycetaceae and the order Schizosaccharomycetales. The genus contains only three species, which inhabit sugar-rich plant materials, such as fruits and honey (Vaughan-Martini and Martini 1998, Barnett et al 2000). The type species, *Schizosaccharomyces pombe*, is saprobic and is characterized by exclusive fission-type of vegetative reproduction and Q-10 as the major ubiquinone system. The circumscription and classification of *S. pombe* and allied taxa were reviewed by Kurtzman and Sugiyama (2001).

Pneumocystis.—The genus *Pneumocystis* has been accommodated in the monotypic family Pneumocys-

tidaceae, order Pneumocystidales (Eriksson 1994). This genus comprises three or more species that can infect humans and other animals; they are unculturable on routine laboratory media. *Pneumocystis* is a principal causal agent of pneumonia in patients with HIV infections. This organism was considered to be a protozoan until Edman et al (1988) found it is a fungus based on nSSU rRNA sequence comparisons. Nishida and Sugiyama (1994b) placed the genus within the Archiascomycetes. The life cycle of *P. carinii*, including mitosis and cytoplasmic fission, was illustrated by Yoshida (1989) and Dei-Cas and Cailliez (1998) on the basis of ultrastructural studies. Taylor et al (1994) and Sugiyama and Nishida (1995) discussed the similarity of life cycles between the fission saprobic yeast *Schizosaccharomyces pombe* (or *S. octosporus*) and the mammal lung pathogen *P. carinii*. Previously known as *Pneumocystis carinii*, the human pathogen has been reclassified in another species, *P. jirovecii* Frenkel (cf. Redhead et al 2006). The nomenclatural problems associated with the genus *Pneumocystis* also were discussed by Redhead et al (2006).

Neolecta.—The genus *Neolecta* has been assigned to the monotypic family Neolectaceae, order Neolectales (Landvik et al 1993). This genus contains three species that are saprobic or weakly parasitic on plant rootlets, but their ecology is little known (Landvik et al 2001). The species are characterized by clavate and stalked apothecia, and cylindrical, aparaphysate and eight-spored asci. *Neolecta* spp. recently have been characterized morphologically, including ultrastructural morphology, by Landvik et al (2003) (cf. TABLE I). Landvik's (1996) cladistic analyses with nSSU and nLSU rDNA sequences demonstrated that both *N. vitellina* and *N. irregularis* grouped together in the branch with the early diverging Ascomycota defined by Berbee and Taylor (1993). Sjamsuridzal et al (1997), Liu et al (1999) and Landvik et al (2001) subsequently confirmed the position of *Neolecta* among the early diverging Ascomycota with nSSU rDNA, RPB2 and β-tubulin genes sequences, respectively. Taxonomically Eriksson and Winka (1997) and Eriksson (2005) have accommodated *Neolecta* in the Neolectomycetes/Taphrinomycotina, whereas Sugiyama (2005) placed it within the Neolectales/''Archiascomycetes''.

The aim of this paper is to shed light on the enigmatic evolutionary relationships of the Taphrinomycotina and to contribute to a taxonomic or systematic framework of the early diverging Ascomycota taxa. We report the use of protein coding genes in addition to rDNA to clarify the relationships of these genera.

MATERIALS AND METHODS

A total of 23 taxa, including 11 early diverging Ascomycota (Taphrinomycotina *sensu* Eriksson 2005), three Saccharomycotina, six Pezizomycotina and three Basidiomycota were sampled (TABLE II). Three species of Basidiomycota were used as outgroup taxa. DNA sequence data were obtained from four loci, nuclear small subunit ribosomal DNA (nSSU rDNA), D1/D2 region of nuclear large subunit ribosomal DNA (nLSU rDNA), the second largest subunit of RNA polymerase (RPB2) and β-tubulin. Because the sequence data for all four loci were not available for some taxa only taxa with at least three sequences were included in the phylogenetic analyses.

Phylogenetic analyses were conducted for the concatenated four-locus dataset under Bayesian, parsimony and distance criteria. Bayesian Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were performed with MrBayes v3.0b4 (Huelsenbeck and Ronquist 2001) with 2 000 000 generations by sampling every 100th generation. For nSSU and nLSU rDNA dataset, the GTR+Γ+I model was used. The same model was applied for the dataset of protein coding genes (RPB2 and β-tubulin), but they were partitioned further according to the codon position. The support for nodes was tested by posterior probabilities obtained from majority rule consensus after deleting the trees during burn-in.

Parsimony analyses (MP) were conducted using PAUP*4.0b10 (Swofford 2002) with the heuristic search option (TBR and MULTREES on) and 1000 replicates of random addition sequence. Support for individual nodes was tested by bootstrapping of 500 replicates with the heuristic search option (TBR and MULTREES on) with five random addition sequences. Neighbor joining (NJ) analyses were conducted using PAUP*4.0b10 with the GTR model and a gamma shape set to 0.5. Support for individual nodes was tested by bootstrap analysis under the same settings. For both MP and NJ criteria, two independent analyses were conducted with or without the third codon position.

RESULTS AND DISCUSSION

We initially compared the representative published nSSU rDNA sequence-based trees (e.g. Berbee and Taylor 1993, 1995, 2001; Nishida and Sugiyama 1994b; Sjamsuridzal et al 1997; Sugiyama 1998; Kurtzman and Sugiyama 2001; Tehler et al 2003; Lutzoni et al 2004) and our trees as inferred from the available sequence data for the selected ascomycete taxa. Trees were generated from NJ with 1000 bootstrap replications and maximum likelihood (ML) with posterior probabilities (300 000 generations) analyses comparing 33 early diverging species (22 *Taphrina* spp., four *Protomyces* spp., one *Saitoella* sp., one *Schizosaccharomyces* sp., two *Pneumocystis* spp. and two *Neolecta* spp.), three hemiascomycete spp. and four euascomycete spp. with *Filobasidiella neoformans* as outgroup (trees not shown). Both distance

TABLE II. Taxon list with associated GenBank accession numbers

Taxon	nSSU rDNA	nLSU rDNA	β-tubulin	RPB2
Neolecta irregularis (Peck) Korf & J.K. Rogers	AY789379	AY789380	AF170962	—
Neolecta vitellina (Bres.) Korf & J.K. Rogers	Z27393	U42695	AF170963	AF107786
Pneumocystis carinii P. Delanoë & Delanoë	X12708	M86760	AF170964	AY485631
Protomyces inouyei Henn.	AY548295	AY548294	AF170967	AY548299
Saitoella complicata Goto et al.	AY548297	AY548296	AF180363	AY548300
Schizosaccharomyces pombe Lindner	X54866	Z19136	AF042828	D13337
Taphrina communis (Sadeb.) Giesenh.	AB000949	AF492032	—	AY641083
Taphrina deformans (Berk.) Tul.	AJ495826	AF492038	L47270	AY485633
Taphrina populina (Fr.) Fr.	D14165	AF492050	AF170968	—
Taphrina virginica Seym. & Sadeb.	AB000960	AF492071	AF170969	—
Taphrina wiesneri (Ráthay) Mix	AY548293	AY548292	—	AY548298
Aleuria aurantia (Pers.) Fuckel	AY544698	AY544654	—	DQ247785
Aspergillus fumigatus Fresen.	AB008401	AY660917	XM741811	AY485610
Chlorociboria aeruginosa (Oeder) Seaver	AFTOL[a]	AFTOL[a]	—	AFTOL[a]
Neurospora crassa Shear & B.O. Dodge	AY046271	AY681158	XM323372	AF107789
Otidea onotica (Pers.) Fuckel	AF006308	AF335121	AY513313	—
Trichoglossum hirsutum (Pers.) Boud.	AY544697	AY533014	AY536845	AY641087
Ashbya gossypii (S.F. Ashby & W. Nowell) Guillierm.	AE016820	AE016820	NM208980	AE016819
Candida albicans (C.P. Robin) Berkhout	NW139715	NW139715	M19398	XM713346
Saccharomyces cerevisiae Meyen ex E.C. Hansen	J01353	AB212638	NC001138	M15693
Filobasidiella neoformans Kwon-Chung	AJ560315	AJ551290	XM569650	AY485620
Suillus sp.	AY662659	AY684154	AY112730	AY786066
Ustilago maydis (DC.) Corda	X62396	AF453938	XM756882	AY485636

[a] Sequences were obtained from Assembling the Fungal Tree of Life Website (http://aftol.org/data.php).

and parsimony analyses divided the Ascomycota into three major clades, Taphrinomycotina, Saccharomycotina and Pezizomycotina. Both trees also clearly demonstrated that all early diverging taxa grouped together as monophyletic, although only moderately well supported. The bootstrap value for the respective nodes between the Taphrinomycotina and other ascomycetes was respectively 84% and 63%. Among the Taphrinomycotina clade all *Taphrina* and *Protomyces* spp. in our analyses formed a monophyletic core group with 100% statistical support in both trees. In this clade *T. vestergrenii* was sister of other *Taphrina* and *Protomyces* spp. The phylogenetic position of *T. vestergrenii* is of special interest, especially in light of the fact that its host plant is a fern. The nLSU rDNA (D1/D2 region) sequence-based NJ analysis also has demonstrated that *T. vestergrenii* appeared to be placed at an intermediate position between the two genera (Rodrigues and Fonseca 2003). The remaining early diverging ascomycete taxa were placed as paraphyletic to the *Taphrina-Protomyces* clade in both trees. However the branching order varied between NJ and ML trees. It is noteworthy that in the ML tree a branch (having 62% bootstrap support) consisting of *Saitoella complicata*, *Neolecta vitellina* and *N. irregularis* was sister of the branch (having 95% bootstrap support) leading to a core group of *Taphrina-Protomyces* spp., *Pneumocystis carinii* and *Schizosaccharomyces pombe*. As a whole the tree topologies in this study were similar to those in previously published studies (e.g. Berbee and Taylor 1993, 2001; Nishida and Sugiyama 1994b; Sjamsuridzal et al 1997; Sugiyama 1998; Kurtzman and Sugiyama 2001; Tehler et al 2003; Lutzoni et al 2004), although the outcome of higher level taxa was different. The results based on our nSSU rRNA dataset from 160 species of Ascomycota are summarized (SUPPLEMENTARY TABLE III) and support a monophyletic Taphrinomycotina as previously suggested by Nishida and Sugiyama (1994b) and Sjamsuridzal et al (1997).

A consensus of eight parsimony trees with 1000 bootstrap replications generated from a dataset of nSSU rDNA+nLSU rDNA (D1/D2 region) sequences (tree not shown) also supported the principal topology resulting from the nSSU rDNA sequence analyses mentioned above; however the branch leading to the Taphrinomycotina received only 56% bootstrap value.

Phylogenetic analysis of various combinations of multigene sequence data available for the Taphrinomycotina included these genes: nSSU rDNA, nLSU rDNA, RPB1, RPB2, EF-1α, ATP6 and β-tubulin. The tree topologies within the Taphrinomycotina lineage varied among trees depending on the combination of genes used in the analyses; these results will be

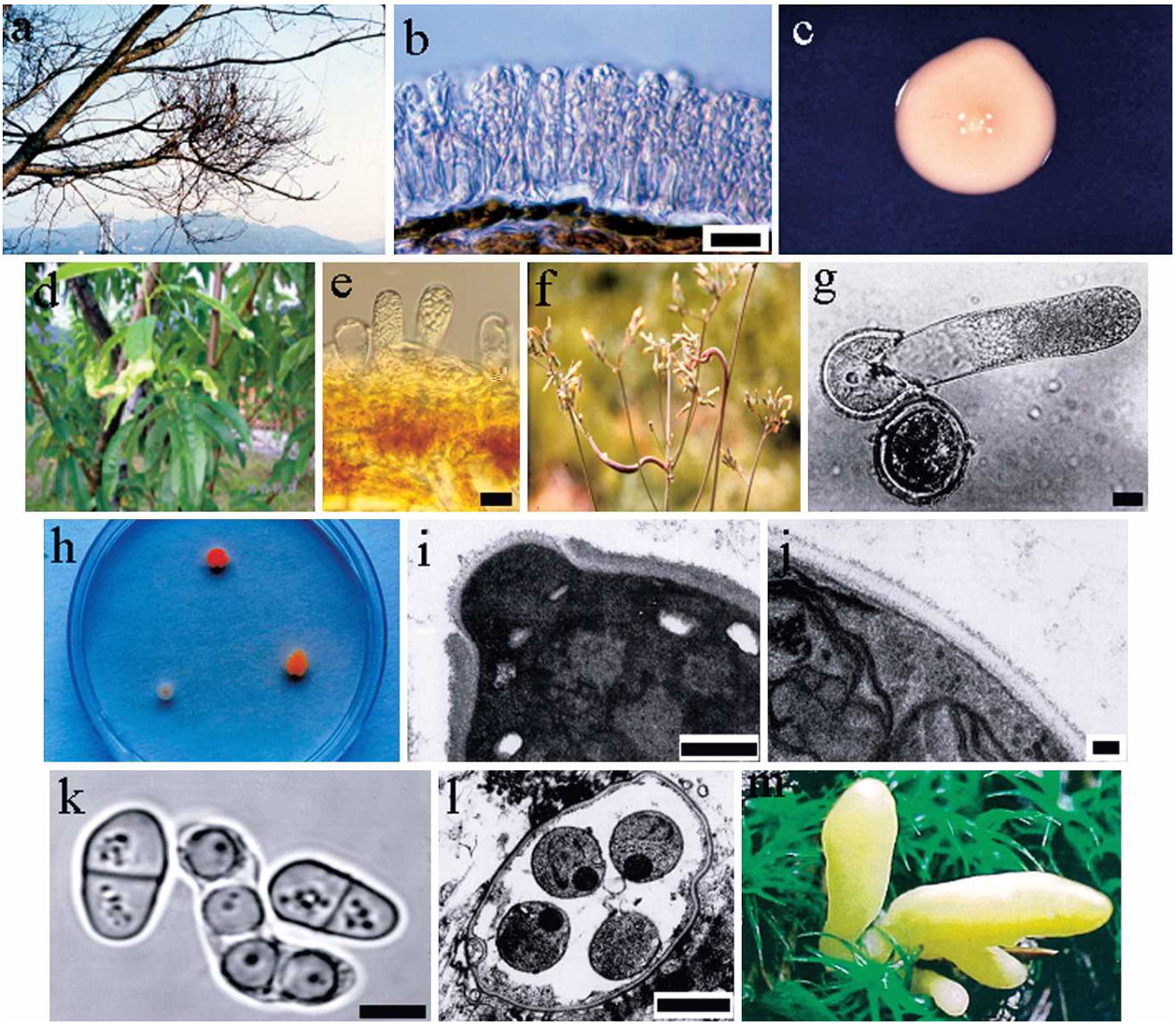

FIG. 1. Macro- and micromorphology showing the diversity of the representative taxa in the early diverging Ascomycota. a. Witches' broom disease of a Japanese cherry tree (''Sakura'': *Cerasus yedoensis*) caused by *Taphrina wiesneri* (photo by Keisuke Tubaki). b. Mature asci of *T. wiesneri* on leaf tissue of a Japanese cherry tree showing ascospore budding (blastoconidium formation) (photo by Keisuke Tubaki). c. A colony on potato dextrose agar (photo by Keisuke Tubaki). d. Symptoms of peach leaf curl disease caused by *Taphrina deformans* (photo courtesy Hideyuki Nagao). e. Hymenium of *Taphrina caerulescens*, causal agent of *Quercus* leaf curl (photo courtesy Hideyuki Nagao). f. Galls induced by *Protomyces inouyei* on stem of *Youngia japonica* (photo by Keisuke Tubaki). g. Germination of a thick-walled resting spore of *P. inouyei* in water (microphotograph by Keisuke Tubaki). h. Colonies of *Rhodosporidium toruloides* (anamorph: *Rhodotorula glutinis*) (top), *Saitoella complicata* (right) and *Taphrina wiesneri* (left) on potato dextrose agar. i. *Saitoella complicata*: TEM showing enteroblastic budding, characteristic of basidiomycetous yeast. j. *Saitoella complicate*. TEM showing the cell wall ultrastructure of the ascomycete type composed of one thin, dark layer and a broad, light inner layer. k. *Schizosaccharomyces pombe*. Fission and an ascus containing four ascospores (microphotograph courtesy Kouzaburo Mikata). l. *Pneumocystis carinii* [sic]. Mature cyst containing intracystic bodies (endospores). (From Dei-Cas and Cailliez 1998; reproduced with permission from Blackwell Publishing). m. *Neolecta vitellina*. Bright yellow fruit-bodies that can grow several cm tall (photo courtesy Sara Landvik). Bars: b, e, g = 20 µm; i = 0.5 µm; j = 0.1 µm; k = 5 µm; l = 1 µm.

published elsewhere. Among the seven genes targeted only the sequences of nSSU rDNA, nLSU rDNA, RPB2 and β-tubulin were available for sufficient numbers of taxa to cover most known genera of the Taphrinomycotina. We therefore performed Bayesian analyses for the combined dataset of the four gene sequences with GTR+Γ+I model for a total of eight data partitions (i.e. nSSU rDNA, nLSU rDNA and the

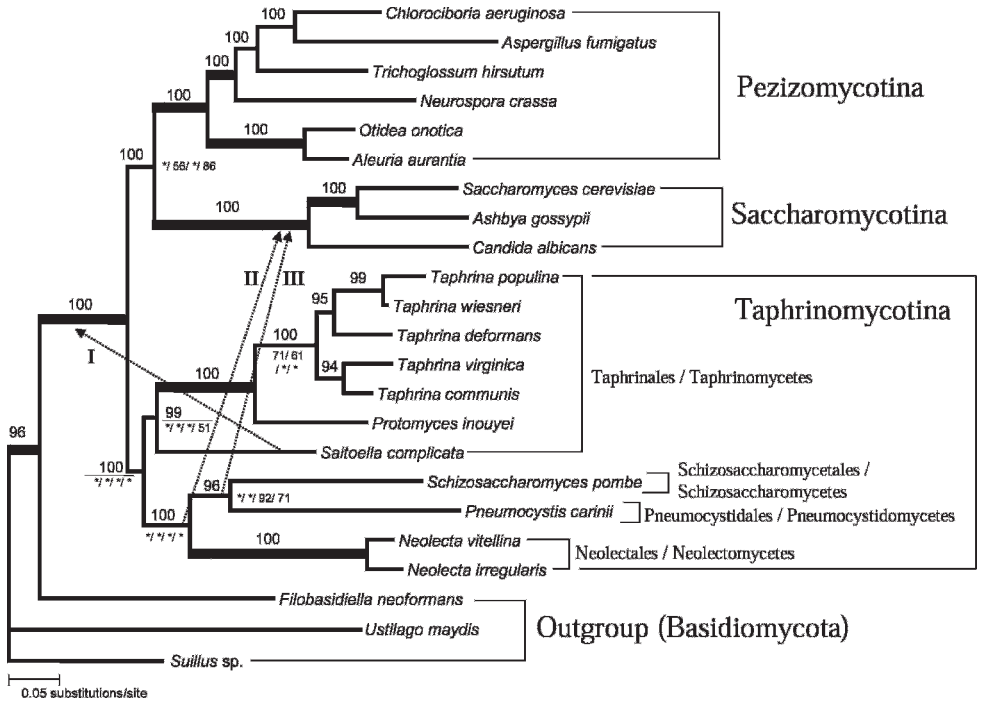

FIG. 2. Phylogeny of the basal Ascomycota. Tree topology is based on the 50% majority rule consensus of 18 000 Bayesian MCMCMC-generated trees. Numbers on branches indicate Bayesian posterior probability. Positions separated by a slash indicate bootstrap values based on parsimony analysis with third codon position/ parsimony analysis without third codon position/ neighbor joining (NJ) analysis with third codon position/NJ analysis without third codon position; an asterisk indicates lack of bootstrap support. Branches supported by ≥95% posterior probability and ≥70% bootstrap value in all analyses (parsimony vs. NJ, third codon included vs. excluded) are indicated by thick lines. Dotted arrows indicate alternative groupings found with ≥70% bootstrap support: I = parsimony analysis with third codon position; II = both parsimony and NJ analyses with third codon position; III = NJ analysis without third codon position.

respective codon positions of RBP2 and β-tubulin). The alignment of 23 nSSU rDNA sequences included 1870 sites, of which 1568 were included, and a total of 194 were potentially parsimony informative. The alignment of 23 nLSU rDNA sequences included 649 sites, of which 395 were included, and a total of 131 were potentially parsimony informative. The final RPB2 dataset included 19 sequences and had a total length of 1539 sites after excluding ambiguously aligned regions and spliceosomal introns, of which 855 sites were potentially parsimony informative. The final β-tubulin gene dataset included 19 sequences and had a total length of 872 sites after excluding ambiguously aligned regions and spliceosomal introns, of which 176 sites were potentially parsimony informative. The tree generated from these data is provided (FIG. 2) with the changes in topology with or without the third codon position of two protein-coding genes noted. The Taphrinomycotina as well as the Saccharomycotina, Pezizomycotina and Saccharomycotina plus Pezizomycotina were monophyletic in the 50% majority rule consensus of 18 000 Bayesian MCMCMC trees. The detection of these three major lineages agreed with those of the previous studies (e.g. Berbee and Taylor 1993, 2001; Nishida and Sugiyama 1994b; Sjamsuridzal et al 1997; Sugiyama 1998; Kurtzman and Sugiyama 2001; Tehler et al 2003). Some nSSU rDNA phylogenies (e.g. Sugiyama 1998, Bacigálová et al 2003, Lopandic et al 2005) showed the monophyly of Taphrinomycotina with 76–94% bootstrap support although the branching order was different among the Taphrinomycotina lineage. On the other hand Landvik et al (2001), Lutzoni et al (2004) and Taylor et al (2004) showed the basal paraphyly of the Taphrinomycotina.

We found it interesting that monophyly of the Taphrinomycotina was supported only by Bayesian analyses based on the four genes mentioned above while the other analyses with different optimality criteria indicated that the Taphrinomycotina is paraphyletic. For example *Neolecta*, *Pneumocystis* and *Schizosaccharomyces* formed a clade with the Saccharomycotina under parsimony and distance criteria when the third codon position was included (arrow II in FIG. 2). This pattern is consistent with the tree topology recovered by Landvik et al (2001) showing the basal paraphyly of the Taphrinomycotina. Of importance is the fact that the tree topology resulting

from parsimony analysis without the third codon position (tree not shown) did not show apparent conflicts (measured by ≥95% posterior probability or ≥70% bootstrap values) with the Bayesian tree (FIG. 2). It is well documented that the third codon positions of RPB1 and EF-1α (Tanabe et al 2004) and RPB2 (P. Brandon Matheny personal communication) are saturated and could produce erroneous results for the higher level phylogeny of fungi. No apparent conflicts between the Bayesian tree (FIG. 2) and parsimony tree (without third codon position) indicate that the saturation problem could be alleviated in Bayesian analysis by applying different substitution models to each codon position.

Our Bayesian analysis showed *Neolecta* as sister taxon of *Schizosaccharomyces* and *Pneumocystis*, which is more or less consistent with that of Landvik et al (2001) although their trees indicated a sister relationship of *Neolecta* and *Pneumocystis*, not including *Schizosaccharomyces*. These relationships were not supported in any other analyses (i.e. parsimony or NJ). On the other hand Sjamsuridzal et al (1997) showed a sister relationship of *Neolecta* and *Saitoella*. Species of *Neolecta* (Redhead 1977) and *Saitoella* (Goto et al 1987) might share the budding state as already suggested by Sugiyama (1998). The newest molecular phylogeny of fungi, based on Bayesian analysis of the combined six gene (nSSU rDNA, nLSU rDNA, ITS, EF-1α, RPB1 and RPB2) dataset by Timothy Y. James (personal communication), suggested the Taphrinomycotina, although lacking taxon sampling from *Saitoella* and *Neolecta*, is monophyletic with strong bootstrap support. However further discussion should wait until the accumulation of more genes and sequences from more taxon samples.

CONCLUSIONS

Early diverging ascomycetes sometimes assigned to the subphylum Taphrinomycotina (= ''Archiascomycetes'') differ markedly in habitat and morphology. Phylogenetic trees using datasets of nSSU rDNA, nSSU rDNA+nLSU rDNA and nSSU rDNA+nLSU rDNA+RPB2+β-tubulin suggested the monophyly of Taphrinomycotina and its sister group relationship with two major ascomycete lineages (i.e. the Saccharomycotina [=Hemiascomycetes] and the Pezizomycotina [=euascomycetes]). At the moment one of the authors (J.S.) supports the monophyly of the Taphrinomycotina for the early diverging ascomycetes in the phylum Ascomycota (Nishida and Sugiyama 1994b, de Hoog et al 2000, Sugiyama 2005) based on a biologist's insight. In addition several recent studies based on a total environmental DNA sampling approach have revealed potentially new basal ascomycete lineages independent of the Taphrinomycotina (Jumpponen and Johnson 2005, Schadt et al 2003, Vandenkoornhuyse et al 2002). Multigene sequence data, especially for protein coding genes, unfortunately are not available for a sufficient number of taxa for a phylogenetic analysis of the group. All the questions on the monophyly, branching order and the origin and evolutionary events of the early diverging Ascomycota remain uncertain. Therefore an integrated analysis of phenotypic and genotypic (molecular) characters from more taxon samples of the archiascomycetes is one of the most fascinating and urgent subjects in fungal systematics and evolution.

ACKNOWLEDGMENTS

We thank Dr Meredith Blackwell, Louisiana State University, and Dr Joseph W. Spatafora, Oregon State University, for improving the English and providing valuable comments and helpful suggestions on the revised version of this manuscript, Dr Tsuyoshi Hosoya, National Science Museum in Tsukuba, for providing the information on *Neolecta* spp., and Mr Tomohiko Kiyuna and Dr Kwang-Deuk An, NCIMB Division, TechnoSuruga Co. Ltd., for preparing tables and figures on the personal computer. We thank all the researchers and the copyright holder mentioned in the legend of FIG. 1 for providing invaluable images. We also acknowledge support from NSF 0090301, Research Coordination Network: a phylogeny for kingdom Fungi, to M. Blackwell, J.W. Spatafora and J.W. Taylor.

LITERATURE CITED

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory mycology. 4th ed. New York: John Wiley & Sons. 869 p.

Bacigálová K, Lopandic K, Rodrigues MG, Fonseca Á, Herzberg M, Pinsker W, Prillinger H. 2003. Phenotypic and genotypic identification and phylogenetic characterization of *Taphrina* fungi on alder. Mycol Prog 2: 179–196.

Barnett JA, Payne RW, Yarrow D. 2000. Yeasts: characteristics and identification. 3rd ed. Cambridge, UK. 1150 p.

Berbee ML, Taylor JW. 1993. Dating the evolutionary radiations of the true fungi. Can J Bot 71:1114–1127.

———, ———. 1995. From 18S ribosomal sequence data to evolution of morphology among the fungi. Can J Bot 73(1):S677–S683.

———, ———. 2001. Fungal molecular evolution: gene trees and geologic time. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol VII Part B (systematic and evolution). Berlin: Springer-Verlag. p 229–245.

Blackwell M, Hibbett DS, Taylor JW, Spatafora JW. 2006. Research Coordination Networks: a phylogeny for kingdom Fungi (Deep Hypha). Mycologia 98:829–837.

Bowen AR, Chen-Wu JL, Momany M, Young R, Szaniszio PJ,

Robbins PW. 1992. Classification of fungal chitin synthases. Proc Natl Acad Sci USA 89:519–523.

Cavalier-Smith T. 1987. The origin of Fungi and pseudofungi. In: Rayner ADM, Brasier CM, Moore D, eds. Evolutionary biology of the fungi. Cambridge: Cambridge University Press. p 339–353.

———. 2001. What are Fungi? In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol VIIA (systematic and evolution). Berlin: Springer-Verlag. p 3–37.

de Hoog GS, Guarro J, Gene J, Figueras MJ. 2000. Atlas of clinical fungi. Reus, Spain: Universitat Rovira i Virgili. 1126 p.

Dei-Cas E, Cailliez J-C. 1998. Editorial. FEMS Immunol Med Microbiol 22:1–4.

Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML. 1988. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. Nature 334:519–522.

Eriksson OE. 1994. *Pneumocystis carinii*, a parasite in lungs of mammals, referred to a new family and order (Pneumocystidaceae, Pneumocystidales, Ascomycota). Systema Ascomycetum 13:165–180.

———. 1999. Outline of Ascomycota—1999. Myconet 3:1–88.

———. 2005. Outline of Ascomycota—2005. Myconet 11:1–113.

———, Svedskog A, Landvik S. 1993. Molecular evidence for the evolutionary hiatus between *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Systema Ascomycetum 11:119–162.

———, Winka K. 1997. Supraordinal taxa of the Ascomycota. Myconet 1:1–16.

Goto S, Sugiyama J, Hamamoto M, Komagata K. 1987. *Saitoella*, a new anamorphic genus in the Cryptococcaceae to accommodate two Himalayan yeast isolates formally identified as *Rhodotorula glutinis*. J Gen Appl Microbiol 33:75–85.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Inácio J, Rodrigues MG, Sorbral P, Fonseca Á. 2004. Characterization and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel *Lalaria* species. FEMS Yeast Res 4:541–555.

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung G-H, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüβler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts R, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schimitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443:818–822.

Jumpponen A, Johnson LC. 2005. Can rDNA analyses of diverse fungal communities in soil and roots detect effects of environmental manipulations? A case study from tall grass prairie. Mycologia 97:1177–1194.

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Dictionary of the fungi. 9th ed. Wallingford: CAB International. 655 p.

Kramer CL. 1973. Protomycetales and Taphrinales. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi, an advanced treatise. Vol IV A. New York: Academic Press. p 33–41.

———. 1987. The Taphrinales. In: de Hoog GS, Smith MTh, Weijman ACM, eds. The expanding realm of yeast-like fungi. Amsterdam: Elsevier. p 151–166.

Kurtzman CP. 1993. Systematics of the ascomycetous yeasts assessed from ribosomal RNA sequence divergence. Antonie van Leeuwenhoek 63:165–174.

———, Sugiyama J. 2001. Ascomycetous yeasts and yeastlike taxa. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. The Mycota. Vol VII Part A (systematic and evolution). Berlin: Springer-Verlag. p 179–200.

Landvik S. 1996. *Neolecta*, a fruit-body-producing genus of the basal ascomycetes, as shown by SSU and LSU rDNA sequences. Mycol Res 100:199–202.

———, Eriksson OE, Gargas A, Gustafsson P. 1993. Relationships of the genus *Neolecta* (Neolectales ordo nov., Ascomycotina) inferred from 18S rDNA sequences. Systema Ascomycetum 11:107–118.

———, ———, Berbee ML. 2001. *Neolecta*—a fungal dinosaur? Evidence from β-tubulin amino acid sequences. Mycologia 93:1151–1163.

———, Schumacher TK, Eriksson OE, Moss ST. 2003. Morphology and ultrastructure of *Neolecta* species. Mycol Res 107:1021–1031.

Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Mol Biol Evol 16:1799–1808.

Lopandic K, Molnár O, Suzuki M, Pinsker W, Prillinger H. 2005. Estimation of phylogenetic relationships within the Ascomycota on the basis 18S rDNA sequences and chemotaxonomy. Mycol Prog 4:205–214.

Lutzoni F, Kauff F, Cox C, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung G-H, Lucking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim Y-W, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R. 2004. Assembling the Fungal Tree of Life: progress, classification and evolution of subcellular traits. Am J Bot 91:1446–1480.

Moore RT. 1990. The genus *Lalaria* gen. nov.: Taphrinales anamorphosum. Mycotaxon 38:315–330.

Nishida H. 2005. Diversity and evolution from the insight for genotypic information. In: Sugiyama J, ed. Diversity and evolution of fungi, bacteria and viruses. Tokyo: Shokabo Publishing. p 121–133. (In Japanese.)

———, Blanz PA, Sugiyama J. 1993. The higher fungus *Protomyces inouyei* has group I introns in the 18S rRNA gene. J Mol Evol 37:25–28.

———, Sugiyama J. 1993. Phylogenetic relationships among *Taphrina*, *Saitoella* and other fungi. Mol Biol Evol 12: 883–886.

———, ———. 1994a. Phylogeny and molecular evolution among higher fungi. Nippon Nogeikagaku Kaishi 68: 54–57. (In Japanese.)

———, ———. 1994b. Archiascomycetes: detection of a major new lineage within the Ascomycota. Mycoscience 35:361–366.

Reddy MS, Kramer CL. 1975. A taxonomic revision of the Protomycetales. Mycotaxon 3:1–50.

Redhead SA. 1977. The genus *Neolecta* (Neolectaceae fam. nov., Lecanorales, Ascomycetes) in Canada. Can J Bot 55:301–306.

———, Cushion MT, Frenkel JK, Stringer JR. 2006. *Pneumocystis* and *Trypanosoma cruzi*: nomenclature and typification. J Eukaryot Microbiol 53:2–11.

Rodrigues MG, Fonseca Á. 2003. Molecular systematics of the dimorphic ascomycete genus *Taphrina*. Int J Syst Evol Microbiol 53:607–616.

Savile DBO. 1955. A phylogeny of the Basidiomycetes. Can J Bot 33:60–104.

———. 1968. Possible interrelationships between fungal groups. In: Ainsworth GC, Sussman AS, eds. The fungi, an advanced treatise. Vol III. New York: Academic Press. p 649–675.

Schadt CW, Martin AP, Lipson DA, Schmidt SK. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. Science 301:1359–1361.

Sjamsuridzal W, Tajiri Y, Nishida H, Thuan TB, Kawasaki H, Hirata A, Yokota A, Sugiyama J. 1997. Evolutionary relationships of members of the genera *Taphrina*, *Protomyces*, *Schizosaccharomyces*, and related taxa within the archiascomycetes: integrated analysis of genotypic and phenotypic characters. Mycoscience 38:267–280.

Sugiyama J. 1998. Relatedness, phylogeny, and evolution of the fungi. Mycoscience 39:487–511.

———. 2005. Classification system of the Fungi *s. lat.* In: Sugiyama J, ed. Diversity and evolution of fungi, bacteria and viruses. Tokyo: Shokabo Publishing. p 396–405. (In Japanese.).

———, Nishida H. 1995. The higher fungi: their evolutionary relationships and implications for fungal systematics. In: Arai R, Kato M, Doi Y, eds. Biodiversity and evolution. Tokyo: The National Science Museum Foundation. p 177–195.

———, ———, Suh S-O. 1993. The paradigm of fungal diagnoses and descriptions in the era of molecular systematics: *Saitoella complicata* as an example. In: Reynolds DR, Taylor JW, eds. The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. Wallingford: CAB International. p 261–269.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

Tanabe Y, Saikawa M, Watanabe MM, Sugiyama J. 2004. Molecular phylogeny of Zygomycota based on EF-1α and RPB1 sequences: limitations and utility of alternative markers to rDNA. Mol Phylogenet Evol 30:438–449.

Taylor JW, Swann EC, Berbee ML. 1994. Molecular evolution of ascomycete fungi: phylogeny and conflict. In: Hawksworth DL, ed. Ascomycete systematics: problems and perspectives in the nineties. New York: Plenum Press. p 201–212.

———, Spatafora J, O'Donnell K, Lutzoni F, James T, Hibbett DS, Geiser D, Bruns TD, Blackwell M. 2004. In: Cracraft J, Donoghue MJ, eds. Assembling the Tree of Life. New York: Oxford University Press. p 171–194.

Tehler A, Little DP, Farris JS. 2003. The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, Fungi. Mycol Res 107:901–916.

Tubaki K. 1957. Biological and cultural studies of three species of *Protomyces*. Mycologia 49:44–54.

Vandenkoornhuyse P, Baldauf SL, Leyval C, Straczek J, Young JPW. 2002. Extensive fungal diversity in plant roots. Science 295:2051.

Vaughan-Martini A, Martini A. 1998. *Schizosaccharomyces* Lindner. In: Kurtzman CP, Fell J, eds. The yeasts, a taxonomic study. 4th ed. Amsterdam: Elsevier. p 391–394.

von Arx JA, van der Walt JP, Liebenberg NVDM. 1982. The classification of *Taphrina* and other fungi with yeast-like cultural states. Mycologia 74:287–296.

Yoshida Y. 1989. Ultrastructural studies of *Pneumocystis carinii*. J Protozool 36:53–60.

SUPPLEMENTARY TABLE III. Taxon specific positions in nSSU rDNA sequences among the three major lineages in the phylum Ascomycota

position[a]	Taphrinomycotina (35 spp.)	Saccharomycotina (59 spp.)	Pezizomycotina (66 spp.)
478–480	ACA	ACG	CTG[c]
883	T	C[b]	C
970	A	A	G

[a] Position of the corresponding residue in the *Saccharomyces cerevisiae* Meyen ex Hansen nSSU rDNA sequences.
[b] The only exception was *Metschnikowia bicuspidata* (Metschnikoff) Kamienski; it was T instead of C.
[c] The exceptions were CTA for *Peziza badia* Pers., CAG for *Cudonia confusa* Bres., and CCG for *Epichloë typina* (Pers.) Tul. & C. Tul. among the species compared.