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Phylogenetic placement of the basidiomycetous yeasts *Kondoa malvinella* and *Rhodosporidium dacryoidum*, and the anamorphic yeast *Sympodiomycopsis paphiopedili* by means of 18S rRNA gene sequence analysis

Sung-Oui Suh* and Junta Sugiyama**

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

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The 18S ribosomal RNA gene sequences of the basidiomycetous yeasts *Kondoa malvinella* and *Rhodosporidium dacryoidum*, and an anamorphic yeast *Sympodiomycopsis paphiopedili* were determined. The 18S rRNA gene of *R. dacryoidum* IAM 13522 (ex type) revealed the presence of an intron-like region with a length of 404 nucleotides, which is presumably assigned to a group I intron. The phylogenetic tree, including 34 published reference sequences, was inferred from 1493 sites which could be unambiguously aligned. The molecular phylogeny, using the ascomycetes as an outgroup, divided the basidiomycetes into three major lineages. The first lineage was composed of the smut fungi (Ustilaginales), represented by *Ustilago maydis, U. hordei*, and *Tilletia caries*, including *S. paphiopedili*. The second lineage included the type species of teliospore-forming yeast genera *Leucosporidium, Rhodosporidium*, and *Sporidiobolus*, and the genera *Erythrobasidium* and *Kondoa*, both previously included in the Filobasidiaceae. *Rhodosporidium dacryoidum* showed a close relationship with *E. hasegawianum*, which was backed by a high bootstrap support. The rust fungi *Cronartium ribicola* and *Peridermium harknessii* were also included in this lineage. The last lineage was formed by the filobasidiaceous yeasts, *Cystofilobasidium capitatum, Mrakia frigida, Filobasidium floriforme*, and *Filobasidiella neoformans*, and the anamorphic yeasts *Bullera alba* (the anamorph of *Bulleromyces albus*) and *Trichosporon cutaneum*. Members of *Tremella* and selected hymenomycetous genera were also included in this lineage.

Key Words——basidiomycetous yeasts; phylogeny; 18S rRNA gene sequence; yeast evolution.

Introduction

Since the publication of "The Yeasts, a Taxonomic Study" (Kreger-van Rij, 1984), several new basidiomycetous yeast genera have been proposed (Hamamoto et al., 1988; Oberwinkler et al., 1983; Yamada and Komagata, 1987; Yamada et al., 1989). According to the system of classification used in "*The Yeasts*", basidiomycetous yeast genera are classified into three taxonomic categories: the teliospore-forming yeasts; Filobasidiaceae; and the Tremellales with a yeast phase. Teliospore formation and basidial morphology have been considered as important taxonomic characters for the classification of the Filobasidiaceae and the teliospore-forming yeasts. However, ultrastructural and molecular studies have revealed that classifying these yeasts by teliospore formation is unsuitable (Suh et al., 1993; Suh and Sugiyama, 1993a, b). The genera *Cystofilobasidium* Oberwinkler et Bandoni, and *Mrakia* Yamada et Komagata, segregates of *Leucosporidium* Fell, Statzell, Hunter et Phaff and *Rhodosporidium* Banno, respectively, have a clear relationship with the Filobasidiaceae based upon septal pore ultrastructure and 18S rRNA gene sequence (Suh et al., 1993; Suh and Sugiyama, 1993 a, b). However, the phylogenetic position of the genus *Kondoa* Yamada, Nakagawa et Banno, separated from the teliospore-forming yeast genus *Rhodosporidium*, has been uncertain (Boekhout et al., 1993; Nakase et al., 1991). Yamada et al. (1989) proposed the genus *Kondoa* in the family Filobasidiaceae on the basis of partial sequences of 18S and 26S rRNA, but they did not show clearly the relationship with the Filobasidiaceae. It was also indicated that *Rhodosporidium dacryoidum* Fell, Hunter et Tallman is a peculier species within the genus based on the comparisons of partial sequences of 18S and 26S rRNA (Yamada et al., 1990). Suh et al. (1993) showed the simple septal pores of *K. malvinella* Yamada, Nakagawa et Banno and *R. dacryoidum*, and suggested that these species should be separated from the members of the Filobasidia-

*Present address: Deep-sea Microorganisms Research group, Japan Marine Science and Technology Center, Yokosuka, Kanagawa 237, Japan.

**Corresponding author.

The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession numbers D13459 (*Rhodosporidium dacryoidum*), D13776 (*Kondoa malvinella*), and D14006 (*Sympodiomycopsis paphiopedili*).

ceae.

Sugiyama et al. (1991) proposed an anamorph-genus *Sympodiomycopsis* Sugiyama, Tokuoka et Komagata for a yeast isolated from orchid nectar. *Sympodiomycopsis paphiopedili* Sugiyama, Tokuoka et Komagata, the only known species, appears to be basidiomycetous based on chemotaxonomic and ultrastructural features. Because the teleomorphic state is not yet known, however, the higher taxonomic placement of this genus is uncertain. Suh et al. (1993) suggested that, based on the septal pores, *S. paphiopedili* is related to the members of Ustilaginales. However, that information alone is not sufficient to decide the correct taxonomic position.

The phylogenetic value of ribosomal RNA (rRNA) sequences has been discussed in some recent reviews (Bruns et al., 1991; Kurtzman, 1992; Kohn, 1992; Hibbett, 1992). In particular, the 18S rRNA gene sequence has been useful in studying the phylogeny of fungi (Berbee and Taylor, 1992a, b, 1993; Bruns et al., 1992; Hen-

Table 1. Organisms analyzed in this study.

Organism	Accession number*	Reference
Ascomycetes		
Saccharomyces cerevisiae	M27607	Mankin et al. (1986)
Candida albicans	X53497	Hendriks et al. (1989)
Basidiomycetes		
Athelia bombacina	M55638	Illingworth et al. (1991)
Auricularia auricula	L22254	Swann and Taylor (1993)
Auricularia polytricha	L22255	Swann and Taylor (1993)
Boletus satanas	M94337	Bruns et al. (1992)
Bullera alba	X60179	van de Peer et al. (1992)
Calocera cornea	L22256	Swann and Taylor (1993)
Coprinus cinereus	M92911	Bruns et al. (1992)
Cronartium ribicola	M94338	Bruns et al. (1992)
Cystofilobasidium capitatum	D12801	Suh and Sugiyama (1993a)
Dacrymyces chrysospermus	L22257	Swann and Taylor (1993)
Dacrymyces stillatus	L22258	Swann and Taylor (1993)
Erythrobasidium hasegawianum	D12803	Suh and Sugiyama (1993a)
Filobasidium floriforme	D13460	Suh and Sugiyama (1993a)
Filobasidiella neoformans	D12804	Suh and Sugiyama (1993a)
Heterotextus alpinus	L22259	Swann and Taylor (1993)
Kondoa malvinella	D13776	This study
Leucosporidium scottii	X53499	Hendriks et al. (1991a)
Mrakia frigida	D12802	Suh and Sugiyama (1993a)
Peridermium harknessii	M94339	Bruns et al. (1992)
Pseudohydnum gelatinosum	L22260	Swann and Taylor (1993)
Rhodosporidium dacryoidum	D13459	This study
Rhodosporidium toruloides	D12806	Suh and Sugiyama (1993a)
Spongipellis unicolor	M59760	Bowman et al. (1992)
Sporobolomyces roseus	X60181	van de Peer et al. (1992)
Sporidiobolus johnsonii	L22261	Swann and Taylor (1993)
Sympodiomycopsis paphiopedili	D14006	This study
*Thanatephorus cucumeris***	M92990	Bruns et al. (1992)
Tilletia caries	U00972	Berbee and Taylor (1993)
Tremella foliacea	L22262	Swann and Taylor (1993)
Tremella globospora	U00976	Berbee and Taylor (1993)
Tremella moriformis	U00977	Berbee and Taylor (1993)
Trichosporon cutaneum	X60182	van de Peer et al. (1992)
Ustilago hordei	U00973	Berbee and Taylor (1993)
Ustilago maydis	X62396	De Wachter et al. (1992)
Xerocomus chrysenteron	M94340	Bruns et al. (1992)

* Accessoin number in EMBL, GenBank, and DDBJ nucleotide libraries
** As *T. praticola*.

driks et al., 1989, 1991a, b, 1992; Nishida and Sugiyama, 1993; Suh and Sugiyama, 1993a; Swann and Taylor, 1993; van de Peer et al., 1992). Therefore, in this paper, we sequenced the nuclear small subunit ribosomal RNA (18S rRNA) gene from three yeast species, *K. malvinella, R. dacryoidum*, and *S. paphiopedili* in an attempt to determine the relationships of the three species and to clarify their classification.

Materials and Methods

Yeast strains The following yeast strains were used for sequencing: *Kondoa malvinella* IAM 13523^T (=CBS 6082, mating type α), *Rhodosporidium dacryoidum* IAM 13522^T (=CBS 6353, mating type A_1B_1), *Sympodiomycopsis paphiopedili* IAM 13459^T. The superscript "T" indicates the strains derived from the holotype. For strain data, see "IAM Catalogue of Strains, 1st Edition, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan, March 1993."

PCR amplification and DNA sequencing The yeasts were all grown in YM broth at 25°C or 17°C (*K. malvinella*) for DNA extraction. DNA was obtained from cells broken by sonication. The polymerase chain reaction (PCR) and sequencing of 18S rDNA were done according to Nishida and Sugiyama (1993).

Phylogenetic analysis We sequenced the 18S rDNA of the three yeasts and analyzed the findings together with

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                PCR primer site
   1  ATCTGGTTGA TCCTGCCAGT NNNNNNNNNC TTGTCTCAAA GATTAAGCCA TGCATGTCTA
  61  AGTATAAACA AATTCATACT GTGAAACTGC GAATGGCTCA TTAAATCAGT TATAGTTTAT
 121  TTGATGGTAC CTTACTACAT GGATAACTGT GGTAATTCTA GAGCTAATAC ATGCTGAAAA
 181  GCCCCGACTT CTGGAAGGGG TGTATTTATT AGATAAAAAA CCAATGGCGG GTAACCGTCT
 241  TGCGTTGATT CATAATAACT TCTCGAATCG CATGGCCTTG CGCCGGCGAT GCTTCATTCA
 301  AATATCTGCC CTATCAACTT TCGATGGTAG GATAGAGGCC TACCATGGTT ATGACGGGTA
 361  ACGGGGAATA AGGGTTCGAT TCCGGAGAGA GGGCCTGAGA AACGGCCCTC AGTCCTAAGG
 421  GACGCAGCAG GCGCGCAAAT TATCCCATCC CGACACGGGG AGATAGTGAC AATAAATAAC
 481  AATATAGGGC TCTTTTGGGT CTTATAATTG GAATGAGTAC AATTTAAATC CCTTAACGAG
 541  GATCAATTGG AGGGCAAGTC TGGTGAACTC TACAGAATTC CTTTACCGGC TTCTGACGCC
 601  AGAGATAGTA GGCAGTTGGG GCAACCCGCT GTATCCCTAC TAGTCGAGGT CACGCAATCA
 661  GTTTTGGGTG TGATCCGGCG AGGTAACCTG GTACGGGGGA ACCTAAGGTC TGGGCAACCA
 721  GGCTATGGTA ATCCCGTGGC GAGCCGAGGA GCAGTGATGC GACTCATTAG GCCGTCGTAA
 781  CGCGCGCTAA GGTACCGGTC AGCTTTGGTT ACTCAAAGTT GGCTCAAGGG ACGTGCTAAT
 841  CCCACCGGAA ACGGTGTTCT GTGCTGGAGC CCCCAAAAGG CAAAGGTGCA GGAGGACGAT
 901  GCTTCAACGA GGAAATGCGA TGAAGATGTC AAGTCCGGTA ATTCTCGATA TCAGATGAGA
 961  AAACAGTGGC CAGCAGCCGC GGTAATTCCA GCTCCAATAG CGTATATTAA AGTTGTTGCC
1021  GTTAAAAAGC TCGTAGTCGA ACTTCGGCCT CTGCCACCCG GTCCGCCTAT TTGGGTGTGT
1081  ACTGGAGTGG TGGAGGCTTA CCTCGTGGTG AGCGGCCATG TCCTTTACTG GGCGTGGTCG
1141  GGAACCATGA CATTTACTTT GAAAAAATTA GAGTGTTCAA AGCAGGCTTA CGCCCGAATA
1201  CATTAGCATG GAATAATAAA ATAGGACGTG CGGTCCTATT TTGTTGGTTT CTAGGATCGC
1261  CGTAATGATT AATAGGGATA GTTGGGGGCA TTCGTATTCA ATTGCTAGAG GTGAAATTCT
1321  TGGATTTATT GAAGACGAAC TACTGCGAAA GCATTTGCCA AGGATGTTTT CATTGATCAA
1381  GAACGAAGGT TAGGGGATCG AAAACGATCA GATACCGTTG TAGTCTTAAC AGTAAACGAT
1441  GCCGACTAGG GATCGGACGA GGATTTTTAA TGACTCGTTC GGCACCTGAA GAGAAATCTT
1501  TAAGTCTAGG TTCGGGGGGG AGTATGGTCG CAAGGCTGAA ACTTAAAGGA ATTGACGGAA
1561  GGGCACCACC AGGTGTGGAG CCTGCGGCTT AATTTGACTC AACACGGGGA AACTCACCAG
1621  GTCCAGACAC GACAAGGATT GACAGATTGA TAGCTCTTTC TTGATTTCGT GGTTGGTGGT
1681  GCATGGCCGT TCTTAGTTGG TGGAGTGATT TGTCTGGTTA ATTCCGATAA CGAACGAGAC
1741  CTTAACCTGC TAAATAGCCC GGCCGACTTT GGTTGGTCGC TGGCTTCTTA GAGGGACTAT
1801  CGGCGTTTAG CCGATGGAAG TTTGAGGCAA TAACAGGTCT GTGATGCCCT TAGATGTTCT
1861  GGGCCGCACG CGCGCTACAC TGACCGAACC AGCGAGTTTA TCACCTTGGC CGGAAGGCTT
1921  GGGTAATCTT GTGAAACTCG GTCGTGATGG GGATAGAGCA TTGCAATTAT TGCTCTTCAA
1981  CGAGGAATAC CTAGTAAGCG TGAGTCATCA GCTCGCGTTG AATTCGTCCC TGCCCTTTGT
2041  ACACACCGCC CGTCGCTACT ACCGATTGAA TGGCTTAGTG AGGTATCCGG ATTGGCATCT
2101  GGGAGCCGGC AACGGCACCT AGTCGCTGAG AAGTTTAACG AACTTGGTCA TTTAGAGGAA
2161  GTAAAAGTCG TAACAAGGTT TCCGTAGGTG AACCTGCGGA AGGATC
                                    PCR primer site
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Fig. 1. Nucleotide sequence of the *Rhodosporidium dacryoidum* nuclear 18S rRNA gene. The sequence of the 404 bp intron-like insertion is boxed, from nucleotide 565 to 968. The sequences in the black boxes are the PCR primer regions.

sequence data for 34 additional species. The organisms analyzed in this study are listed in Table 1 with their accession numbers in nucleotide sequence databases (GenBank, EMBL, and DDBJ). All sequences were aligned using the multialignment program CLUSTAL V (Higgins et al., 1992). Distances between the sequences were calculated using the two-parameter model of Kimura (1980). Sites where gaps existed in any of the sequences were excluded. A phylogenetic tree was constructed using the neighbour-joining method (Saitou and

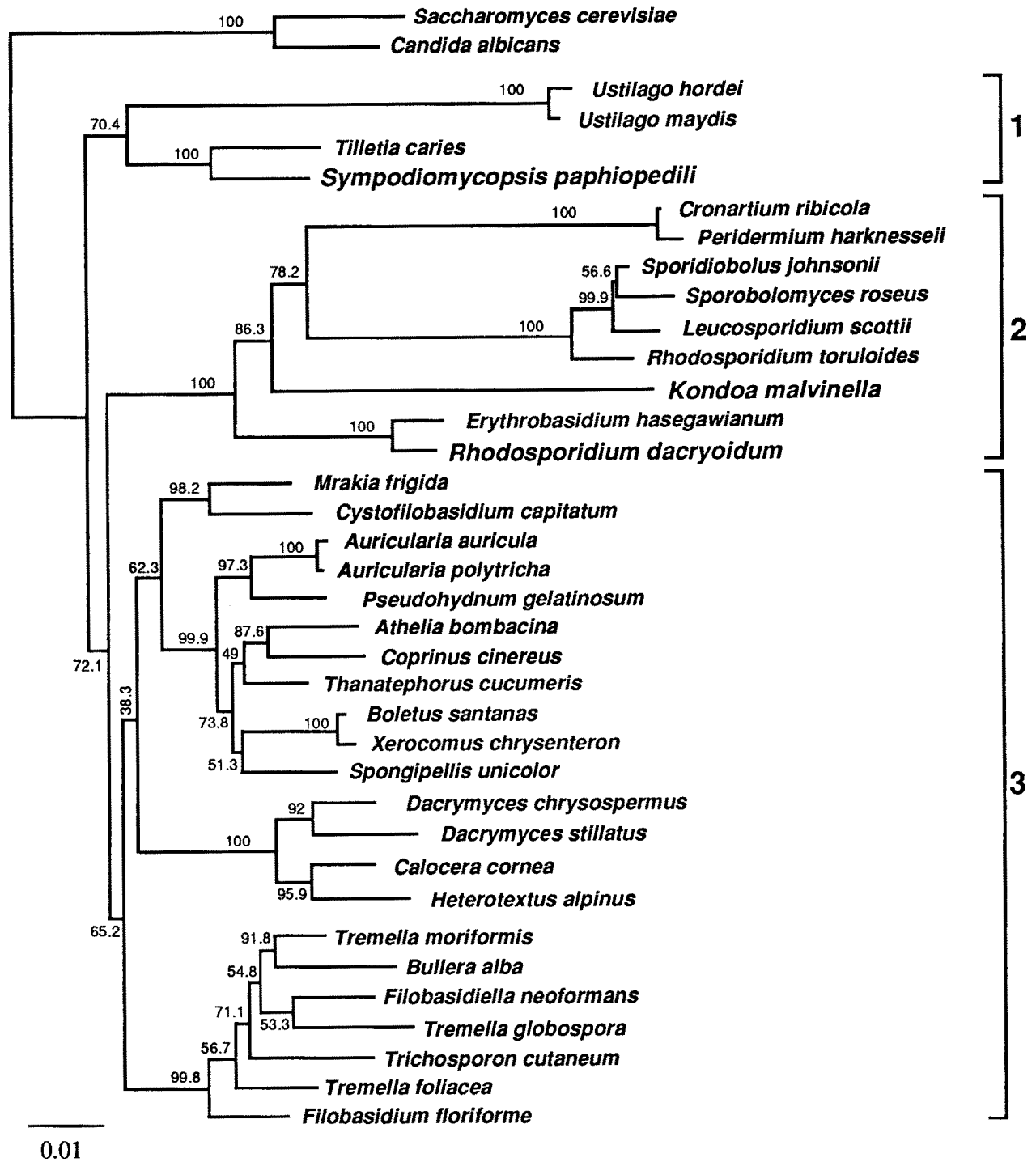

Fig. 2. Evolutionary tree of 37 species based on 18S rDNA sequence. The tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbour-joining method (Saitou and Nei, 1987). Each number indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches. The distance corresponding to one base change per hundred nucleotide positions is indicated by a bar.

Nei, 1987) with bootstrap analysis (Felsenstein, 1985) of 1000 random resamplings.

Results and Discussion

An intron-like region in 18S rDNA of *Rhodosporidium dacryoidum* As the result of sequencing, the we found a long insertion of 404 nucleotides in the gene encoding 18S rRNA of *Rhodosporidium dacryoidum* (Fig. 1). Because the region containing the insertion is highly conserved, as compared with other 18S rDNA sequence data, we presumed it to be an intron. Recently, several reports of introns in 18S rDNA have been published (Dávila-Aponte et al., 1991; De Wachter et al., 1992; DePriest and Been, 1992; Nishida et al., 1993; Sogin and Edman, 1989; Wilcox et al., 1992). Most of these were group I introns and some were messenger RNA introns (Rogers et al., 1993). The intron-like region in *R. dacryoidum* is presumed to be a group I intron according to Cech's definition (1988), because it starts next to a thymine (T) and finishes at guanine (G) as shown in Fig. 1. However, we could not find the conserved regions of group I introns, P, Q, R, and S, in the intron-like region we determined. Further studies are needed of the secondary structure and other molecular biological approaches to clarifying the evolutionary implications of this intron-like region.

Evolutionary relationships among basidiomycetous yeasts based on 18S rDNA sequence data We sequenced about 1750 bases of PCR amplification products of 18S rDNA of *Kondoa malvinella* and *Sympodiomycopsis paphiopedili*, and about 2200 bases from that of *Rhodosporidium dacryoidum*, which has an insertion of 404 bases. The sequence data were well aligned for all 37 fungi, and a phylogenetic tree (Fig. 2) was constructed by the neighbour-joining method (Saitou and Nei, 1987) based on the 1493 sites compared. The phylogenetic tree divided the basidiomycetes used in this analysis into three major lineages: 1) the smut fungi (Ustilaginales) parasitic on monocots, represented by *Ustilago maydis* (DC.) Corda, *U. hordei* (Pers.: Pers.) Lagerh. (Ustilaginaceae), and *Tilletia caries* (DC.) Tul. (Tilletiaceae), with the anamorph *Sympodiomycopsis paphiopedili*; 2) the smut-like, teliospore-forming yeast species and *Erythrobasidium hasegawianum* Hamamoto, Sugiyama et Komagata with the two rusts *Cronartium ribicola* Fischer and *Peridermium harknessii* Moore; and 3) the filobasidiaceous yeasts, represented by *Filobasidium floriforme* Olive, *Filobasidiella neoformans* Kwon-Chung, *Cystofilobasidium capitatum* Oberwinkler et Bandoni and *Mrakia frigida* Yamada et Komagata, some species of Tremellaceae, and related anamorphs *Bullera alba* Derx (the anamorph of *Bulleromyces albus* Boekhout et Fonseca; Boekhout et al., 1991) and *Trichosporon cutaneum* Ota with selected species of Hymenomycetes. These three major lineages agreed well with the result of Swann and Taylor (1993), who compared the 18S rDNA sequence of 19 basidiomycetes and using the ascomycetes as an outgroup, clarified the existence of three major lineages in basidiomycetes: the Ustilaginales smuts, simple septate basidiomycetes, and hymenomycetes, which formed a basal unresolved trifurcation.

The taxonomic characteristics of the smut-like, teliospore-forming yeast and filobasidiaceous species used in this study are summarized in Table 2. As shown in Fig. 2, these species were accommodated within two major groups, which correlated well with the septal pore type of the mycelium (simple pore or dolipore) and the presence or absence of cellular xylose. One major group (i.e., in the second major lineage in Fig. 2) is characterized by having simple pores and no xylose in the cells; these are the type species of *Sporidiobolus* Nyland, *Leucosporidium, Rhodosporidium, Kondoa*, and *Erythrobasi-*

Table 2. Taxonomic characteristics of the smut-like, teliospore-forming yeast and filobasidiaceous species used in this study.

Species	Septal pore structure*	Xylose in the cells**	Major ubiquinone system***	Teliospore****	Basidial form****
Leucosporidium scottii	Simple	−	9, 10	+	Phragmo
Rhodosporidium toruloides	Simple	−	9	+	Phragmo
Rhodosporidium dacryoidum	Simple	−	10	+	Phragmo
Sporidiobolus johnsonii	Simple	−	10	+	Phragmo
Kondoa malvinella	Simple	−	9	+	Phragmo
Erythrobasidium hasegawianum	Simple	−	10 (H_2)	−	Holo
Cystofilobasidium capitatum	Dolipore	+	8	+	Holo
Mrakia frigida	Dolipore	+	8	+	Holo
Filobasidium floriforme	Dolipore	+	10	−	Holo
Filobasidiella neoformans	Dolipore	+	10	−	Holo

*Data from Johnson-Reid and Moore (1972), Kwon-Chung and Popkin (1976), Moore (1972), Moore and Kreger-van Rij (1972), Nakagiri and Tubaki (1983), Oberwinkler et al. (1983), Suh and Sugiyama (1993b), and Suh et al. (1993).

**Data from Sugiyama et al. (1985), Suh and Sugiyama (1993b).

***Data from Nakase and Suzuki (1986), Sugiyama et al. (1985), Suh and Sugiyama (1993b), and Yamada and Kondo (1972a, b, 1973).

****Data from Hamamoto et al. (1988), and Kreger-van Rij (1984).

dium Hamamoto, Sugiyama et Komagata, and *Rhodosporidium dacryoidum*. Another major group (i.e., in the third major lineage in Fig. 2) is characterized by having dolipores and xylose in the cells: these are the type species of *Mrakia, Cystofilobasidium, Filobasidiella* Kwon-Chung, and *Filobasidium* Olive. This reinforces the results presented by Suh and Sugiyama (1993a).

Boekhout et al. (1993) concluded that the basidiomycetous yeasts can be accommodated in at least three orders of Heterobasidiomycetes, i.e., Ustilaginales, Tilletiales, and Tremellales. They accommodated the teliospore-forming yeast genera (e.g., *Leucosporidium, Rhodosporidium*, and *Sporidiobolus*), and related anamorphs within Ustilaginales, and members of the Filobasidiaceae within Tremellales. However, the phylogeny based on 18S rDNA sequence (Fig. 2) indicates that the teliospore-forming yeast species are closely related to each other but not to *Ustilago maydis* and *U. hordei* of the Ustilaginales. Swann and Taylor (1993) have reported the same result based on the comparison of 18S rDNA and concluded that the Sporidiales should be separated from the Ustilaginales. More molecular data are needed for species of *Ustilago* (Pers.) Roussel and related taxa in order to discuss relationships of the Ustilaginales and other teliospore-forming yeast species.

Phylogenetic placement of *Kondoa malvinella* Blanz and Gottschalk (1984) suggested transfer of *Rhodosporidium malvinellum* Fell et Hunter to another genus, because of the high degree of dissimilarity in the 5S rRNA sequence between this species and *R. toruloides* Banno. Yamada et al. (1989) placed the genus *Kondoa* within the Filobasidiaceae, and transferred *R. malvinellum* to *Kondoa* as the type species *K. malvinella* on the basis of comparisons of 18S and 26S rRNA partial sequences among species of the genera *Leucosporidium* and *Rhodosporidium*. However, Suh et al. (1993) suggested the exclusion of *Kondoa* from the Filobasidiaceae because *K. malvinella* has simple pores which are slightly inflated near the center, whereas the members of Filobasidiaceae have dolipores. In addition, other taxonomic characters of this species do not match with the Filobasidiaceae; e.g., the cellular carbohydrates profiles (Table 2). Boekhout et al. (1993) accommodated *Kondoa* in *Rhodosporidium* in their system for heterobasidiomycetous yeasts. Our phylogenetic tree inferred from 18S rDNA sequences showed that *Kondoa malvinella* was included in the lineage composed of *Leucosporidium scottii, Rhodosporidium toruloides, R. dacryoidum, Sporidiobolus johnsonii* Nyland, *Sporobolomyces roseus* Kluyver et van Niel, *Cronartium ribicola, Peridermium harknessii*, and *Erythrobasidium hasegawianum*. It showed clearly that *K. malvinella* is phylogenetically closer to these species than the members of Filobasidiaceae. However, the evolutionary distance between *K. malvinella* and *Rhodosporidium toruloides*, the type species of *Rhodosporidium*, was possibly enough to separate these two taxa taxonomically.

Phylogenetic placement of *Rhodosporidium dacryoidum* Suh et al. (1993) found simple pores in *Rhodosporidium dacryoidum*, and concluded that the inclusion of this species in the genus *Rhodosporidium* was justified because the septal structure, basidial form and chemotaxonomic data indicate a good correlation with other *Rhodosporidium* spp. Phylogeny from this study, however, showed a close relationship with *R. dacryoidum* and *Erythrobasidium hasegawianum*. The bootstrap analysis supported the topologies of these two yeasts at the 100% confidence level. The genus *Erythrobasidium* was tentatively placed in the Filobasidiaceae by Hamamoto et al. (1988), but a relationship to the teliospore-forming yeast species was suggested from our phylogenetic studies of septal ultrastructure and 18S rDNA sequences (Suh et al., 1993; Suh and Sugiyama, 1993a). Tani and Ohshima (1991) suggested a phylogenetically close relationship between *R. dacryoidum* and *E. hasegawianum*. They examined the U6 small nuclear RNA genes of several species of the genera *Rhodosporidium* and *Rhodotorula* using the PCR analysis and found only two, *R. dacryoidum* and *Rhodotorula hasegawae* (≡*E. hasegawianum*), which have mRNA-type introns in the gene. We also found an intron-like region in the 18S rDNA of *R. dacryoidum* as mentioned above, but not in *E. hasegawianum* (Suh and Sugiyama, 1993a). The introns inserted in several ribosomal RNA genes have been studied by many molecular biologists as mentioned above, but the evolutionary implications of introns are still uncertain. Nevertheless, the results of Tani and Ohshima (1991) suggest a potential area of study concerning the relationship between *R. dacryoidum* and *E. hasegawianum*. As seen in Fig. 2, the evolutionary distance between *R. dacryoidum* and *R. toruloides*, the type species, is probably sufficient to separate the former from the latter generically. Very recently, Yamada et al. (1994) proposed the new genus *Sakaguchia* for *R. dacryoidum* from the comparisons of 18S and 26S rRNA partial sequences. However, the result of Yamada et al. (1994) was not conclusive enough to justify the phylogenetic position of *R. dacryoidum*, because they compared only a few related species with no statistical test. We require more 18S rDNA data of other *Rhodosporidium* spp. and related taxa in order to justify the phylogenetic position of *R. dacryoidum*.

Phylogenetic placement of *Sympodiomycopsis paphiopedili* Sugiyama et al. (1991) proposed the anamorphic yeast genus *Sympodiomycopsis* for a yeast isolated from orchid nectar (*Paphiopedilum primurinum*). *Sympodiomycopsis paphiopedili*, the type species, appears to be basidiomycetous based on chemotaxonomic and ultrastructural studies (Sugiyama et al., 1991; Suh et al., 1993). The higher taxonomic placement and phylogenetic relationship remain uncertain, because the teleomorphic state is not yet known. Suh et al. (1993) suggested that *Sympodiomycopsis* could be accommodated in the Sporobolomycetaceae according to the van der Walt's system (1987) on the basis of its septal pore structure and chemotaxonomic data. In this study, we determined the sequence of 18S rDNA of *S. paphiopedili*

and compared it with those of 24 other basidiomycetes. As a result, our phylogenetic tree (Fig. 2) showed *S. paphiopedili* to be phylogenetically close to *Tilletia caries*, a smut fungus. The topology containing *T. caries* and *S. paphiopedili* is well supported by bootstrapping (Fig. 2). Phylogenetic analysis of 5S rRNA sequences has indicated that *Ustilago* and related smut fungi do not form a monophyletic group (Blanz and Unseld, 1987; Wolters and Erdmann, 1986). At the moment, we cannot fully explain the relationship between *T. caries*, whose septa have dolipores with multiperforate pore caps (Deml, 1977), and *S. paphiopedili*, whose septa have simple pores (Suh et al., 1993). However, phylogenetically, it is clear that *S. paphiopedili* is separated from the teliospore-forming yeast species and the Filobasidiaceae based on the comparison of 18S rDNA sequence.

In conclusion, the inferred phylogeny based on 18S rDNA sequence divided 35 selected species of basidiomycetes into three major lineages as mentioned above. The anamorphic yeast *Sympodiomycopsis paphiopedili*, together with the smut fungi *Tilletia caries, Ustilago maydis* and *U. hordei*, form the first major lineage. The second major lineage is composed of the smut-like, teliospore-forming yeast species, which are characterized by simple septal pores, and no xylose in the whole cell hydrolysates: it includes the type species of genera *Rhodosporidium, Leucosporidium, Sporidiobolus, Kondoa*, and *Erythrobasidium*, and *R. dacryoidum*. In addition to these yeasts, it accommodates the two rust fungi *Cronartium ribicola* and *Peridermium harknessii*. The third major lineage is composed of the filobasidiaceous yeasts which are characterized by dolipores, and presence of xylose in the whole cell hydrolysates: it includes the type species of the genera *Cystofilobasidium, Mrakia, Filobasidium*, and *Filobasidiella*. Anamorphic yeasts *Trichosporon cutaneum* and *Bullera alba* (the anamorph of *Bulleromyces albus*), some *Tremella* spp., and selected hymenomycetous genera are also included in this lineage.

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