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Molecular phylogeny of onygenalean fungi based on small subunit ribosomal DNA (SSU rDNA) sequences

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Phylogenetic analysis of nucleotide data from small subunit ribosomal DNA (SSU rDNA) sequences (ca. 1685 bp.) was performed on 19 taxa of the Onygenales and three related mitosporic fungi. Phylogenetic trees were constructed by the neighbor-joining method with the sequence data of related taxa obtained from DNA databases. The species in the Onygenales form two clusters and seven subclusters, and the tree topology reflects the traditional classification by Currah (1985) with some exceptions. The Myxotrichaceae is placed in the different lineage, separate from other plectomycetous taxa and among the Leotiales and the Erysiphales. Furthermore, two separate lineages in the Myxotrichaceae were found. Tree topology suggested the Onygenaceae is polyphyletic and composed of three subgroups; 1) most members of Onygenaceae, 2) *Spiromastix warcupii*, and 3) pathogenic dimorphic fungi classified in *Ajellomyces*.

Key Words——Onygenales; phylogeny; small subunit rDNA.

The Onygenales is an order of Ascomycota that produces gymnothecial or cleistothecial ascomata. In "Dictionary of the Fungi" (Hawksworth et al., 1995), 90 species of 36 genera are included in the order. The diagnostic characteristics of the family are as follows: 1) peridia are various in morphology, such as undifferentiated hyphae surrounding asci loosely or tightly, peridia with mesh-like, disarticulating wall, and membranous wall or cleistothecia formed in stipitate stroma; 2) asci are spherical, thin-walled, deliquescent, and formed freely on the mycelium or within peridia; 3) ascospores are small, single-celled and hyaline or pale in color with various surface ornamentations; 4) anamorph is aleurio- or arthroconidia that seceding rhexolytically; 5) several taxa degrade keratin and are associated with animals or their products including dung, feathers and bones.

In classifying of the Onygenales, ascoma structure was traditionally emphasized to circumscribe the taxa (Benjamin, 1956; Kuehn, 1958, 1959; Apinis, 1964; Benny and Kimbrough, 1980). Currah (1985) revised this order based on a large number of collections and pointed out that the resemblance of peridial structure does not reflect the phylogenetic lineage because many of them might result from convergent evolution. He introduced physiological characteristics in addition to morphological differences of ascospore and conidia for the higher classification in the Onygenales, and established four families in this order, viz., Arthrodermataceae, Gymnoascaceae, Myxotrichaceae, and Onygenaceae. His system has been widely accepted among mycologists, though von Arx (1987) proposed another system based mainly on the morphology of ascospores. Von Arx placed the onygenalean taxa into the Eurotiales, dividing the order among four families, i.e., the Amauroascaceae, Eurotiaceae, Gymnoascaceae and Onygenaceae.

After Currah's monograph, new taxa or new combinations have been proposed, and physiological, ecological and molecular data related to the order have been accumulated. Based on new information about the order, reconsideration of Currah's system has been attempted.

Currah (1994, 1995) himself pointed out that the Myxotrichaceae is dissimilar to other onygenalean families because of their affinity to plant debris and cellulolytic characters. Some species of the family are also known to form mycorrhizas with ericaceous host plants (Dalpé, 1989). Currah (1995) suggested that the family should be treated as an independent order and that the phylogenetic relatedness to other families should be examined by molecular methods.

He also suggested the Gymnoascaceae is an assemblage lacking the characteristics of the other families (Currah, 1985, 1994), and this family is possibly polyphyletic. If one were to accept his opinions, some species in this family would be transferred to the Eurotiales.

Recently, Udagawa (1997) tentatively rearranged the Amauroascaceae von Arx as a group of the Onygenales and suggested that *Amauroascus* J. Schroet., *Amaurascopsis* Guarro et al., *Auxarthron* Orr et Kuehn and *Spiromastix* Kuehn et Orr might be placed in the Amauroascaceae rather than in the Onygenaceae based on the characters of Q-10(H_2) ubiquinone system and less keratinolytic ability.

Molecular techniques also have been introduced for the systematics of Onygenales and have proved to be a

reliable tool for clarifying the phylogenetic relationships among the Onygenales (Bowman and Taylor, 1993). The data of Bowman et al. (1996) supported the monophyletic nature of the Onygenaceae, Gymnoascaceae and Arthrodermataceae by analyzing SSU rDNA sequences. Landvik et al. (1996) also analyzed SSU rDNA sequence of *Onygena equina* (Willd.) Pers., and suggested that two other plectomycetous ascomycetes, the Eremascaceae and the Ascosphaeraceae, form a monophyletic group together with the Onygenales. LeClerc et al. (1994) analyzed 25S rRNA partial sequences of 16 onygenalean taxa and 12 mitosporic species recognized as dermatophytes or pathogenic dimorphic fungi and suggested the pathogenic Onygenales separated into two groups: one group consisting of geophilic species and dermatophytes, and the other of systemic human pathogens. Their sequence analysis also suggested that the Myxotrichaceae had no close phylogenetic relationships with the other Onygenales, although only one species (*Byssoascus striatosporus* (Barron et Booth) von Arx) was analyzed. Most of these studies (LeClerc et al., 1994; Pan et al., 1994) were limited to the pathogenic species (dermatophytes or systemic human pathogens) and phylogenetic relationships among a broader selection of onygenalean taxa and their systematic position among the ascomycetes are still unclear.

In this study, we have used SSU rDNA sequence data to determine the taxonomic and phylogenetic relationships among some onygenalean taxa and related ascomycetous taxa. We determined the sequences of 19 species of the Onygenales belonging mainly to nonpathogenic groups, and three related mitosporic species, and compared resulting tree topology with the traditional classification system of the Onygenales.

Materials and Methods

Strains examined Representative strains of 22 species in 20 genera were newly sequenced by the following method (Table 1).

PCR amplification, cloning, and sequencing of SSU rDNA gene The strains were cultivated in a malt extract liquid medium (2% malt extract, 2% glucose) at 27°C for 7 d. The fungal cells were collected by centrifugation (3,000 rpm, 15 min), packed into aluminum foil, frozen to −80°C and then crushed mechanically with a hammer. The extraction and purification of DNA were performed

Table 1. Species and strains examined.

Family	Species[a]	Strain No.[b]	Accession No.
Amauroascaceae	*Amauroascus kuehnii* von Arx	CBS 539.72^{T}	AB015766
	Auxarthron compactum Orr et Plunkett	CBS 200.64^{T}	AB015767
	Spiromastix warcupii Kuehn et Orr	CBS 576.63^{T}	AB015768
Arthrodermataceae	*Arthroderma cifferrii* Varsavsky et Ajello (deposited as *Chrysosporium georgiae*)	CBS 272.66^{T}	AB015769
	Arthroderma incurvatum (Dawson et Gentles) Weitzman et al.	S 1477	AB015770
	Ctenomyces serratus Eidam	CBS 187.61^{NT}	AB015771
Gymnoascaceae	*Gymnascella aurantiaca* Peck (deposited as *Arachniotus verruculosus*)	CBS 655.71^{T}	AB015772
	Gymnoascoideus petalosporus Orr et al. (deposited as *Gymnoascus petalosporus*)	CBS 252.72	AB015773
	Gymnoascus reessii Baranetzky	CBS 410.72	AB015774
	Rollandina hyalinospora Kuehn et al. (deposited as *Gymnascella hyalinospora*)	CBS 548.72	AB015775
Myxotrichaceae	*Byssoascus striatosporus* (Barron et Booth) von Arx	CBS 642.66^{T}	AB015776
	Myxotrichum deflexum Berk.	CBS 228.61^{NT}	AB015777
	Pseudogymnoascus roseus Raillo	CBS 395.65^{NT}	AB015778
Onygenaceae	*Aphanoascus mephitalis* (Malloch et Cain) Cano et Guarro (deposited as *Neoxenophila foetida*)	CBS 453.75	AB015779
	Aphanoascus terreus (Randhawa et Sandhu) Apinis (deposited as *Chrysosporium indicum*)	CBS 342.64^{T}	AB015780
	Apinisia graminicola La Touche	CBS 721.68^{T}	AB015781
	Ascocalvatia alveolata Malloch et Cain	CBS 777.70	AB015782
	Pectinotrichum llanense Varsavsky et Orr	CBS 882.71^{T}	AB015783
	Renispora flavissima Sigler et al.	CBS 708.79	AB015784
Mitosporic fungi related to Onygenales	*Geomyces pannorum* (Link) Sigler et Carmichael var. *pannorum*	CBS 108.14	AB015785
	Malbranchea aurantiaca Sigler et Carmichael	CBS 127.77^{T}	AB015786
	Oidiodendron tenuissimum (Peck) Hughes	CBS 238.31	AB015787

a T, ex Type; NT, ex Neotype.

b Strain derived from: CBS, Centraalbureau voor Schimmelcultures, Baarn, the Netherlands; S, soil isolate in this study.

as described by Yotsumoto et al. (1995).

The SSU rDNA sequences were determined by amplifying the gene using the polymerase chain reaction (PCR) with Taq polymerase (AmpliTaq® DNA polymerase, PE Applied Biosystems) and primers; AO5 (5′-GAAACTGCGAATGGCTCATTAAATC-3′) and AO11 (5′-CCTTGTTACGACTTTTACTTCCTC-3′). The PCR reaction as performed with a DNA thermal cycler (PTC-200, MJ Research) by 30 amplification cycles consisting of denaturation at 94°C for 1 min, primer annealing at 55°C for 1.5 min, primer extension at 72°C for 2.5 min followed by 10 min extension.

The PCR products (corresponding to *Saccharomyces cerevisiae* Meyen ex E. C. Hansen position 83-1766, approximately 1685 bp.), were cloned by using the plasmid vector pT7Blue(R) (Novagen cloning kit), then transformed into competent DH5α *Escherichia coli* cells (TOYOBO).

Both strands of cloned SSU rDNA were sequenced by the dideoxy method with ABI PRISM™ Cycle Sequencing Kits and ABI PRISM® 377 automated sequencers (PE Applied Biosystems). The following primers were used for sequencing; AO5, AO6 (5′-CGGAGAGGGAGCCTGAGAAACGGC-3′), AO8 (5′-CTACTGCGAAAGCATTTGCCAAGG-3′), AO9 (5′-TGGTGGTGCATGGCCGTTCTTAG-3′), AO11, AO12 (5′-CATCTAAGGGCATCACAGACCTG-3′), AO13 (5′-CAATTCCTTTAAGTTTCAGCCTTG-3′) and AO15 (5′-ATTTATTGTCACTACCTCCCCG-3′).

Phylogenetic analysis Overlapping sequence data for each primer were aligned and connected by using the sequence editing software GENETYX-SV/R ver. 3.1 for Windows. An intron that was found in the sequences of *Geomyces pannorum* (Link) Sigler et Carmichael var. *pannorum* (1086–1451 bp.) was excluded manually.

The SSU rDNA sequences retrieved from the nucleotide sequence databases (GenBank/EMBL/DDBJ) are listed in Table 2 with their accession numbers. All sequences were aligned using CLUSTAL W ver. 1.7, the multiple sequence alignment program. Subsequent phylogenetic analysis was performed with the PHYLIP package ver. 3.5. Using the two-parameter model of Kimura (1980), distances between the sequences (K_{nuc} values) were calculated. Phylogenetic trees were constructed by the neighbor-joining (NJ) method (Saitou and Nei, 1987) based on the comparison of 1685 sites of partial sequence data sets. The topology of the trees was evaluated by a bootstrap analysis (Felsenstein, 1985) of 1,000 random resamplings. The DDBJ homology search system, FASTA was used (Pearson and Lipman, 1988) for revealing close relatives to *Myxotrichum deflexum* Berk. and *Pseudogymnoascus roseus* Raillo.

Results

For analyzing phylogenetic relationships of onygenalean fungi and the other cleistothecial fungi (Ascosphaerales, Eurotiales and Elaphomycetales), the new sequence data were aligned with 18 previously published sequences and the phylogenetic tree was constructed by the NJ method (Fig. 1). *Saccharomyces cerevisiae* was employed as an outgroup.

As shown in Fig. 1, plectomycetous taxa were divided into three major clusters, I, II and III. The members of cluster I corresponded to the Eurotiales and the Elaphomycetales, while the Ascosphaerales and the Onygenales were included in cluster II. Among the cluster II, which corresponded to the three families of the Onygenales, five subclusters were distinguished (subcluster IIb~IIf). The Myxotrichaceae diverged into a monophyletic group (cluster III).

The Ascosphaerales, *Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltoir and *Eremascus albus* Eidam, located in the cluster II and formed a sister group with a part of Onygenaceae (subcluster IIb). However, the bootstrap confidence (BS) indicating the monophyly of subcluster IIa and IIb was low (33.1%).

In the cluster II, the species in the Onygenaceae sensu Currah were separated into three subgroups. There are 13 saprophytic or geophilic species in the Onygenaceae and *Coccidioides immitis* Rixford et Gilchrist in the subcluster IIb. Three species in the *Ajellomyces* McDonough et Lewis constituted subcluster IIe though they were classified into the Onygenaceae by Currah (1985). *Spiromastix warcupii* Kuehn et Orr was located outside the subcluster IIb and constituted a branch of its own (subcluster IId).

The Arthrodermataceae was clearly individualized as subcluster IIc (BS of 97.1%), though *Apinisia graminicola* La Touche in the Onygenaceae was also included in this subcluster (BS of 66.3%).

The species in Gymnoascaceae also formed a monophyletic group (subcluster IIf) with high bootstrap confidence (99.1%).

As shown in Fig. 1, three genera of Myxotrichaceae and two related anamorphic genera formed a separate cluster from other plectomycetous taxa with high bootstrap confidence (96.7%). In a search for sequences similar to those of *Myxotrichum deflexum* and *Pseudogymnoascus roseus*, the species in Loculoascomycetes and Discomycetes were indicated.

Figure 2 shows a phylogenetic tree focusing on the phylogenetic position of the Myxotrichaceae with respect to the Loculoascomycetes and Discomycetes. The Myxotrichaceae formed a monophyletic group with some Leotiales (e.g., *Monilinia laxa* (Aderhold et Ruhland) Honey and *Neobulgaria premnophila* F. Roll-Hansen et H. Roll-Hansen) with high bootstrap support (96.0%). It is much notable that completely different taxa, *Blumeria graminis* (DC.) Speer (≡*Erysiphe graminis* DC., Erysiphales, Erysiphaceae) are also included in this cluster. In addition, two different lineages were found in this cluster (subcluster IIIa and IIIb). Subcluster IIIa contained *Pseudogymnoascus roseus* and *Geomyces pannorum* var. *pannorum*, while IIIb contained *Myxotrichum deflexum*, *Byssoascus striatosporus* and *Oidiodendron tenuissimum* (Peck) Hughes.

Table 2. Reference SSU rDNA sequences obtained from the databases.

Class	Order	Family	Species	Accession No.
Plectomycetes	Onygenales	Onygenaceae	*Ajellomyces capsulatus* (Kwon-Chung) McGinnis et Katz	Z75306
			Ajellomyces crescens Sigler	U29390
			Ajellomyces dermatitidis McDonough et Lewis	X59420
			Auxarthron zuffianum (Morini) Orr et Kuehn	L28062
			Onygena equina (Willde.) Pers.	U45442
			Uncinocarpus reesii Sigler et Orr	U29394
	Mitosporic fungi related to Onygenales		*Coccidioides immitis* Rixford et Gilchrist	M55627
			Malbranchea albolutea Sigler et Carmichael	L28063
			Malbranchea dendritica Sigler et Carmichael	L28064
			Trichophyton rubrum (Castellani) Sabour.	X58570
	Ascosphaerales	Ascosphaeraceae	*Ascosphaera apis* (Maassen ex Claussen) Olive et Spiltoir	M83264
		Eremascaceae	*Eremascus albus* Eidam	M83258
	Eurotiales	Trichocomaceae	*Byssochlamys nivea* Westling	M83256
			Coonemeria crustacea (Apinis et Chesters) Mouchacca	M83263
			Eupenicillium javanicum (van Beyma) Stolk et Scott	U21298
			Talaromyces macrosporus (Stolk et Samson) Frisvad et al.	M83262
	Mitosporic fungus related to Eurotiales		*Aspergillus fumigatus* Fresenius	M55626
Pyrenomycetes	Hypocreales	Hypocreaceae	*Hypocrea lutea* (Tode) Petch	D14407
	Erysiphales	Erysiphaceae	*Blumeria graminis* (DC.) Speer f. sp. *hordei*	L26253
Discomycetes	Caliciales	Caliciaceae	*Cyphelium inquinans* (Sm.) Trevis	U86695
		Sphinctrinaceae	*Sphinctrina turbinata* (Pers.) De Not.	U86693
	Cyttariales	Cyttariaceae	*Cyttaria darwinii* Berk.	U53369
	Elaphomycetales	Elaphomycetaceae	*Elaphomyces leveillei* Nees	U45441
	Lecanorales	Stereocaulaceae	*Pilophorus acicularis* (Ach.) Nyl.	U70960
	Leotiales	Geoglossaceae	*Spathularia flavida* Fr.: Fr.	Z30239
		Leotiaceae	*Neobulgaria premnophila* F. Roll-Hansen et H. Roll-Hansen	U45445
		Sclerotiniaceae	*Monilinia laxa* (Aderhold et Ruhland) Honey	Y14210
		Orbiliaceae	*Orbilia auricolor* (Bloxam ex Berk.) Sacc.	U72598
	Neolectales	Neolectaceae	*Neolecta vitellina* (Bres.) Korf et J.K. Rogers	Z27393
	Pezizales	Helvellaceae	*Gyromitra montana* Harmaja	U42652
			Rhizina undulata Fr.: Fr.	U42664
		Morchellaceae	*Verpa bohemica* (Krombholz) Schroeter	U42645
Loculoascomycetes	Dothideales	Botryosphaeriaceae	*Botryosphaeria ribis* Grossenbacher et Duggar	U42477
		Dothideaceae	*Dothidea hippophaes* (Passerini) Fuckel	U42475
			Dothidea insculpta Wallroth	U42474
		Leptosphaeriaceae	*Leptosphaeria maculans* (Desm.) Cesati et De Not.	U04238
		Lophiostomataceae	*Herpotrichia juniperi* (Duby) Petrak	U42483
			Lophiostoma crenatum (Pers.: Fr.) Fuckel	U42485
		Mycosphaerellaceae	*Mycosphaerella mycopappi* Funk et Dorworth	U43463
		Sporormiaceae	*Sporormia lignicola* Phillips et Plowright	U42478
	Mitosporic fungus related to Loculoascomycetes		*Aureobasidium pullulans* (de Bary) Arnaud	M55639
Ascomycota (Incertae sedis)			*Symbiotaphrina kochii* Jurzitza ex W. Gams	D49656
Hemiascomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces cerevisiae* Meyen ex E. C. Hansen	M27607

Discussion

Phylogenetic analysis using SSU rDNA sequences clearly showed the phylogenetic relatedness among the onygenalean taxa. The families proposed by Currah (1985) can be recognized in the tree topology although there are some exceptions (Fig. 1). Currah (1995) also suggested the Myxotrichaceae should be treated as 'Myxotrichales' because of its ecological differences from other onygenalean taxa. The species in this family are characterized by cellulolytic ability and affinity to plant materials and most of them are known to be psychrophilic. Our results support his opinion (Fig. 1) and also confirmed the results from 25S rRNA se-

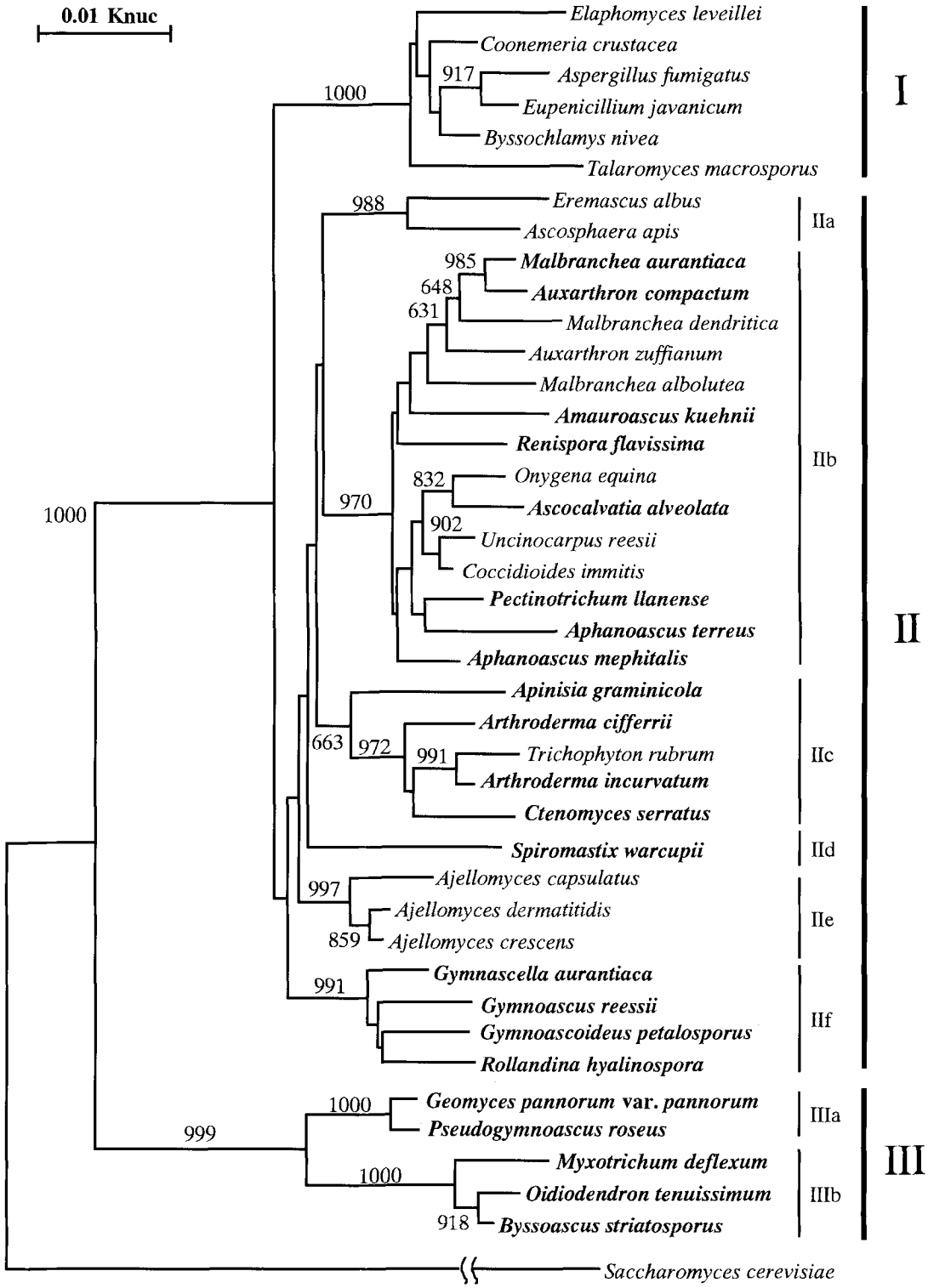

Fig. 1. Phylogenetical tree based on SSU rDNA sequences for the Onygenales and related plectomycetous taxa. The tree was constructed by the NJ method. The numerals represent the confidence level from 1,000 replicate bootstrap samplings (frequencies less than 50% are not indicated).

quences analysis by LeClerc et al. (1994), although they analyzed only one species (*Byssoascus striatosporus*). In our analysis, two lineages were recognized in the Myxotrichaceae (subclusters IIIa and IIIb). These subclusters were finely corresponded to different groups distinguished by ascospore ornamentation, i.e., the lon-

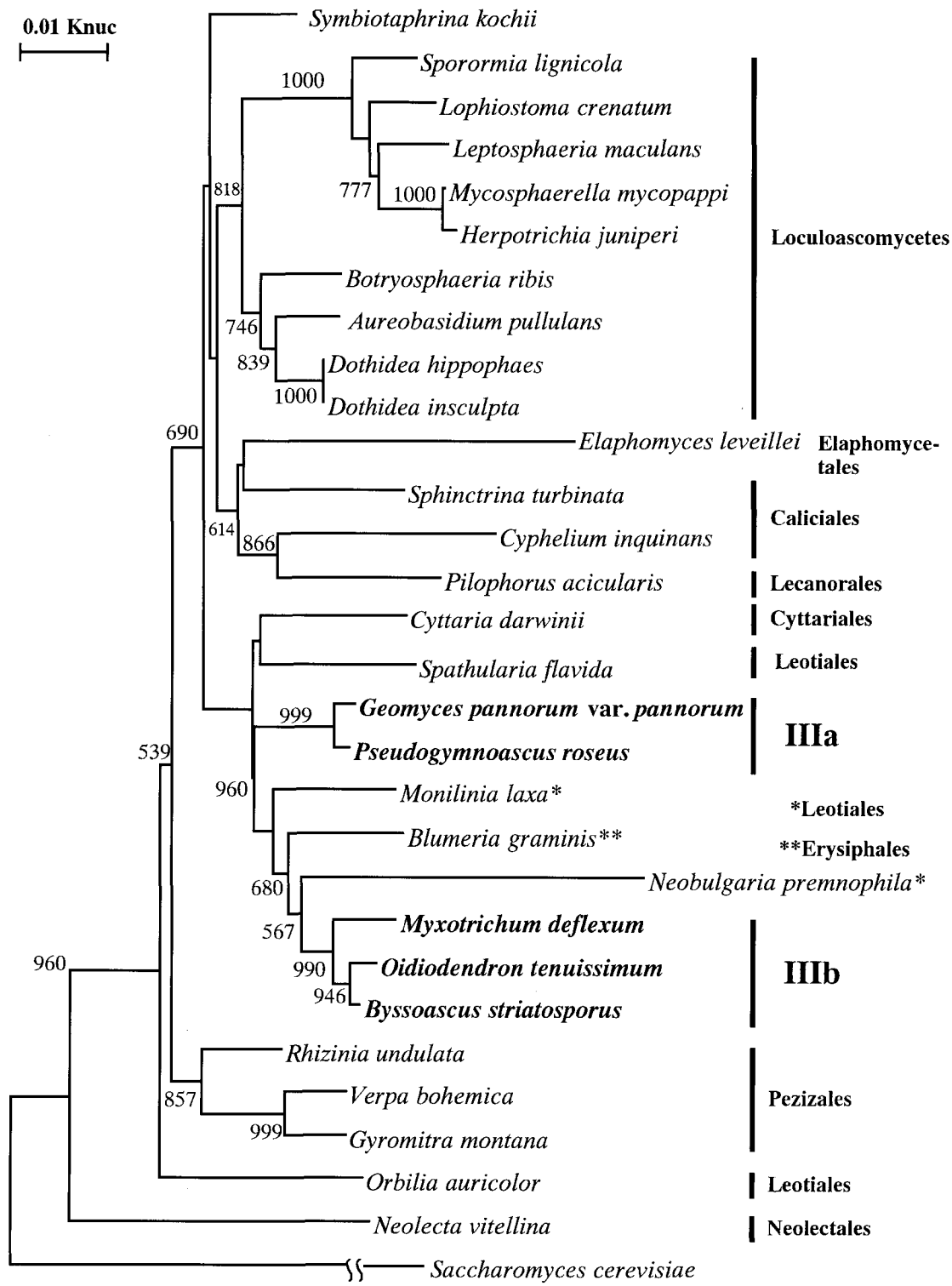

Fig. 2. Phylogenetical tree based on SSU rDNA sequences showing the disposition of myxotrichaceous species.

gitudinally striate ascospore one is common in subcluster IIIb and the non-striate ascospore group (*Pseudogymnoascus roseus* and its anamorphic genus *Geomyces*) is in subcluster IIIa. Our tree topology (Fig. 1) is consistent with the results of ITS region of rDNA analysis by Hambleton et al. (1998b). It may be an interesting subject that the phylogenetic location of *Gymnostellatospora* Udagawa et al. (Udagawa et al., 1993) which produces ascospores with longitudinal crests.

In Fig. 2, the relatedness among the Myxo-

trichaceae, Leotiales and Erysiphales is shown. These results are very interesting because these three taxa are quite different from each other in morphologiclal and/or ecological-physiological aspects.

The species of the discomycete order, Leotiales, form clavate, inoperculate asci with paraphyses on the hymenium of apothecia which are morphologically dissimilar to the cleistothecial ascomata of the Myxotrichaceae. Currah (1994, 1995) suggested the phylogenetic relationships between the Myxotrichaceae and *Hymenoschyphus ericae* (Read) Korf et Kernan in the Leotiales because some species in the Myxotrichaceae are known to form ericoid mycorrhizae in the same manner as *H. ericae*. Recently, Hambleton et al. (1998a) analyzed 18SrDNA sequences (data not shown) of root endophytic fungi in the Ericaceae, including *Myxotrichum arcticum* Udagawa et al., *Oidiodendron maius* Barron and *H. ericae*. Their results support the close relationships of the species in the Myxotrichaceae and *H. ericae*.

Blumeria graminis, which is classified in the Erysiphales, produces cleistothecia and an oidium anamorph, but the species in the Erysiphales are commonly obligate biotrophs known as powdery mildews of cereals and grasses, they can not grow saprophytically, and in this respect differ from the species in the Myxotrichaceae.

Our results proved the independency of the Myxotrichaceae from other onygenalean taxa (Fig. 1). Hambleton et al. (1998a) also described the same results. We consider that this family should be separated from other onygenalean taxa in the phylogenetic viewpoint. However, as shown in Fig. 2, it would be inadequate to treat them in a single taxonomic group such as 'Myxotrichales' as suggested by Currah (1995). In any case, accumulation and comparison of more sequence data is necessary among the Leotiales, Erysiphales and two groups of the Myxotrichaceae to clarify their phylogenetic relationships.

Currah (1985, 1994, 1995) pointed out the Gymnoascaceae is an artificial assemblage and probably polyphyletic. In our analysis, however, the four species in the Gymnoascaceae formed a monophyletic group in Fig. 1 with high bootstrap support. Although Currah (1994) suggested that *Gymnoascoideus petalosporus* Orr et al. is better disposed in the Arthrodermataceae, SSU rDNA sequence data did not support it. Currah (1994) also suggested that *Gymnascella* Peck is a large genus within the family and the species with pronounced equatorial modifications [e.g., *G. confluens* (Sartory et Bainier) Currah, *G. marginospora* (Kuehn et Orr) Currah, *G. punctata* (Dutta et Ghosh) Currah] would be better accommodated in the Eurotiales. In this study, macromolecular sequences of these species were not analyzed. Further sequencing study including many gymnoascaceous species is essential for a better understanding of the phylogenetic relationships in the Gymnoascaceae.

In our analysis, the species in Onygenaceae sensu Currah are separated into three subgroups. This family is characterized by pitted ascospores and by having at least some ability to degrade keratin. Peridial and anamorph morphology are variable among the genera. The ubiquinone system also varies in this family, so that Udagawa (1997) described the Amauroascaceae for the species possessing Q-10(H_2) ubiquinone system and less keratin degradation ability.

The Amauroascaceae is recognizable as a monophyletic group in the subcluster IIb (Fig. 1), but the bootstrap confidence was low (36.3%). Therefore, the phylogenetic divergence between the Amauroascaceae and the Onygenaceae is still unclear and further morphological and nonmorphological analyses study will be required to determine the best classification for these species.

Spiromastix warcupii is placed in the Amauroascaceae by Udagawa (Udagawa, 1997). This species sited apart from the subcluster IIb although it also has Q-10(H_2) ubiquinone system (Sugiyama, unpublished data). Recently, several new species of this genus were reported from animal dung and soils in tropical or temperate regions (Currah and Locquin-Linard, 1988; Guarro et al., 1993; Uchiyama et al., 1995). Therefore, we will reserve the phylogenetic conclusion on *Spiromastix* until the SSU rDNA sequences of these additional species are analyzed.

Currah (1994) recently transferred *Pectinotrichum llanense* Varsavsky et Orr to the genus *Auxarthron*. Our tree topology did not support his treatment. This species resembles to the other *Auxarthron* species in the peridium and ascospore morphology, but differs in having pectinate structures associated with the peridium and a *Chrysosporium*-type anamorph. Consequently, the data do not support Currah's placement of *P. llanense* in the genus *Auxarthron*.

The species in the Arthrodermataceae form a monophyletic group with high bootstrap support and corresponded well with morphological classification by Currah (1985). The species in this family are characterized keratin degrading ability, smooth ascospores and ossiform peridial cells. In addition, most species of this family produce macroconidia (*Microsporum* Gruby and *Trichophyton* Malmsten) with microconidia. These characterstics are thought to reflect their phylogenetic relatedness.

Trichophyton rubrum (Castellani) Sabouraud formed more consistent group (99.0% of bootstrap confidence) with *Arthroderma incurvatum* (Dawson et Gentles) Weitzman et al. (anamorph: *Microsporum*) rather than *Arthroderma cifferrii* Varsavsky et Ajello (anamorph: *Chrysosporium*). *Arthroderma incurvatum* was classified as the genus *Nannizzia* Stockdale (=*N. incurvata*) because of their anamorphic difference. This result supports the notion *Arthroderma* and *Nannizzia* are congeneric in spite of the morphological differences in macroconidia (Weitzman et al., 1986) as confirmed previously by RFLP analysis of mitochondrial DNA by Kawasaki et al. (1992).

Apinisia graminicola is placed in subcluster IIc though it is classified in the Onygenaceae (Currah, 1985). Our result showed the possibility that *A. graminicola* was more closely related to the Arthrodermataceae than the Onygenaceae in spite of the low boot-

strap confidence (66.3%). However, ascospores of *A. graminicola* are globose and reticulate, and clearly different from lenticular and smooth ascospores of all members in the Arthrodermataceae. Therefore, molecular analysis of other species of this genus is necessary to clarify whether *Apinisia* form a monophyletic group independent from the other Onygenaceae.

In conclusion, we have examined the molecular phylogeny of some onygenalean taxa based on the sequences of SSU rDNA. Our results suggest that the higher classification system in the Onygenales proposed by Currah (1985) mostly reflects the phylogenetic relationships. The Myxotrichaceae, however, is placed in an independent phylogenetic position compared to other families in the Onygenales. In addition, the tree topology suggests that the Onygenaceae sensu Currah is polyphyletic, with three subgroups which are composed of the most members of the traditional Onygenaceae, *Spiromastix warcupii*, and pathogenic dimorphic fungi classified as *Ajellomyces*.

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Literature cited

Apinis, A. E. 1964. Revison of British Gymnoascaceae. Mycol. Pap. Commonw. Mycol. Inst. **96**: 1–56.

Arx, J. A. von. 1987. A re-evaluation of the Eurotiales. Persoonia **13**: 273–300.

Benjamin, R. K. 1956. A new genus of the Gymnoascaceae with a review of the other genera. El Aliso **3**: 301–328.

Benny, G. L. and Kimbrough, J. W. 1980. A synopsis of the orders and families of plectomycetes with keys to genera. Mycotaxon **12**: 1–91.

Bowman, B. H. and Taylor, J. W. 1993. Molecular phylogeny of pathogenic and non-pathogenic Onygenales. In: The fungal holomorph: Mitotic, meiotic and pleomorphic speciation in fungal systematics, (ed. Reynolds, D. R. and Taylor, J. W.), pp. 169–178. CAB International, Wallingford.

Bowman, B. H., White, J. T. and Taylor, J. W. 1996. Human pathogenic fungi and their close nonpathogenic relatives. Mol. Phylogenet. Evol. **6**: 89–96.

Currah, R. S. 1985. Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotrichaceae and Onygenaceae. Mycotaxon **24**: 1–216.

Currah, R. S. 1994. Peridial morphology and evolution in the prototunicate ascomycetes. In: Ascomycete systematics: Problems and perspectives in the nineties, (ed. Hawksworth, D. L.), pp. 281–293. Plenum Press, New York.

Currah, R. S. 1995. Ecological data and the systematics of the Onygenales. Proc. 3rd Intern. Symp. Mycol. Soc. Japan, Nov. 30-Dec.1, 1995, pp. 68–72. Nat. Hist. Mus. Inst., Chiba, Japan.

Currah, R. S. and Locquin-Linard, M. 1988. *Spiromastix grisea* sp. nov. and its relationship to other Onygenaceae with helical appendages. Can. J. Bot. **66**: 1135–1137.

Dalpé, Y. 1989. Ericoid mycorrhizal fungi in the Myxotrichaceae and Gymnoascaceae. New Phytol. **113**: 523–527.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**: 783–791.

Guarro, J., Gené, J. and de Vroey, C. H. 1993. A new species of *Spiromastix* from Africa. Mycotaxon **46**: 307–313.

Hambleton, S., Currah, R. S. and Egger, K. N. 1998a. Phylogenetic relationships of ascomycetous root endophytes of the Ericaceae inferred from 18S rDNA sequence analysis. Programme and Abs. 2nd Intern. Conf. Mycorrhiza, July 5–10, 1998, pp. 78–79. Swed. Univ. Agri. Sci., Uppsala, Sweden.

Hambleton, S., Egger, K. N. and Currah R. S. 1998b. The genus *Oidiodendron*: species delimitation and phylogenetic relationships based on nuclear ribosomal DNA analysis. Mycologia **90**: 854–869.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. 1995. Ainsworth & Bisby's dictionary of the fungi, 8th ed. CAB International, Wallingford, U.K.

Kawasaki, M., Aoki, M., Ishizaki, H., Nishio, K., Mochizuki, T. and Watanabe, S. 1992. Phylogenetic relationships of the genera *Arthroderma* and *Nannizzia* inferred from mitochondrial DNA analysis. Mycopathologia **118**: 95–102.

Kimura, M. 1980. A simple method for estimation of evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120.

Kuehn, H. H. 1958. A preliminary survey of the Gymnoascaceae. I. Mycologia **50**: 417–439.

Kuehn, H. H. 1959. A preliminary survey of the Gymnoascaceae. II. Mycologia **51**: 665–692.

Landvik, S., Shailer, N. F. J. and Eriksson, O. E. 1996. SSU rDNA sequence support for a close relationship between the Elaphomycetales and the Eurotiales and Onygenales. Mycoscience **37**: 237–241.

LeClerc, M. C., Philippe, H. and Guého, E. 1994. Phylogeny of dermatophytes and dimorphic fungi based on large subunit ribosomal RNA sequence comparisons. J. Med. Vet. Mycol. **32**: 331–341.

Pan, S., Sigler, L. and Cole, G. T. 1994. Evidence for a phylogenetic connection between *Coccidioides immitis* and *Uncinocarpus reesii* (Onygenaceae). Microbiology **140**: 1481–1494.

Pearson, W. R. and Lipman, D. J. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**: 2444–2448.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.

Uchiyama, S., Kamiya, S. and Udagawa, S. 1995. *Spiromastix saturnispora*, a new species from Indonesian soil. Mycoscience **36**: 353–357.

Udagawa, S., Uchiyama, S. and Kamiya, S. 1993. *Gymnostellatospora*, a new genus of the Myxotrichaceae. Mycotaxon **48**: 197–205.

Udagawa, S. 1997. Taxonomic studies on Plectomycetes (Cleistothecial ascomycetes). Nippon Kingakukai Kaiho **38**: 143–157. (In Japanese.)

Weitzman, I., McGinnis, M. R., Padhye, A. A. and Ajello, L. 1986. The genus *Arthroderma* and its later synonym *Nannizzia*. Mycotaxon **25**: 505–518.

Yotsumoto, Y., Ohara, A. and Mikawa, T, 1995. A convenient method for extraction and purification of DNA from filamentous fungi. Microbiol. Cult. Coll. **11**: 19–21. (In Japanese.)