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Phylogenetic Evaluation of Stereoid Fungi

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Abstract Phylogenetic relationships of stereoid fungi were examined by comparing nuclear small subunit ribosomal RNA gene sequences. Stereoid taxa were scattered into several groups and the traditional Stereaceae proved to be polyphyletic. *Stereum* and *Xylobolus* were classified in the Stereaceae as the core group of stereoid fungi, and *Amylostereum* was grouped with *Echinodontium* of the Echinodontiaceae. *Chondrostereum* and *Cystostereum* were clustered in the Stereaceae *sensu* Donk and *Cymatoderma* and *Podoscypha* in the Podoscyphaceae Reid. *Columnocystis abietinum* and *C. ambigua* were grouped with *Meripilus giganteus* and proved to be not included in the Chaetodermataceae *sensu* Nakasone. *Lopharia cinerascens* and *L. mirabilis* were grouped together but *L. spadicea* was unrelated to them, indicating that *Lopharia* is heterogeneous at a generic level.

Key words: *Amylostereum*, *Columnocystis*, *Lopharia*, *Xylobolus*

Stereoid fungi are well-known wood-inhabiting members of the Aphyllophorales (Hymenomycetes, Basidiomycota) and typically have leathery bracket basidiocarps with smooth hymenophores. Historically, stereoid fungi were first described as a tribe Resupinatus of the genus *Thelephora* by Fries [6] for a group having smooth hymenophores. Fries' concept of *Stereum* also included species of *Hymenochaete* and *Dendrothele*, and dominated the classification system until the early twentieth century [7]. However, the concept of *Stereum* was increasingly narrowed as microscopic characters became extensively studied since the mid-twenties. Pouzar [36] narrowed the concept of *Stereum* and segregated *Hematostereum*, *Laurilia*, *Lloydellopsis*, *Columnocystis*, *Chondrostereum*, and *Cystostereum* from *Stereum*. The truly stipitate genera such as *Podoscypha*, *Aquascypha*, *Cotylidia*, *Cyphellostereum*, *Inflatostereum*, *Stereopsis*, and *Cymatoderma* were excluded from *Stereum* and elevated to a family level by Reid [37]. *Laxitextum* was segregated from *Stereum* due to differences in sterile elements, asperulate spores, and hyphal construction [7, 37]. *Boreostereum* was separated from *Stereum* by the green reaction of the hyphal encrustation in KOH, a slightly folded rusty brown hymenophore with a dark brown tomentum and a distinct black subiculum in section [35]. *Amylostereum* and *Dendrophora* were also segregated from *Stereum* [7]. Chamuris [6] divided *Stereum* into 3 subgenera, *Stereum*, *Aculeatostereum*, and *Acanthostereum*, based on the presence or absence of acanthohyphidia and pseudoacanthohyphidia.

According to Chamuris [7], *Stereum* and *Xylobolus* form a core group among genera that have been included in the Stereaceae. However, many genera of the family show distinct characters of their own, different from those of *Stereum*, and were often regarded as distantly related to *Stereum* by many authors. For instance, members of *Amylostereum* with amyloid spores and a dimitic hyphal system consisting of skeletal hyphae have similar characters, which are closely related to those of *Stereum*, but they differ by the brown color of the entire basidiocarp caused by intramembranal pigmentation of skeletal hyphae and cystidia [36]. *Chondrostereum* differs from *Stereum* in its cartilaginous consistency of trama, vesicular bodies, and inamyloid spores [36]. *Cystostereum* has a great number of gloeocystidia, a dimitic hyphal system with very scarce light-colored skeletal hyphae, hard consistency of trama, and inamyloid spores [36]. *Columnocystis* has cystidia of generative origin and inamyloid spores [36] and develops brown rot like *Veluticeps* [13, 33].

As many genera of the Stereaceae are so different to one another as stated above, various controversies existed on the familial assignment of genera among taxonomists [7, 8, 25, 33, 40]. Donk [8] included *Chondrostereum* in the Stereaceae, while Parmasto [35] and Talbot [40] included

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it in the Corticiaceae. *Laxitextum* was included in the Hericiaceae by Donk [8] but was later placed in the Corticiaceae by Parmasto [35] and Talbot [40]. Jülich [25] suggested a new family Chaetodermataceae that includes *Chaetoderma* of the Corticiaceae and also *Columnocystis* which was classified in the Stereaceae by Donk [8], Talbot [40], and Parmasto [35]. Nakasone [33] suggested that *Columnocystis* and *Veluticeps* were congeneric and, with *Chaetoderma* and *Crustoderma*, should be grouped in the Chaetodermataceae. *Cystostereum* used to be assigned to the families Stereaceae [8, 40], Steccherinaceae [35], and Cystostereaceae [25]. Stipitate genera such as *Podoscypha* and *Cymatoderma* were included in the family Stereaceae [8] or Podoscyphaceae [25, 40]. As pointed out by Donk [8] and Jülich [25], the limit between the Stereaceae and the Corticiaceae often seems to be indistinct, although the two families were recently proved to be not synonymous by the analysis of nuclear large subunit ribosomal DNA [44]. Such examples as above explain why there have been so many debates on the relationships of stereoid fungi, and the scope of the Stereaceae.

Recently, phylogenetic studies using molecular markers have been applied to various taxonomic situations for solving taxonomic problems, and molecular techniques are becoming increasingly important as a means to obtain appropriate characters and to study taxonomic and phylogenetic relationships among fungi [1, 2, 5, 19, 24, 28]. Some species in the Stereaceae have been sequenced as part of phylogenetic studies on the Aphyllophorales. Hibbett and Donoghue [18], Hibbett [17], and Hibbett *et al.* [19] have determined sequences of nuclear and mitochondrial ribosomal RNAs from some stereoid fungi. Boidin *et al.* [3] extensively studied internal transcribed spacer (ITS) regions of numerous genera and species in the Aphyllophorales, including many taxa of the Stereaceae. In these studies, various interesting phylogenetic conclusions have been made on the systematics of the Aphyllophorales, but few facts have yet been established about the relationships of taxa in the Stereaceae. Wu *et al.* [45] recently analyzed *Aleurodiscus s.l.*, taxonomically equivalent to *Aleurodiscus sensu* Núñez and Ryvarden [34], using nuclear large subunit ribosomal DNA data, and discussed the phylogenetic relationships of aleurodiscoid fungi in relation to stereoid fungi.

For phylogenetic analyses of stereoid fungi, this study was accomplished to see specific phylogenetic relationships among genera of the traditional Stereaceae, find taxa that constitute the core group of stereoid fungi, and evaluate the present status of the Stereaceae and other related families. Nuclear small subunit ribosomal RNA gene regions were used as an adequate molecular marker to explain the present taxonomic subject at a family level. Traditional stereoid genera that were found to be unrelated to the Stereaceae *s.s.* were discussed from the point of view of phylogenetics.

MATERIALS AND METHODS

Sources and DNA Preparations

Thirteen strains and two herbarium specimens (*Stereum ostrea* SFC 960921-8, *Lopharia mirabilis* SFC 991030-8) and their sources used in this study are listed with 42 compared taxa in Table 1. Total DNAs were extracted

Table 1. Fungal taxa used in this study, their families, and GenBank accession numbers.

Species name	Family	Source[a]	GenBank
Aleurodiscus botryosus Burt	Corticiaceae Herter		F026603
Amylostereum areolatum (Fr.) Boid.	Stereaceae Pilát	CBS[b] 334.66	AF082845
Amylostereum chailletii (Fr.) Boid.	Stereaceae Pilát	CBS 480.83	AF082846
Antrodia carbonica (Overh.) Ryv. & Gilbn.	Polyporaceae Corda		U59059
Athelia bombacina (Pers.) Jül.	Corticiaceae Herter		M55638
Auriscalpium vulgare S. F. Gray	Auriscalpiaceae Maas G.		U59060
Bjerkandera adusta (Willd.: Fr.) Karst.	Polyporaceae Corda		U59061
Bondarzewia berkeleyi (Fr.) Bond. & Sing.	Bondarzewiaceae Kotl. & Pouz.		U59062
Boreostereum radiatum (Peck) Parm.	Stereaceae Pilát	CBS 417.61	AF082847
Botryobasidium subcoronatum (v. Höhn. & Litsch.) Donk	Corticiaceae Herter		AF026609
Ceriporia purpurea (Fr.) Donk	Polyporaceae Corda		U59065
Chondrostereum purpureum (Pers.: Fr.) Pouz.	Stereaceae Pilát	CBS 427.72	AF082851
Clavariadelphus pistillaris (Fr.) Donk	Clavariaceae Chev.		AF026639
Clavicorona pyxidata (Fr.) Doty	Clavicoronaceae Corner		U59066
Clavulina cristata (Fr.) Schroet.	Clavulinaceae Donk		AF026640
Columnocystis abietina (Fr.) Pouz.	Stereaceae Pilát	HHB[c]-12622-Sp	AF082848
Columnocystis ambigua (Peck) Pouzar	Stereaceae Pilát	CBS 136.63	AF303530
Cymatoderma caperatum (Berk. & Mont.) Reid	Stereaceae Pilát	CBS 201.62	AF082849
Cystostereum murraii (Berk. & Curt.) Pouz.	Stereaceae Pilát	CBS 257.73	AF082850

Table 1. Continued.

Species name	Family	Source[a]	GenBank
Dentocorticium sulphurellum (Peck) M.J. Larsen & Gilbn.	Corticiaceae Herter		AF026604
Echinodontium tinctorium (Ell. & Ev.) Ell. & Ev.	Echinodontiaceae Donk		AF026578
Fistulina hepatica Schaeff.: Fr.	Fistulinaceae Maire		U59070
Fomes fomentarius (L.: Fr.) Fr.	Polyporaceae Corda		U59069
Fomitopsis pinicola (Swartz: Fr.) Karst.	Polyporaceae Corda		U59071
Gloeocystidiellum leucoxanthum (Bres.) Boid.	Corticiaceae Herter		AF026602
Gloeophyllum sepiarium (Fr.) Karst.	Polyporaceae Corda		AF026608
Gomphus floccosus (Schw.) Sing.	Gomphaceae Donk		AF026637
Hericium ramosum (Bull.: Mérat) Let.	Hericiaceae Donk		U59073
Heterobasidion annosum (Fr.) Bref.	Polyporaceae Corda		U59072
Hydnellum sp.	Thelephoraceae Chev.		AF026626
Hydnum repandum L.: Fr.	Hydnaceae Chev.		AF026641
Hyphodontia alutaria (Burt) J. Erikss.	Corticiaceae Herter		AF026615
Inonotus hispidus (Bull.: Fr.) Karst.	Hymenochaetaceae Donk		U59074
Laxitextum bicolor (Fr.) Lentz	Corticiaceae Herter		AF026605
Lentinellus ursinus (Fr.) Kühn.	Auriscalpiaceae Maas G.		U59076
Lopharia cinerascens (Schw.) Cunn.	Stereaceae Pilát	CBS 486.62	AF082852
Lopharia mirabilis (Berk. & Br.) Pat.	Stereaceae Pilát	SFC[d] 991030-8	AF303529
Lopharia spadicea (Pers.: Fr.) Boid.	Stereaceae Pilát	CBS 474.48	AF082853
Meripilus giganteus (Pers.: Fr.) Karst.	Polyporaceae Corda		U59082
Panus rudis Fr.	Pleurotaceae Kühner		U59086
Peniophora nuda (Fr.) Bres.	Corticiaceae Herter		U59085
Phanerochaete chrysosporium Burds.	Corticiaceae Herter		U59084
Phlebia radiata Fr.	Corticiaceae Herter		AF026649
Podoscypha elegans (Meyer: Fr.) Pat.	Stereaceae Pilát	CBS 322.66	AF082854
Pulcherricium caeruleum (Fr.) Parm.	Corticiaceae Herter		U59083
Ramaria stricta (Fr.) Quél.	Ramariaceae Corner		AF026638
Russula compacta Frost & Peck	Russulaceae Roze		U59093
Schizophyllum commune Fr.: Fr.	Schizophyllaceae Roze		X54865
Schizopora paradoxa (Schrad.: Fr.) Donk	Polyporaceae Corda		AF026612
Spongipellis unicolor (Schw.) Murr.	Polyporaceae Corda		M59760
Stereum gausapatum Fr.: Fr.	Stereaceae Pilát	CBS 348.39	AF082855
Stereum hirsutum (Willd.: Fr.) S. F. Gray	Stereaceae Pilát		U59095
Stereum ostrea (Bl. & Nees) Fr.	Stereaceae Pilát	SFC 960921-8	AF082856
Thelephora sp.	Thelephoraceae Chev.		AF026627
Tremella foliacea Pers.: Fr.	Tremellaceae Fr.		L22262
Veluticeps berkeleyi (Berk. & Curt.) Cooke	Stereaceae Pilát	CBS 725.68	AF082857
Xylobolus annosus (Berk. & Br.) Boid.	Stereaceae Pilát		U59089

[a]Sources of thirteen strains and two herbarium specimens sequenced in this study.
[b]Centraalbureau voor Schimmelcultures.
[c]H. H. Burdsall, Jr.
[d]Seoul National University Fungus Collection.

from cultured mycelia, maintained on malt extract agar (MEA), and dried specimens by a rapid method for nucleic acid extraction [4, 31] with some modification [32].

PCR Amplification, DNA Sequencing, and Sequence Analyses

The region of the nuclear small subunit ribosomal RNA gene was amplified using NS1 and NS8 primers [43]. PCR products were purified through Wizard PCR preps (Promega) and directly sequenced by the thermal cyclic termination method with ^{35}S-labeled ATP [21] using the *Top*™ DNA sequencing kit (Bioneer). Sequencing reactions were carried out using primers NS1 and NS8 [43] for both strands.

Previously published sequences were retrieved from GenBank's database and were aligned with newly obtained sequences for comparison, using an alignment algorithm CLUSTALX [41]. The multiple aligned sequences were visually optimized. To analyze data, the most parsimonious trees were sought using PAUP* 4.0b4a [39]. *Tremella foliacea* was used as an outgroup taxon to root trees, and all characters

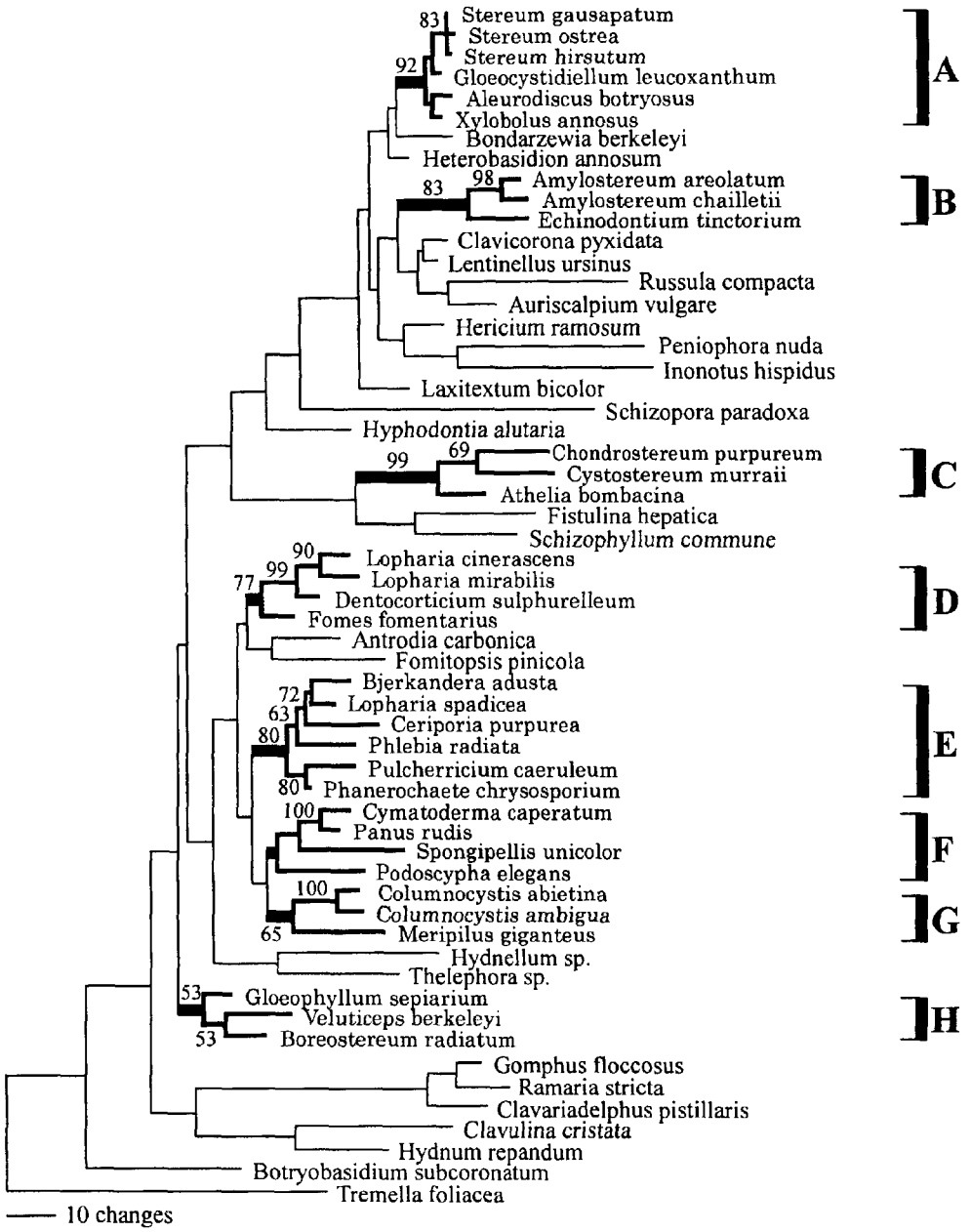

Fig. 1. Parsimony tree inferred from the analysis of nuclear small subunit rRNA gene sequences of 57 taxa.
Total of 24 most parsimonious trees (tree length=1205 steps, CI=0.4639) were yielded using the stepwise addition option of the heuristic method of PAUP*4.0b4a. *Tremella foliacea* was used as an outgroup to root the tree. Bootstrap frequencies in percentages are shown by the nodes of phylogenetically interesting branches. Bold lines were used for the eight clades where stereoid fungi were involved.

were equally weighted. Due to the size of taxa, searching was limited to heuristic searches with simple addition sequence, TBR branch swapping, MAXTREES unrestricted, and MULPARS on. To evaluate the strength of support for branches in most parsimonious trees, 1000 replicates of bootstrap resampling (simple addition sequence, TBR swapping, MAXTREES 1000) were performed [12]. Alternative topologies were tested to confirm the circumscription of the core group of stereoid fungi (designated Group A in Fig. 1) and to see whether the monophyly of the core group could be rejected, using the Kishino-Hasegawa test [29].

Results and Discussion

The parsimony analysis produced 24 most parsimonious trees with 1205 steps and a consistency index of 0.4639. One of

these trees is shown in Fig. 1. Statistical support from 1000 bootstrap resamplings is numbered on appropriate branches. In this tree, species in the Stereaceae *sensu* Donk were scattered into several branches, which had been suggested by previous authors [7, 8, 25, 36, 37]. In this study, eight phylogenetically distinct or interesting groups were identified.

Group A, the Core Group of Stereoid Fungi

Group A made a clade at the 92% confidence level and contained *Stereum gausapatum*, *S. ostrea*, *S. hirsutum*, and *Xylobolus annosus* of the Stereaceae along with *Gloeocystidiellum leucoxanthum* and *Aleurodiscus botryosus* of the Corticiaceae. These members have a monomitic hyphal system and amyloid spores in common. Clamps may be present or absent on generative hyphae. *Xylobolus* and *Stereum* have a hyphal system with simple-septate generative hyphae and acanthohyphidia in a number of species [7, 9]. *Xylobolus* was once treated as a taxon included in *Stereum* [8] but differs from *Stereum* in its hard, perennial basidiomata, lack of a detectable extracellular phenoloxidase system, and multiple clamp connections in culture [7]. But *Xylobolus* is similar to the subgenus *Acanthostereum* of *Stereum* in that both have all three types of hyphidia (simple hyphidia, acantho- and pseudoacanthohyphidia) [6, 7]. *Gloeocystidiellum leucoxanthum* has generative hyphae with clamps and gloeoplerous hyphae [11]. *Aleurodiscus botryosus* has a hyphal system with simple-septate generative hyphae, gloeocystidia, and ornamented amyloid spores [26, 34].

As indicated in Fig. 1, *Stereum* and *Xylobolus* of the Stereaceae, and *Aleurodiscus* and *Gloeocystidiellum* of the Corticiaceae, form a monophyletic clade that could be grouped in a single family, the prospect of which has been supported by recent phylogenetic studies [3, 19, 20, 27, 45]. In the analyses based on nuclear and mitochondrial ribosomal DNA sequences [19, 20], *S. hirsutum*, *S. annosum* (=*X. annosus*), *G. leucoxantha* (=*G. leucoxanthum*), and *A. botryosus* formed a fully supported clade by 100% bootstrap value. In the study of nuclear ribosomal DNA sequences [27], *S. hirsutum*, *S. gausapatum*, *S. ostrea*, *X. annosus*, *G. leucoxantha*, and *A. botryosus* formed a strongly supported clade by 93% bootstrap value. The study of Boidin *et al.* [3] based on ITS regions again demonstrated that *Stereum*, *Xylobolus*, *Aleurodiscus*, and *Gloeocystidiellum* formed a monophyletic group together with *Acanthophysium*, *Conferticium*, *Megalocystidium*, and *Aleurobotrys* within the Hériciales.

Boidin *et al.* [3] showed that, in the Acanthophysiaceae-Stereaceae-Gloiothelaceae clade, *Acanthophysium* was polyphyletic and clustered *pro parte* with *Stereum* and part of *Aleurodiscus*, and *pro parte* with *Xylobolus* and *Conferticium*. In the work of Hallenberg and Parmasto [16], *Acanthophysium* again clustered with *Stereum* and *Aleurodiscus*. The recent study by Wu *et al.* [45] also indicated the similar result that *Acanthophysellum*, which is partly equivalent to *Acanthophysium*, is polyphyletic. However, as far as the current nomenclatural point of view is concerned, *Acanthophysium* (Acanthophysiaceae Boidin) and *Aleurobotrys* (Aleurodiscaceae Pilát emend. Boidin) are all considered as synonyms of *Aleurodiscus s.l.* [34]. *Megalocystidium* erected by Jülich is restricted only to the *Gloeocystidiellum luridum* group, among seven *Gloeocystidiellum* groups of Eriksson and Ryvarden [10]. These groups are characterized by clamped hyphae, clavate basidia, and smooth or minutely verrucose basidiospores [44]. *Conferticium* was segregated from *Gloeocystidiellum* by Hallenberg [15] and is equivalent to the *G. ochraceum* group [10] which is characterized by simple-septate hyphae, very dense pseudoparenchymatic context consisting of vertical and cyanophilous hyphae, and internal basidial repetition [44].

Thus, the present study's Group A, based on nuclear small subunit ribosomal DNA, eventually proves to be in accordance with the results of Boidin *et al.* [3] based on ITS DNA and Wu *et al.* [45] based on nuclear large subunit ribosomal DNA. However, the monophyletic relationship of the taxa belonging to the Hériciales based on the analysis of ITS sequences by Boidin *et al.* [3] may need further verification using more slowly evolving molecules applied to ranks higher than genera. This is because the ITS has been mostly used to examine phylogenetic positions or relationships at specific ranks of fungi. Based on the above recent phylogenetic results of ribosomal DNA studies, Group A evidently constitutes the core group of the traditional Stereaceae.

Groups B and C and Cystostereaceae

Group B is well supported by 83% bootstrap value and is composed of *Amylostereum areolatum* and *A. chailletii* of the Stereaceae, and *Echinodontium tinctorium* of the Echinodontiaceae. They are characterized by a dimitic hyphal system with skeletal hyphae, clamped generative hyphae, smooth or asperulate amyloid spores, and thick-walled encrusted cystidia [7, 26]. They differ from the species of Group A in having encrusted cystidia instead of gloeocystidia. In the analysis of Boidin *et al.* [3], *Amylostereum* and *Echinodontium* formed a monophyletic group with *Boidinia* and *Gloeodontia*. There seems to be no appropriate family or taxonomic group equivalent to Group B and it is also questionable if this group could receive enough support when more taxa are added to the present data.

Group C is strongly supported by bootstrap frequencies of 99% and consists of *Chondrostereum* and *Cystostereum* of the Stereaceae *sensu* Donk [8] and *Athelia* of the Corticiaceae. Common features of this group are resupinate or effused-reflexed basidiomata, clamped generative hyphae

and smooth inamyloid basidiospores [7, 22]. *Athelia bombacina* has a monomitic hyphal system and lacks cystidial structures [10]. *Chondrostereum purpureum* has a monomitic hyphal system and smooth or encrusted cystidia. *Cystostereum murraii* has a dimitic hyphal system with skeletal hyphae and numerous vesicles with yellow oily or resinous contents [7]. Although *Cho. purpureum* and *Cys. murraii* differ in the miticity, they have important common characters such as white rot and abundant vesicles in zones throughout the thickened hymenium [37]. However, in the study of Boidin *et al.* [3], *Cystostereum* is separated by itself and grouped in the Phanerochaetales instead of the Hériciales where the Stereaceae is placed. Group C seems to be partly comparable to the Cystostereaceae *sensu* Jülich in which *Cystostereum* is placed [25], but has enough capacity to serve as a basis for a new family. Nevertheless, to establish an independent family, it is advisable that more taxa related to Group C be supplemented to it.

Groups D and E and the Heterogeneity of *Lopharia*

Groups D and E reproduced moderate bootstrap values of 77% and 80%, respectively. In Group D, *Lopharia cinerascens* and *L. mirabilis* are clustered with *Dentocorticium sulphurellum* of the Corticiaceae by 99% bootstrap support, and then with *Fomes fomentarius* of the Polyporaceae. Macroscopically and microscopically, these three genera are quite different. *Lopharia cinerascens* has an even to warted hymenophore, a dimitic hyphal system with skeletal hyphae, clamped generative hyphae, and thick-walled cystidia [7, 26, 42]. *Fomes fomentarius* has a poroid hymenophore and a trimitic hyphal system with clamped generative hyphae and no sterile elements [14]. *Dentocorticium sulphurellum* has a smooth to warted hymenophore, a monomitic hyphal system with clamped generative hyphae, and abundant dendrohyphidia [26]. On the other hand, they all have smooth, thin-walled, medium-sized to large, and inamyloid spores in common. The study of Boidin *et al.* [3] indicated that *L. cinerascens* and *L. mirabilis* formed a monophyletic group with *Lenzites* and *Trametes* which were known to be phylogenetically related to *Fomes* [18, 38]. Apart from morphological features, the monophyly of two species of *Lopharia* and *F. fomentarius* does not seem to be a puzzling result in view of phylogeny.

In Group E, *L. spadicea* was grouped with *Bjerkandera adusta*, *Ceriporia purpurea*, *Phlebia radiata*, *Pulcherricium caeruleum*, and *Phanerochaete chrysosporium*. These species have morphologically different characters even though they were phylogenetically grouped together, which makes it rather difficult to correlate them. *Lopharia spadicea* has a dimitic hyphal system with skeletal hyphae, clamped generative hyphae, cystidia originated from generative and skeletal hyphae [26, 42]. However, *B. adusta*, *Phl. radiata*, and *Pul caeruleum* have a monomitic hyphal system with clamped generative hyphae, while *C. purpurea* and *Pha. chrysosporium* have a monomitic hyphal system with simple-septate generative hyphae [14, 26].

Species of *Lopharia*, separated into Groups D and E, proved to be unrelated and heterogeneous to one another. It is evident that *Lopharia* itself is polyphyletic at a generic level [30]. Taxonomically, *Lopharia* has been a subject of concern as an unnatural taxon and used to be divided into three subgeneric groups according to the presence of a cuticle on the surface and clamps on generative hyphae [7, 42]. By this criterion, *L. cinerascens* was assigned to the *L. cinerascens* group having clamps and a well-developed cuticle, and *L. spadicea* to the *L. spadicea* group having clamps but lacking a cuticle [42]. Hjortstam and Ryvarden [23] then transferred some species of *Lopharia* including *L. spadicea* to another genus, *Porostereum*, and left *L. cinerascens* and *L. mirabilis* in *Lopharia*, which is concordant with the discussion of Ko *et al.* [30] and the present result.

Groups F to H, Podoscyphaceae and Chaetodermataceae

Group F, which is poorly supported by bootstrap analysis, is composed of *Cymatoderma* and *Podoscypha* of the Stereaceae *sensu* Donk, and *Spongipellis unicolor* of the Polyporaceae in the Aphyllophorales, and *Panus* of the Pleurotaceae in the Agaricales, according to the current classification. *Cymatoderma caperatum* and *Pod. elegans* were regarded as unrelated to the true *Stereum* and separated into a family Podoscyphaceae of their own by Reid [37]. Similarly, *Panus* was shown to be not close to *Pleurotus* and stood outside the euagarics inferred from ribosomal DNA sequences by Hibbett *et al.* [19]. Jülich [25] classified the Podoscyphaceae and the Pleurotaceae in the order Polyporales. These three species have similar stipitate basidiocarps, a dimitic hyphal system with skeletal hyphae, generative hyphae with clamps, and smooth inamyloid thin-walled basidiospores. However, Boidin *et al.* [3] placed *Podoscypha*, *Cymatoderma*, *Hypochnicium*, and *Sarcodontia* together in their new order Podoscyphales. Group F morphologically corresponded to a part of the Podoscyphaceae Reid, which has been recognized as a well-characterized family but now needs to be reconsidered in terms of molecular data. To be more specific and conclusive about phylogenetic relationships within Group F, additional analyses of more strains with taxonomic significance are definitely needed.

Group G comprised *Meripilus* of the Polyporaceae and *Columnocystis* of the Stereaceae *sensu* Donk, but was weakly supported statistically. There is no reference material to any detailed connection between these two genera. They appear to have no comparable morphological features in common, but microscopically have a few similar characters such as smooth and hyaline spores, cylindrical to clavate basidia with 4 sterigmata, and septa with clamps on generative hyphae. Besides, *Columnocystis* proved to be not related to

Table 2. Results of the Kishino-Hasegawa test.

Topology	Tree length	- ln L	-ln L difference	SD[a]	t[b]	Significantly worse?[c]
Group A monophyletic	1205	- 11095.0	best			No
Groups A and B monophyletic	2070	- 14969.3	- 3874.3	389.3	9.95	Yes
Groups A and C monophyletic	2127	- 15200.8	- 4105.8	424.6	9.67	Yes
Groups A and D monophyletic	2206	- 15601.9	- 4506.9	469.0	9.61	Yes
Groups A and E monophyletic	2201	- 15599.6	- 4504.6	470.7	9.57	Yes
Groups A and F monophyletic	2201	- 15567.0	- 4472.0	466.8	9.58	Yes
Groups A and G monophyletic	2166	- 15390.4	- 4295.4	444.2	9.67	Yes
Groups A and H monophyletic	2162	- 15397.2	- 4302.2	446.7	9.63	Yes

[a]The standard deviation in log-likelihood.

[b]The t-value is determined by dividing the difference in log-likelihood by the standard deviation.

[c]The topology is considered to be significantly worse if the difference in log-likelihood is more than twice the standard deviation.

Veluticeps (of Group H), contrary to the emendation by Nakasone [33] on the Chaetodermataceae.

Finally, Group H is a newly discovered clade and includes *Boreostereum* of the Stereaceae *sensu* Parmasto [35], *Veluticeps* of the Stereaceae *sensu* Donk [8], and *Gloeophyllum sepiarium* of the Polyporaceae. This group is poorly supported by the bootstrap value of 53%. *Boreostereum radiatum* has a dimitic hyphal system with simple-septate generative hyphae, *V. berkeleyi* a monomitic hyphal system of sclerified generative hyphae with clamps, and *G. sepiarium* a trimitic hyphal system with clamped generative hyphae. In other words, these members are quite different to each other in the hyphal system. All three species are characterized by brown-colored skeletal hyphae or sclerified generative hyphae and brown rot on attacked hosts, with the occasional exception of *B. radiatum* in which white rot has been reported at the same time [7].

The present result partly agrees with the view of Nakasone [33], who broadened the definition of the Chaetodermataceae Jülich by accepting *Veluticeps* (inclusive of *Columnocystis* of Group G), *Chaetodermella* (the new name for *Chaetoderma*), and *Crustoderma* in it. Nakasone [33] suggested that *Veluticeps berkeleyi* and *Columnocystis abietinum* are congeneric and need to be united in the family Chaetodermataceae in which brown hyphae and brown rot are common characters. Nakasone also indicated that *Gloeophyllum* would be the closest relative to the Chaetodermataceae, which coincides with the result of Group H where *Veluticeps* is grouped with *Gloeophyllum*. Current results sustain the scheme of Nakasone to some extent even though the bootstrap analysis rather poorly supports the present clade. In this case, the nutrition habit of brown rot and the pigmentation of brown-colored hyphae apparently play an important role in the phylogenetic characterization of the clade.

Phylogenetic Conclusions on the Traditional Stereaceae

Based on the results inferred from nuclear small subunit ribosomal RNA gene sequences, it became clear that the Stereaceae *s.l.* was phylogenetically polyphyletic and its genera were scattered into many groups, each of which was comparable or equal to a family level or a new family rank. Group A is composed of *Stereum*, *Xylobolus*, *Gloeocystidiellum*, and *Aleurodiscus* and, based on most recent phylogenetic studies, constitutes the core group of stereoid fungi. In terms of bootstrap values, Group A forms a clade at significant confidence level, and is possibly composed of phylogenetically homogeneous taxa.

The results of the Kishino-Hasegawa test [29] shown in Table 2 confirmed the monophyletic circumscription of the Stereaceae assigned to Group A. In the studies by Hibbett *et al.* [19] and Hibbett and Thorn [20], the Stereaceae was included in the russuloid clade where most of its families have distinct characters and are probably monophyletic. With the addition of two more genera, *Gloeocystidiellum* and *Aleurodiscus*, to the core concept of Chamuris [7], it is taxonomically essential that the traditional Stereaceae should be evaluated in a strict sense as a phylogenetically distinct taxon based on the members of Group A [27] and those of Hibbett *et al.* [19] and Wu *et al.* [45]. For a more all-inclusive confirmation, some further species related to the genera of Group A may need to be added to the present molecular data.

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