Bull. Natl. Mus. Nat. Sci., Ser. B, **34**(4), pp. 161–173, December 22, 2008

Molecular Phylogenetics of Geastrales with Special Emphasis on the Position of *Sclerogaster*

Kentaro Hosaka[1] and Michael A. Castellano[2]

[1] Department of Botany, National Museum of Nature and Science,
4–1–1 Amakubo, Tsukuba 305–0005, Japan
[2] USDA, Forest Service, Northern Research Station, Forestry Sciences Laboratory,
3200 Jefferson Way, Corvallis, OR 97331, USA

Abstract Molecular phylogenetic analyses for the order Geastrales (Agaricomycetes, Basidiomycota, Fungi) were conducted based on a two gene dataset with increased taxon sampling of the genus *Sclerogaster*. The placement of *Sclerogaster* in Geastrales, and the monophyly of the genus were strongly supported in all analyses. *Sclerogaster* forms one of the distinct lineages within Geastrales, but its exact position within Geastrales remains unresolved. Bayesian analyses support the sister relationship between Geastraceae and *Sclerogaster* without significant posterior probabilities. Most analyses under parsimony criterion supported the sister relationship between *Sclerogaster* and *Schenella*, but lack significant bootstrap values. The topology of Geastrales indicated that the truffle-like form may be an ancestral morphology of the earthstar fruit body-type. Biogeographical patterns of *Sclerogaster* indicate that long distance dispersal may be the most important factor, and this may be associated with its saprotrophic habit.

Key words: *atp6*, Basidiomycota, fungi, LSU, Phallomycetidae, Phylogeny, *Sclerogaster*, Systematics.

Introduction

The subclass Phallomycetidae (Agaricomycetes, Basidiomycota) is commonly known as the gomphoid-phalloid fungi (Hibbett and Thorn, 2001). Because of its morphological diversity, traditional taxonomy failed to recognize the gomphoid-phalloid fungi as a single entity. Hibbett *et al*. (1997) first demonstrated the monophyly of the gomphoid-phalloid fungi, which were subsequently supported by additional studies (Binder and Hibbett, 2002; Hibbett and Binder, 2002; Hibbett and Thorn, 2001; Hosaka *et al*., 2006; Humpert *et al*., 2001; James *et al*., 2006; Lutzoni *et al*., 2004; Matheny *et al*., 2007; Moncalvo *et al*., 2002; Pine *et al*., 1999). The first attempt to incorporate these results into a formal classification scheme by treating the gomphoid-phalloid fungi as a single order Phallales was made by Kirk *et al*. (2001). Later Hosaka *et al*. (2006) analyzed a 5-gene dataset of the gomphoid-phalloid fungi. Their results clearly showed that there are four major clades within the larger gomphoid-phalloid clade, and each of the smaller clades were well-supported and in two cases, i.e., Phallales and Gomphales, with a long history of ordinal status. Hosaka *et al*. (2006) elevated Phallales sensu Kirk *et al*. to subclass status, and proposed the new subclass Phallomycetidae. Four major clades within the Phallomycetidae each received ordinal status; Phallales, Gomphales, Hysterangiales, and Geastrales.

These phylogenetic analyses revealed many previously unexpected lineages, and taxonomic revisions for several taxa were made accordingly. However, the taxonomic revisions for many taxa have not been conducted yet. The order Geastrales is especially problematic because several genera, such as *Phialastrum* and *Trichaster*, were not included in the phylogenetic analyses although they traditionally have been placed in Geastraceae (Sunhede, 1989). In addition, Zeller

(1948) described Broomeiaceae in Lycoperdales, but it is unclear whether this family is more closely related to Geastrales or to other homobasidiomycetes.

One of the unexpected lineages within Geastrales includes the genus *Sclerogaster* Hesse, which possesses hypogeous (truffle-like) basidiomata with a yellow or green tinted gleba (Fig. 3) and yellow to brown, warty basidiospores (Fig. 4) (Castellano *et al*., 1989). When the genus was described by Hesse (1891), it was placed in the "Hymenogastreen", which was considered an artificial assemblage of truffle-like basidiomycetes (Clémençon *et al*., 2007). Fischer (1900) later placed the genus in the family Hymenogastraceae, but its phylogenetic affinity remained unresolved. Since then, several attempts were made to phylogenetically place the genus. Some authors (Jülich, 1981; Castellano *et al*., 1989) considered *Sclerogaster* to be closely related to *Octaviania* (Boletales), but others (Dodge and Zeller, 1936; Zeller and Dodge, 1935; Kreisel, 1969) implied close affinity of *Sclerogaster* with *Hydnangium* (Agaricales). Furthermore, Malençon (1931) and Heim (1971) implied a close affinity of the genus with Russulales. Therefore, *Sclerogaster* have been placed in at least three distinct orders of Basidiomycota.

Molecular phylogenetic analyses strongly suggested placement of the genus in Geastrales with close affinity to the Geastraceae and Schenellaceae (Pyrenogastraceae) (Hosaka *et al*., 2006). This was surprising because a close relationship between *Sclerogaster* and Geastraceae had never been postulated previously. Morphologically, *Sclerogaster*, Geastraceae and Schenellaceae all possess globose spores with a surface ornamented with spines or warts (Domínguez de Toledo and Castellano, 1996; Sunhede, 1989; Castellano *et al*., 1989). *Sclerogaster* differs from Geastraceae and Schenellaceae in having a green to yellow or orange gleba (Fig. 3) versus brown to black gleba that turns powdery at maturity. However, only one species (*Sclerogaster xerophilus*) was sampled from approximately 15 described species, and more critically, no type species were included. Additional taxon sampling is necessary for a more definitive placement of the genus. This study attempts to answer the questions of: (1) monophyly of the genus *Sclerogaster*, and (2) phylogenetic placement of the genus.

Materials and Methods

Taxon sampling, PCR, and DNA sequencing

Taxa sampled, along with GenBank accession numbers, are listed in Table 1. A total of 51 taxa (four outgroup and 47 ingroup taxa) were sampled for this study. The selection of ingroup and outgroup taxa was based on the phylogeny of previous studies (Hosaka *et al*., 2006) to cover the diversity of Geastrales.

DNA was extracted from glebal tissue of fresh or dried fruiting bodies. The protocol generally follows that of Doyle and Doyle (1987) but with the following modifications. For fresh materials, immature glebal tissue was soaked in DMSO buffer (Seutin *et al*., 1991) with an addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfite (Na_2SO_3) under 4°C until extraction was conducted. For dried materials, immature glebal tissue was soaked overnight in modified DMSO buffer under room temperature.

Soaked tissue samples were then ground in liquid nitrogen using mortar and pestle. After grinding, samples were immediately transferred to 1.5 mL tubes with 1,000 mL of 2X CTAB buffer (Doyle and Doyle, 1987) with an addition of 0.1M Na_2SO_3. Some taxa of Geastrales have a powdery spore mass at maturity, and the powdery spore mass cannot easily be ground in liquid nitrogen. If the spore mass was the only tissue available for DNA extraction, spores were ground using the bead beating protocol modified from Munkacsi *et al*. (2006). Approximately 30 milligrams of dry basidiospores were mixed with 0.2 mg of 0.1 mm glass beads (BioSpec), 20 g of 1 mm chrome steel beads (BioSpec) and two 3 mm chrome steel beads (BioSpec), and frozen in liquid nitrogen for 5 minutes. The mixture was beaten at 30 Hz for 1 minute using the TissueLyser (Qiagen), frozen in liquid nitrogen for 3

minutes, and then beaten again at 30 Hz for 1 minute. After grinding, 1,000 mL of 2X CTAB buffer with an addition of 0.1 M Na_2SO_3 was added directly to the sample tubes.

Samples were incubated at 55°C overnight, and centrifuged at 12,000 rpm for 5 minutes. Only the aqueous phase was transferred to a new tube, and precipitated tissue debris were discarded. The equal volume of the mixture of chloroform: isoamylalcohol (24 : 1) was added to the buffer, mixed vigorously for two minutes, and centrifuged at 12,000 rpm for 15 minutes. The aqueous phase was pipetted out and transferred to a new tube. This step of using chloroform was conducted only once.

After transferring ca. 300 mL of the aqueous phase, 1,000 mL of 6M sodium idodine buffer (6 M NaI, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.1 M Na_2SO_3) was added and mixed gently for 1 minute. Silica mixture was prepared following the protocol of Rogstad (2003), and 25 mL of the mixture was added to the samples. Samples were incubated at 55°C for 1 hour, and centrifuged at full speed for ca. 10 seconds. The supernatant was discarded and 750 mL of washing buffer (10 mL Tris-HCl (pH 7.4), 1mM EDTA, 100 mM NaCl, 50% EtOH) was added, mixed briefly, and centrifuged at full speed for ca. 5 seconds. This washing step was repeated twice. After finishing the washing step, samples were centrifuged one more time at full speed for 10 seconds, the remaining washing buffer was pipetted out, and precipitated silica was dried at room temperature for 30 minutes to 1 hour. Final elution was performed by adding 100 mL of ultra pure water, mixed briefly, and incubated at 65°C for 15 minutes. Samples were centrifuged at 12,000 rpm for 1 minute, and supernatant layer was transferred to a new tube and stored at −20°C until PCR was performed.

DNA sequence data were obtained from two independent loci: LR0R–LR3 region for nuclear large subunit of rDNA (LSU) and ATPase subunit 6 (*atp6*). The primers and PCR protocols were described previously (Kretzer and Bruns 1999; Vilgalys and Hester 1990). Cycle sequencing was performed following the manufacturer's instructions (Big Dye ver. 3.1, Applied Biosystems) using the same primers described above.

Phylogenetic analyses

DNA sequences were initially aligned using Muscle v.3.6 (Edgar, 2004a, b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall 1999). Ambiguously aligned regions and introns were excluded from the analyses. To test for incongruence between the two individual datasets, parsimony analyses of individual loci were compared. First, 70 % bootstrap (BS) trees were calculated (100 BS replicates with five random addition sequences, TBR and Multrees options off) including only the taxa with sequences from both loci (36 taxa). These trees were used as constraints in a different dataset (for example, parsimony analysis of the *atp6* dataset with the LSU tree as a constraint), using the 'Load Constraints' option in PAUP version 4.0b10 (Swofford 2002). Parsimony analyses were conducted under these constraints, keeping only the trees that are compatible with these constraints. Comparisons of constraint and unconstraint trees were made using the 'Tree Scores' option in PAUP version 4.0b10 (Swofford 2002). Parsimony based comparisons were performed by the Templeton test (Templeton 1983), using nonparametric pairwise tests option. Likelihood-based comparisons were performed by the Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999), using RELL optimization with 1,000 BS replicates. Significance of results was determined by a P-value of less than 0.05. After testing for incongruence, the individual gene datasets were combined and phylogenetic (both parsimony and Bayesian) analyses were conducted with a combined dataset of two loci as described above.

Parsimony analyses (Analyses 1–6, Table 2) were conducted under the equally weighted parsimony criterion using PAUP version 4.0b10 (Swofford 2002). Phylogenetic analyses were conducted with a combined dataset of LSU and *atp6* either with (51 taxa = Analysis 1, Table 1) or without (36 taxa = Analysis 2, Table 1) missing

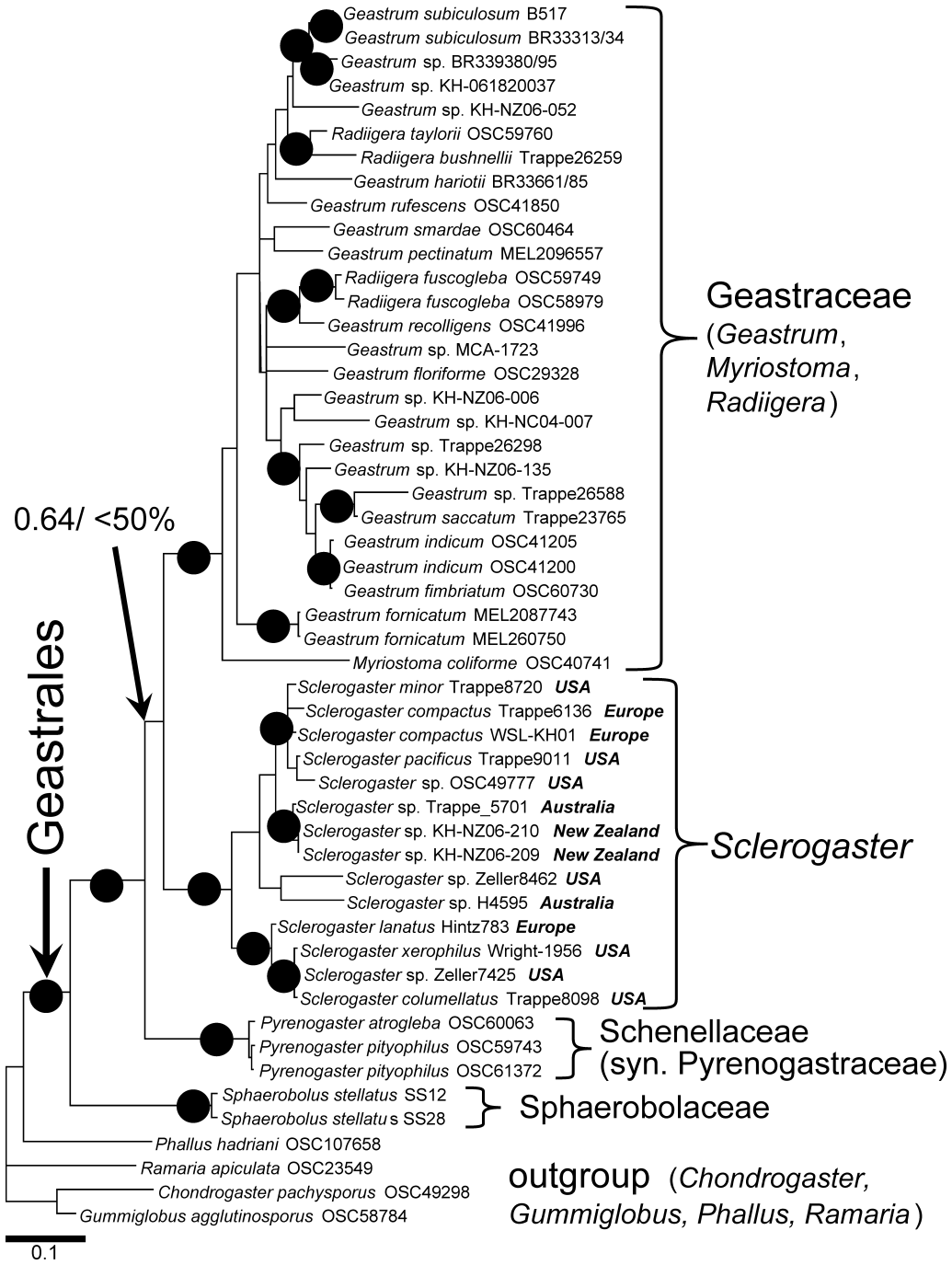

Fig. 1. A 50% majority rule consensus of Geastrales phylogeny derived from Bayesian analysis using combined *atp6* and LSU dataset for a supermatrix of 51 taxa (Analysis 1). Taxon names followed by voucher numbers, and by area of distribution in bold face (only for the Sclerogastraceae clade). Nodes supported by ≥0.95 Bayesian posterior probability (PP) and by ≥70% parsimony bootstrap (BS) values are indicated by black circles. Numbers on branches are nodal supports (Bayesian PP/parsimony BS values).

Table 1. Sequence data newly generated for this study and associated GenBank accession numbers.

Taxon	Herbarium	Specimen No.	LSU	*atp6*
Geastrum hariottii	BR	33661/85	——	FJ435985
Geastrum saccatum	OSC	Trappe 23765	FJ435966	FJ435986
Geastrum subiculosum	BR	33313/34	FJ435967	FJ435987
Geastrum subiculosum	BR	B517	FJ435968	FJ435988
Geastrum sp.	BR	339380/95	FJ435969	FJ435989
Geastrum sp.	CUW	MCA 1723	——	FJ435990
Geastrum sp.	OSC	Trappe 26298	FJ435970	FJ435991
Geastrum sp.	TNS	KH-6182003-7	——	FJ435992
Geastrum sp.	TNS	KH-NC04-007	——	FJ435993
Geastrum sp.	TNS	KH-NZ06-006	FJ435971	FJ435994
Geastrum sp.	TNS	KH-NZ06-052	——	FJ435995
Geastrum sp.	TNS	KH-NZ06-135	——	FJ435996
Sclerogaster columellatus	OSC	Trappe 8098	FJ435972	——
Sclerogaster compactus	TNS	WSL-KH01	FJ435973	FJ435997
Sclerogaster compactus	OSC	Trappe 6136	FJ435974	——
Sclerogaster lanatus	OSC	Hintz 783	FJ435975	——
Sclerogaster minor	OSC	Trappe 8720	FJ435976	FJ435998
Sclerogaster pacificus	OSC	Trappe 9011	FJ435977	——
Sclerogaster xerophilus	NY	Wright 1956	FJ435978	——
Sclerogaster sp.	OSC	H4595	FJ435979	——
Sclerogaster sp.	OSC	Trappe 15701	FJ435980	FJ435999
Sclerogaster sp.	NY	Zeller 7425	FJ435981	——
Sclerogaster sp.	NY	Zeller 8462	FJ435982	——
Sclerogaster sp.	TNS	KH-NZ06-209	FJ435983	FJ436000
Sclerogaster sp.	TNS	KH-NZ06-210	FJ435984	FJ436001

data. The analyses 3–6 were conducted with a single locus dataset with a minimum number of taxa (36 taxa=Analyses 4 & 6) or more taxa (Analyses 3 & 5). The analyses were performed under the heuristic search option with tree bisection–reconnection (TBR) and Multrees option on, and 1,000 replicates of random addition sequence were conducted. Support for the individual nodes was tested with BS analysis under the equally-weighted parsimony criterion. BS analysis was based on 500 BS replicates using the heuristic search option (TBR and Multrees options on), with five random addition sequences.

Bayesian analyses (Analyses 7–12, Table 3) were conducted using MrBayes version 3.0b4 (Huelsenbeck and Ronquist 2001). Phylogenetic analyses were conducted with a combined datasets of LSU and *atp6* either with (51 taxa=Analysis 7, Table 1) or without (36 taxa=Analysis 8, Table 1) missing data. The analyses 9?12 were conducted with a single locus dataset with a minimum number of taxa (36 taxa=Analyses 10 & 12) or more taxa (Analyses 9 & 11). Four data partitions, including LSU and one for each codon position for the protein-coding locus (*atp6*), were delimited for Bayesian analyses. The GTR+G+I model was employed separately for each of the four data partitions. Bayesian analyses were run with 2 million MCMCMC generations with four chains, sampling trees every 100th generation. The log-likelihood scores of sample points against generation time were plotted using TRACER version 1.3 (http://evolve.zoo.ox.ac.uk/software.html) to determine if the run reached stationarity. We also observed the average standard deviation of split frequencies (ASDSF) and verified that the values dropped below 0.01. The support of nodes was tested by posterior probabilities (PP), obtained from a 50 % majority rule consensus after deleting the trees in the burn-in period.

Results and Discussion

PCR amplification and sequencing

Most DNA samples produced a clear, single

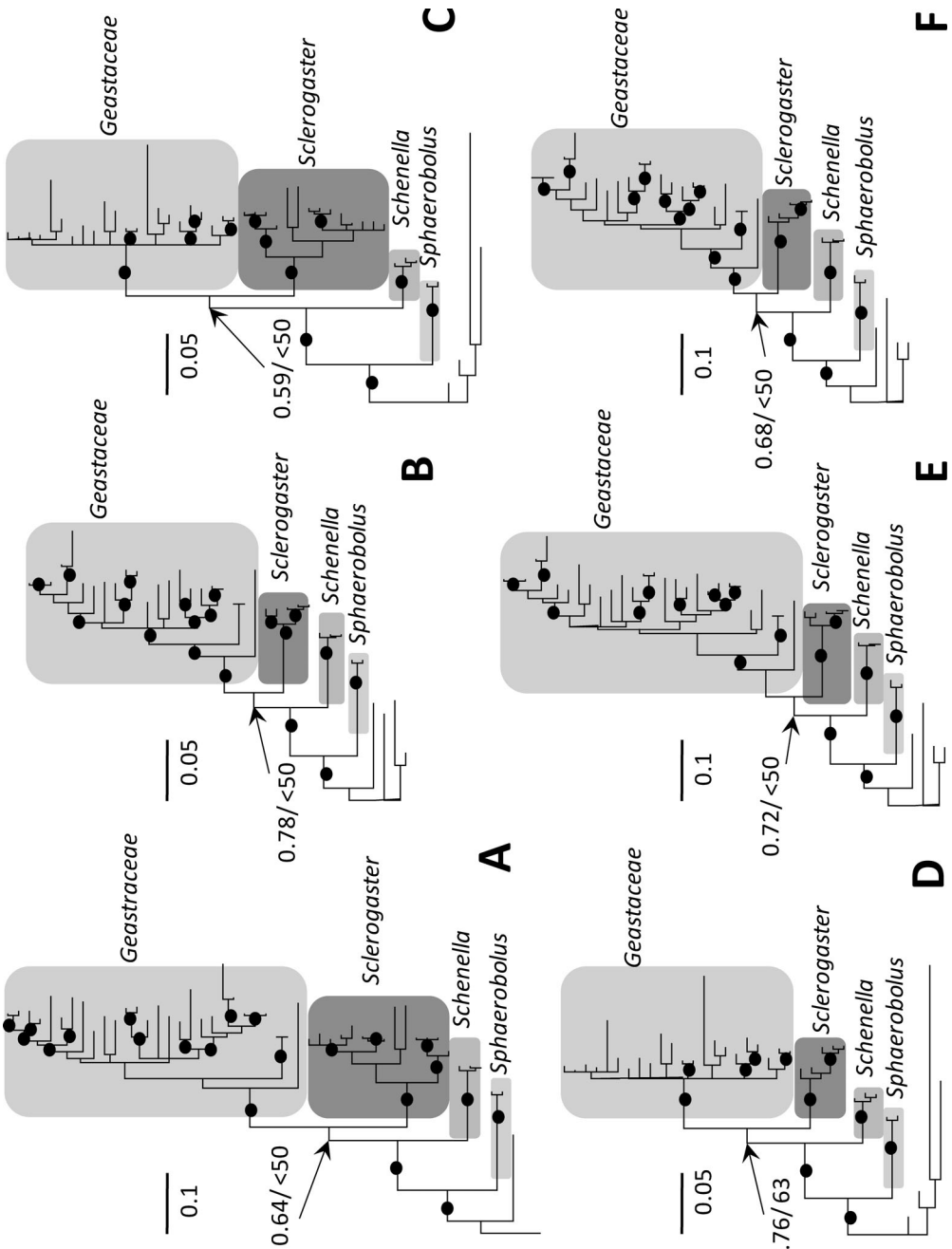

Fig. 2. Phylogeny of Geastrales. Each tree is a 50% majority consensus based on analysis of: A. Analyses 1 & 7, B. Analyses 2 & 8, C. Analyses 3 & 9, D. Analyses 4 & 10, E. Analyses 5 & 11, and F. Analyses 6 & 12. Nodes supported by ≥0.95 Bayesian PP and by ≥70% parsimony BS values are indicated by black circles. Numbers on branches are nodal supports (Bayesian PP/parsimony BS values).

band of LSU (ca. 650 bp). However, direct sequencing of some PCR products resulted in ambiguous peaks, suggesting the presence of heterogeneous copies of LSU. This needs to be resolved by cloning the PCR products. On the other hand, PCR of the *atp6* failed to amplify eight DNA samples of *Sclerogaster* spp. Because the primers for the *atp6* successfully amplified most species of Phallomycetidae (Hosaka *et al*., 2006), this suggests substitutions of nucleotide sequences at the primer regions for *Sclerogaster*. Taxon specific primers for *Sclerogaster* need to be designed to obtain sequences of *atp6*. In addition, some *Sclerogaster* species (not included in this study) resulted in PCR products longer than 1000 bp. Because most PCR products of *atp6* were ca. 700 bp long for Geastrales, this suggests the presence of introns in the *atp6*. Further research is necessary to confirm these findings.

Test of dataset incongruence

The Templeton test and Shimodaira-Hasegawa test did not reveal any major conflicts between datasets. This suggests the two datasets of LSU and *atp6* are combinable. However, not all species in this study have sequences for both genes, and the presence of missing data may cause conflicts between datasets. To test this, the individual dataset of LSU and *atp6*, as well as the combined dataset, were analyzed using different numbers of taxa (Table 2, 3). Phylogenetic analyses under different taxon and character sampling schemes (analyses 1–12) produced almost identical topologies (Fig. 2), suggesting the presence of missing data did not cause any major conflicts between datasets. All analyses showed the monophyly of Geastrales, and four clades within the order (Geastraceae, *Sclerogaster*, *Schenella*, and *Sphaerobolus*) were also strongly supported as monophyletic (Figs. 1, 2).

Parsimony analyses

Although the number of included characters are almost the same between the LSU (572 bp) and *atp6* (648 bp) datasets, the number of parsimony informative characters were almost three times more for the *atp6* dataset than LSU dataset (Table 2). This implies that the *atp6* dataset has more resolution power for reconstructing the phylogeny of Geastrales. This is consistent with the fact that the LSU dataset produced much more equally parsimonious trees than the *atp6* dataset (Table 2).

However, both datasets strongly supported (BS≥70%) the monophyly of Geastrales, as well as the monophyly of four major clades (Geastraceae, *Sclerogaster*, *Schenella*, and *Sphaerobolus*) within it. This suggests that the lack of resolution power of the LSU dataset is problematic only for the lower level phylogeny (e.g., within the Geastraceae clade). Parsimony analyses, except the analysis 4, supported a sister relationship of *Sclerogaster* and *Schenella*, but no significant bootstrap values were observed. More data and taxa need to be sampled to resolve this potential conflict between parsimony and Bayesian analyses.

Bayesian analyses

After 2 million generations of MCMCMC

Table 2. Parsimony analyses using various combinations of datasets and taxon numbers (Analyses 1 to 6).

Analysis	Dataset	number of taxa	number of included characters	number of parsimony informative characters	number of trees generated	Tree Length	CI	RI	RC
1	*atp6*+LSU	51	1,220	434	4	1,831	0.3790	0.6783	0.2571
2	*atp6*+LSU	36	1,220	420	4	1,550	0.4226	0.6901	0.2916
3	LSU	45	572	127	12	388	0.4459	0.7806	0.3481
4	LSU	36	572	119	450	331	0.4864	0.7447	0.3622
5	*atp6*	42	648	307	2	1,414	0.3685	0.6504	0.2396
6	*atp6*	36	648	301	1	1,190	0.4151	0.6868	0.2851

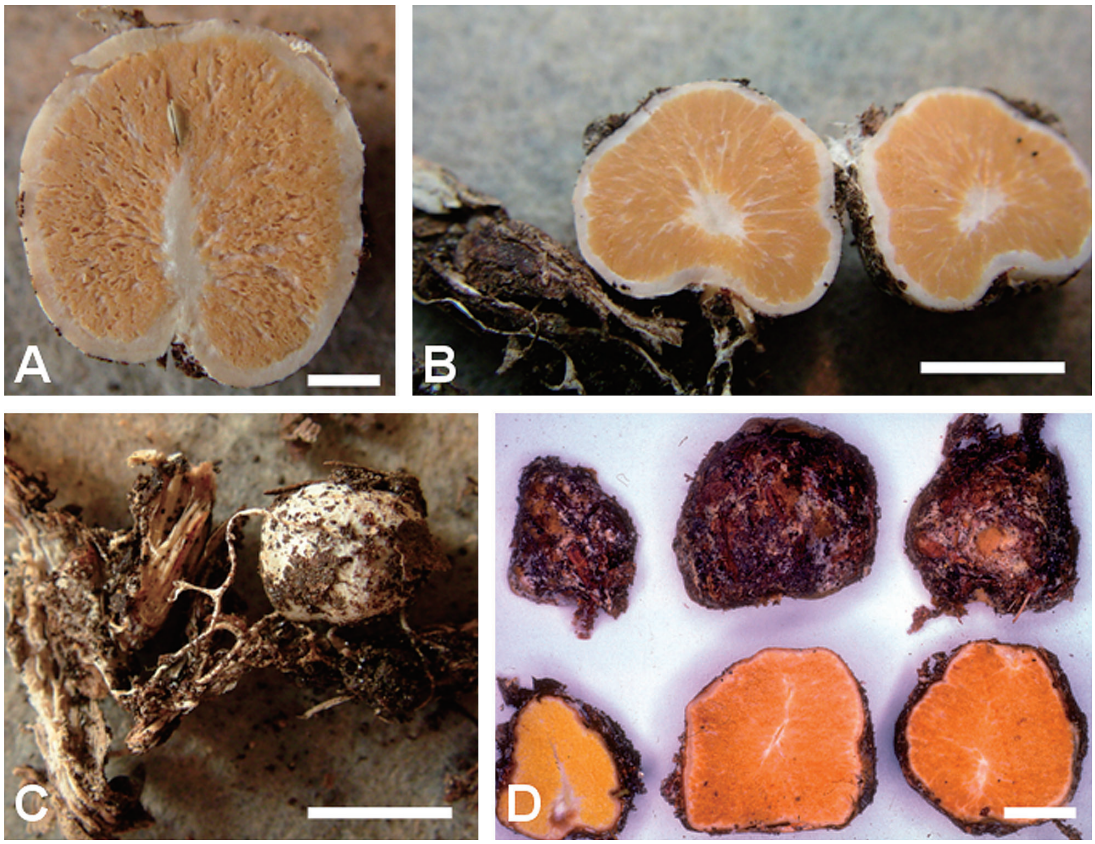

Fig. 3. Basidiomata of *Sclerogaster* spp. A. *S.* sp. from New Zealand (KH-NZ06-209). Note yellowish gleba. B. Immature basidiomata of *S.* sp. (KH-NZ06-209). C. *S.* sp. (KH-NZ06-209). Note thick, white rhizomorphs attaching to woody debris. D. *S. xerophilus* from USA (Trappe 7346). Bars＝0.5 cm.

runs, all analyses reached stationarity. The average standard deviation of split frequencies (ASDSF) dropped below 0.01 after ca. 1 million generations for the analyses 7 and 9, ca. 800,000 generations for the analysis 8, ca. 580,000 generations for the analysis 11, and ca. 350,000 generations for the analyses 10 and 12. Accordingly, the number of trees discarded as the burn-in phase are 10,000 for the analyses 7 and 9, 8,000 for the analysis 8, 6,000 for the analysis 11, and 4,000 for the analyses 10 and 12 (Table 3). After discarding the trees in the burn-in phase, the potential scale reduction factor was 1.000–1.002 for all parameters, indicating that the analyses were run for a sufficient number of generations.

All analyses strongly supported (PP≥0.95) the monophyly of Geastrales, as well as the monophyly of four major clades (Geastraceae, *Sclerogaster*, *Schenella*, and *Sphaerobolus*) within it (Figs. 1, 2). In contrast to the parsimony analyses, however, all Bayesian analyses (analyses 7–12) supported a monophyly of Geastraceae and *Sclerogaster* (Fig. 2). The result is inconsistent with the previous study by Hosaka *et al*. (2006), which showed *Sclerogaster* was sister to Geastraceae and *Schenella* (as *Pyrenogaster*) combined. However, both studies failed to obtain significant (PP≥0.95) support for these relationships, and therefore more data are necessary to make a final determination.

Taxonomic implication

Although significant supports are lacking, Bayesian analyses consistently showed that *Scle-*

Table 3. Bayesian analyses using various combinations of datasets and taxon numbers (Analyses 7 to 12).

Analysis	Dataset	number of taxa	number of included characters	number of trees retained after the burn-in	Arithmetic mean (log-likelihood)	Harmonic mean (log-likelihood)
7	*atp6*+LSU	51	1,220	10,000	−10646.84	−10730.00
8	*atp6*+LSU	36	1,220	12,000	−9358.01	−9436.54
9	LSU	45	572	10,000	−3092.83	−3168.34
10	LSU	36	572	16,000	−2750.82	−2811.69
11	*atp6*	42	648	14,000	−7236.25	−7280.97
12	*atp6*	36	648	16,000	−6294.62	−6330.75

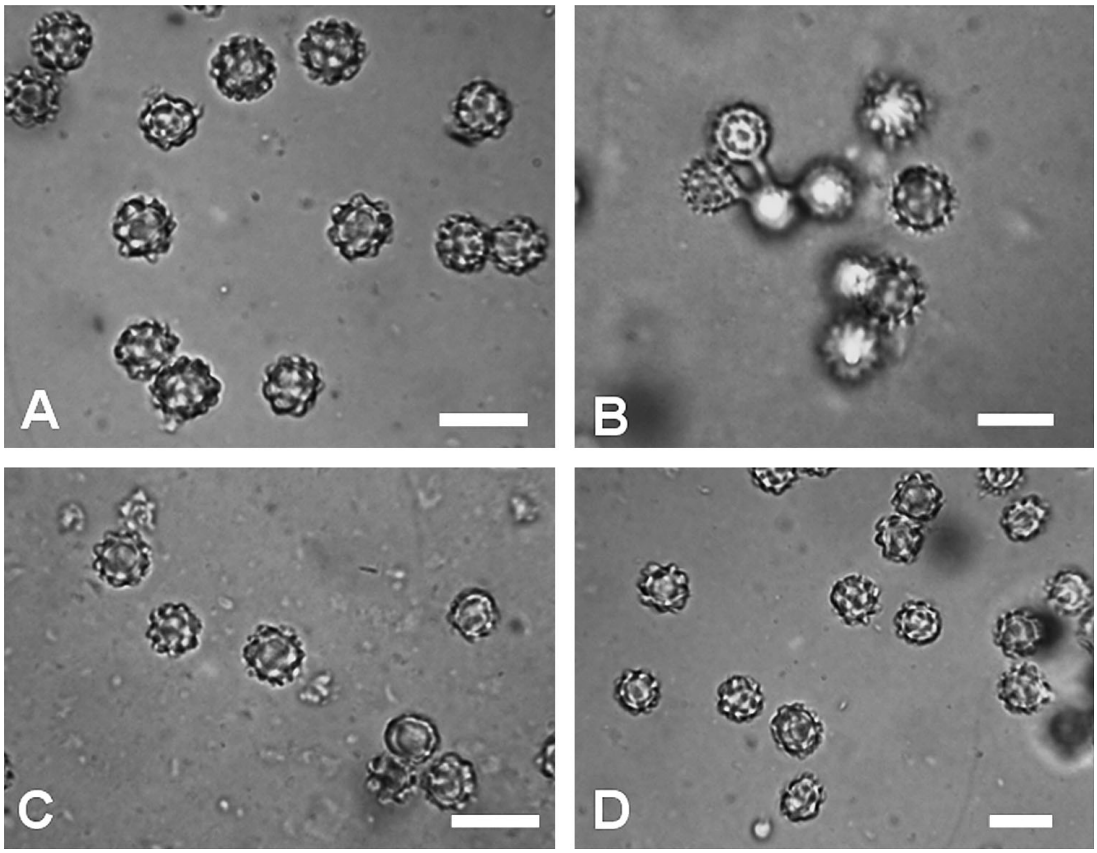

Fig. 4. Basidiospores of *Sclerogaster* spp. A. *S. columellatus* from USA (Trappe 8098). Note warty ornaments. B. *S. minor* from USA (Trappe 8720). C. *S. pacificus* from USA (Trappe 9011). D. *S. xerophilus* from USA (Wright 1956). Bars=10 μm.

rogaster is a distinct lineage within Geastrales. This supports the recognition of family status for this group. In fact, Sclerogastraceae was published by Locquin (1974), but it was without a Latin diagnosis and therefore regarded as invalid in accordance with Article 36.1 of the ICBN. More recently, Kirk *et al*. (2008) validated the family Sclerogastraceae. The family is monotypic, containing the genus *Sclerogaster* Hesse.

Traditionally, *Sclerogaster* has been considered to be closely related to *Octaviania* (Jülich, 1981; Castellano *et al*., 1989; Watling, 2006), *Hyd-*

nangium (Dodge and Zeller, 1936; Zeller and Dodge, 1935), or Russulales (Malençon, 1931; Heim, 1971) but this study strongly suggests placement in the Geastrales with close affinity to the Geastraceae and Schenellaceae (Figs. 1, 2). This is consistent with the fact that Sclerogastraceae, Geastraceae and Schenellaceae all possess globose spores with a surface ornamented with spines or warts (Fig. 4; Domínguez de Toledo and Castellano, 1996; Sunhede, 1989; Castellano *et al*., 1989). In addition, Agerer and Iosifidou (2004) and Clémençon *et al*. (2007) demonstrated the similarity of rhizomorph structure between *Sclerogaster* and Geastraceae.

This study strongly showed the monophyly of the genus, including the type species, *S. lanatus*. The exact placement of *Sclerogaster* in Geastrales, however, remains unresolved. According to the parsimony analyses, *Sclerogaster* is potentially sister to Schenellaceae, which was known as Pyrenogastraceae until recently, but the former name has a nomenclatural priority (Estrada–Torres *et al*., 2005). If *Sclerogaster* and Schenellaceae are demonstrated as the sister groups, it may be preferable to recognize only one family name because both Schenellaceae and Sclerogastraceae are monotypic. More taxon sampling is necessary for a definitive placement of these taxa.

Trends in morphological evolution of fruiting bodies

It is generally believed that sequestrate (truffle-like) fruiting bodies were derived from nonsequestrate ancestors (Thiers, 1984). It is usually attributed to the loss of forcible spore discharge (ballistospory), and clearly demonstrated to be true for Russulales (Miller *et al*., 2001) and Cortinariaceae (Peintner *et al*., 2001). For Phallomycetidae, however, this general theory does not hold because all representative taxa in Phallales, Hysterangiales and Geastrales are gastroid (including sequestrate) and lack a forcible spore discharge mechanism. Hosaka *et al*. (2006) reconstructed the ancestral character state of fruiting body morphology, and demonstrated that sequestrate fruiting bodies are ancestral to above-ground, stinkhorn morphology. They also tried to reconstruct the ancestral character state for Geastrales, but failed to reconstruct the basal nodes unambiguously. To obtain unambiguous reconstruction, more robust phylogeny is necessary. However, presence of two major sequestrate lineages (*Sclerogaster* and Schenellaceae) at a basal position within Geastrales (Figs. 1, 2) suggests that sequestrate fruiting bodies may be ancestral to above-ground, earthstar morphology.

Biogeographical implication

Although only 14 taxa were included in the analyses, biogeographical patterns of *Sclerogaster* (Fig. 1) clearly show some differences from those of Hysterangiales (Hosaka *et al*., 2008). Phylogeny of Hysterangiales strongly supported basal paraphyletic assemblage of the Southern Hemisphere taxa, suggesting the Southern Hemisphere (Gondwanan) origin of the group, with subsequent range expansion to the Northern Hemisphere (Hosaka *et al*., 2008). On the other hand, *Sclerogaster* did not show such a clear pattern (Fig. 1). However, extensive taxon sampling has not been conducted from the Southern Hemisphere for this study, and it may therefore be premature to draw any biogeographical conclusions at this time.

As expected, taxa from New Zealand (KH-NZ06-209, 210) and Australia (Trappe 15701) were shown to be sister taxa (Fig. 1). It is again premature to draw any conclusions at this time, but this relationship may be due to long distance dispersal between Australia and New Zealand, which was demonstrated for both some ectomycorrhizal fungi (Hosaka *et al*., 2008; Moyersoen *et al*., 2003) and saprotrophic fungi (Hibbett, 2001). One of the intriguing relationships was the sister relationship of taxa from Australia (H4595) and USA (Zeller 8462) (Fig. 1). Although this relationship was not strongly supported, this may be due to long distance dispersal between the Northern and Southern Hemispheres. Although it seems unlikely for truffle-like fungi to have the ability to disperse such a long distance, the im-

portance of oceanic dispersal for many organisms has recently been revisited (de Queiroz, 2005).

It is noteworthy to mention that *Sclerogaster* is presumably saprotrophic. Although direct evidence is lacking, their habitat on rotten wood and branches (Fig. 3), or in thick humus layers indicates that they are not ectomycorrhizal fungi. In addition, nearly all taxa within Geastrales are reported as saprotrophic (Sunhede, 1989; Domínguez de Toledo and Castellano, 1996; Flegler, 1984; but see Agerer and Beenken, 1998). Saprotrophic fungi are generally more widespread in distribution than ectomycorrhizal fungi because they do not require the presence of compatible host plants. The study of *Sphaerobolus*, which belongs to Geastrales and therefore is closely related to *Sclerogaster*, showed little phylogeographic pattern within the Northern Hemisphere, suggesting on-going long distance dispersal (Geml *et al*., 2005). *Sphaerobolus* is undoubtedly saprotrophic because they easily produce fruiting bodies on artificial media (Flegler, 1984). A similar nutritional mode (saprotrophic) shared by *Sphaerobolus* and *Sclerogaster* may also indicate that they might have a similar phylogeographical pattern. More extensive taxon sampling for *Sclerogaster* is required to understand the biogeography of the genus.

References

Agerer, R. and Beenken, L. 1998. *Geastrum fimbriatum* Fr.+*Fagus sylvatica* L. *Descriptions of Ectomycorrhizae* **3**: 13–18.

Agerer, R. and Iosifidou, P. 2004. Rhizomorph structures of Hymenomycetes: a possibility to test DNA-based phylogenetic hypotheses? *In*: Frontiers in Basidiomycote Mycology. Agerer, R., Piepenbring, M. and Blanz, P. (eds), pp. 249–302. IHW-Verlag, Eching.

Binder, M. and Hibbett, D. S. 2002. Higher-level phylogenetic relationships of Homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. *Molecular Phylogenetics and Evolution* **22**: 76–90.

Castellano, M. A., Trappe, J. M., Maser, Z. and Maser, C. 1989. Key to Spores of the Genera of Hypogeous Fungi of North Temperate Forests with Special Reference to Animal Mycophagy. 186 pp. Mad River Press, Eureka, California.

Clémençon, H., Hosaka, K. and Taylor, A. S. F. 2007. Rhizomorph anatomy confirms the systematic position of *Sclerogaster* (Phallomycetidae, Basidiomycota). *Mycotaxon* **100**: 85–95.

de Queiroz, A. 2005. The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution* **20**: 68–73.

Dodge, C. W. and Zeller, S. M. 1936. *Hydnangium* and related genera. *Annals of the Missouri Botanical Garden* **23**: 565–598.

Domínguez de Toledo, L. S. and Castellano, M. A. 1996. A revision of the genus *Radiigera* and *Pyrenogaster*. *Mycologia* **88**: 863–884.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19**: 11–15.

Edgar, R. C. 2004a. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

Edgar, R. C. 2004b. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**: 113.

Estrada-Torres, A., Gaither, T. W., Miller, D. L., Lado, C. and Keller H. W. 2005. The myxomycete genus *Schenella*: morphological and DNA sequence evidence for synonymy with the gasteromycete genus *Pyrenogaster*. *Mycologia* **97**: 139–149.

Fischer, E. 1900. Phallineae, Hymenogastrineae, Lycoperdineae, Nidulariineae, Plectobasidiineae. *In*: Engler, A. and Prantl, K. (eds.), Die Natürlichen Pflanzenfamilien. Teil I, Abt. 1**. pp. 276–346.

Flegler, S. L. 1984. An improved method for production of *Sphaerobolus* fruit bodies in culture. *Mycologia* **76**: 944–946.

Geml, J., Davis, D. D. and Geiser, D. M. 2005. Systematics of the genus *Sphaerobolus* based on molecular and morphological data, with the description of *Sphaerobolus* ingoldii sp. nov. *Mycologia* **97**: 680–694.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95–98.

Heim, R. 1971. The interrelationships between the *Agaricales* and Gasteromycetes. *In*: Petersen, R.H. (ed.), Evolution of the Higher *Basidiomycetes*. pp. 505–534. University Tennessee Press, Knoxville.

Hesse, R. 1891. Die Hypogaeen Deutschlands. Band I, Die Hymenogastreen. Verlag Hofstetter, Halle a.S.

Hibbett, D. S. 2001. Shiitake mushrooms and molecular clocks: historical biogeography of *Lentinula*. *Journal of Biogeography* **28**: 231–241.

Hibbett, D. S. and Binder, M. 2002. Evolution of complex fruiting-body morphologies in homobasidiomycetes.

Proceedings of the Royal Society of London, B **269**: 1963–1969.

Hibbett, D. S. and Thorn, R. G. 2001. Basidiomycota: Homobasidiomycetes. *In*: McLaughlin, D.J., McLaughlin, E.G. and Lemke, P.A. (eds.), The Mycota, volume 7. Systematics and evolution. pp. 121–168. Springer-Verlag, Berlin.

Hibbett, D. S., Pine, E. M., Langer, E., Langer, G. and Donoghue, M. J. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proceedings of the National Academy of Sciences, USA* **94**: 12002–12006.

Hosaka, K., Bates, S. T., Beever, R. E., Castellano, M. A., Colgan, W., Dominguez, L. S., Geml, J., Giachini, A. J., Kenney, S. R., Nouhra, E. R., Simpson, N. B., Spatafora, J. W. and Trappe, J. M. 2006. Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass *Phallomycetidae* and two new orders. *Mycologia* **98**: 949–959.

Hosaka, K., Castellano, M. A. and Spatafora, J. W. 2008. Biogeography of the *Hysterangiales* (Phallomycetidae, Basidiomycota). *Mycological Research* **112**: 448–462.

Huelsenbeck, J. P. and Ronquist, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Humpert, A. J., Muench, E. L., Giachini, A. J., Castellano, M. A. and Spatafora, J. W. 2001. Molecular phylogenetics of *Ramaria* and related genera: evidence from nuclear large subunit and mitochondrial small subunit rDNA sequences. *Mycologia* **93**: 465–477.

James, T. Y., Kauff, F., Schoch, C., Matheny, P. B., Hofstetter, V., Cox, C. J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H. T., Rauhut, A., Reeb, V., Arnold, A. E., Amtoft, A., Stajich, J. E., Hosaka, K., Sung, G.-H., Johnson, D., OÅfRourke, B., Binder, M., Curtis, J. M., Slot, J.C., Wang, Z., Wilson, A.W., Schüßler, A., Longcore, J. E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P. M., Powell, M. J., Taylor, J. W., White, M. M., Griffith, G. W., Davies, D. R., Sugiyama, J., Rossman, A. Y., Rogers, J. D., Pfister, D. H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R. A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R., Serdani, M., Crous, P. W., Hughes, K. W., Matsuura, K., Langer, E., Langer, G., Untereiner, W. A., Lücking, R., Büdel, B., Geiser, D. M., Aptroot, A., Buck, W. R., Cole, M. S., Diederich, P., Hillis, D. M., Printzen, C., Schmitt, I., Schultz, M., Yahr, R., Zavarzin, A., Hibbett, D. S., Lutzoni, F., McLaughlin, D. J., Spatafora, J. W. and Vilgalys, R. 2006. Reconstructing the early evolution of the fungi using a six gene phylogeny. *Nature* **443**: 818–822.

Jülich, W. 1981. Higher taxa of basidiomycetes. *Bibliotheca Mycologica* **85**: 1–485.

Kirk, P. M., Cannon, P. F, David, J. C. and Stalpers, J. A. 2001. Ainsworth and Bisby's Dictionary of the Fungi, 9th edition. 624 pp. CABI Publishing, Wallingford.

Kirk, P. M., Cannon, P. F., Minter, D. W. and Stalpers, J. A. 2008. Ainsworth and Bisby's Dictionary of the Fungi, 10th edition. 771 pp. CABI Publishing, Wallingford.

Kreisel, H. 1969. Grundzüge eines natürlichen Systems der Pilze. Jena, Germany: Gustav Fischer Verlag/Cramer. 245 pp.

Kretzer, A. M. and Bruns, T. D. 1999. Use of *atp6* in fungal phylogenetics: an example from the Boletales. *Molecular Phylogenetics and Evolution* **13**: 483–492.

Locquin, M.V. 1974. De Taxia Fungorum 1: 1–62.

Lutzoni, F., Kauff, F., Cox, C. J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T. Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A. E., Miadlikowska, J., Spatafora, J. W., 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, R. C., 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. and Vilgalys, R. 2004. Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *American Journal of Botany* **91**: 1446–1480.

Malençon, G. 1931. La série des Astérosporés. *Travaille Cryptogamie dédiés à L. Mangin* **1**: 337–396.

Matheny, P. B., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Nilsson, R. H., Hughes, K. W., Hofstetter, V., Ammirati, J. F., Schoch, C., Langer, E., Langer, G., McLaughlin, D. J., Wilson, A. W., Froslev, T., Ge, Z. W., Kerrigan, R. W., Kerrigan, R., Slot, J., Yang, Z. L., Baroni, T. J., Fischer, M., Hosaka, K., Matsuura, K., Seidl, M., Vauras, J. and Hibbett, D. S. 2007. Contributions of *rpb2* and *tef1* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* **43**: 430–451.

Miller, S. M., McClean, T. M., Walker, J. F. and Buyck, B. 2001. A molecular phylogeny of the Russulales including agaricoid, gasteroid and pleurotoid taxa. *Mycologia* **93**: 344–354.

Moncalvo, J.-M., Vilgalys, R., Redhead, S. A., Johnson, J. E., James, T. Y., Aime, M. C., Hofstetter, V., Verduin, S. J. W., Larsson, E., Baroni, T. J., Thorn, R. G., Jacobsson, S., Clemençon, H. and Miller, O.K. 2002. One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* **23**: 357–400.

Moyersoen, B., Beever, R. E. and Martin, F. 2003. Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist* **160**: 569–579.

Munkasci, A. B., Kawakami, S., Pan, J. J., Lee, K., Stox-

en, S., Hang, J. and May, G. 2006. Genome-wide assessment of tandem repeat markers for biogeographical analyses of the corn smut fungus, *Ustilago maydis*. *Molecular Ecology Notes* **6**: 221–223.

Peintner, U., Bougher, N. L., Castellano, M. A., Moncalvo, J. M., Moser, M. M., Trappe, J. M. and Vilgalys, R. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae). *American Journal of Botany* **88**: 2168–2179.

Pine, E. M., Hibbett, D. S. and Donoghue, M. J. 1999. Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia* **91**: 944–963.

Rogstad, S. H. 2003. Plant DNA extraction using silica. *Plant Molecular Biology Reporter* **21**: 463a–463g.

Seutin, G., White, B. N. and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* **69**: 82–90.

Shimodaira, H. and Hasegawa, M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114–1116.

Sunhede, S. 1989. Synopsis Fungorum 1: Geastraceae (Basidiomycotina); Morphology, Ecology, and Systematics with Special Emphasis on the North European species. Fungiflora, Oslo.

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

Templeton, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**: 221–244.

Thiers, H. D. 1984. The secotioid syndrome. *Mycologia* **76**: 1–8.

Vilgalys, R. and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

Watling, R. 2006. The sclerodermatoid fungi. *Mycoscience* **47**: 18-24.

Zeller, S. M. 1948. Notes on certain Gasteromycetes, including two new orders. *Mycologia* **40**: 639–668.

Zeller, S. M. and Dodge, C. W. 1935. New species of Hydnangiaceae. *Annals of the Missouri Botanical Garden* **22**: 365–373.