AN ABSTRACT OF THE DISSERTATION OF

Kentaro Hosaka for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on October 26, 2005.

Title: Systematics, Phylogeny, and Biogeography of the Hysterangiales and Related Taxa (Phallomycetidae, Homobasidiomycetes).

Abstract approved:

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Monophyly of the gomphoid-phalloid clade was confirmed based on multigene phylogenetic analyses. Four major subclades (Hysterangiales, Geastrales, Gomphales and Phallales) were also demonstrated to be monophyletic. The interrelationships among the subclades were, however, not resolved, and alternative topologies could not be rejected statistically. Nonetheless, most analyses showed that the Hysterangiales and Phallales do not form a monophyletic group, which is in contrast to traditional taxonomy. The higher-level phylogeny of the gomphoid-phalloid fungi tends to suggest that the Gomphales form a sister group with either the Hysterangiales or Phallales. Unweighted parsimony character state reconstruction favors the independent gain of the ballistosporic mechanism in the Gomphales, but the alternative scenario of multiple losses of ballistospory could not be rejected statistically under likelihood-based reconstructions. This latter hypothesis is consistent with the widely accepted hypothesis that the loss of ballistospory is irreversible. The transformation of fruiting

body forms from nongastroid to gastroid was apparent in the lineage leading to *Gautieria* (Gomphales), but the tree topology and character state reconstructions supported that truffle-like taxa of the Phallales are ancestral to stinkhorns, which possess more complex, epigeous fruiting bodies. Importantly all taxa within the Phallales are statismosporic and thus the derived stinkhorn morphology does not require an independent gain of ballistospory.

Biogeographical analyses of the Hysterangiales strongly suggest that the ectomycorrhizal lineages within the Hysterangiales originated in the East Gondwana. The synonymous substitution rate indicated a Paleozoic origin of the Hysterangiales although a possibility of a Cretaceous origin could not be discarded. Because modern ectomycorrhizal plants were absent during the Paleozoic era, a potential existence of the Hysterangiales during this time must be explained either by novel ectomycorrhizal association of the Hysterangiales with unknown plant lineages, or multiple, independent gains of ectomycorrhizal habit. The Paleozoic origin of the Hysterangiales also indicates that mycophagous animals may not be the most important factor for range expansions of the Hysterangiales.

Taxonomic revisions are made for the gomphoid-phalloid fungi. One subclass (Phallomycetidae), two orders (Hysterangiales and Geastrales), four families (Gallaceaceae, Phallogastraceae, Trappeaceae and Sclerogastraceae), 7 genera (*Austrohysterangium, Cribbangium, Rodwayomyces, Beeveromyces, Cazomyces, Insulomyces* and *Viridigautieria*) and 22 new combinations are proposed.

Systematics, Phylogeny, and Biogeography of the Hysterangiales and Related Taxa (Phallomycetidae, Homobasidiomycetes)

by

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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I do not trust Occam's razor. The simplest explanations are not necessarily the right ones in biogeography. To choose the simplest explanation because it is simple is like a surgeon choosing to cut a patient's throat with one razor stroke rather than to perform a complex operation. Occam's razor should be used to make an exploratory cut into a problem, not to solve it.

P.J. Darlington, 1965

Systematics, Phylogeny, and Biogeography of the Hysterangiales and Related Taxa (Phallomycetidae, Homobasidiomycetes)

CHAPTER 1

General Introduction

THE DISCOVERY OF THE GOMPHOID-PHALLOID CLADE

With the advent of DNA sequence data, efficient phylogenetic algorithms, and enhanced computational power, numerous novel evolutionary relationships have been revealed over the last few decades. The kingdom Fungi is no exception. For the higher-level phylogeny, the sister relationship between Fungi and Animals (collectively called Opisthokonta by Cavalier-Smith, 1987), the sister relationship between Opisthokonta and plasmodial/cellular slime molds, and the polyphyletic nature of the 'fungi', with Oomycetes being more closely related to brown algae, were all confirmed based on multigene sequence data (Baldauf *et al.*, 2000). For lower-level phylogeny, the results from initial studies were largely congruent with traditional classifications; Basidiomycota and Ascomycota are sister groups, and Basidiomycota could be divided into at least two higher groups corresponding to basidial morphology (Bruns *et al.*, 1992; Swann & Taylor, 1993, 1995). As more and more taxa were

sequenced and analyzed, however, numerous conflicts with traditional classifications became apparent.

One of the pioneer molecular studies for mushroom-forming fungi was that of Hibbett *et al.* (1997). They demonstrated that gilled mushrooms and gasteromycetes, which have traditionally been treated in separate groups, are represented in many clades, suggesting that both morphologies evolved independently multiple times. One of the most surprising relationships revealed in this study was a discovery of a monophyletic group containing the genera *Pseudocolus, Ramaria, Gomphus, Geastrum,* and *Sphaerobolus*, which was later referred to as "gomphoid-phalloid" clade (Hibbett & Thorn, 2001). The gomphoid-phalloid clade contains morphologically very diverse groups of fungi. The fruiting body morphology includes earthstars, stinkhorns, cannon ball fungi, coral fungi, club fungi, gilled mushrooms, tooth fungi, and false truffles. Because of its diversity, traditional morphology-based taxonomy has classified the fungi belonging to the gomphoid-phalloid clade into several unrelated orders, including the Lycoperdales, Phallales, Nidulariales, Gomphales, Hysterangiales, and Gautieriales (Zeller, 1949; Jülich, 1981), many of which were not supported as monophyletic.

THE TAXONOMIC HISTORY OF THE GOMPHOID-PHALLOID FUNGI

The nomenclatural history for several taxa in the gomphoid-phalloid clade began in 1801 when Christiaan Hendrik Persoon published Synopsis Methodica Fungorum, in which he described or sanctioned many taxa. Persoon (1801) placed the gomphoid-phalloid fungi in two separate classes: 1) "Angiocarpi" which contained some gomphoid-phalloid taxa (*Sphaerobolus* and *Geastrum*) along with *Puccinia*, *Pilobolus*, and myxomycetes, and 2) "Gymnocarpi" which contained other gomphoid-phalloid taxa (*Phallus*, *Clathrus*, *Ramaria*, *Gomphus*) along with *Agaricus*, *Boletus*, and discomycetes. Later Elias Magnus Fries, sometimes called "the Linnaeus of Mycology" (Hawksworth *et al.*, 1995), used a slightly different system in the series of Systema Mycologicum published during 1821-1832. He divided the fungi into three classes, among which the gomphoid-phalloid fungi are represented in "Hymenomycetes" and "Gasteromycetes". The "Hymenomycetes" included some gomphoid-phalloid fungi (*Ramaria, Gomphus*) along with *Agaricus* and *Boletus*. The other gomphoid-phalloid fungi (*Phallus, Clathrus, Geastrum* and *Sphaerobolus*) were included in "Gasteromycetes" along with *Pilobolus*, pyrenomycetes, and myxomycetes. During this time, fungi were still grouped based on macroscopic characters alone, with no distinctions between Ascomycota and Basidiomycota, or even Myxomycetes (Jülich, 1981). Nonetheless the classification systems developed by Persoon and Fries, sometimes called 'Friesian systems', became a foundation for the taxonomy of mushroom-forming fungi.

In his 1831 publication entitled Monographia Tuberacearum, Carlo Vittadini established two truffle-like genera of the gomphoid-phalloid clade, *Hysterangium* and *Gautieria*. Both genera were placed in the family Tuberaceae. Vittadini followed the Friesian system by including all truffle-like taxa in the Tuberaceae under class Gasteromycetes. In Vittadini's concept, the family Tuberaceae contains both ascomycetous (e.g., *Tuber* and *Elaphomyces*) and basidiomycetous truffles.

Basidiomycetes and ascomycetes were finally differentiated when Heinrich Georg Winter (1881) described the classes Basidiomycetes and Ascomycetes in Rabenhorst's Kryptogamen–Flora. Microscopic characters became increasingly important for the taxonomy of fungi around this time. In 1900, Narcisse Théophile Patouillard further elaborated the taxonomic system of basidiomycetes on the basis of basidial characters. Patouillard divided the basidiomycetes into two groups, heterobasidiomycetes (as "Basidiomycètes hétérobasidiés") and homobasidiomycetes (as "Basidiomycètes homobasidiés"). Since then, all the gomphoid-phalloid fungi were correctly placed in the homobasidiomycetes, although gastroid and nongastroid taxa of the gomphoid-phalloid clade have never been considered in the same context until DNA sequence data became available.

Arguably the most seminal publication for the taxonomy of gasteromycetes was Edward Fischer's system in Die Natürlichen Pflanzenfamilien (published in 1900, later revised in 1933). He established five (1900) or six (in 1933) orders with numerous families based mostly on the structure of the basidia and development of the hymenium. Many order and family names are still recognized today. Especially

important for the gomphoid-phalloid fungi is the establishment of the order Phallales and the family Hysterangiaceae (Fischer, 1900). Fischer's system was largely followed with minor modifications by subsequent mycologists, including Gordon Herriot Cunningham, Sanford Myron Zeller, Hanns Kreisel and Walter Jülich. The contribution of Cunningham to the taxonomy of the gomphoid-phalloid fungi includes the establishment of the family Claustulaceae, Mesophelliaceae (as Lycoperdaceae tribe Mesophelliae), and the genus *Phallobata* (Cunningham, 1926, 1931, 1932). Zeller provided a comprehensive key to the orders, families and genera of gasteromycetes (Zeller, 1949) and described numerous taxa of gomphoid-phalloid fungi including the families Protophallaceae, Gelopellaceae, and Gautieriaceae (Zeller, 1939, 1948). Zeller (1939) also recognized the order Hysterangiales as a separate order from the Phallales although he did not provide a Latin diagnosis. Kreisel (1969) recognized the order Geastrales, segregating it from the order Lycoperdales. Jülich (1981) synthesized all of these past classification systems, and provided a comprehensive treatment of ordinal and familial classification systems for the Basidiomycota. It is essentially this classification of Jülich (1981) that provided the working classification for the gomphoid-phalloid fungi at the initiation of molecular phylogenetics of the Basidiomycota as discussed in Hibbett *et al.* (1997).

THE COMPOSITION OF THE DISSERTATION

This dissertation consists of 6 chapters. Chapter 2 discusses the evolutionary relationships of the gomphoid-phalloid fungi based on 3-gene sequences (nuc-LSU-rDNA, mt-SSU-rDNA, and *ATP6*) with emphasis on the evolution of ballistospory. One interesting feature of the gomphoid-phalloid clade is that it contains a disproportionate number of gastroid taxa, which all lack a forcible spore discharge mechanism (called ballistospory) and release their spores passively (statismospory). The phylogenies of the gomphoid-phalloid fungi could challenge the traditional view of statismospory being a derived state from a ballistospory (McLaughlin *et al.*, 1985; Thiers, 1984). Ancestral character states for the spore discharge mechanism were reconstructed based on parsimony and likelihood criteria. Also addressed are the issues of dataset combinability, which have been discussed extensively in the last few decades (Buckley *et al.*, 2002; Cunningham, 1997; Farris *et al.*, 1995; Goldman *et al.*, 2000; Kishino & Hasegawa, 1989; Mason-Gamer & Kellogg, 1996; Shimodaira & Hasegawa, 1999; Templeton, 1983).

Chapter 3 discusses the biogeography of the Hysterangiales. Because spore dissemination of truffle-like fungi, including that of Hysterangiales, is mostly dependent on animal mycophagy (fungal consumption by other organisms), long distance (such as intercontinental) dispersal of spores of truffle-like fungi is arguably less likely. The distribution of Hysterangiales, however, is worldwide, both in the Northern and Southern Hemisphere (Castellano, 1990, 1999). This is consistent with

Hysterangiales being an old taxon, and the current distribution is a result of the ancient vicariant events of the supercontinent Pangaea. This hypothesis was tested by comparing the biogeographical patterns, molecular clock age estimates, ectomycorrhizal host association patterns, geological history, and biogeographical patterns of other organisms.

Chapter 4 discusses the higher-level phylogenetic hypotheses of the gomphoid-phalloid fungi based on 5-gene sequences (3 genes described above and *RPB2* and *EF1α*). The analyses were conducted with the most extensive taxon sampling ever for the gomphoid-phalloid fungi. Comparisons were made between the past classification systems and the phylogenetic hypotheses of the gomphoid-phalloid fungi. Also discussed is a new classification scheme for ordinal and subclass level taxonomy.

Chapter 5 is a taxonomic revision for the gomphoid-phalloid fungi based on the phylogenetic hypotheses from the previous chapters. All recognized families and genera are listed for the Hysterangiales, Phallales, and Geastrales, with emphasis on a familial-level revision for the Geastrales and Phallales. Familial- and generic-level revisions are made for the Hysterangiales. Finally, chapter 6 is a conclusion, synthesizing the results from all chapters.

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CHAPTER 2

Molecular phylogenetics of the gomphoid-phalloid fungi (Homobasidiomycetes, Fungi) with special emphasis on the evolution of ballistospory

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ABSTRACT

Molecular phylogenetic analyses of gomphoid-phalloid fungi (mushroom-forming fungi, Homobasidiomycetes) were conducted based on a 3-gene-dataset (nuc-LSU-rDNA, mt-SSU-rDNA, and *ATP6*). The monophyly of the gomphoid-phalloid clade was strongly supported and four well-supported major clades were recognized within the gomphoid-phalloid clade. Although alternative tree topologies could not be rejected statistically, both parsimony and Bayesian analyses suggest the sister relationship of the Hysterangiales and Gomphales clades. Parsimony-based ancestral character state reconstructions for the spore discharge mechanism (ballistospory/ statismospory) favored an independent gain of ballistospory in the Gomphales, a result which is contradictory to the generally accepted hypothesis for the evolution of this spore discharge mechanism. Maximum-likelihood reconstructions favored the hypothesis that the loss of ballistospory is more likely than a gain, although neither hypothesis could be statistically rejected. This latter character state reconstruction as well as the polyphyletic origins of gastroid taxa and complex mechanism of ballistospory favors the loss of ballistospory as the evolutionary scenario.

INTRODUCTION

The class Homobasidiomycetes (Basidiomycota) contains most of the mushroom-forming, fleshy fungi. Based on phylogenetic analyses using ribosomal DNA sequence data, 8 major clades have been defined in the Homobasidiomycetes (Binder & Hibbett, 2002; Hibbett & Thorn, 2001). One of them and the focus of this study, the gomphoid-phalloid clade, is particularly interesting because of its morphological and ecological diversity. The fruiting body morphology of the taxa in this clade includes earthstars, stinkhorns, cannon ball fungi, coral fungi, club fungi, gilled mushrooms, tooth fungi, and false truffles. Because of their morphological diversity, traditional morphology-based taxonomy has classified the fungi belonging to the gomphoid-phalloid clade into several different orders, including the Lycoperdales, Phallales, Nidulariales, Gomphales, Hysterangiales, and Gautieriales (Zeller, 1939, 1947, 1948, 1949; Jülich, 1981). It is now clear, however, that many of these orders are artificial, polyphyletic groups; for example, two genera in the order Lycoperdales, *Geastrum* (earthstars) and *Lycoperdon* (puffballs) are not supported in molecular phylogenetic studies as being closely related; *Geastrum* with its earthstar fruiting body morphology belongs to the gomphoid-phalloid clade, whereas *Lycoperdon* with its puffball fruiting body morphology is a member of the euagarics clade and is closely related to the gilled mushroom, i.e., *Agaricus* (Binder & Hibbett, 2002; Hibbett & Thorn, 2001). Although mycologists have yet to discover a morphological synapomorphy of the gomphoid-phalloid clade, numerous studies have repeatedly

shown strong support of this clade and the inclusion of the aforementioned taxa (Binder & Hibbett, 2002; Hibbett & Thorn, 2001; Moncalvo *et al.*, 2002). Most studies, however, included only a few taxa representing the gomphoid-phalloid clade, and analyses were restricted to ribosomal DNA sequences. As a result, current understanding of the systematics of the gomphoid-phalloid clade is arguably preliminary and the exact relationships among gomphoid-phalloid fungi remain unresolved.

One of the interesting features of the gomphoid-phalloid clade is that it contains a disproportionate number of gastroid taxa. The term gastroid is defined as a lack of forcible spore discharge mechanism due to the development and maturation of spores occurring within an enclosed spore-producing tissue or gleba (Miller & Miller, 1988). In the gomphoid-phalloid clade, earthstars, stinkhorns, cannon ball fungi, and false truffles are all gastroid taxa. This lack of a forcible spore discharge mechanism (called statismospory) is in contrast to the other members of this clade, including gilled, tooth, coral and club fungi, which are non-gastroid, producing their spores on exposed spore-producing tissue and possessing the typical forcible spore discharge mechanism (called ballistospory) of the Basidiomycota. The mechanism of the ballistospory is complex, and because of its much simpler form, gastroid (hence statismosporic) taxa have often been considered as derived from non-gastroid, ballistosporic taxa (McLaughlin *et al.*, 1985; Thiers, 1984). This view was supported by the study of Hibbett *et al.* (1997), which showed the multiple origins of gastroid taxa and the concurrent multiple and independent losses of ballistospory during the

evolution of Homobasidiomycetes.

The goal of this study was to conduct a more thorough phylogenetic analysis of the gomphoid-phalloid clade and thereby develop a better understanding of large scale morphological and ecological evolutionary patterns of the Basidiomycota. In this study, we expanded the taxon sampling for the gomphoid-phalloid clade to cover the breadth of taxonomic diversity of this clade. Nucleotide sequences were determined from three genes, including two ribosomal (nuc-LSU rDNA and mt-SSU rDNA) and one protein coding locus (*ATP6*), and analyzed by both parsimony and Bayesian approaches. Ancestral character state evolution of the spore discharge mechanism was inferred using both parsimony- and likelihood-based methods. Understanding the phylogeny and character state evolution in this clade will further facilitate our overall understanding of the evolution of the spore discharge mechanism and nutritional mode in the Homobasidiomycetes.

MATERIALS AND METHODS

Taxon sampling, PCR, and DNA sequencing

A total of 68 species, four outgroup and 64 ingroup taxa, were sampled for this study (Table 2.1). The ingroup taxa were selected based on the phylogenetic hypotheses of previous studies (Humpert *et al.*, 2001; Villegas *et al.*, 1999) and

traditional morphology-based classifications (Dominguez de Toledo & Castellano, 1996; Dring, 1980; Marr & Stuntz, 1973; Zeller, 1949; Jülich, 1981) to cover the diversity of the gomphoid-phalloid fungi. Among the ingroup taxa, 54 taxa are the gastroid taxa, and coded as statismosporic. The detailed coding scheme for spore discharge mechanism is shown in Fig. 2.2 & 2.5. The outgroup taxa were chosen so that DNA sequences of all three target loci were available from GenBank.

Sequence data were obtained from nuclear large subunit (nuc-LSU-rDNA) and mitochondrial small subunit ribosomal RNA genes (mt-SSU-rDNA), and one mitochondrial protein coding gene, *ATP6*. The primers and PCR protocols have been described in previous studies. For nuc-LSU-rDNA, the primer combination of LR0R and LR3 (Vilgalys & Hester, 1990) was used. For mt-SSU-rDNA, three different primer combinations were used. Most samples amplified well with MS1 and MS2 (White *et al.*, 1990). If not, the other primer combinations, U1 (primer sequences available from the webpage of Tom Brun's lab; http://plantbio.berkeley.edu/%7Ebruns/primers.html) and MS2, or Phal1 and MS2, were used. The Phal1 primer sequence is 5'-CCAKAAGACTCGGTAAKG-3'. The PCR conditions follow the protocol described by Humpert *et al.* (2001). For *ATP6*, the primer combination atp6-3 and atp6-2 (Kretzer & Bruns, 1999) was used, and the PCR protocol followed that of Kretzer and Bruns (1999). Sequencing reactions were done using the DYEnamic™ ET terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc.) following the manufacture's protocol. Sequencing was run on an ABI 373XL automated DNA sequencer. Sequences were edited using the SeqEd version

Table 2.1. Taxon list.

Taxon	Herbarium	Specimen numbers	GenBank accession number		
			nuc-LSU	mt-SSU	ATP6
INGROUP					
Austrogautieria chlorospora E.L. Stewart & Trappe	OSC	46596	DQ218477	DQ218652	DQ218761
Austrogautieria manjimupana E.L. Stewart & Trappe	OSC	59545	DQ218478	DQ218653	DQ218762
Austrogautieria sp.	OSC	80139	DQ218479	DQ218654	DQ218763
Austrogautieria sp.	OSC	80140	DQ218480	DQ218655	DQ218764
Beenakia fricta Maas Geest.	K	2083	AY574693	AY574766	AY574833
Clavariadelphus ligula (Schaeff.) Donk	OSC	67068	AY574650	AY574723	AY574793
Dictyophora duplicata (Bosc) E. Fisch.	OSC	38819	DQ218481	DQ218656	DQ218765
Gallacea eburnea Castellano & Beever	OSC	59601	DQ218482	DQ218657	DQ218766
Gallacea scleroderma (Cooke) Lloyd	OSC	59621	AY574645	AY574719	AY574787
Gautieria caudata (Harkn.) Zeller & C.W. Dodge	OSC	59201	DQ218483	DQ218658	DQ218767
Gautieria crispa (Vittad.) Bougher & Castellano	OSC	61308	DQ218484	DQ218659	DQ218768
Gautieria monticola Harkn.	OSC	65121	AY574651	AY574724	AY574794
Geastrum floriforme Vittad.	OSC	29328	DQ218485	DQ218660	DQ218769
Geastrum recolligens (With.) Desv.	OSC	41996	DQ218486	DQ218661	DQ218770
Gloeocantharellus purpurascens (Hesler) Singer	TENN	12793	AY574683	AY574756	AY574823
Gomphus clavatus (Pers.) Gray	OSC	97587	DQ218487	DQ218662	DQ218771
Gomphus floccosus (Schwein.) Singer	OSC	69167	AY574656	AY574729	AY574799
Gummiglobus joyceae Trappe, Castellano & Amar.	OSC	59485	DQ218488	DQ218663	DQ218772
Hysterangium aggregatum J.W. Cribb	OSC	H4262	DQ218489	DQ218664	DQ218773
Hysterangium album Zeller & C.W. Dodge	OSC	T15139	DQ218490	DQ218665	DQ218774
Hysterangium aureum Zeller	OSC	56988	DQ218491	DQ218666	DQ218775
Hysterangium calcareum R. Hesse	M	Gross97	DQ218492	DQ218667	DQ218776
Hysterangium cistophilum (Tul. & Tul.) Zeller & Dodge	OSC	T1088	DQ218493	DQ218668	DQ218777
Hysterangium coriaceum R. Hesse	OSC	64939	AY574686	AY574759	AY574826

Table 2.1. Taxon list (continued).

Hysterangium crassirhachis Zeller & C.W. Dodge	OSC	58056	DQ218494	DQ218669	DQ218778
Hysterangium crassum (Tul. & C. Tul.) E. Fisch.	OSC	110447	AY574687	AY574760	AY574827
Hysterangium epiroticum Pacioni	OSC	T6116	DQ218495	DQ218670	DQ218779
Hysterangium fragile Vittad.	OSC	Kers3971	DQ218496	DQ218671	DQ218780
Hysterangium hallingii Castellano & J.J. Muchovej	OSC	Halling5741	DQ218497	DQ218672	DQ218781
Hysterangium membranaceum Vittad.	OSC	T12836	DQ218498	DQ218673	DQ218782
Hysterangium occidentale Harkn.	OSC	47048	AY574685	AY574758	AY574825
Hysterangium pompholyx Tul. & C. Tul.	OSC	Gross495	DQ218499	DQ218674	DQ218783
Hysterangium rugisporum Castellano & Beever	OSC	59662	DQ218500	DQ218675	DQ218784
Hysterangium salmonaceum Beaton, Pegler & Young	K	Beaton33	DQ218501	DQ218676	DQ218785
Hysterangium separabile Zeller	OSC	69030	DQ218502	DQ218677	DQ218786
Hysterangium spegazzinii Castellano & J.J. Muchovej	OSC	Singer3426	DQ218503	DQ218678	DQ218787
Hysterangium strobilus Zeller & C.W. Dodge	OSC	T5285	DQ218504	DQ218679	DQ218788
Hysterangium youngii Castellano & Beever	OSC	59645	DQ218505	DQ218680	DQ218789
Hysterangium sp.	K	K & G Beaton	DQ218506	DQ218681	DQ218790
Kavinia alboviridis (Morgan) Gilb. & Budington	O	102140	AY574692	AY574765	AY574832
Lentaria pinicola (Burt) R.H. Petersen	SUC	M89	AY574688	AY574761	AY574828
Lysurus mokusin f. *sinensis* (Lloyd) Kobayasi	CUW	MB02-012	DQ218507	DQ218682	DQ218791
Malajczukia amicorum Trappe & Castellano	OSC	59295	DQ218508	DQ218683	DQ218792
Malajczukia ingratissima (Berk.) Trappe & Castellano	OSC	59296	DQ218509	DQ218684	DQ218793
Malajczukia viridigleba Trappe & Castellano	OSC	59287	DQ218510	DQ218685	DQ218794
Mesophellia clelandii Trappe, Castellano & Malajczuk	OSC	59292	DQ218511	DQ218686	DQ218795
Mesophellia oleifera Trappe, Castellano & Malajczuk	OSC	79923	DQ218512	DQ218687	DQ218796
Mutinus elegans (Mont.) E. Fisch.	OSC	107657	AY574643	AY574717	AY574785
Phallus costatus Vent.	CUW	MB02-040	DQ218513	DQ218688	DQ218797
Phallus hadriani Vent.	OSC	107658	DQ218514	DQ218689	DQ218798
Phallus ravenelii Berk. & M.A. Curtis	CUW	s.n.	DQ218515	DQ218690	DQ218799
Protubera borealis S. Imai	OSC	OKM21898	DQ218516	DQ218691	DQ218800

Table 2.1. Taxon list (continued).

Protubera hautuensis Castellano & Beever	OSC	59673	DQ218517	DQ218692	DQ218801
Protubera maracuja A. Möller	OSC	Garido2550A	DQ218518	DQ218693	DQ218802
Protubera nothofagi Castellano & Beever	OSC	59699	AY574644	AY574718	AY574786
Pyrenogaster pityophilus Malençon & Riousset	OSC	59743	DQ218519	DQ218694	DQ218803
Radiigera taylorii (Lloyd) Zeller	OSC	59760	DQ218520	DQ218695	DQ218804
Ramaria rainierensis Marr & D.E. Stuntz	SUC	M231	AF213115	AF213135	AY574834
Ramaria stricta (Pers.:Fr) Quél.	SUC	M405	AF213117	AF213138	DQ218805
Ramaria stuntzii Marr	SUC	M214	AF213102	AF213134	AY574850
Simblum sphaerocephalum Schlechtendal	CUW	MB02-016	DQ218521	DQ218696	DQ218806
Sphaerobolus stellatus Tode	PSU*	SS12	AF393077	AF026662	AY574789
Sphaerobolus stellatus Tode	PSU*	SS28	AY574647	AY488024	AY574790
Trappea phillipsii (Harkn.) Castellano	OSC	56042	DQ218522	DQ218697	DQ218807
OUTGROUP					
Chamonixia caespitosa Rolland	———	———	AF336245	AF213145	AF114444
Phylloporus rhodoxanthus (Schwein.) Bres.	———	———	AF071533	M91013	AF114443
Tapinella atrotomentosa (Batsch) Šutara	———	———	AY177261	M91012	AF114448
Xerocomus chrysenteron (Bull.) Quél.	———	———	AF071537	M91018	AF002143

Herbarium code: OSC = Oregon State University Herbarium; CUW = Clark University Herbarium; SUC = State University of New York Herbarium; K = Royal Botanic Gardens, Kew, UK; M = Herbarium at Botanische Staatssammlung München, Germany; TENN = University of Tennessee Herbarium; PSU = The Pennsylvania State University Mushroom Culture Collection.

1.0.3. (Applied Biosystems, Inc. 1992), and deposited in GenBank (GenBank numbers are listed in Table 2.1).

Phylogenetic analyses

DNA sequences were aligned by visual examination in the data editor of PAUP*4.0b10 (Swofford, 2003). Ambiguously aligned regions were excluded from analyses. Phylogenetic analyses were conducted using equally-weighted parsimony and six-parameter parsimony (Moncalvo *et al.*, 2000) in PAUP*. For the *ATP6* dataset, phylogenetic trees were inferred from the heuristic search option (with TBR and Multrees on) and 500 replicates of random addition sequence. Due to computational intensity and dense taxon sampling of terminal clades, a two-step search approach was conducted for nuc-LSU-rDNA and mt-SSU-rDNA datasets. In the first step, the heuristic search option (with TBR, no Multrees) and 100 replicates of random addition sequence were performed, keeping only up to two shortest trees per replicate. In the second step, all of the shortest trees from the first step were used as starting trees for heuristic search option (with TBR and Multrees on) and 500 replicates of random addition sequence, with MAXTREES set to 10,000. These approaches were followed in all parsimony analyses. Support for individual nodes was tested with bootstrap analysis under the parsimony criterion. Bootstrap analysis was based on 500 bootstrap replicates using heuristic search option (TBR and Multrees off), with 5 random

addition sequences.

Bayesian analyses of individual gene datasets and various combinations of datasets were performed using MrBayes ver. 3.0b4 (Huelsenbeck, 2000). For nuc-LSU-rDNA and mt-SSU-rDNA datasets, a GTR+Γ+I model was used. The same model was used for *ATP6* dataset, but it was further partitioned according to the codon position for a total of 5 partitions. Bayesian analyses were run with 2 million MCMCMC generations, sampling every 10^{th} tree. The support of nodes was tested by posterior probabilities, obtained from majority rule consensus after deleting trees from the burn-in period.

Test of dataset combinability

Before combining the three datasets, tests of dataset combinability were conducted. First, the most parsimonious trees from each dataset were imported to MacClade ver. 4.06 (Maddison & Maddison, 2003) and all nodes, except the nodes with 70% or higher bootstrap support, were manually collapsed. These new trees were used as constraints in a different dataset (for example, parsimony analysis of *ATP6* dataset with nuc-LSU-rDNA tree as constraint), using the "Load Constraints" option in PAUP*. Parsimony analyses were conducted under these constraints, keeping only the trees that are compatible with these constraints. A total of six constraint parsimony analyses were conducted for all pairwise gene comparisons.

Comparisons of constraint and unconstraint trees were made using the "Tree Scores" option in PAUP*. 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 (SH-test; Shimodaira & Hasegawa, 1999), using RELL optimization with 1000 bootstrap replicates. Significance of results was determined by a *p*-value less than 0.05. When significance was observed, the constraint test described above was repeated, but keeping only one node each time, until the nodes of significant conflict were determined. After the nodes of conflict were determined, taxa causing the conflict were deleted, and the same constraint analyses were repeated until no conflict was observed. After testing for combinability, individual gene datasets were combined and phylogenetic analyses were conducted as described above. Analyses were performed for all 3 combinations of the two-gene dataset, and one with the combined dataset for all three genes.

Test of alternative topologies

For the combined dataset, constraint analyses of alternative topologies were conducted. First, the most parsimonious trees were imported in MacClade ver. 4.06 (Maddison & Maddison, 2003), and all nodes, except nodes supporting four major clades (node D, E, F, and G of Fig. 2.5) were collapsed manually. Three unrooted

networks without an outgroup (61 taxa) and 15 rooted trees (65 taxa) were made by manually swapping the branches, and used for the constraint parsimony analyses. These new trees were used as the constraints in a combined dataset, and comparisons were made between the most parsimonious trees and the constraint trees using SH-test and Templeton test as described above.

Ancestral character state reconstructions

Ancestral character state reconstructions under the parsimony criterion were performed for the spore discharge mechanism (statismospory or ballistospory) using MacClade. Character coding was based on the literature. All characters were coded in binary form. Character states on all nodes shown in Fig. 2.5 were reconstructed for the most parsimonious trees derived from unconstrained analyses. The same analyses were also performed on 15 alternative topologies shown in Fig. 2.4. Analyses were conducted using a range of gain: loss cost ratios. These reconstructions were made either with or without an outgroup. Because the outgroup taxa in this study were chosen simply to root the tree, the reconstructions were conducted by artificially using a different character state for the outgroup taxa to test if different coding scheme for the outgroup taxa affect the overall character state reconstructions. For example, one analysis was conducted with all outgroups as statismosporic, and the other with all outgroups as ballistosporic. Likelihood-based reconstructions of ancestral character

state were also performed using Bayesian-Multistate version 1.1 (Buschbom *et al.*, 2003). First, every 100th trees from the initial Bayesian analyses were sub-sampled for Bayesian-Multistate analyses, resulting in a total of 1950 trees. Character state of each node was reconstructed using the "Node reconstruction, local" option. The scale parameter was fixed to 1 (no scaling) for all trees. Character states were reconstructed for all 11 nodes (Fig. 2.5) present in the majority rule consensus obtained from Bayesian analyses. The likelihood of each state on a particular node was averaged, and the significance of the difference in likelihood was determined by difference in 2 or more of -ln likelihood of each state, following Pagel (1999). These reconstructions were made either with or without outgroups, and also with different coding schemes for the outgroup taxa as described above.

RESULTS

Important notes

For the rest of this paper, all clade names shown in Fig. 2.2 are capitalized to distinguish them from the actual taxon names or traditional taxonomic ranks. For example, HYSTERANGIUM corresponds to the "Hysterangium" clade in Fig. 2.2 whereas *Hysterangium* indicates the genus name. Also MESOPHELLIACEAE indicates the clade name, but Mesophelliaceae indicates the family name.

Characteristics of three target loci

PCR amplifications resulted in ca. 700 bp for nuc-LSU-rDNA and *ATP6*. The length of amplified products for mt-SSU-rDNA varied, ranging from ca. 500 bp to over 2000 bp. The length variations were due to the presence/absence of the hypervariable regions 2, 4, and 6, as designated by Hibbett & Donoghue (1995). Because these hypervariable regions were not alignable across taxa, they were excluded from the phylogenetic analyses. The final alignment after exclusion of ambiguously aligned regions was 1632 bp, including 539 bp of nuc-LSU rDNA, 439 bp of mt-SSU rDNA, and 654 bp of *ATP6*. The numbers of parsimony informative characters for 65 taxa (without problematic taxa; discussed below) were 168 for nuc-LSU-rDNA, 180 for mt-SSU-rDNA, and 386 for *ATP6* dataset.

Test of dataset combinability

When all 68 taxa were included, the SH-test detected significant levels of conflict between the nuc-LSU-rDNA and mt-SSU-rDNA datasets ($P = <0.05$, Fig. 2.1a). When topologies based on either nuc-LSU-rDNA or mt-SSU-rDNA were used as constraints for the *ATP6* dataset, SH-tests were not significant ($P = >0.09$, Fig. 2.1a), suggesting that those datasets are combinable. However, *ATP6* topologies used for constraint tests on either nuc-LSU-rDNA or mt-SSU-rDNA datasets resulted in a

significant measure of conflict (P = <0.05, Fig. 2.1a). After examining the individual and constraint analyses, three taxa, *Austrogautieria chlorospora* OSC46596, *Austrogautieria manjimupana* OSC59545, *and Protubera borealis* OKM21898, proved to be problematic (Fig. 2.1b). After deleting the three problematic taxa, the SH-test showed high *p*-value for at least one direction for any combinations although one direction (forcing *ATP6* topology to the other datasets) still received small *p*-values (P = <0.05). Therefore, we combined the three datasets for subsequent analyses without the three problematic taxa.

Phylogeny of the gomphoid-phalloid fungi

The analyses based on a combined dataset of three genes suggested four well-supported clades, HYSTERANGIALES, GOMPHALES, PHALLALES, and GEASTRALES, within the gomphoid-phalloid clade. All of the 4 major clades were supported by 100% posterior probability in Bayesian analyses regardless of inclusion or deletion of the three problematic taxa (Fig. 2.2, 2.3, Table 2.2). Parsimony analyses also showed high bootstrap support (>70%) for all major clades, except the GEASTRALES clade.

When all 68 taxa (including the three problematic taxa based on the combinability test) were included, parsimony analyses showed the sister relationship of the HYSTERANGIALES and GOMPHALES clades with moderate bootstrap

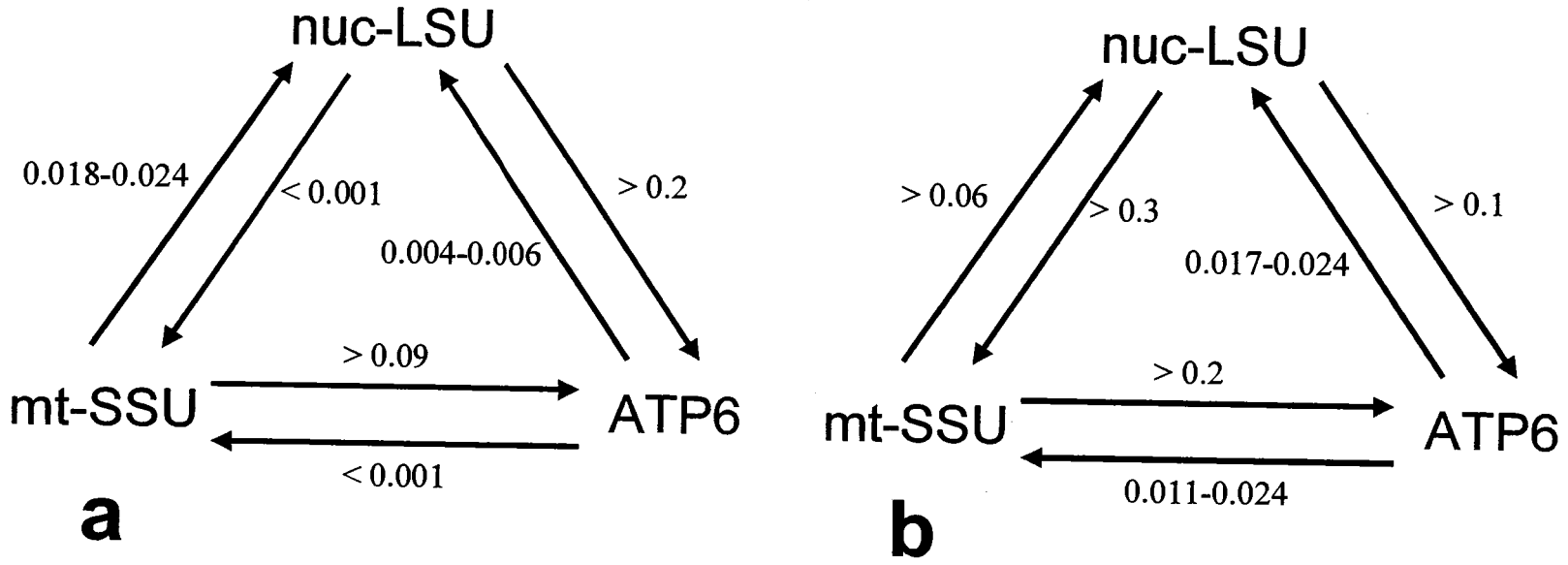

Fig. 2.1. The results from the test of dataset combinability. The numbers on arrows indicate the *p*-value based on the SH-test. nuc-LSU = nuclear large subunit of ribosomal DNA; mt-SSU = mitochondrial small subunit of ribosomal DNA; ATP6 = mitochondrial atp6 gene dataset. a) The results of the SH-test using all (68) taxa; b) 65 taxa (without the 3 problematic taxa). Arrows indicate the direction of constraint; for example, mt-SSU → ATP6 reads "when tree topologies based on the mt-SSU dataset are used as constraint in the ATP6 dataset".

support (Fig. 2.2, 2.3, Table 2.2). Bayesian analysis produced a different topology, showing a monophyly of HYSTERANGIALES + PHALLALES + GEASTRALES, but this was not supported by posterior probability (Fig. 2.3, Table 2.2). When the three problematic taxa were deleted from the analyses, both parsimony and Bayesian analyses showed an identical topology for the higher-level phylogeny (Fig. 2.3). The deep nodes (node B and C; Fig. 2.5) were well supported in parsimony analysis whereas they were only poorly supported in Bayesian analysis (Fig. 2.3, Table 2.3). Because the three problematic taxa based on the SH-test could affect the overall topologies (Fig. 2.3), subsequent analyses of topology constraint (Fig. 2.4) and the ancestral character state reconstructions (Fig. 2.5, Table 2.3, 2.4, 2.5) were all based on 65 taxa (excluding the three problematic taxa).

Within the HYSTERANGIALES clade, the HYSTERANGIUM and MESOPHELLIACEAE clades were shown to be sister clades with a good bootstrap value and posterior probability (Fig. 2.2). The genus *Austrogautieria* is supported as a member of the HYSTERANGIALES, and it is only distantly related to the genus *Gautieria*, which belongs to the GOMPHALES (Fig. 2.2). The genus *Protubera* was polyphyletic, being placed in the PHALLALES, TRAPPEA, and AUSTROGAUTIERIA clades (Fig. 2.2). Within the GEASTRALES clade, the position of *Radiigera taylorii* differed in Bayesian and parsimony analyses. Bayesian analyses showed the sister relationship of *R. taylorii* and *Geastrum recolligens* (Fig. 2.5d), but *G. recolligens* and *G. floriforme* were sister species in parsimony analysis (Fig. 2.5e). Within the GOMPHALES clade, the position of *Ramaria stricta* differed

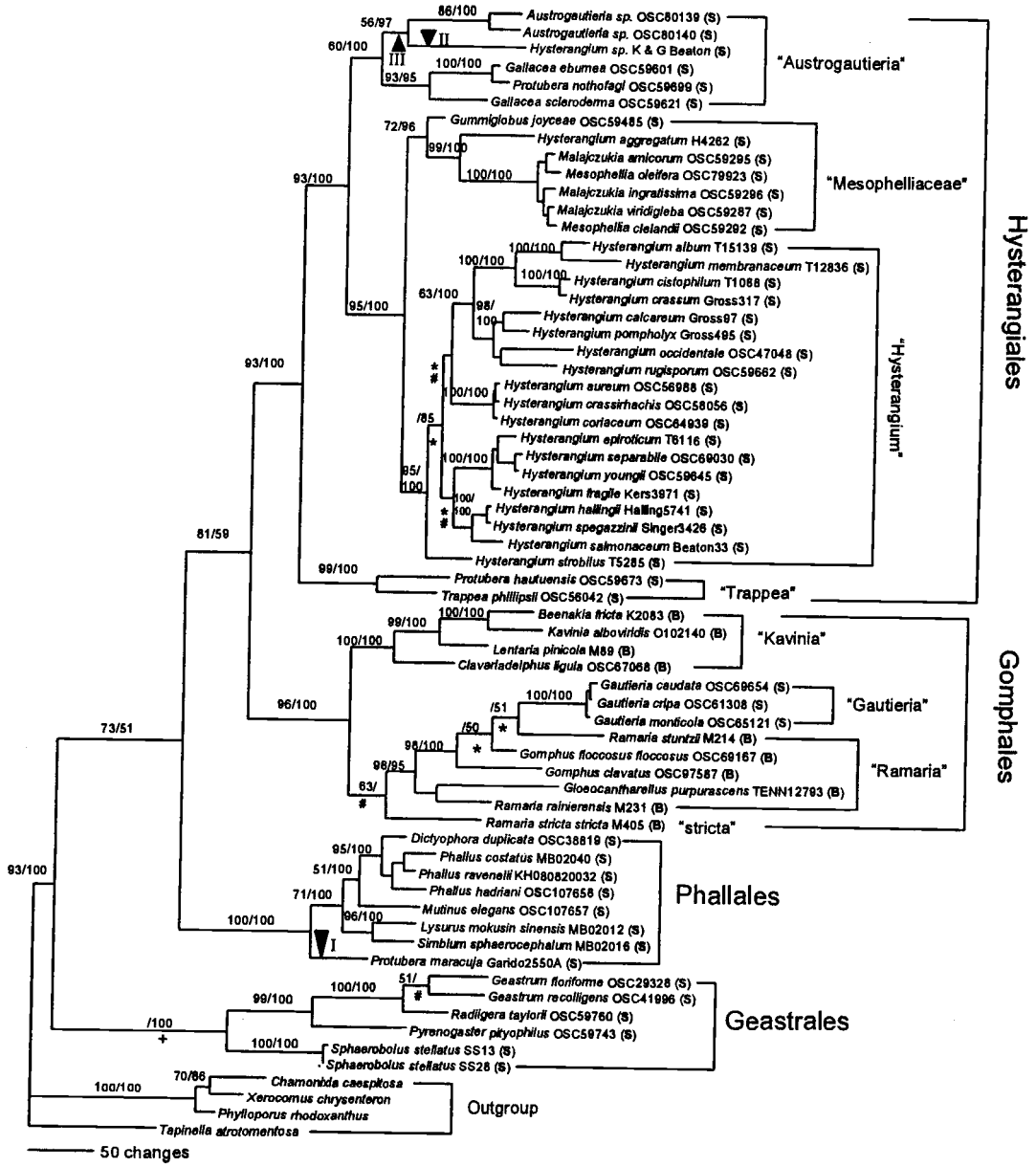

Fig. 2.2. One of the most parsimonious trees based on the combined dataset of all three genes with 65 taxa (excluding the three problematic taxa). Taxon names are followed by spore discharge mechanism in parentheses: S = statismospory; B = ballistospory. The numbers on branches indicate the parsimony bootstrap value/ Bayesian posterior probabilities. Posterior probabilities are based on 50% majority consensus from 2 million generations of MCMCMC. The symbols below branches: + = nodes present in strict consensus without bootstrap support; * = nodes collapsed in strict consensus; # = nodes not present in 50% majority consensus of Bayesian trees. Black triangles indicate the position of the three problematic taxa: I = *Protubera borealis*; II = *Austrogautieria chlorospora*; III = *Austrogautieria manjimupana*.

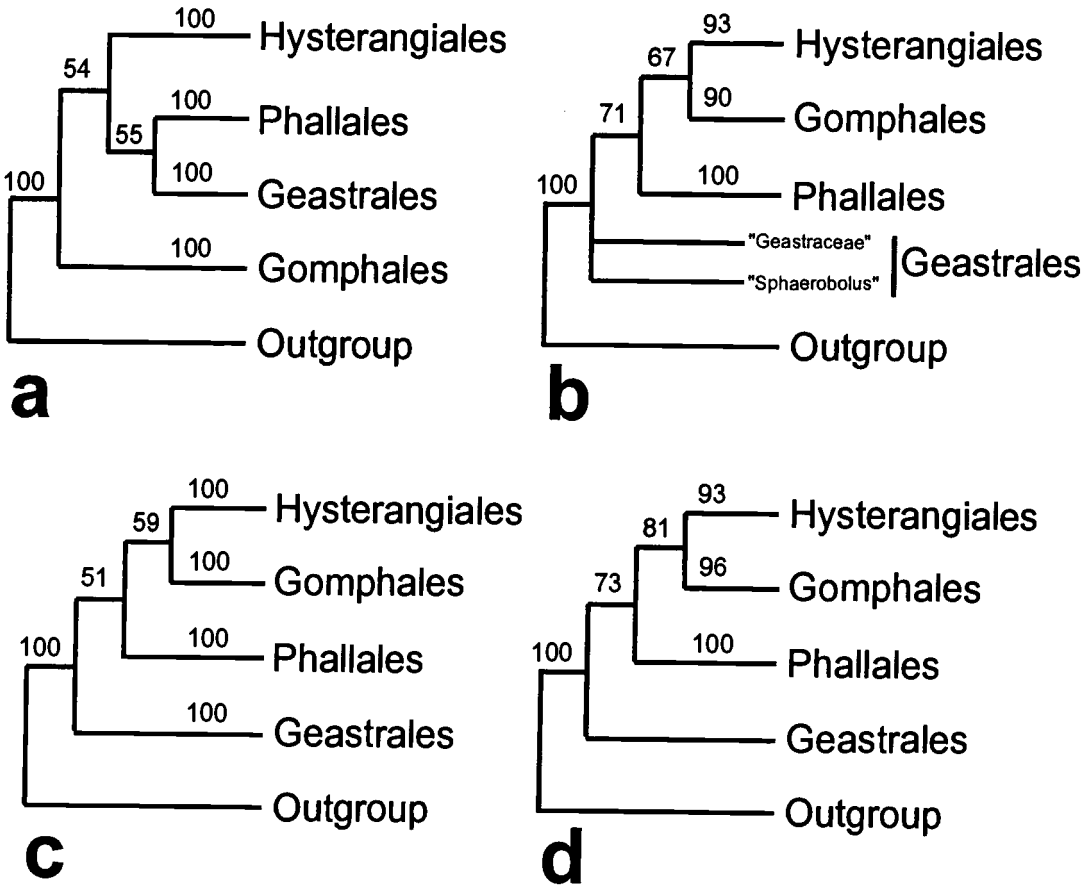

Fig. 2.3. The results of the phylogenetic analyses based on the combined dataset of all three genes. Only the interrelationships of the four major clades are shown. The names of clades correspond to those of Fig. 2.2. The numbers on branches indicate either parsimony bootstrap values or Bayesian posterior probabilities. a) Bayesian analysis using 68 taxa; b) Parsimony analysis using 68 taxa; c) Bayesian analysis using 65 taxa (without the three problematic taxa); d) Parsimony analysis using 65 taxa.

in Bayesian and parsimony analyses. Bayesian analysis showed a basal split of *R. stricta* and the rest of the GOMPHALES (Fig. 2.5b), but parsimony analysis showed that *R. stricta* was nested within the GOMPHALES clade (Fig. 2.5c).

Nodal support with individual and various combinations of datasets

In parsimony analyses, neither nuc-LSU-rDNA nor mt-SSU-rDNA dataset were able to resolve the relationships within the gomphoid-phalloid clade (Table 2.2). The only clade supported by nuc-LSU-rDNA dataset was the PHALLALES clade, while the HYSTERANGIALES and GOMPHALES clades and node C were never strongly supported regardless of inclusion or exclusion of the problematic taxa (Table 2.2). Although it was not supported by bootstrap value, the PHALLALES clade formed a monophyletic group with some taxa of the MESPHELLIACEAE clade and was nested within the HYSTERANGIALES clade, making HYSTERANGIALES paraphyletic. Similarly mt-SSU-rDNA dataset resolved only the GEASTRALES and/or GOMPHALES clade with high bootstrap value (Table 2.2). Most of the higher bootstrap values were obtained by the *ATP6* dataset. The *ATP6* dataset alone recovered most of the major clades, which were also recovered by the combined dataset, except for the GEASTRALES clade (Table 2.2). When datasets were combined, either more clades were resolved or higher bootstrap supports were obtained in most cases (Table 2.2). There were some examples with opposite results.

Table 2.2. Nodal support based on the individual and various combinations of datasets. The numbers indicate either parsimony bootstrap values or Bayesian posterior probabilities. The names of clades correspond to those of Fig. 2.2. The letters (B, C, D, E, F, and G) indicate the nodes designated in Fig. 2.5. lsu/L = nuclear large subunit of ribosomal DNA; ssu/S = mitochondrial small subunit of ribosomal DNA; atp/A = atp6 dataset. 68UW = unweighted parsimony analysis using 68 taxa; 68-6p = 6-parameter weighted parsimony using 68 taxa. 65UW and 65-6p also indicate unweighted and 6-parameter parsimony, respectively, using 65 taxa (without the 3 problematic taxa). Bayes indicate Bayesian analysis. X = clade/node not present; ! = node present at least in one of the most parsimonious trees; + = node present in a strict consensus, but without bootstrap support.

[68UW]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	x	88	x	93	94	93
Phallales (F)	99	x	96	99	100	100	100
Gomphales (G)	x	x	79	60	83	81	90
C	!	68	x	!	x	x	!
B	x	x	65	x	55	65	67
A	+	x	58	+	68	64	71
"Austrogautieria"	x	x	94	x	66	74	!
"Mesophelliaceae"	x	x	89	x	100	+	73
"Hysterangium"	x	x	93	+	94	94	96

[68-6p]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	x	97	x	89	92	98
Phallales (F)	96	x	98	98	100	100	100
Gomphales (G)	x	x	80	65	85	79	85
C	+	64	x	56	x	x	x
B	x	x	60	x	69	59	64
A	x	x	62	59	79	66	80
"Austrogautieria"	x	x	90	x	77	74	!
"Mesophelliaceae"	x	x	82	x	100	x	72
"Hysterangium"	x	x	95	+	95	96	96

Table 2.2. (Continued).

[65UW]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	+	90	+	93	94	93
Phallales (F)	99	x	93	100	100	95	100
Gomphales (G)	x	56	84	53	87	89	96
C	!	+	x	54	x	50	+
B	x	x	73	+	76	76	81
A	+	x	56	x	66	63	73
"Austrogautieria"	x	x	89	x	66	86	60
"Mesophelliaceae"	x	x	92	x	98	!	72
"Hysterangium"	x	x	91	+	94	99	95

[65-6p]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	+	99	+	89	94	97
Phallales (F)	99	+	99	100	100	96	100
Gomphales (G)	x	+	86	71	90	76	91
C	+	70	X	(55)	x	(52)	+
B	x	x	77	x	73	71	74
A	x	x	62	+	72	59	66
"Austrogautieria"	x	x	88	x	69	85	+
"Mesophelliaceae"	x	x	83	x	97	x	73
"Hysterangium"	x	x	90	+	92	98	99

[68-Bayes]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	x	92	100	100	100	100
Phallales (F)	83	x	100	100	100	100	100
Gomphales (G)	61	x	98	99	100	100	100
C	x	92	100	86	99	100	100
B	x	x	53	x	92	x	x
A	77	x	x	84	47	x	x
"Austrogautieria"	x	x	100	x	100	99	99
"Mesophelliaceae"	x	x	74	x	99	x	93
"Hysterangium"	x	x	100	x	100	100	100

Table 2.2. (Continued).

[65-Bayes]

clade	lsu	ssu	atp	L+S	L+A	S+A	L+S+A
Hysterangiales (D)	x	95	87	99	100	100	100
Phallales (F)	93	53	100	100	100	100	100
Gomphales (G)	69	48	99	99	100	100	100
C	x	96	100	90	99	100	100
B	x	x	57	x	86	x	59
A	63	x	x	89	42	x	51
"Austrogautieria"	x	x	100	x	100	100	100
"Mesophelliaceae"	x	x	79	69	99	x	96
"Hysterangium"	x	x	100	40	100	100	100

For example, the AUSTROGAUTIERIA and MESOPHELLIACEAE clades received a high bootstrap value in the *ATP6* dataset, but when datasets were combined, the bootstrap value became significantly lower. This result was in contrast to the Bayesian analyses, which showed higher posterior probabilities for most clades, including the AUSTROGAUTIERIA and MESOPHELLIACEAE clades, when datasets were combined.

Test of alternative topologies

To test if alternative topologies are significantly worse explanations, the SH-test was applied. Three unrooted networks had a very small difference in tree lengths, ranging from 3358 (the most parsimonious tree) to 3362, and none of them showed a significant *p*-value (Fig. 2.4). Therefore, all of 15 possible rooted trees, 5 for each of three unrooted networks, were tested for significance. Tree length varied from 3905 (the most parsimonious tree = I-1) to 3928 (II-4) (Fig. 2.4). Six of 15 topologies showed marginal *p*-value, but none of them showed *p*-value unambiguously smaller than 0.05.

Fig. 2.4. The results from the tests of alternative tree topologies. Taxon names are abbreviated based on the clade names shown in Fig. 2.2: H = Hysterangiales; P = Phallales; Go = Gomphales; Ge = Geastrales; OG = outgroup. TL = tree length; p = *p*-value based on the SH-test. Arrows and numbers on unrooted networks indicate the position for rooting. *1 = $p < 0.05$ when the Templeton test was applied; *2 = $p > 0.05$ when the Templeton test was applied.

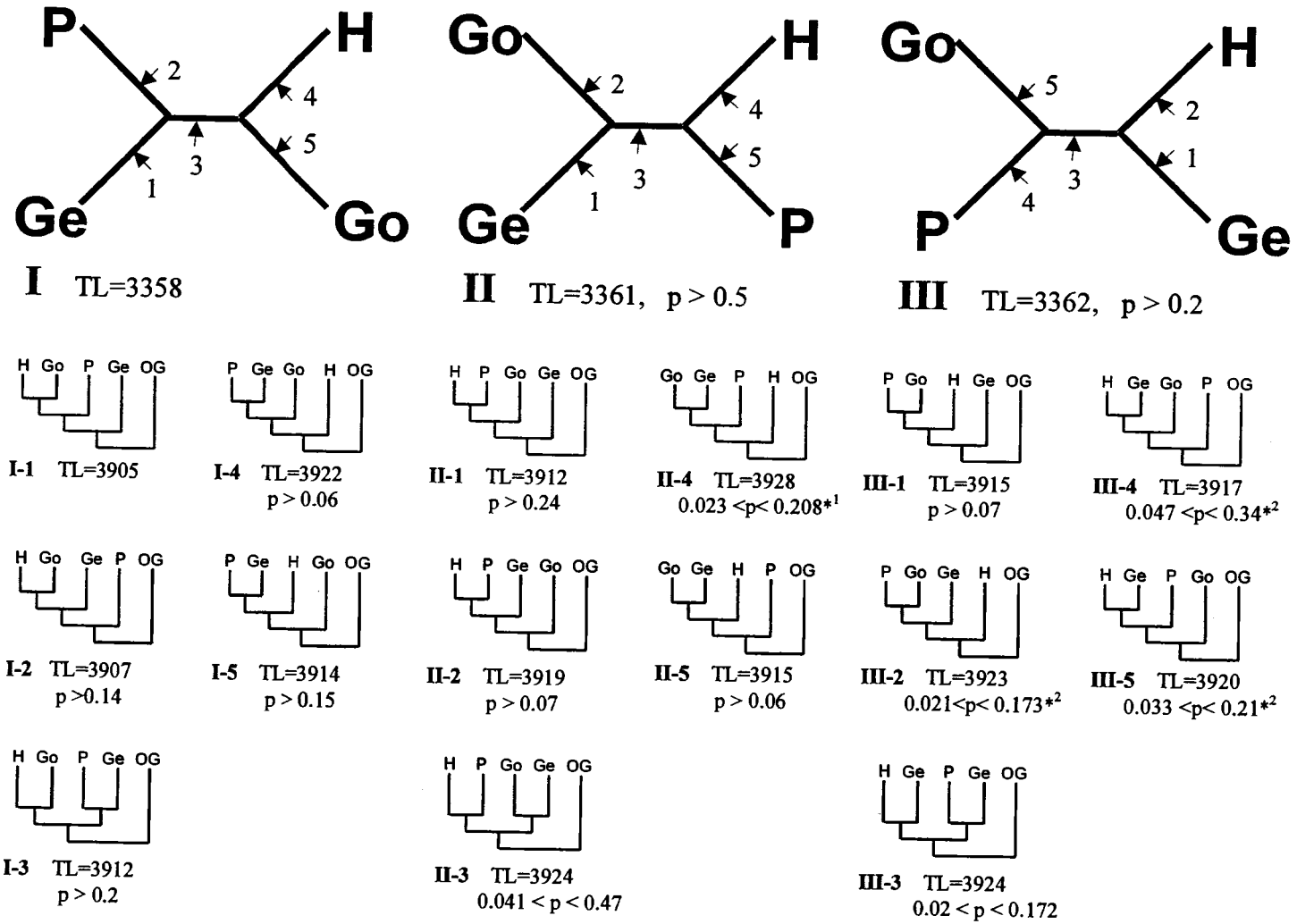

Fig. 2.4. The results from the tests of alternative tree topologies.

Ancestral character state reconstructions

Under parsimony criterion using the most parsimonious trees with equal or higher weight for loss of ballistospory, three deep nodes (node A, B, and C in Fig. 2.5) were unambiguously reconstructed as statismospory (Table 2.3). The character states of those three nodes changed with higher weight for gain of ballistospory and/or different state for outgroup (Table 2.3). Reconstructions for the other nodes were consistent throughout the different gain: loss cost ratios. Under the Bayesian-Multistate analysis, character states for nodes A, B, and C could not be determined. The results did not change with different treatments for outgroup. Character states for the other nodes were consistent with parsimony based reconstructions. This analysis also showed that the average rate of losses of ballistospory is 4.7 (± 0.97) times higher than gains (mean rate of gain = 0.70 ± 0.44; mean rate of loss = 2.96 ± 1.38).

Because most of the 15 alternative topologies were not significantly worse explanations than the most parsimonious trees, parsimony based character state reconstructions for all of these topologies were also conducted. Like reconstructions using the most parsimonious trees, the character state of node K was always unambiguously statismosporic. Because all of the other nodes within the GOMPHALES clade were ballistosporic, at least one unambiguous loss of ballistospory was inferred. The GOMPHALES clade is the only one with ballistosporic taxa, and node G of this clade was unambiguously ballistosporic in all reconstructions, so a question is whether the ballistospory of node G is independently

gained for the GOMPHALES, or simply a plesiomorphy of the entire gomphoid-phalloid clade.

Most reconstructions with equal weight for the gain: loss cost resulted in the independent gain of ballistospory for the gomphoid-phalloid clade. Six topologies (I-3, 4, II-1, 3, III-3, 4 in Fig. 2.4) showed ambiguous reconstructions, but results changed to the independent gain of ballistospory when the outgroup was excluded (Table 2.4). Three topologies (I-5, II-2, III-5) resulted in the loss of ballistospory as the most parsimonious evolutionary scenario (Table 2.4). The reconstruction became ambiguous when no outgroup was used. When higher weights for gain of ballistospory were applied, more topologies favored the loss of ballistospory as the preferred reconstruction, but results changed with different treatment of outgroup. In summary, inferred ancestral character state varied across different topologies and different gain: loss cost ratios.

DISCUSSION

Outgroup

There are several phylogenetic studies based on DNA sequence data of the Homobasidiomycetes (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Hibbett *et al.*, 1997; Hibbett & Thorn, 2001). They show that the gomphoid-phalloid clade could be

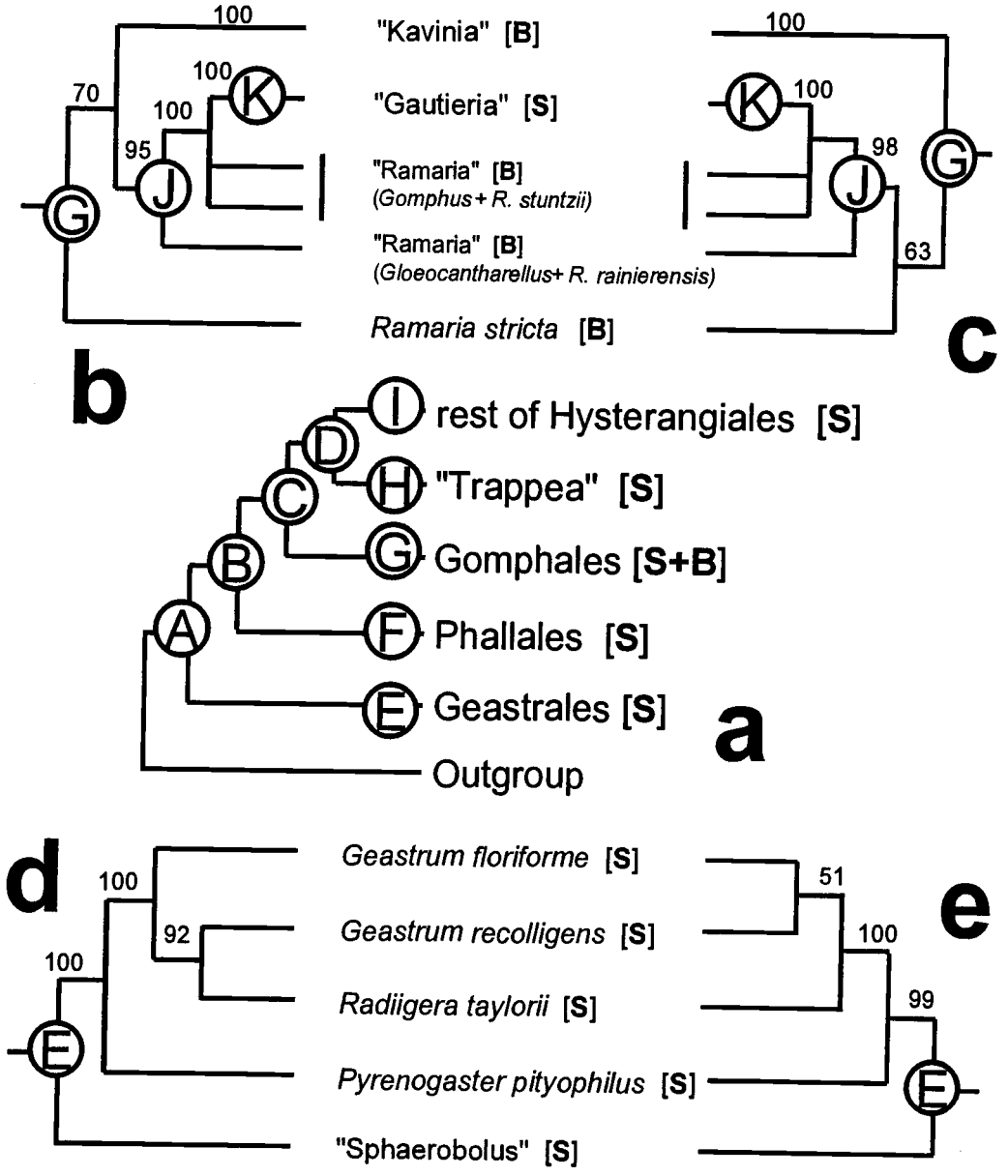

Fig. 2.5. Nodes used for the ancestral character state reconstructions of the spore discharge mechanism (A~K). a) Higher-level phylogeny of the gomphoid-phalloid clade; b) Phylogeny within the Gomphales clade based on Bayesian analysis; c) Phylogeny within the Gomphales clade based on parsimony analysis; d) Phylogeny within the Geastrales clade based on Bayesian analysis; e) Phylogeny within the Geastrales clade based on parsimony analysis. The names of taxa and clades correspond to those of Fig. 2.2. The numbers on branches indicate either parsimony bootstrap values or Bayesian posterior probabilities. The letters after the taxon/clade names indicate the character state of the spore discharge mechanism: S = statismospory; B = ballistospory.

Table 2.3. The results of the ancestral character state reconstructions of the spore discharge mechanism. Node names are based on the Fig. 2.5. BM = results from Bayesian-Multistate analyses; MacClade = results from parsimony-based reconstructions using MacClade with various gain: loss cost ratios. Character state of the spore discharge mechanism: S = statismospory; B = ballistospory. Two character states on single node indicate that the reconstructions were equivocal. Results are based on the outgroup coded as ballistospory. *1 = unambiguously S (statismospory) when outgroup was coded as statismospory or no outgroup was used; *2 = unambiguously S when outgroup was coded as statismospory, but ambiguous when no outgroup was used.

		MacClade				
		Gain (S→B) : Loss (B→S) cost ratios				
node	**BM**	**1:1**	**1:2**	**1:3**	**2:1**	**3:1**
A	S/B	S	S	S	S/B *1	B *2
B	S/B	S	S	S	S/B *1	B *2
C	S/B	S	S	S	S/B *1	B *2
D	S	S	S	S	S	S
E	S	S	S	S	S	S
F	S	S	S	S	S	S
G	B	B	B	B	B	B
H	S	S	S	S	S	S
I	S	S	S	S	S	S
J	B	B	B	B	B	B
K	S	S	S	S	S	S

Table 2.4. The results of ancestral character state reconstructions of the spore discharge mechanism using 15 alternative topologies in Fig. 2.4. BM = results from Bayesian-Multistate analyses; MacClade = results from parsimony-based reconstructions using MacClade with various gain: loss cost ratios. OG=B indicates that the outgroup was coded as ballistospory; OG=S indicates the outgroup was coded as statismospory; no OG indicates no outgroup was used. All reconstructions unambiguously showed the loss of ballistospory at the "*Gautieria*" clade (or node K in Fig. 2.5). G = independent gains of ballistospory without subsequent losses (except for the *Gautieria* clade); L = losses of ballistospory without independent gains; E = equivocal (or ambiguous) reconstructions.

[OG =B]

		MacClade				
		Gain (S→B) : Loss (B→S) cost ratios				
topology	**BM**	**1:1**	**1:2**	**1:3**	**2:1**	**3:1**
I-1	E	G	G	G	E	L
I-2	/	G	G	G	E	L
I-3	/	E	G	G	L	L
I-4	/	E	G	G	L	L
I-5	/	L	E	G	L	L
II-1	/	E	G	G	L	L
II-2	/	L	E	G	L	L
II-3	/	E	G	G	L	L
II-4	/	G	G	G	E	L
II-5	/	G	G	G	E	L
III-1	/	G	G	G	E	L
III-2	/	G	G	G	E	L
III-3	/	E	G	G	L	L
III-4	/	E	G	G	L	L
III-5	/	L	E	G	L	L

Table 2.4. (Continued).

[OG = S]

		MacClade Gain (S→B) : Loss (B→S) cost ratios				
topology	**BM**	**1:1**	**1:2**	**1:3**	**2:1**	**3:1**
I-1	E	G	G	G	G	G
I-2	/	G	G	G	G	G
I-3	/	G	G	G	G	E
I-4	/	G	G	G	G	E
I-5	/	G	G	G	E	L
II-1	/	G	G	G	G	E
II-2	/	G	G	G	E	L
II-3	/	G	G	G	G	E
II-4	/	G	G	G	G	G
II-5	/	G	G	G	G	G
III-1	/	G	G	G	G	G
III-2	/	G	G	G	G	G
III-3	/	G	G	G	G	E
III-4	/	G	G	G	G	E
III-5	/	G	G	G	E	L

[no OG]

		MacClade Gain (S→B) : Loss (B→S) cost ratios				
topology	**BM**	**1:1**	**1:2**	**1:3**	**2:1**	**3:1**
I-1	E	G	G	G	G	E
I-2	/	G	G	G	G	E
I-3	/	G	G	G	E	L
I-4	/	G	G	G	E	L
I-5	/	E	G	G	L	L
II-1	/	G	G	G	E	L
II-2	/	E	G	G	L	L
II-3	/	G	G	G	E	L
II-4	/	G	G	G	G	E
II-5	/	G	G	G	G	E
III-1	/	G	G	G	G	E
III-2	/	G	G	G	G	E
III-3	/	G	G	G	E	L
III-4	/	G	G	G	E	L
III-5	/	E	G	G	L	L

one of the most basal clades within the Homobasidiomycetes, along with the cantharelloid-, thelephoroid, and hymenochaetoid clades, but deep nodes tend to be only poorly supported. In this study, we selected members of the bolete clade for the outgroup because all 3-gene sequences were available. This may not be the best choice, but using other outgroups (with missing data) did not change the overall topology of phylogeny of the gomphoid-phalloid fungi (data not shown).

Phylogeny of the gomphoid-phalloid clade

Hibbett *et al.* (1997) first demonstrated the existence of the gomphoid-phalloid clade based on nuclear and mitochondrial ribosomal DNA. This relationship has been repeatedly supported by subsequent studies with different genes and/or different taxa. However, most studies used only a few taxa for this clade, so detailed relationships within the gomphoid-phalloid clade remain uncertain (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Hibbett *et al.*, 1997; Hibbett & Thorn, 2001; Moncalvo *et al.*, 2002; Humpert *et al.*, 2001; Pine *et al.*, 1999). The most extensive taxon sampling in this clade was made by Humpert *et al.* (2001), focusing on the genus *Ramaria sensu lato*. They demonstrated that *Gautieria* was nested within the *Ramaria s.l.*, which is also strongly supported in this study. Humpert *et al.* (2001) also sampled two species of *Hysterangium* and stinkhorns, but the position of *Hysterangium* and stinkhorn taxa within the Gomphales was not supported by bootstrap values, and the results remained

inconclusive. In this study, with more taxa from the Hysterangiales, Phallales, and Geastrales, and a complete dataset of three genes, we demonstrate that there are distinct clades of the HYSTERANGIALES, GOMPHALES, PHALLALES, and GEASTRALES, each supported by high Bayesian posterior probability and/or bootstrap values. The HYSTERANGIALES clade is particularly interesting, because it is exclusively composed of truffle-like taxa. The truffle-like fruiting body morphology is often considered as derived from more complex morphologies, such as the agaric form, and in some groups, it is shown that truffle-like morphologies are derived independently multiple times (Miller *et al.*, 2001; Peintner *et al.*, 2001).

The relationships within the gomphoid-phalloid clade, however, remain inconclusive. After deleting the three problematic taxa, both parsimony and Bayesian analyses showed the same topology, which supported the sister relationships of the HYSTERANGIALES and GOMPHALES. The recognition of the Hysterangiales as a distinct taxon from the Phallales is consistent with the classifications of Zeller (1939) and Jülich (1981), which treated the order Hysterangiales as distinct from the Phallales. The alternative topologies, however, could not be rejected statistically. There are some trends in *p*-value, tree lengths, and the tree topologies. For example, all three topologies showing the sister relationship of the HYSTERANGIALES and GEASTRALES (Fig. 2.4, III-3, 4, 5), a topology never inferred from any parsimony or Bayesian analyses, had marginal *p*-values, making it the least probable. Other topologies of emphasis are I-5, II-2, and III-5 because they show the basal split of the GOMPHALES and the rest of the gomphoid-phalloid fungi, which favors the loss of

ballistospory as indicated in the discussion of ancestral character state reconstructions. Although topology III-5 showed a marginal *p*-value, the other two topologies showed no significance. Although it was only poorly supported, the Bayesian analyses showed the topology I-5 when three problematic taxa were included. These results clearly indicate that the possibility of alternative higher topologies for the gomphoid-phalloid fungi should not be discarded.

Several novel phylogenetic relationships were revealed by this study. First, the results show that members of the family Mesophelliaceae formed a sister clade to *Hysterangium*. Unlike the fruiting bodies of the genus *Hysterangium*, which has a gelatinous tissue in a gleba with a dendroid columella (Castellano & Beever, 1994; Zeller, 1949), Mesophelliaceae have a powdery gleba with a central sterile core (Beaton & Weste, 1983, 1984; Trappe *et al.*, 1992, 1996a, b). Because the genus *Radiigera* also has a powdery gleba with similar glebal arrangement, it has been often hypothesized that Mesophelliaceae and *Radiigera*, as well as the other members of the family Geastraceae, were closely related (Singer *et al.*, 1963; Zeller, 1944, 1949). Some authors suggested that the Mesophelliaceae and Geastraceae are not closely related (Askew & Miller, 1977; Sunhede, 1989; Jülich, 1981; Trappe *et al.*, 1996b), but the relationship of Mesophelliaceae to the other Homobasidiomycetes remained uncertain. This is the first study to show that the Mesophelliaceae are more closely related to *Hysterangium*. Besides a truffle-like fruiting body morphology, shared characters between the Mesophelliaceae and *Hysterangium* include elongated spores with a utricle, ectomycorrhizal habit, and formation of a dense mycelial mat (Beaton

& Weste, 1983, 1984; Trappe *et al.*, 1992, 1996a, b).

Second, the genus *Austrogautieria*, which is traditionally classified as a member of the family Gautieriaceae (Beaton *et al.*, 1985; Stewart & Trappe, 1985), was shown to be a member of the Hysterangiales and only distantly related to *Gautieria*, which is a member of Gomphales. It is well documented that *Gautieria* and *Ramaria* (specifically, subgenus *Ramaria*) are closely related both morphologically and molecularly (Humpert *et al.*, 2001). One of the most obvious morphological similarities is spore ornamentation, having longitudinally striate spores. Within the Hysterangiales, *Austrogautieria* is the only taxon with longitudinally striate spores, so the similarity between *Gautieria* and *Austrogautieria* is an example of convergent evolution.

Third, the genus *Protubera* is resolved as polyphyletic. Microscopically all species of *Protubera* are similar, having small (mostly smaller than 6 μm), smooth, ellipsoid spores (Malloch, 1989; Beaton & Malajczuk, 1986; Castellano & Beever, 1994) that strikingly resemble those of stinkhorns. Macroscopically, however, fruiting body morphology of *Protubera* varies significantly. The most common type, including that of *Protubera maracuja* (type species), is characterized by a very thick gelatinous layer beneath the peridium (Malloch, 1989) similar to that of stinkhorns and consistent with its phylogenetic position. The other extreme is observed in *P. nothofagi* and *P. hautuensis*, which have a very thin gelatinous layer (Castellano & Beever, 1994). Ecologically, *P. nothofagi* forms fruiting bodies below-ground under ectomycorrhizal trees, whereas the other species of *Protubera* in this study usually grow above-ground

without ectomycorrhizal trees (Malloch, 1989; Castellano & Beever, 1994).

Nodal supports

It is interesting to note that the bootstrap supports for the nodes of the higher-level phylogeny (node B and C, Fig. 2.5) were at least 73%, which is usually considered strong support (Alfaro *et al.*, 2003; Hillis & Bull, 1993), whereas Bayesian posterior probability were only 59% or less. A number of studies showed that Bayesian posterior probability tends to be much higher than bootstrap value (Alfaro *et al.*, 2003; Douady *et al.*, 2003; Huelsenbeck *et al.*, 2002; Suzuki *et al.*, 2002), and the Bayesian approach is sometimes criticized as an overestimation (Suzuki *et al.*, 2002). It is well-known that Bayesian posterior probability and bootstrap values are not equivalent measures of confidence and cannot be directly compared (Alfaro *et al.*, 2003; Douady *et al.*, 2003; Huelsenbeck *et al.*, 2002; Huelsenbeck *et al.*, 2001), but most studies based on simulation data showed that Bayesian posterior probability and bootstrap values are well-correlated (Alfaro *et al.*, 2003; Douady *et al.*, 2003; Huelsenbeck *et al.*, 2002; Suzuki *et al.*, 2002). This might indicate the conservative nature of bootstrap values (Hillis & Bull, 1993; Suzuki *et al.*, 2002), and that posterior probability and bootstrap support could potentially be interpreted as upper and lower bound of node support (Douady *et al.*, 2003). This study along with the others based on real data (Miller *et al.*, 2002; Leache & Reeder, 2002), however, showed that there

are exceptions to this interpretation. Although many nodes with high posterior probability (defined as 95% or higher) also received strong bootstrap support (defined as 70% or more), some (such as node B and C) had strong bootstrap support but low posterior probability. Although it is usually a conservative measure of support, bootstrapping may overestimate or underestimate the phylogenetic accuracy (Alfaro *et al.*, 2003; Hillis & Bull, 1993). If this is the case, the bootstrap support of node B and C could be interpreted as an example of overestimation. Other reasons of low posterior probability include 1) the use of misfit models of evolution (Castoe *et al.*, 2004; Huelsenbeck *et al.*, 2002; Larget & Simon, 1999), 2) insufficient taxon and/or character sampling (Alfaro *et al.*, 2003; Simmons *et al.*, 2004), 3) rapid radiation among the major clades (Poe & Chubb, 2004; Binder & Hibbett, 2002), or 4) other analytical artifacts, including sequencing or alignment error and computer algorithms. Scenario 3 is unlikely because the internodes are not short (unlike the case of Poe & Chubb, 2004 or Binder & Hibbett, 2002), and the phylogenies resulting from each individual dataset were not incongruent (Poe & Chubb, 2004). Scenario 4 is equally unlikely because both parsimony and Bayesian analyses produced similar topology. Importantly nodes B and C are two of the basal nodes, to which the Bayesian approach tends to assign high (potentially positively misleading) support when character sampling is insufficient (Alfaro *et al.*, 2003). Because we believe our taxon sampling is sufficient to represent the phylogenetic diversity of the gomphoid-phalloid fungi, more character sampling would help resolve the higher relationships within the gomphoid-phalloid clade.

When the datasets are combined, support of each node is expected to be higher, because more phylogenetic signal with less noise becomes available (Baldauf *et al.*, 2000; de Queiroz *et al.*, 1995). This seems to be exactly the case for some clades, such as the GOMPHALES and HYSTERANGIALES clades (Table 2.2). However, some clades such as the AUSTROGAUTIERIA and MESOPHELLIACEAE clades, which received relatively high bootstrap support when the individual *ATP6* dataset was analyzed, showed much lower support when the datasets were combined (Table 2.2). This trend did not change before and after the exclusion of the three problematic taxa revealed by the SH-test. A similar trend was observed by Binder & Hibbett (2002) analyzing the Homobasidiomycetes ribosomal DNA sequences (including nuc-LSU-rDNA and mt-SSU-rDNA). Therefore it might indicate that there are real conflicts among fungal ribosomal gene phylogenies, which could not be detected by the SH-test. If this is the case, one of the likely reasons for conflict is the different stochastic processes between datasets (de Queiroz *et al.*, 1995), because there were strong heterogeneities of the branch length in the individual datasets. Two clades with relatively long branches, the AUSTROGAUTIERIA and MESOPHELLIACEAE clades, received higher Bayesian posterior probability when the datasets were combined, which could be explained by the relative robustness of the likelihood-based methods to the substitution rate heterogeneity compared to parsimony analyses (Huelsenbeck *et al.*, 2001; Huelsenbeck *et al.*, 2002). Although the real reason for the decrease of bootstrap support is unclear, the node supports for the AUSTROGAUTIERIA and MESOPHELLIACEAE clades were consistent with the

idea of overestimation by Bayesian posterior probability (Suzuki *et al.*, 2002) and/or conservative estimation by parsimony bootstrapping (Hillis & Bull, 1993; Suzuki *et al.*, 2002).

Dataset combinability

A number of different tests have been developed and used for comparing datasets, and different tests typically show different strengths and weaknesses (Buckley *et al.*, 2002; Cunningham, 1997; Farris *et al.*, 1995; Goldman *et al.*, 2000; Kishino & Hasegawa, 1989; Mason-Gamer & Kellogg, 1996; Shimodaira & Hasegawa, 1999; Templeton, 1983). In this study, we used the SH-test (Shimodaira & Hasegawa, 1999) and Templeton test (Templeton, 1983) for testing dataset combinability and alternative tree topologies, and both tests produced similar results. The tests showed that datasets are in conflict due to the three problematic taxa; when these taxa are excluded, the datasets seem to be combinable. However, the tests still showed a small *p*-value when the topology based on the *ATP6* dataset was used for constraint in the other two datasets. Because the other direction (nuc-LSU-rDNA or mt-SSU-rDNA topology as constraint in the *ATP6* dataset) did not show conflict, we considered this was not the evidence for significant conflict among datasets. This discrepancy is probably due to the heterogeneous distribution of parsimony informative characters among datasets. The *ATP6* dataset had more parsimony

informative characters than the other two datasets combined, and it resolved more nodes with higher bootstrap support.

In this study, strong support was defined as a bootstrap value of 70% or higher based on the empirical study of Hillis & Bull (1993). As discussed above, this value could be overestimated or underestimated depending on the data (Alfaro *et al.*, 2003; Hillis & Bull, 1993). It is therefore possible that some nodes with strong support in the *ATP6* dataset are simply overestimated and should not be used for the constraint topology. Using higher bootstrap values as a cutoff value would definitely change the results, but more fundamentally, the meanings of bootstrapping for testing the phylogenetic accuracy should be questioned (Alfaro *et al.*, 2003). Several tests using posterior probability under the Bayesian framework have been proposed (Buckley *et al.*, 2002; Goldman *et al.*, 2000). Comparisons of parsimony-based and Bayesian-based tests should be conducted using both simulated and empirical data.

Another potential problem is the use of a p-value = 0.05 as significance. Cunningham (1997), using the ILD test (Farris *et al.*, 1995), reported that combined data improved the phylogenetic accuracy when the p-value was larger than 0.01, but suffered from a decrease in accuracy when the p-value was less than 0.001. Although the results from different tests cannot be compared directly, it implies that the conventional cut-off value should not automatically be used as a sign of significance. In fact, our results showed that there were no conflicts among datasets if the p-value = 0.01 was used as significance (Fig. 2.1b). Nonetheless, it is still possible that there are real conflicts among datasets due to sampling error, different stochastic processes, or

different histories (de Queiroz *et al.*, 1995). For example, the fungal mitochondrial genes are shown to be strongly AT-biased, and the nucleotide substitution rate of mt-SSU-rDNA is significantly higher than that of nuclear counterpart (Bruns & Szaro, 1992). However, our data showed that the combined dataset produced higher support for most nodes. Therefore we consider conflicts in our dataset, if any, are negligible.

Ancestral character state reconstructions

Besides the gomphoid-phalloid clade, the cantharelloid, hymenochaetoid, and thelephoroid clades also tend to be the most basal clades of Homobasidiomycetes (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Hibbett & Thorn, 2001), and importantly, there are no known gastroid (hence statismosporic) taxa in those clades (Hibbett & Thorn, 2001). Also most members of the potential sister groups to the Homobasidiomycetes, the orders Auriculariales, Dacrymycetales, and Tremellales are ballistosporic (Wells & Bandoni, 2001; McLaughlin *et al.*, 1985; Webster & Chien, 1990). Furthermore, there are ballistosporic taxa in two other classes within the Basidiomycota, the Ustilaginomycetes and Urediniomycetes (Bauer *et al.*, 2001; McLaughlin *et al.*, 1985; Swan *et al.*, 2001). It is therefore reasonable to assume that ballistospory is the plesiomorphic character state for Homobasidiomycetes (McLaughlin *et al.*, 1985).

Within the gomphoid-phalloid clade, only the GOMPHALES clade contains

ballistosporic taxa. The ballistospory of the GOMPHALES was inferred to be derived independently from statismospory in the ancestral character state reconstructions under parsimony criterion with equal weights of gain: loss ratio (or higher weight for loss of ballistospory). However, this finding is contradictory to the general idea for the evolution of ballistospory. Many mycologists have hypothesized that the complex mechanism of ballistospory has been repeatedly lost (McLaughlin *et al.*, 1985; Thiers, 1984). The mechanism of typical ballistospory includes asymmetric shape and attachment of spores to the sterigma, and formation of a liquid droplet prior to spore discharge (Burk *et al.*, 1982, 1983; McLaughlin *et al.*, 1985; Money, 1998; Webster & Chien, 1990). Although spores are usually discharged for only a short distance (but still much longer than their own length), one estimate showed that discharged spores are subject to an acceleration of 25,000 *g* (Money, 1998). The exact mechanisms of ballistosporic discharge still remain unanswered (McLaughlin *et al.*, 1985; Webster & Chien, 1990), but it is hard to imagine that this complex system was derived multiple times independently in several different lineages of Basidiomycota.

The other related subject to the spore discharge mechanism is the evolution of the gastroid fruiting body. Some mycologists assumed that gastroid morphology was ancestral (Smith, 1971), but others have argued that gastroid morphology was derived from non-gastroid (such as agaricoid) forms (Heim, 1948, 1971; Thiers, 1984). It is now well-documented in some groups of Homobasidiomycetes that gastroid (hence statismosporic) taxa are nested within non-gastroid taxa, suggesting they have been derived from non-gastroid (hence ballistosporic) ancestors (Hibbett *et al.*, 1997; Miller

et al., 2001; Moncalvo *et al.*, 2002; Peintner *et al.*, 2001; Humpert *et al.*, 2001). Other studies indicated that apparently a huge morphological transformation from non-gastroid to gastroid fruiting bodies require relatively small genetic differentiation (Baura *et al.*, 1992; Bruns *et al.*, 1989; Hibbett *et al.*, 1994; Kretzer & Bruns, 1997), which implies a relative ease of loss of ballistospory. Some mushroom-forming fungi are known to produce polymorphic fruiting bodies, forming both agaricoid and secotioid ones. For example, the secotioid form of usually agaricoid taxon, *Lentinus tigrinus*, is caused by a recessive allele at a single locus (Hibbett *et al.*, 1994). Importantly, however, the secotioid form of *L. tigrinus* still retains ballistospory (Hibbett *et al.*, 1994). This is consistent with the hypothesis that transformations from agaricoid to secotioid form proceed with the loss of ballistospory (Thiers, 1984). As far as we know, no studies have shown the transformation from statismospory to ballistospory. All of the above evidence favors loss of ballistospory as the evolutionary scenario of the spore discharge mechanism, despite results from the parsimony reconstructions.

Although the ancestral character state reconstruction based on parsimony seems to favor an independent gain of ballistospory in the GOMPHALES clade, it was sensitive to the choice of the gain: loss cost ratios, the tree topology, and taxon sampling. The only node consistently supporting the loss of ballistospory across gain: loss cost ratios was in the GAUTIERIA clade. Although most alternative topologies produced similar results of ancestral character state reconstructions, topology I-5, II-2, and III-5 (Fig. 2.4) favored the loss of ballistospory. If one of these topologies

represented the true phylogeny of the gomphoid-phalloid fungi, the ancestral character state reconstruction would be less controversial, suggesting that the loss of ballistospory, instead of an independent gain of ballistospory, is the likely scenario in the gomphoid-phalloid clade. Those ambiguities are inevitable using the parsimony-based methods. It is well-known that parsimony-based character state reconstructions are sensitive to gain: loss cost ratios and tree topologies (Hibbett & Donoghue, 2001), and this study was consistent with that argument.

In contrast, likelihood-based reconstructions using Bayesian-Multistate did not favor the independent gain of ballistospory. The character state for three critical nodes (node A, B, C of Fig. 2.5) could not be assigned unambiguously. Despite these ambiguities, the results showed that the average rate of losses of ballistospory is 4.7 times higher than gains, and importantly, this ratio was not set *a priori* like parsimony-based method, but resulted from averaging of all reconstructions for each of the MCMCMC-sampled trees. There are several advantages for using Bayesian-Multistate reconstructions over the parsimony-methods. Because it is based on pools of the MCMCMC-sampled trees, the uncertainty associated with trees, such as branch length and tree topology, is statistically taken into account, and is not based on any particular gain: loss cost ratios (Lutzoni *et al.*, 2001, Hibbett & Donoghue, 2001). Therefore, we believe that the parsimony based reconstruction of an independent gain of ballistospory is not an accurate reconstruction for the evolution of spore discharge mechanism. Obviously, the topological uncertainty (Fig. 2.4) is one of the big caveats of our results, but another possible reason for the ambiguous reconstructions may be

insufficient taxon sampling. Although we are confident in sampling the representatives to cover the diversity of the gomphoid-phalloid fungi, its morphological and ecological diversity leaves the possibility that there are unsampled ballistosporic taxa within the gomphoid-phalloid clade, both extant and extinct. Importantly, simply adding a single hypothetical ballistosporic taxon to many positions of the parsimony tree changes the reconstruction dramatically. For example, adding such a hypothetical ballistosporic taxon to five different nodes makes the reconstructions of nodes A, B and C (Fig. 2.5) ambiguous when an equal weight of gain: loss ratio was applied (data not shown).

Judging from the above results, the character state reconstructions of the spore discharge mechanism in the gomphoid-phalloid clade still remain ambiguous. But we propose that multiple, parallel losses, instead of independent gains, of ballistospory in the gomphoid-phalloid clade is the preferred working hypothesis for evolution of the spore discharge mechanism in the gomphoid-phalloid clade.

CONCLUSIONS

Four major clades, including the GEASTRALES, GOMPHALES, HYSTERANGIALES, and PHALLALES, are resolved within the gomphoid-phalloid clade. Although interrelationships among those major clades remained unresolved, the sister relationship of the HYSTERANGIALES and GOMPHALES was suggested

both by parsimony and Bayesian analyses. This relationship is in contrast to the traditional classification, in which the HYSTERANGIALES and PHALLALES have been considered more closely related to each other. Because alternative topologies could not be rejected, more taxon and/or character sampling is necessary to clarify the higher-level phylogeny of the gomphoid-phalloid fungi. For the lower-level phylogeny, several previously unrecognized relationships have been discovered. This is the first study showing the polyphyly of the genus *Protubera*, a close relationship between *Hysterangium* and Mesophelliaceae, and the homoplastic origin of *Gautieria*-like spore morphology.

The ancestral character state of the spore discharge mechanism was reconstructed using both parsimony and likelihood methods. The results of parsimony-based reconstructions varied across different tree topologies and gain: loss cost ratios. Bayesian-Multistate analyses showed ambiguous reconstructions of the basal nodes, but also indicated that the average rate of losses of ballistospory is 4.7 times higher than the gains. This fact as well as the polyphyletic origins of gastroid taxa and complex mechanism of ballistospory favors the multiple, parallel losses of ballistospory within the gomphoid-phalloid clade.

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CHAPTER 3

Biogeography of the Hysterangiales (Basidiomycota)

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ABSTRACT

To understand the biogeography of truffle-like fungi, DNA sequences were collected from representative taxa of the order Hysterangiales. Multigene phylogenies resolved three major clades within the order that are composed exclusively of the Southern Hemisphere taxa, and they form a basal paraphyletic grade, supporting an origin of the Hysterangiales in the Southern Hemisphere. The results of ancestral area reconstructions are consistent with the hypothesis of an East Gondwanan, i.e., Australian, origin of the order, with subsequent range expansions to the Northern Hemisphere. Although the topologies of some more terminal clades are consistent with vicariance (e.g., a sister relationship of New Zealand and New Caledonian taxa), some areas (e.g., Australia) are in several different subclades of the order, which is in conflict with a strict vicariant scenario. Therefore the importance of long distance dispersal, though probably a rare event, could not be discarded. Although a Cretaceous origin remains as a possibility, age estimates based on the synonymous substitution rates indicated a Paleozoic origin of the Hysterangiales, which is much older than the oldest fossils for mushroom-forming fungi. This also indicates that the Hysterangiales could exist prior to the origin of the currently recognized ectomycorrhizal plants, as well as the arrival of mycophagous animals in Australia. This inconsistency between the estimated age of the Hysterangiales and the fossil record of its extant hosts suggest that either the three ectomycorrhizal clades of Hysterangiales represent parallel

evolution of the ectomycorrhizal symbiosis or that the Hysterangiales was mycorrhizal with members of the extinct flora of Gondwana.

INTRODUCTION

The Hysterangiales is an order of phylum Basidiomycota (Fungi) that forms hypogeous (subterranean) fruiting bodies commonly referred to as false-truffles (Beaton *et al.*, 1985; Castellano, 1990a, 1999; Castellano *et al.*, 1989; Zeller & Dodge, 1929). Because of its hypogeous habit, the spores of Hysterangiales cannot be disseminated by wind, as is the case of many above-ground (epigeous) mushroom-forming fungi that are capable of long distance spore dispersal (Bruns *et al.*, 1989; Thiers, 1985). Instead, they produce a unique aroma that attracts small animals, which rely on hypogeous fungi as a large part of their diet (Castellano *et al.*, 1989; Thiers, 1985). Hypogeous fruiting bodies are eaten by small animals and the fungal spores are disseminated with the animal feces (Castellano *et al.*, 1989; Cazares & Trappe, 1994; Claridge & Lindenmayer, 1998; Claridge *et al.*, 1992; Currah *et al.*, 2000; Fogel & Trappe, 1978; Green *et al.*, 1999; Johnson & McIlwee, 1997; Malajczuk *et al.*, 1997; Maser & Maser, 1988; Maser *et al.*, 1985; Maser *et al.*, 1978; Reddell *et al.*, 1997). Because spore dissemination of hypogeous fungi, including that of the Hysterangiales, depends on such mycophagy (fungal consumption by other organisms), long distance (such as intercontinental) dispersal of spores of hypogeous fungi is arguably less likely.

Despite its hypogeous habit and high dependence on animal mycophagy, the Hysterangiales is distributed worldwide, both in the Northern and Southern Hemisphere (Castellano, 1990a, 1999). This is consistent with the Hysterangiales

being an old taxon, and the current distribution being the result of ancient vicariant events associated with the supercontinent Pangaea. Alternatively, it could be explained by the Hysterangiales being a much more efficient disperser than predicted by morphology with a more recent origin. So far, Australia, North America, and Europe are documented centers of diversity for the Hysterangiales (Castellano, 1999), and recent studies also revealed relatively high diversity in New Zealand (Castellano & Beever, 1994) and South America (Castellano & Muchovej, 1996). The other known distribution for the Hysterangiales include Africa, India, temperate and tropical Asia, New Caledonia, and Papua New Guinea (Castellano, 1990a; Castellano *et al.*, 2000) although the numbers of taxa are relatively small. Importantly, the distribution of each species appears to be restricted to a single continent or island (Castellano, 1990a, 1999). Therefore, areas of endemism (Harold & Mooi, 1994; Linder, 2001; Szumik *et al.*, 2002) can be easily defined as each continent (e.g., North America) or island (e.g., New Caledonia).

Another important aspect is that most species of the Hysterangiales are ectomycorrhizal fungi. As ectomycorrhizal fungi, species of Hysterangiales form symbiotic associations with host trees. For the Hysterangiales, this ectomycorrhizal habit is considered obligate. That is, species of the Hysterangiales cannot undertake sexual reproduction, hence are unable to produce fruiting bodies, without associating with host trees. A wide range of trees, including both gymnosperms and angiosperms, are known as ectomycorrhizal hosts for the Hysterangiales, i.e., Fagaceae, Nothofagaceae, Myrtaceae, and Pinaceae (Castellano, 1990a, 1999), but other plant

groups, such as Dipterocarpaceae and caesalpinioid legumes (Caesalpinioideae) are also demonstrated to be ectomycorrhizal hosts for the Hysterangiales (Castellano *et al.*, 2002). Despite its wide host range as an order, any one species of the Hysterangiales only associates with hosts from one plant family and often only one genus or species (Castellano, 1990a, 1999) and the host association closely correlates with current geographic distribution. That is, most Northern Hemisphere species associate with hosts from the Fagaceae or Pinaceae and Southern Hemisphere species associate with hosts from the Myrtaceae or Nothofagaceae (Castellano, 1990a, 1999; Castellano & Beever, 1994; Castellano & Muchovej, 1996; Malajczuk *et al.*, 1987).

Recent studies support that the gomphoid-phalloid clade, to which the Hysterangiales belongs, as being one of the basal clades of the homobasidiomycetes (mushroom-forming fungi; Binder & Hibbett, 2002; Hibbett *et al.*, 1997b, 2000; Moncalvo *et al.*, 2002; Hibbett & Binder, 2002; Hibbett, 2004; Lutzoni *et al.*, 2004; Hosaka Chapter 2). Although the age estimates of fungal lineages vary, Precambrian origin of major fungal lineages are postulated by some molecular clock studies (Heckman *et al.*, 2001; Wang *et al.*, 1999; Hedges *et al.*, 2004), and ectomycorrhizal fungi are thought to have diversified in the Jurassic or even older before the break-up of Pangaea (Halling, 2001; Martin *et al.*, 2001). These data are again consistent with the potentially ancient and vicariant origin of the Hysterangiales, making it an excellent fungal system that could contribute to our overall knowledge and understanding of the biotic evolution and global biogeography of fungi.

All of the above features of global distribution with well-defined areas of endemism, ectomycorrhizal host range and specificity, hypogeous habit, and phylogenetic position make the Hysterangiales an attractive system for testing numerous evolutionary hypotheses including dispersal vs. vicariance, host-tracking vs. host-shifting, and the ancient origin of extant fungal lineages. To address the overall goals of this study we sampled all available species of the Hysterangiales with an emphasis on geographical distribution and ectomycorrhizal host association. As far as we know, this is the first study dealing with global biogeography of truffle-like fungi.

MATERIALS AND METHODS

Taxon sampling, PCR, and DNA sequencing

A total of 114 taxa, 2 outgroup and 112 ingroup taxa, were sampled for this study (Table 3.1). The selection of ingroup and outgroup taxa was based on the phylogeny of previous studies (Humpert *et al.*, 2001; Giachini *et al.*, unpublished data) and traditional morphology-based classifications (Zeller & Dodge, 1929; Zeller, 1949; Jülich, 1981; Castellano & Beever, 1994; Castellano, 1990a, b; Castellano & Muchovej, 1996; Castellano *et al.*, 2000) to cover the diversity of the Hysterangiales. Among the ingroup taxa, 107 taxa are ectomycorrhizal, and 5 taxa were coded as

saprobic, based on the habitat information, or direct observation (morphological or molecular) of ectomycorrhizae.

Sequence data were obtained from two ribosomal RNA genes, nuclear large subunit (nuc-LSU-rDNA) and mitochondrial small subunit ribosomal RNA genes (mt-SSU-rDNA), two nuclear protein coding genes, the second largest subunit of RNA polymerase (*RPB2*) and translation elongation factor subunit 1α (*EF1α*), and one mitochondrial protein coding gene, ATPase subunit 6 (*ATP6*). The primers and PCR protocols have been described in previous studies. For nuc-LSU-rDNA, the primer combination of LR0R and LR3 (Vilgalys & Hester, 1990) was used, and the amplified PCR products were approximately 600 bp. For mt-SSU-rDNA, three different primer combinations were used. Most samples amplified well with MS1 and MS2 (White *et al.*, 1990). If not, the other primer combinations, U1 and MS2, or Phal1 and MS2, were used. The Phal1 primer sequence is 5'-CCAKAAGACTCGGTAAKG-3'. The primer sequence for U1 is available at http://plantbio.berkeley.edu/%7Ebruns/primers.html. The amplified products varied in length, ranging from 500 bp. to over 2000 bp. The PCR conditions follow the protocol described by Humpert *et al.* (2001). For *ATP6*, the primer combination atp6-3 and atp6-2 (Kretzer & Bruns, 1999) was used, and the PCR protocol followed that of Kretzer & Bruns (1999). The amplified products were approximately 700 bp. For *RPB2*, the primer combination bRPB2-6F and bRPB2-7R (Liu *et al.*, 1999; http://faculty.washington.edu/benhall/) was used, and the amplified PCR products were approximately 800 bp. For *EF1α*, the primer combination EF1-983F and EF1-

Table 3.1. Taxon list.

Genus	species	Herbarium	specimen number	GenBank#				
				nucLSU	mtSSU	ATP6	RPB2	EF
INGROUP								
Andebbia	*pachythrix*	OSC	58809	DQ218523	———	DQ218808	DQ218940	DQ219117
Aroramyces	*gelatinosporus*	OSC	H4010	DQ218524	DQ218698	DQ218809	DQ218941	DQ219118
Aroramyces	*radiatus*	OSC	A. Verbeken 99-062	DQ218525	DQ218699	DQ218810	DQ218942	DQ219119
Aroramyces	*sp.*	OSC	T15013	DQ218526	———	DQ218811	DQ218943	DQ219120
Aroramyces	*sp.*	OSC	T9930	DQ218527	DQ218700	DQ218812	DQ218944	DQ219121
Aroramyces	*sp.*	OSC	122858	DQ218528	———	DQ218813	DQ218945	DQ219122
Aroramyces	*sp.*	OSC	122590	DQ218529	DQ218701	DQ218814	DQ218946	DQ219123
Aroramyces	*sp.*	RMS	S. Miller 10030	DQ218530	DQ218702	DQ218815	DQ218947	DQ219124
Austrogautieria	*chlorospora*	OSC	46596	DQ218477	———	DQ218761	DQ218948	DQ219125
Austrogautieria	*clelandii*	OSC	62178	DQ218531	———	DQ218816	DQ218949	DQ219126
Austrogautieria	*clelandii*	OSC	80012	DQ218532	———	DQ218817	DQ218950	DQ219127
Austrogautieria	*manjimupana*	OSC	55900	DQ218533	———	DQ218818	DQ218951	DQ219128
Austrogautieria	*manjimupana*	OSC	59545	DQ218478	———	DQ218762	DQ218952	DQ219129
Austrogautieria	*sp.*	OSC	80139	DQ218479	DQ218654	DQ218763	DQ218953	DQ219130
Austrogautieria	*sp.*	OSC	80140	DQ218480	DQ218655	DQ218764	DQ218954	DQ219131
Austrogautieria	*sp.*	OSC	122637	DQ218534	———	DQ218819	DQ218955	DQ219132
Austrogautieria	*sp.*	MELU	Beaton 66	DQ218535	———	DQ218820	———	DQ219133
Castoreum	*sp.*	OSC	122814	DQ218536	———	DQ218821	DQ218956	DQ219134
Chondrogaster	*angustisporus*	OSC	62041	DQ218537	DQ218703	DQ218822	DQ218957	DQ219135
Chondrogaster	*pachysporus*	OSC	49298	DQ218538	DQ218704	DQ218823	DQ218958	DQ219136
Gallacea	*dingleyae*	OSC	59606	DQ218539	DQ218705	DQ218824	DQ218959	DQ219137
Gallacea	*eburnea*	OSC	59601	DQ218482	DQ218657	DQ218766	DQ218960	DQ219138
Gallacea	*scleroderma*	OSC	59621	AY574645	AY574719	AY574787	DQ218961	DQ219139
Gallacea	*sp.*	PDD	REB2364	DQ218540	DQ218706	DQ218825	DQ218962	DQ219140
Gallacea	*sp.*	OSC	T25038	DQ218541	———	DQ218826	DQ218963	DQ219141
Gallacea	*sp.*	OSC	80855	———	DQ218707	DQ218827	DQ218964	DQ219142
Gallacea	*sp.*	OSC	122728	DQ218542	DQ218708	DQ218828	DQ218965	DQ219143

Table 3.1. (Continued).

Gallacea	*sp.*	OSC	122813	DQ218543	DQ218709	DQ218829	DQ218966	DQ219144
Gummiglobus	*agglutinosporus*	OSC	58784	DQ218544	DQ218710	DQ218830	DQ218967	———
Gummiglobus	*joyceae*	OSC	59485	DQ218488	DQ218663	DQ218772	DQ218968	———
Hallingea	*purpurea*	OSC	Garido 418-A	DQ218545	———	———	DQ218969	DQ219145
Hysterangium	*affine*	OSC	T6884	DQ218546	———	DQ218831	DQ218970	———
Hysterangium	*aggregatum*	OSC	H4262	DQ218489	DQ218664	DQ218773	DQ218971	DQ219146
Hysterangium	*album*	OSC	T15139	DQ218490	DQ218665	DQ218774	DQ218972	DQ219147
Hysterangium	*aureum*	OSC	56988	DQ218491	DQ218666	DQ218775	DQ218973	DQ219148
Hysterangium	*calcareum*	M	Gross 97	DQ218492	DQ218667	DQ218776	DQ218974	DQ219149
Hysterangium	*cistophilum*	OSC	T1088	DQ218493	DQ218668	DQ218777	DQ218975	DQ219150
Hysterangium	*clathroides*	MPU	Szemere 11-SEPT-1955	DQ218547	DQ218711	DQ218832	DQ218976	DQ219151
Hysterangium	*coriaceum*	MICH	Kers 4984	———	———	DQ218833	———	———
Hysterangium	*coriaceum*	OSC	55265	———	———	DQ218834	———	———
Hysterangium	*coriaceum*	OSC	64939	AY574686	AY574759	AY574826	DQ218977	DQ219152
Hysterangium	*crassirhachis*	OSC	58056	DQ218494	DQ218669	DQ218778	DQ218978	DQ219153
Hysterangium	*crassum*	OSC	110447	AY574687	AY574760	AY574827	DQ218979	DQ219154
Hysterangium	*epiroticum*	OSC	T6116	DQ218495	DQ218670	DQ218779	DQ218980	DQ219155
Hysterangium	*fragile*	OSC	Kers 3971	DQ218496	DQ218671	DQ218780	DQ218981	DQ219156
Hysterangium	*gardneri*	OSC	T6950	DQ218548	DQ218712	DQ218835	DQ218982	DQ219157
Hysterangium	*hallingii*	OSC	R. Halling 5741	DQ218497	DQ218672	DQ218781	DQ218983	DQ219158
Hysterangium	*inflatum*	OSC	H4035	DQ218549	———	DQ218836	DQ218984	DQ219159
Hysterangium	*membranaceum*	OSC	T12836	DQ218498	DQ218673	DQ218782	DQ218985	DQ219160
Hysterangium	*neotunicatum*	OSC	T15545	DQ218550	———	DQ218837	DQ218986	DQ219161
Hysterangium	*occidentale*	OSC	47048	AY574685	AY574758	AY574825	DQ218987	DQ219162
Hysterangium	*pompholyx*	OSC	Gross 495	DQ218499	DQ218674	DQ218783	———	DQ219163
Hysterangium	*rugisporum*	OSC	59662	DQ218500	DQ218675	DQ218784	DQ218988	DQ219164
Hysterangium	*rupticutis*	OSC	59667	DQ218551	DQ218713	DQ218838	———	———
Hysterangium	*salmonaceum*	K	Beaton 33	DQ218501	DQ218676	DQ218785	DQ218989	DQ219165
Hysterangium	*separabile*	OSC	69030	DQ218502	DQ218677	DQ218786	DQ218990	DQ219166
Hysterangium	*setchellii*	OSC	58071	DQ218552	———	DQ218839	DQ218991	DQ219167

Table 3.1. (Continued).

Hysterangium	*sp.*	OSC	T591	———	DQ218714	DQ218840	DQ218994	DQ219170
Hysterangium	*sp.*	OSC	T17501	DQ218553	DQ218715	DQ218841	———	DQ219171
Hysterangium	*sp.*	OSC	T3296	DQ218554	DQ218716	DQ218842	DQ218995	DQ219172
Hysterangium	*sp.*	MEL	2078287	DQ218555	DQ218717	DQ218843	DQ218996	DQ219173
Hysterangium	*sp.*	K	K. & G. Beaton	DQ218506	DQ218681	DQ218790	DQ218997	DQ219174
Hysterangium	*sp.*	MEL	2049882	DQ218556	DQ218718	DQ218844	DQ218998	DQ219175
Hysterangium	*sp.*	OSC	H4123	DQ218557	DQ218719	DQ218845	DQ218999	DQ219176
Hysterangium	*sp.*	OSC	T4794	DQ218558	DQ218720	DQ218846	DQ219000	DQ219177
Hysterangium	*sp.*	OSC	Garcia 3779	DQ218559	DQ218721	DQ218847	DQ219001	DQ219178
Hysterangium	*sp.*	MEL	2057692	DQ218560	DQ218722	DQ218848	DQ219002	DQ219179
Hysterangium	*sp.*	OSC	T19263	DQ218561	DQ218723	DQ218849	DQ219003	DQ219180
Hysterangium	*sp.*	OSC	T22832	DQ218562	DQ218724	DQ218850	DQ219004	DQ219181
Hysterangium	*sp.*	OSC	122857	DQ218563	DQ218725	DQ218851	DQ219005	DQ219182
Hysterangium	*sp.*	OSC	T3328	DQ218564	DQ218726	DQ218852	DQ219006	DQ219183
Hysterangium	*sp.*	OSC	59629	DQ218565	———	DQ218853	DQ219007	DQ219184
Hysterangium	*sp.*	OSC	AHF602	DQ218566	———	DQ218854	DQ219008	DQ219185
Hysterangium	*sp.*	OSC	T6923	DQ218567	———	DQ218855	DQ219009	DQ219186
Hysterangium	*sp.*	OSC	H2022	DQ218568	———	DQ218856	DQ219010	DQ219187
Hysterangium	*sp.*	OSC	T17856	DQ218569	DQ218727	DQ218857	DQ219011	DQ219188
Hysterangium	*sp.*	OSC	T6889	DQ218570	DQ218728	DQ218858	DQ219012	DQ219189
Hysterangium	*sp.*	OSC	122859	DQ218571	———	DQ218859	DQ219013	DQ219190
Hysterangium	*sp.*	OSC	122860	DQ218572	DQ218729	DQ218860	DQ219014	DQ219191
Hysterangium	*sp.*	OSC	H4749	DQ218573	DQ218730	DQ218861	DQ219015	DQ219192
Hysterangium	*sp.*	OSC	H5057	DQ218574	———	DQ218862	DQ219016	DQ219193
Hysterangium	*sp.*	OSC	H5573	DQ218575	DQ218731	DQ218863	DQ219017	DQ219194
Hysterangium	*sp.*	OSC	H6105	DQ218576	DQ218732	DQ218864	DQ219018	DQ219195
Hysterangium	*sp.*	OSC	122836	DQ218577	———	DQ218865	DQ219019	DQ219196
Hysterangium	*sp.*	OSC	122721	DQ218578	DQ218733	DQ218866	DQ219020	DQ219197
Hysterangium	*sp.*	OSC	122483	DQ218579	———	DQ218867	DQ219021	DQ219198
Hysterangium	*sp.*	PDD	82853	DQ218580	DQ218734	DQ218868	DQ219022	DQ219199

Table 3.1. (Continued).

Hysterangium	*sp.*	RMS	S. Miller 10007	DQ218581	———	DQ218869	DQ219023	DQ219200
Hysterangium	*sp.*	RMS	S. Miller 10100	DQ218582	DQ218735	DQ218870	DQ219024	DQ219201
Hysterangium	*sp.*	RMS	S. Miller 10166	DQ218583	DQ218736	DQ218871	DQ219025	DQ219202
Hysterangium	*sp.*	OSC	T13345	DQ218584	DQ218737	DQ218872	DQ219026	DQ219203
Hysterangium	*sp.*	OSC	T26347	DQ218585	———	DQ218873	DQ219027	DQ219204
Hysterangium	*sp.*	OSC	T26367	DQ218586	———	DQ218874	DQ219028	DQ219205
Hysterangium	*sp.*	OSC	T27921	DQ218587	DQ218738	DQ218875	DQ219029	DQ219206
Hysterangium	*sp.*	OSC	T8997	DQ218588	———	DQ218876	———	DQ219207
Hysterangium	*strobilus*	OSC	T5285	DQ218504	DQ218679	DQ218788	DQ218992	DQ219168
Hysterangium	*youngii*	OSC	59645	DQ218505	DQ218680	DQ218789	DQ218993	DQ219169
Malajczukia	*amicorum*	OSC	59295	DQ218508	DQ218683	DQ218792	DQ219030	DQ219208
Malajczukia	*ingratissima*	OSC	59296	DQ218509	DQ218684	DQ218793	DQ219031	DQ219209
Mesophellia	*arenaria*	OSC	59306	DQ218589	———	DQ218877	DQ219032	DQ219210
Mesophellia	*clelandii*	OSC	59292	DQ218511	DQ218686	DQ218795	DQ219033	DQ219211
Mesophellia	*glauca*	OSC	56986	DQ218590	———	DQ218878	DQ219034	DQ219212
Mesophellia	*sabulosa*	OSC	55918	DQ218591	DQ218739	DQ218879	DQ219035	DQ219213
Mesophellia	*trabalis*	OSC	59282	DQ218592	———	DQ218880	DQ219036	DQ219214
Nothocastoreum	*cretaceum*	OSC	79832	DQ218593	———	DQ218881	———	DQ219215
Nothocastoreum	*cretaceum*	OSC	79925	DQ218594	———	———	DQ219037	DQ219216
Phallogaster	*saccatus*	OSC	T13202	DQ218595	DQ218740	DQ218882	DQ219038	DQ219217
Protubera	*hautuensis*	OSC	59673	DQ218517	DQ218692	DQ218801	DQ219039	DQ219218
Protubera	*nothofagi*	OSC	59699	AY574644	AY574718	AY574786	DQ219040	DQ219219
Protubera	*sp.*	OSC	T20068	DQ218596	———	DQ218883	DQ219041	DQ219220
Trappea	*phillipsii*	OSC	56042	DQ218522	DQ218697	DQ218807	DQ219042	———
Trappea	*pinyonensis*	OSC	AHF530	DQ218597	———	DQ218884	DQ219043	DQ219221
OUTGROUP								
Phallus	*hadriani*	OSC	107658	DQ218514	DQ218689	DQ218798	DQ219044	DQ219222
Ramaria	*flavobrunnescens*	SUC	M7	AF213082	AF213140	DQ220790	DQ219045	DQ219223

Table 3.1. (Continued).

Herbarium code: OSC = Oregon State University Herbarium; SUC = State University of New York Herbarium; K = Royal Botanic Gardens, Kew, UK; M = Herbarium at Botanische Staatssammlung München, Germany; MEL = Herbarium at Royal Botanic Gardens, Australia; MELU = University of Melbourne Herbarium; PDD = Herbarium at Landcare Research, New Zealand; RMS = University of Wyoming Herbarium; MPU = Herbarium at Université Montpellier II; MICH = University of Michigan Herbarium.

1567R (http://ocid.nacse.org/research/deephyphae/EF1primer.pdf) was used, and the amplified PCR products were approximately 600 bp.

Sequencing reactions were performed using the DYEnamic™ ET terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc.) following the manufacture's protocol. All sequencing reactions were conducted using the same primers used for PCR reactions, except for *EF1α*. For *EF1α*, the primer EF1-1567Ra (http://ocid.nacse.org/research/deephyphae/EF1primer.pdf) was used as a reverse primer. Sequencing was run on an ABI 373XL automated DNA sequencer. Sequences were edited using the SeqEd version 1.0.3. (Applied Biosystems, Inc. 1992), and deposited in GenBank.

Phylogenetic analyses

DNA sequences were initially aligned using Clustal X (Thompson *et al.*, 1997), 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. Phylogenetic analyses were conducted using equally-weighted parsimony under PAUP* ver. 4.0b10 (Swofford, 2003). Because of dense taxon sampling of terminal clades and limited resolution power, a two-step search approach was conducted for all individual datasets. In the first step, the heuristic search option (with TBR, no Multrees) and 100 replicates of random addition sequence were performed, keeping

only up to two shortest trees per replicate. In the second step, all of the shortest trees from the first step were used as starting trees for the heuristic search option (with TBR and Multrees on) and 500 replicates of random addition sequence, with MAXTREES set to 10,000. These approaches were followed in all parsimony analyses. Support for individual nodes was tested with bootstrap analysis under the parsimony criterion. Bootstrap analysis was based on 500 bootstrap replicates using the heuristic search option (TBR and Multrees off), with 5 random addition sequences.

Bayesian analyses of individual gene datasets and various combinations of datasets were also performed using MrBayes ver. 3.0b4 (Huelsenbeck, 2000). For nuc-LSU-rDNA and mt-SSU-rDNA dataset, the GTR+Γ+I model was used. The same model was used for the dataset of protein coding gene, but they were further partitioned according to the codon position. Bayesian analyses were run with 2 million MCMCMC generations, sampling trees every 10^{th} generation. The support of nodes was tested by posterior probabilities, obtained from majority rule consensus after deleting the trees in burn-in period.

Test of dataset combinability

Before combining the 5 datasets, tests of dataset combinability were conducted. First, the most parsimonious trees from each dataset were imported to MacClade (Maddison & Maddison, 2003) and all nodes, except the nodes with 70% or

higher bootstrap support, were manually collapsed. These new trees were used as constraint in a different dataset (for example, parsimony analysis of *ATP6* dataset with nuc-LSU-rDNA tree topology as a constraint), using "Load Constraints" option in PAUP* ver. 4.0b10 (Swofford, 2003). Parsimony analyses were conducted under these constraints, keeping only the trees that are compatible with these constraints. A total of 20 constraint parsimony analyses were conducted.

Comparisons of constraint and unconstraint trees were made using "Tree Scores" option in PAUP*. Parsimony based comparisons were performed by Templeton test (Templeton, 1983), using nonparametric pairwise tests option. Likelihood based comparisons were performed by Shimodaira-Hasegawa test (SH-test; Shimodaira & Hasegawa, 1999), using RELL optimization with 1000 bootstrap replicates. Significance of results was determined by a p-value less than 0.05. When significance was observed, the constraint test described above was repeated, but keeping only one node each time, until the nodes of significant conflict were determined. After the nodes of conflict were determined, taxa causing the conflict were deleted, and the same constraint analyses were repeated until no conflict was observed. After testing for combinability, individual gene datasets were combined and phylogenetic analyses were conducted as described above.

Biogeographical analyses

Areas of endemism

The programs DIVA (Ronquist, 1996), TreeFitter (Ronquist, 2002) and TreeMap (Page, 1995) were used for testing and refining biogeographic hypotheses of the Hysterangiales. DIVA (Ronquist, 1996) can deal with up to 15 unit areas, and one of its main advantages is that it can be applied even when area relationships are not hierarchical. Analyses of TreeFitter and TreeMap (see below) require the topology of area relationships, which implicitly assumes that area relationships are hierarchical. A total of 10 areas of endemism were considered for assessing the biogeography of the Hysterangiales. Although some areas, especially the Holarctic (Northern Hemisphere) could be further divided into several smaller areas, these subdivisions were not used for analyses. This is mainly because the area relationships for the Holarctic are not necessarily hierarchical. For example, the land connection between North America and Eurasia was connected and disconnected several times (Sanmartín *et al.*, 2001). Nonetheless, area relationships within the Holarctic could give us very important information on the biogeography of the Hysterangiales. Therefore, to produce comparable inferences for the biogeography of the Hysterangiales, not more than 10 unit areas were used for the DIVA and TreeFitter analyses when the entire Hysterangiales was taken into account, but additional unit areas were used only when particular subclades of Hysterangiales (i.e., clade 1~4 in the Hysterangium s.s. clade;

Fig. 3.2 & 3.4) are discussed. The following 10 areas of endemism were used throughout this study.

1) Australia (AUS): This is one of the better represented areas for Hysterangiales. A few species occur in Tasmania, and could be treated separately. However, relatively recent separation of Tasmania from the main continent (McLaughlin, 2001) makes it unnecessary. Most Australian Hysterangiales are represented in Victoria and its vicinity, but some are from Western Australia and Queensland. In this study, Australia including Tasmania was treated as one unit area.

2) New Guinea (PNG): Two species were represented, and both are from Papua New Guinea. Although several areas of New Guinea Island could be treated separately due to its complex geological history, the whole island was treated as one area for this study.

3) New Zealand (NZ): Both North and South Islands were treated as the same area. Many animals and plants show a distribution not restricted to either one of these island. One species of *Hysterangium* (*H. youngii*) was collected in New Zealand, and it was demonstrated to be very closely related to the other Holarctic taxa. Although we do not have a detail vegetation record of the collecting site, we suspect that this species is introduced from the Northern Hemisphere with either Pinaceae or Fagaceae plantation. Therefore for this study, *H. youngii* was treated as a Holarctic taxon.

4) New Caledonia (NC): Two species were represented for this study, and both are from the main island of New Caledonia (Grand Terre).

5) Southern South America (SSAM): It is used here as the southern temperate region of South America, following the treatment of Sanmartín & Ronquist (2004). All known Hysterangiales distributed in the southern South America are from Argentina or Chile.

6) Northern South America (NSAM): It is defined here as north-central South America east of the Andes, following the treatment of Sanmartín & Ronquist (2004). All known Hysterangiales distributed in the northern South America are from Guyana.

7) Africa (AF): One species was represented for this study, and it is from Zimbabwe.

8) India (IND): One species was represented for this study, and it is from Karnataka Province (southwest India).

9) Southeast Asia (SEA): It is defined here as Malaysian Peninsula. New Guinea was treated as independent area, and not included in the Southeast Asia. Three species were represented for this study. Two of them are from southern part of Thailand, and the other is from Singapore.

10) Holarctic (HOL): It is defined here as combination of Palearctic and Nearctic. One species is from Central America (Costa Rica), and it was treated as a Holarctic taxon, following the treatment of Sanmartín & Ronquist (2004). Holarctic was further divided into four regions for dispersal-vicariance analysis; western North America (WNAM), eastern North America (ENAM), Asia, and Europe (EUR). These subdivisions of unit areas were used for dispersal-vicariance analyses when only one particular clade (*Hysterangium s.s.* clade; Fig. 3.2) was taken into account. WNAM includes the area west of the Rocky Mountains, including Mexico and Costa Rica. ENAM includes the entire region east of Rocky Mountains. Asia is represented by three species, all from Japan. Europe is defined here as the entire region of the Eurasia continent west of the Ural Mountains.

Geological scenario

Because the analyses of TreeFitter and TreeMap (see below) require the topology of area relationships, generally accepted sequences of Pangaea breakup were used for constructing area cladograms (Fig. 3.1). We used two slightly different cladograms as a representation for Pangaea breakup. The only difference between these two cladograms is the relative position of India.

One cladogram (Fig. 3.1a) was based on Sanmartín & Ronquist (2004), where they used the opening of the South Atlantic Ocean (135 million years ago = MYA) as

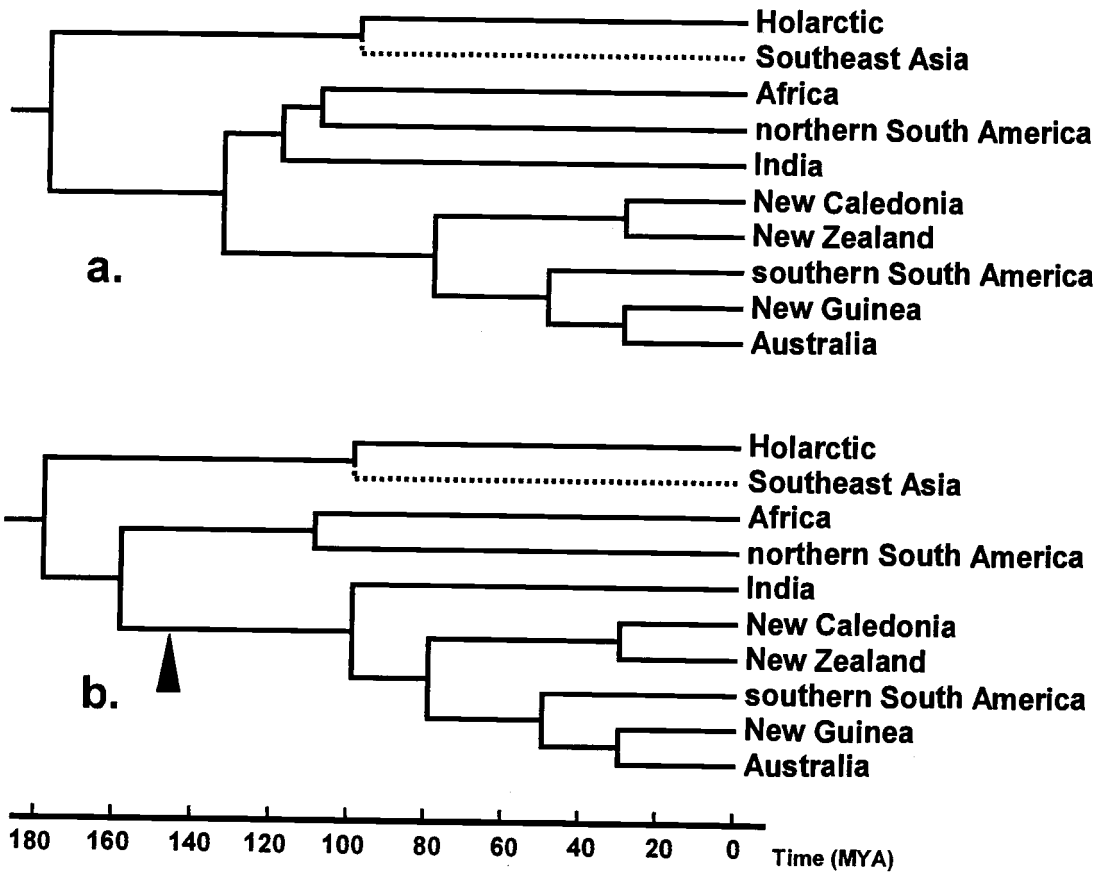

Fig. 3.1. Area relationships based on geological data. (a) Based on Sanmartín & Ronquist (2004); (b) Based on McLaughlin (2001). Two cladograms only differ in the relative position of India. Position of Southeast Asia was arbitrarily determined because of its complicated history (see Materials and Methods). Time is in million years ago from present (MYA). Black triangle in b) indicates an alternative position of Southern South America based on Hallam (1994), showing the initial separation of Southern South America from the rest of Gondwana (see Discussion).

a basis of the initial breakup of Gondwana. This separated the region including India, northern South America and Africa from the rest of Gondwana. However, several studies (McLaughlin, 2001; Hallam, 1994) suggest that the opening of the Weddell Sea (ca. 160 MYA) separated East (including Australia, New Zealand, and India) and West Gondwana (including Africa and northern South America). This event was reflected in the alternative geological scenario (Fig. 3.1b). Timing of the separation of India from the rest of Gondwana and collision to the Northern continents is another controversial issue. Sanmartín & Ronquist (2004) used 120 MYA as the timing of separation of India (and Madagascar) from Africa. However, some studies suggest that India has been connected to the rest of East Gondwana via Antarctica and the Kerguelen Plateau for a long time. Although it is currently submerged, the Kerguelen Plateau emerged above sea level during the Cretaceous and might have been an important corridor for terrestrial organisms (McLaughlin, 2001). Some studies suggest that India had a direct land connection to the rest of East Gondwana up to 80 MYA (Sampson *et al.*, 1998).

Relative position of Southeast Asia was somewhat arbitrarily reflected in Fig. 3.1 because of its hybrid nature. Geological evidences suggest that the present Southeast Asia was once located in the northern periphery of East Gondwana. The separation of Southeast Asia from Gondwana happened multiple times during the Paleozoic and Early Mesozoic. The geological history of Malaysian Peninsula is of particular interest herein, because all three Southeast Asian species of Hysterangiales sampled in this study are from this region. The present Malaysian Peninsula was

largely composed of continental terrane known as Sibumasu, which was separated from Gondwana by the Late Permian and collided into Asia by the Late Triassic (Metcalfe, 1998). Because these events occurred prior to the major breakup of Pangaea, Southeast Asia was treated as a sister area of the Holarctic.

The area relationships for the other areas are much less controversial. However, biogeographical studies often show conflicting patterns to the geological scenario, and several alternative geological scenarios were proposed accordingly. Swenson *et al.* (2001b) summarized two such alternatives. One suggests the possible sister relationships between New Guinea and New Caledonia. The other suggests that New Zealand and New Caledonia are not sister areas, and that New Caledonia is more closely related to Australia, New Guinea, and South America. These and the other alternative geological scenarios are generally not supported by geological evidence, so for this study, we followed the traditional hypothesis of Pangaean breakup depicted in Fig. 3.1.

Ancestral area reconstructions

Ancestral areas were reconstructed for every node within the Hysterangiales using dispersal-vicariance analysis (Ronquist, 1997). Outgroup taxa were used for rooting, but not included in the analyses of ancestral area reconstructions. All taxa in the Hysterangiales were coded according to their areas of endemism. A total of 10 unit

areas (Australia, New Guinea, New Zealand, New Caledonia, southern South America, northern South America, Africa, India, Southeast Asia, and Holarctic) were used. As far as we know, no species of Hysterangiales are distributed in more than one unit areas, and thus no species was treated as widespread.

Reconstructions were made using DIVA 1.1 (Ronquist, 1996). The topology of the Hysterangiales used for this analyses are based on the Bayesian analysis (Fig. 3.2). An initial attempt of ancestral area reconstructions without constraint of maximum numbers of ancestral areas resulted in highly unresolved reconstructions. Therefore, the following reconstructions were made constraining the numbers of ancestral areas using "maxareas" command. The numbers of maximum ancestral areas were constrained from 2 to 10, and each result was compared.

Dispersal-vicariance analysis was conducted separately only for the *Hysterangium* s.s. clade (Fig. 3.2). This clade was demonstrated to be exclusively composed of the Holarctic (Northern Hemisphere) taxa. So Holarctic was subdivided into 4 unit areas: western North America (WNAM), eastern North America (ENAM), Asia, and Europe (EUR), as described above. Ancestral area reconstructions were made without "maxareas" specified.

Maximum vicariance analysis

Although results of dispersal-vicariance analysis could be used to identify potential vicariant nodes, highly unresolved reconstructions for some nodes made it extremely complicated. Therefore, a different approach was also attempted. TreeMap ver. 1.0a (Page, 1995) was used for identifying potential vicariant nodes in the Hysterangiales tree (Fig. 3.2). The same 10 unit areas used for DIVA analyses were applied. Because the program requires known geological trees, the hypothesized scenario of Pangaean breakup (Fig. 3.1b) was used. We are very aware that reconstructions of TreeMap are based on maximum vicariance, a completely different approach from DIVA, which is based on a total cost of dispersal and extinction. Reconstructions by TreeMap were basically used for visual aid.

Searches for the optimal area cladograms

Searches for the best area cladograms were conducted for the Hysterangiales using TreeFitter 1.3b1 (Ronquist, 2002). The same 10 unit areas used for DIVA analyses were applied. The topology of the Hysterangiales used for this analyses are based on Bayesian analysis (Fig. 3.2). Searches were conducted using the "exhaustive" option with the default setting for event costs (0.01 for vicariance and duplication, 1.0 for extinction, and 2.0 for dispersal).

To assess the statistical significance of the fit between the best area cladograms and the Hysterangiales phylogeny, randomization tests were conducted. Randomized tests were based on 10000 permutations of the terminals in the area cladograms. The percentage of area cladograms obtained from permutations with a lower cost than the original area cladograms was used as the significance value. Same randomization tests were also conducted between the area cladograms based on geological evidence (Fig. 3.1b) and Hysterangiales phylogeny, and the significance of fit was calculated.

Age estimate based on synonymous substitution rates

To obtain a rough estimate of node age, synonymous substitution rates of *RPB2* were calculated across Opisthokonta (Fungi and Animals). *RPB2* was selected for this comparison because of its relative resolution power and sequence availability across kingdoms and phyla. Taxa were selected to represent the phylogeny of Opisthokonta (Baldauf *et al.*, 2000; Liu *et al.*, 1999). From the Animal Kingdom, four taxa (human, mice, fly, and nematode) were selected (Fig. 3.7). Only Ascomycota and Basidiomycota were selected for Fungi. From Ascomycota, 2 Taphrinomycotina, 1 Saccharomycotina, 2 Pezizomycetes, 3 Eurotiomycetes, and 4 Sordariomycetes were selected (Fig. 3.7). From Basidiomycota, 2 Ustilaginomycetes and 11 Hymenomycetes, of which 7 Hysterangiales to represent the phylogeny of Hysterangiales, were selected (Fig. 3.7). Comparisons were also made independently

for several nodes within the Hysterangiales. Only coding regions were kept for alignment, and all ambiguously aligned regions were removed from the analysis.

The numbers of synonymous substitutions were estimated using MEGA 2.0 (Kumar *et al.*, 2000) under the modified Nei-Gojobori method, with uncorrected *p*-distance and the default transition/transversion ratio (= 2) options. All combinations of pairwise distances were calculated. Comparisons between two groups were made by averaging all pairwise distances from each group. Comparisons were made for the following pairs of major groups: Fungi vs. Animals, Basidiomycota vs. Ascomycota; Sordariomycetes vs. Eurotiomycetes; *Microascus* vs. all the other Sordariomycetes; Taphrinomycotina vs. the other Ascomycota; Saccharomycotina vs. Pezizomycotina; Ustilaginomycetes vs. Homobasidiomycetes; Hysterangiales vs. all the other Homobasidiomycetes; Phallogastraceae vs. all the other Hysterangiales. Calibrations of all these comparisons are possible using the age estimate from the previous studies, and/or the fossil record, except for the node of the Hysterangiales vs. all the other Homobasidiomycetes split.

Because the age estimate for each node typically varies, multiple different age estimates were used as calibration for each node (Table 3.2). For the Fungi vs. Animal split, 1500 MYA (Heckman *et al.*, 2001; Wang *et al.*, 1999; Hedges *et al.*, 2004) and 900 MYA (Berbee & Taylor, 2001) were used as the oldest and youngest age estimates, respectively. For the Basidiomycota vs. Ascomycota split, 1200 MYA (Heckman *et al.*, 2001) and 550 MYA (Berbee & Taylor, 2001) were used as the oldest and youngest age estimates, respectively, and 960 MYA (Hedges *et al.*, 2004)

was used for the intermediate estimate. For both the Taphrinomycotina vs. all the other Ascomycota split and Saccharomycotina vs. Pezizomycotina split, the age estimate of 1000 MYA was used. There are much younger age estimates available (Berbee & Taylor, 2001), but it is unreasonably young given the fossil records, and therefore ignored. For the Sordariomycetes vs. Eurotiomycetes split, the age estimates of 670 MYA (Heckman *et al.*, 2001) and 550 MYA (Hedges *et al.*, 2004) were used. As the most conservative estimate, the fossil record of Sordariomycetes from 400 MYA Rhynie cherts (Taylor *et al.*, 1999; Taylor *et al.*, 2005) was also used as the node age. This fossil record was also used for calibrating the node for *Microascus* vs. all the other Sordariomycetes split. For the Ustilaginomycetes vs. Homobasidiomycetes split, 960 MYA (Heckman *et al.*, 2001) and 500 MYA (Berbee & Taylor, 2001) was used as the oldest and youngest age estimates, respectively. The fossil record of clamp connections from 300 MYA (Dennis, 1970) was used for calibrating the node for the Hysterangiales vs. all the other Homobasidiomycetes split.

Using the above calibration points and pairwise distance between two groups in comparisons, the averaged synonymous substitution rates (per site per year) were calculated. The age estimates for the Hysterangiales were calculated using the synonymous substitution rates obtained above. Age estimates for lineages within the Hysterangiales were also obtained using the TreeEdit ver.1.0a10 (Rambaut & Charleston, 2002). Branch lengths in the original phylogram (based on all 5-gene sequences) were transformed based on nonparametric rate smoothing (Sanderson,

1997), and ages for each node were superimposed by fixing the age of the deepest node. The averaged synonymous substitution rate obtained from the Eurotiomycetes (3×10^{-9}; Kasuga *et al.*, 2002) was also used as an independent comparison.

Ancestral ectomycorrhizal host reconstructions

Ancestral ectomycorrhizal hosts were reconstructed for every node within the Hysterangiales. Because only the Phallogastraceae clade (Fig. 3.2) contains non-mycorrhizal (saprobic) taxa, and the rest of the Hysterangiales are all ectomycorrhizal, the Phallogastraceae clade was not included for reconstructions.

Each taxon was coded for its known ectomycorrhizal host plant. Although for some taxa, more specific host information was available (genus or species of host plants), only familial-level information was used for coding. A total of 8 host families (Myrtaceae, Nothofagaceae, Fagaceae, Pinaceae, Caesalpinioideae, Casuarinaceae, Dipterocarpaceae, and Ericaceae) were used for coding. For some taxa, presumable ectomycorrhizal hosts could not be identified to a single host family because their fruiting bodies were collected from mixed stands of more than one ectomycorrhizal host family. In this case, taxa were coded as polymorphic.

MacClade ver. 4.06 (Maddison & Maddison, 2003) was used for reconstructing the ancestral hosts. All reconstructions are based on unweighted parsimony criterion. Because some nodes were reconstructed only ambiguously, both

maximum and minimum numbers of all possible changes from one host family to the others were recorded using "state changes and stasis" option.

RESULTS

Phylogenetic analyses

The Shimodaira-Hasegawa test detected a minor conflict among datasets when the mt-SSU-rDNA dataset was compared with the other datasets. However, including or excluding the mt-SSU-rDNA dataset did not change the overall topology (data not shown). Therefore, the following results are all based on the topology obtained from the phylogenetic analyses with a combined 5-gene dataset. The monophyly of the Hysterangiales was strongly supported by both Bayesian and parsimony analyses (100% posterior probability and bootstrap value; Fig. 3.2). Six major clades within the Hysterangiales were recognized, all of which, except for the *Hysterangium s.s.* clade and *Aroramyces* clade, were well-supported by both the Bayesian and parsimony analyses (Fig. 3.2). All saprotrophic taxa within the Hysterangiales were confined to the Phallogastraceae clade, and the rest of the Hysterangiales were all ectomycorrhizal taxa (ECM-Hysterangiales clade in Fig. 3.2).

Higher-level phylogeny of the Hysterangiales revealed that strong biogeographical patterns exist (Fig. 3.2). While the Phallogastraceae clade was

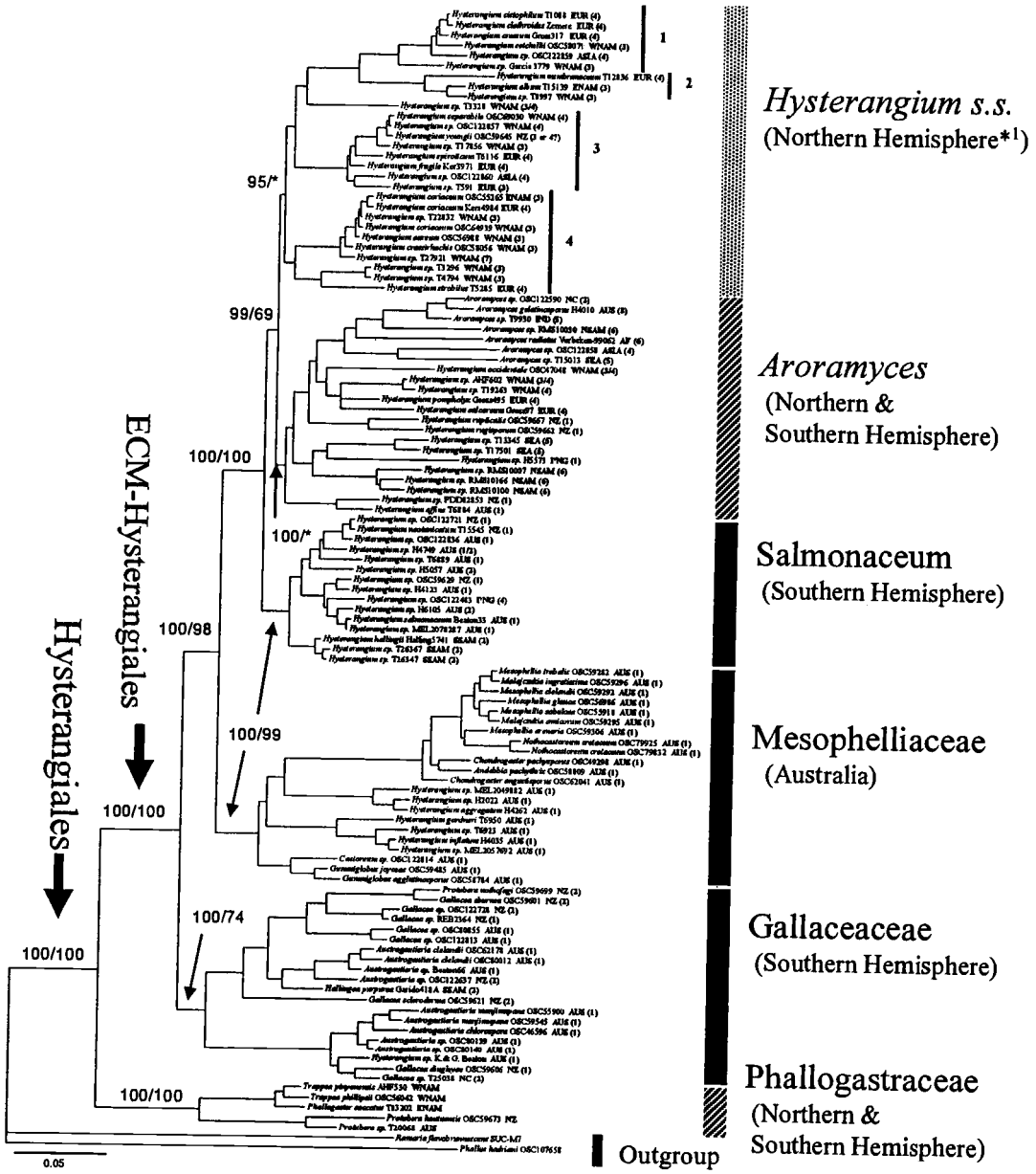

Fig. 3.2. 50% majority consensus of the Hysterangiales phylogeny derived from Bayesian analysis. Taxon names followed by areas of distributions (for abbreviation of areas, see Materials and Methods), and by presumable ECM host in parentheses (1=Myrtaceae, 2=Nothofagaceae, 3=Pinaceae, 4=Fagaceae, 5=Dipterocarpaceae, 6=Caesalpinioideae, 7=Ericaceae, 8=Casuarinaceae). Numbers on branches are nodal supports (Bayesian posterior probability/ Parsimony bootstrap values; * indicates no bootstrap support; only deep nodes are labeled, and no terminal nodes are labeled). *1 = One taxon distributed in New Zealand, but treated as Holarctic taxon (see text in Materials and Methods).

composed of both Northern and Southern Hemisphere taxa, the basal 3 clades within the ECM-Hysterangiales clade were all composed strictly of Southern Hemisphere taxa (Fig. 3.2). Northern Hemisphere taxa were restricted in more terminal clades, *Hysterangium s.s.* and *Aroramyces* clades (Fig. 3.2). Neither Northern Hemisphere nor Southern Hemisphere taxa formed monophyletic groups. Southern Hemisphere taxa comprised a basal paraphyletic assemblage, and Northern Hemisphere taxa were nested within Southern Hemisphere taxa. One major clade, the *Hysterangium s.s.*, was strictly composed of Northern Hemisphere taxa (Fig. 3.2).

DIVA & TreeMap analyses

Dispersal-vicariance analyses using DIVA showed that the ancestral areas of the Hysterangiales are widespread in Australia, New Zealand and Holarctic, suggesting the vicariant origin of the Hysterangiales (Fig. 3.3). However, the ancestral area for the higher nodes within the ECM-Hysterangiales clade was demonstrated to be restricted to Australia (Fig. 3.3). These patterns were consistent through different settings for the maximum numbers of ancestral areas from 2 to 5. When the maximum numbers of ancestral areas were specified to 6 or more, results were largely unresolved with equally parsimonious solutions for numerous combinations of ancestral areas. Besides the Phallogastraceae clade, the first appearance of the non-Southern Hemisphere area was at the common ancestor of the *Hysterangium s.s.* and

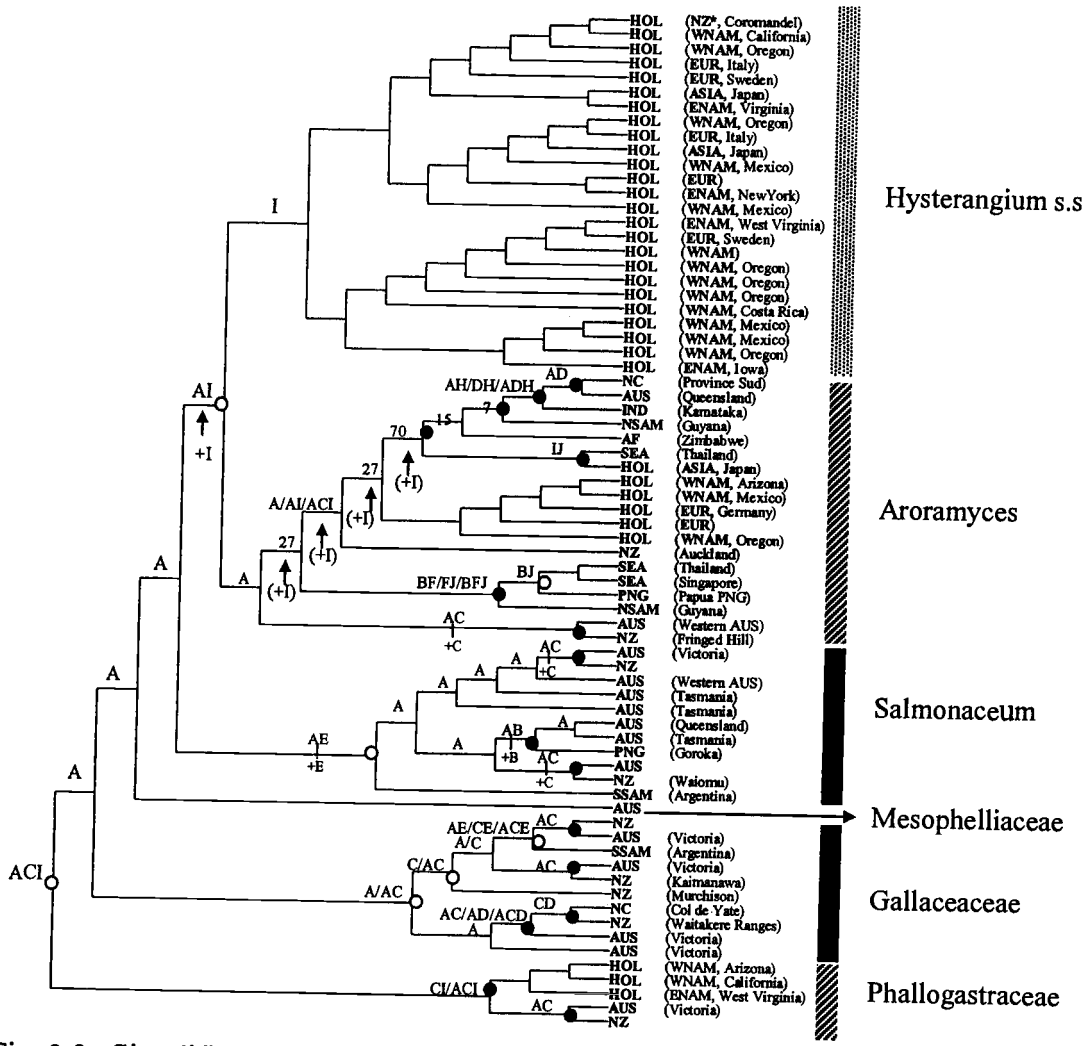

Fig. 3.3. Simplified taxon-area cladogram used for DIVA and TreeFitter. Taxon names were replaced by areas of endemism (for abbreviation of areas, see Materials and Methods). Areas are followed by more specific locality information if available. Holarctic is subdivided into 4 unit areas, but these were not used for analyses (for abbreviations, see Materials and Methods). Clade names follow Fig. 3.1. Characters on node indicate the results of the ancestral area reconstructions using DIVA (A=Australia, B=New Guinea, C=New Zealand, D=New Caledonia, E=southern South America, F=Africa, G=northern South America, H=India, I=Holarctic, J=Southeast Asia). Two or more characters without a space indicate that ancestors were widespread across those areas. Two or more characters separated by a slash indicate the alternative equally parsimonious reconstructions. Numbers on the node indicate the numbers of equally parsimonious reconstructions. Characters below branches with '+' indicate a range expansion to the new areas. Black circles indicate the potential vicariant nodes based on TreeMap. White circles indicate additional nodes potentially corresponding to vicariance based on DIVA. Black triangles indicate the nodes with a potential range expansion to the Holarctic.

Aroramyces clades (Fig. 3.3). This node was unambiguously reconstructed as Australia & Holarctic for ancestral areas, consistent with vicariant events. The same pattern was observed in the Phallogastraceae clade, showing the potential Laurasia/Gondwana vicariant pattern (Fig. 3.3).

The ancestral area for the common ancestor of the *Aroramyces* clade was demonstrated to be Australia. However, except for some very terminal nodes, ancestral areas for many nodes could not be reconstructed unambiguously (Fig. 3.3). Numerous equally parsimonious solutions were possible and depending on the reconstructions, at least one more appearance of the Holarctic as an ancestral area was observed in the *Aroramyces* clade (nodes shown with black circles, Fig. 3.3). TreeMap showed 6 vicariant nodes within the *Aroramyces* clade (Fig. 3.3). One node was consistent with a Laurasia/Gondwana split, and subsequent nodes were consistent with the East and West Gondwana split, the India/East Gondwana split, and the Australia/New Caledonia split.

The reconstructions using TreeMap showed a total of 16 potential vicariant nodes (including 6 nodes within the *Aroramyces* clade described above), some of which were corresponding to the results of DIVA analyses (Fig. 3.3). Most potential vicariant nodes were located at or near tips of trees. Of the 16 potential vicariant nodes, the most frequently encountered pattern was the sister relationship of Australia and New Zealand. Southern South America appeared in two different clades, and both nodes could be vicariant nodes according to DIVA analyses, but they never formed a sister relationship with Australia, as geological history suggested. The sister

relationship to New Caledonia was also inconsistent, one node showing the sister relationship to New Zealand, and the other showing a New Caledonia/Australia sister relationship. The sister relationship of New Guinea was also inconsistent, with one taxa being a sister to Australia, and the other being a sister to Southeast Asia.

DIVA reconstructions for the Holarctic clade

Dispersal-vicariance analysis for the *Hysterangium s.s.* clade showed a relatively well-resolved reconstruction (Fig. 3.4). The ancestral areas for the whole clade were shown to be widespread for all four unit areas. Subsequently, one clade (clade 4 in Fig. 3.4) became restricted to western North America, followed by dispersal to eastern North America and Europe, with possible vicariance. The other clades showed more complex patterns with a few more potential vicariant nodes. Clade 3 is the only clade showing a dispersal event to western North America from the other areas.

Repeated vicariance and dispersal events were observed. For example in clade 1, 2, and 3, initially widespread ancestors experienced a vicariance (A for clade 1 and 2, and BCD for clade 3; Fig. 3.4). In clade 1 and 2, areas B (eastern North America), C (Europe), and D (Asia) all reappeared, followed by potential vicariance (Fig. 3.4). In clade 3, area A (western North America) reappeared, followed by potential vicariance. Only two nodes showed sister relationships for continental areas, western and eastern

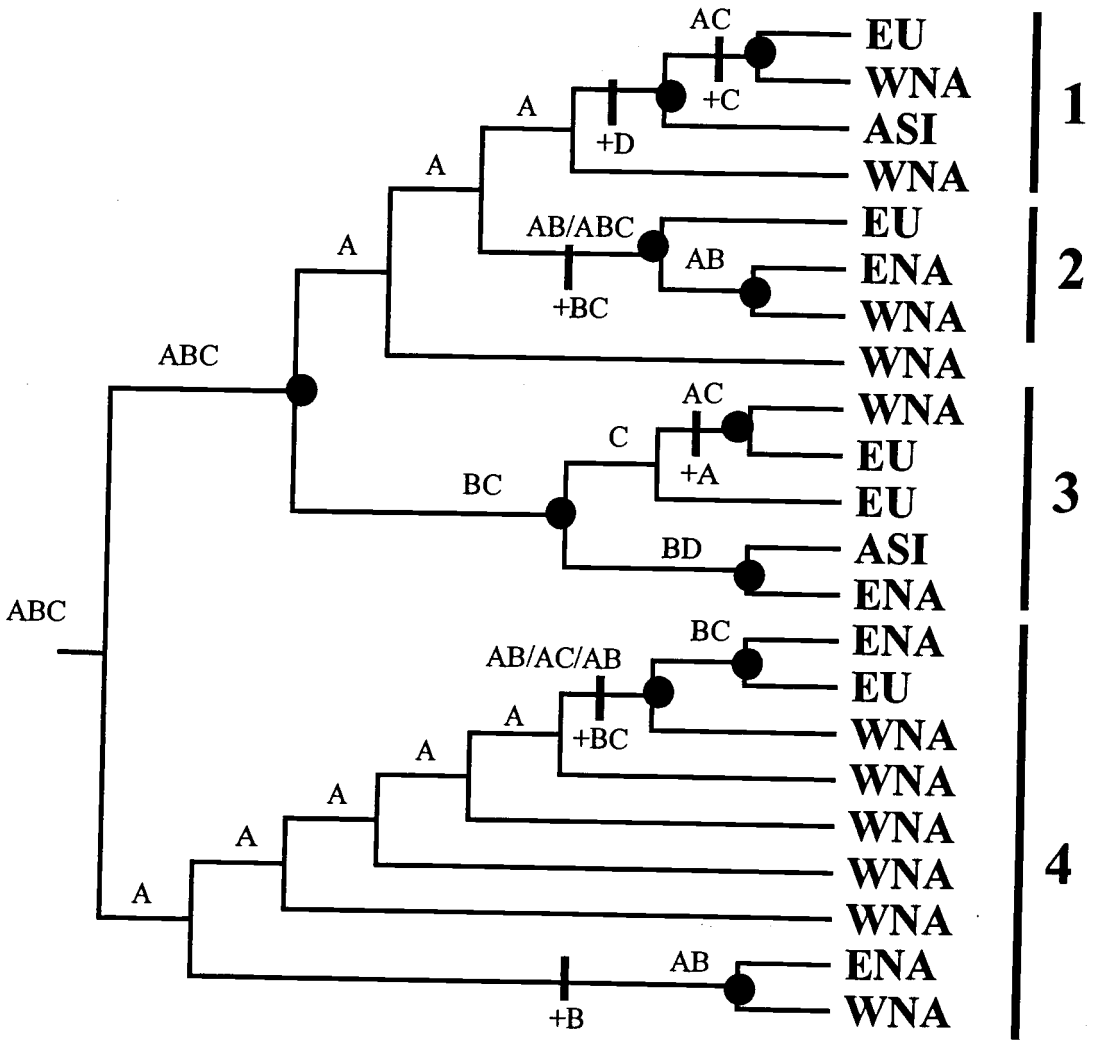

Fig. 3.4. Ancestral area reconstructions for the *Hysterangium s.s.* clade based on DIVA. Clade 1~4 are corresponding to Fig. 3.2. Four areas of endemism (Western North America, Eastern North America, Europe, Asia) were used (for abbreviations, see Materials and Methods). Characters on nodes indicate the results of the ancestral area reconstructions using DIVA (A=Western North America, B=Eastern North America, C=Europe, D=Asia). Two or more characters without separation indicate that ancestors were widespread across those areas. Two or more characters separated by a slash indicate the alternative equally parsimonious reconstructions. Characters below branches with a '+' indicate one of the most parsimonious reconstructions showing range expansion to the new areas. Black circles indicate the potential vicariant node.

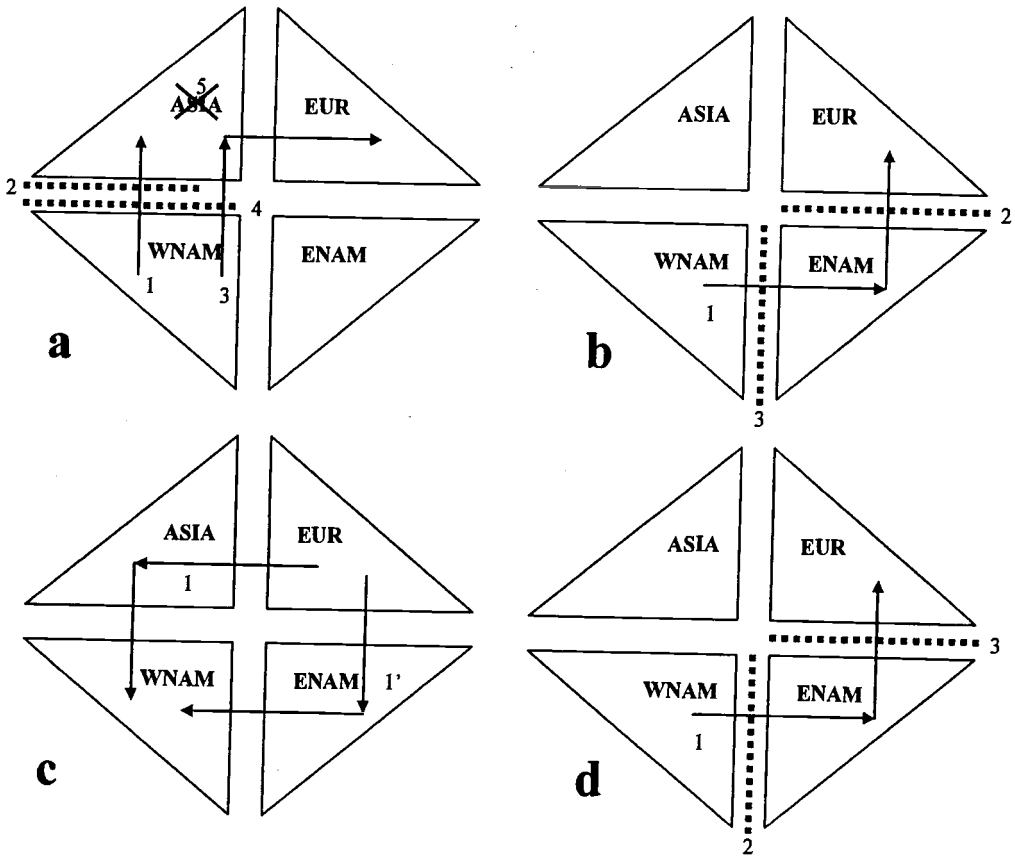

Fig. 3.5. One of the most parsimonious reconstructions for Holarctic biogeography. a) Reconstructions for clade 1 in Fig. 3.4; b) Reconstructions for clade 2 in Fig. 3.4.; c) Reconstructions for clade 3 in Fig. 3.4; d) Reconstructions for clade 4 in Fig. 3.4. Abbreviations for four areas of endemism follow Fig. 3.4 (also see Materials and Methods). Arrows indicate range expansion or dispersal to the new areas. Dotted lines indicate the establishment of dispersal barrier. Cross marks on an area indicates extinction in that area. Numbers on arrows, dotted lines, and cross marks indicate the sequences of those events. Two arrows with 1 and 1' in c) indicate two alternative dispersal routes. See text for detailed discussion.

North America. No node showed a sister relationship of Europe and Asia. Disjunct patterns were observed in some nodes. Two nodes showed a sister relationship of western North America and Europe, and one node showed a sister relationship of eastern North America and Asia (Fig. 3.4).

Searches for the optimal area cladograms

Thirteen equally parsimonious area cladograms were recovered from the Hysterangiales phylogeny using TreeFitter, but none corresponded to a topology identical to geologic history (Fig. 3.6). Among the 13 area cladograms, the only consistent patterns were a sister relationship of Australia and New Zealand, as well as New Caledonia and India. The topology varied mostly due to various positions of southern and northern South America, New Guinea, and Southeast Asia. Randomization tests show that these area cladograms had a statistically significant fit to the Hysterangiales phylogeny ($p < 0.0001$). The same randomization tests were implemented for assessing the fit between the Hysterangiales phylogeny and the geological tree (Fig. 3.1b), and was demonstrated to be statistically not significant, indicating that the fit between the Hysterangiales phylogeny and geological history could happen by chance.

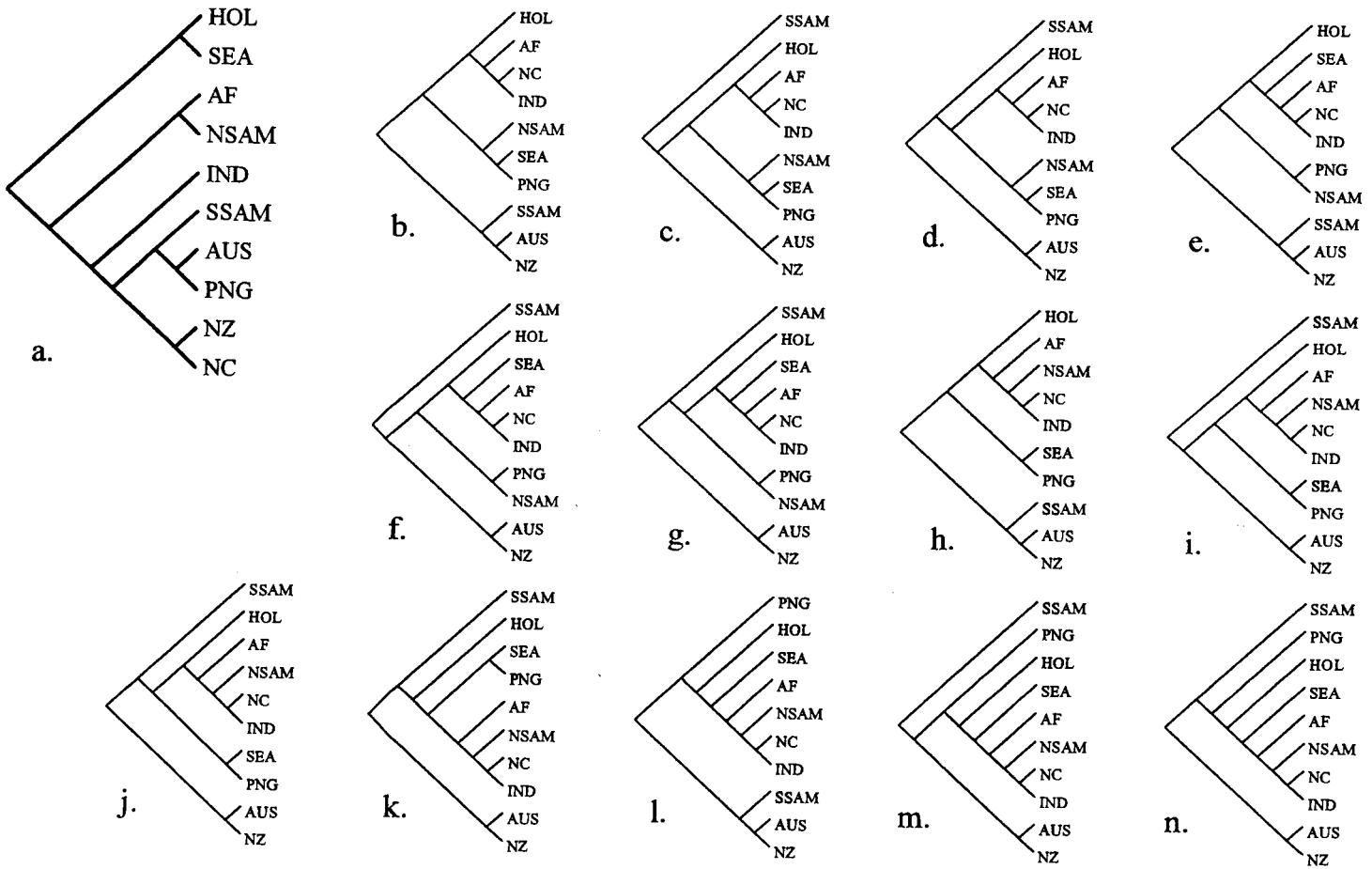

Fig. 3.6. Results of the search for the optimal area cladograms using TreeFitter based on the previous taxon-area cladogram in Fig. 3.3. (a) Area relationships based on geological records (also Fig. 3.1b); (b~n) Equally parsimonious area cladograms derived from the Hysterangiales phylogeny (Fig. 3.3). For abbreviations of areas, see Materials and Methods.

Estimates for synonymous substitution rates

The alignment was comprised of 630 base pairs, of which 201 base pairs were estimated to be synonymous sites. Despite very different calibration points being used, the estimated synonymous substitution rates varied only between 0.15 and 1.0×10^{-9} (per site per year; Table 3.2). The fastest rate was obtained using 300 MYA as the age of the Hysterangiales and all the other Homobasidiomycetes split.

Using 1.0×10^{-9} (per site per year) as a synonymous substitution rate, the age of Hysterangiales was estimated as 260 (± 16) MYA (Table 3.2). 260 MYA was used as a conservative age estimate of the Hysterangiales. There were some consistencies and inconsistencies for the age estimates of potential vicariant nodes. For example, the youngest age estimate for the Australia/New Zealand split (in the Salmonaceum clade) was less than 30 MYA, whereas the oldest one (in the Gallaceaceae clade) was more than 120 MYA (Fig. 3.8, left). A node for the split of the *Hysterangium s.s.* and *Aroramyces* clades, which was suggested as one of the vicariant nodes for the Laurasia/Gondwana split according to DIVA analysis (Fig. 3.3), was estimated to be ca. 160 MYA (Fig. 3.8, left). The other node consistent with the Laurasia/Gondwana split, a node within the *Aroramyces* clade, was estimated to be ca. 100 MYA. One node within the *Hysterangium s.s.* clade, which resolved a sister relationship of East Asia and eastern North America, was estimated to be ca. 40 MYA (in clade 3 of Fig. 3.8, left). The ages for the Europe and North America split varied, from ca. 30 MYA (clade 1) to ca. 50 MYA (clade 2 and 3) (Fig. 3.8, left). The age of the eastern

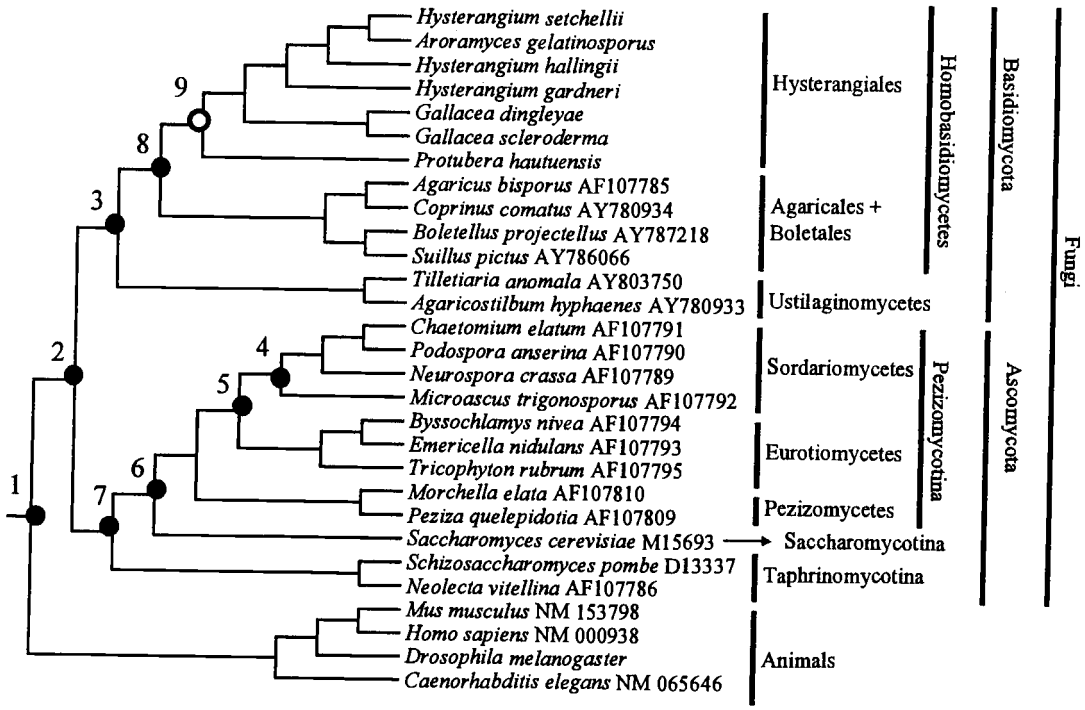

Fig. 3.7. Schematic representation of Opisthokonta (Animals & Fungi) phylogeny. Topology was based on Liu *et al.* (1999) and Baldauf *et al* (2000). Node 1~8 were used as calibration point based on previous studies. Ages for the node with white circle, which corresponds to the origin of the Hysterangiales (node 9) was estimated based on the fastest synonymous substitution rates obtained (1.0×10^{-9} substitution per site per year; see Fig. 3.8 & Table 3.2). Numbers after taxon names are GenBank accession numbers.

Table 3.2. Synonymous substitution rates for RPB2 obtained by pairwise comparison between major groups in Opisthokonta. Names of groups follow Fig. 3.7. Calibration points were based on previous studies (See Materials & Methods). Synonymous substitution rates (per site per year) were obtained dividing the average distances between groups based on synonymous substitution by estimated ages (in million years ago = MYA). Errors associated with the age estimates were based on the standard deviations associated with the average distance. *1 = based on Kasuga *et al.* (2002).

Comparisons	# of pairwise comparison	Average *p*-distance	Calibration (MYA)	Synonymous substitution rate (10^{-9})	Age of the Hysterangiales (MYA)
Fungi vs. Animals	100	0.63 ± 0.05	1500	0.15 ± 0.02	2543 ± 153
			900	0.35 ± 0.03	763 ± 46
Basidiomycota vs. Ascomycota	156	0.61 ± 0.04	1200	0.26 ± 0.02	1045 ± 63
			960	0.32 ± 0.02	836 ± 51
Taphrinomycotina vs. other Ascomycota	20	0.65 ± 0.05	1000	0.32 ± 0.03	825 ± 50
Saccharomycotina vs. Pezizomycotina	9	0.64 ± 0.04	1000	0.32 ± 0.02	828 ± 50
Sordariomycetes vs. Eurotiomycetes	12	0.58 ± 0.06	670	0.44 ± 0.04	612 ± 37
			550	0.53 ± 0.05	502 ± 30
			400	0.73 ± 0.07	365 ± 22
Microascus vs. other Sordariomycetes	3	0.52 ± 0.02	400	0.65 ± 0.03	409 ± 25
Ustilaginomycetes vs. Homobasidiomycetes	11	0.59 ± 0.04	960	0.31 ± 0.02	858 ± 52
			500	0.59 ± 0.04	447 ± 27
Hysterangiales vs. other Homobasidiomycetes	28	0.62 ± 0.04	300	1.0 ± 0.06	260 ± 16
Phallogastraceae vs. other Hysterangiales	6	0.53 ± 0.03	—	—	—
Eurotiomycetes *1	—	—	—	3.0 ± 1.3*1	89 ± 5

Fig. 3.8. Age estimates for the representative nodes within the Hysterangiales. Branch lengths were transformed from the original phylogram (Fig. 3.2) based on the nonparametric rate smoothing using the TreeEdit ver. 1.0a10. Taxon names are replaced by areas (coding scheme as previously). Nodes with particular interests (such as nodes potentially correspond to vicariant events) are indicated by black circles. Clade names follow Fig. 3.1 and 3.4. (Left): Estimates based on the fastest synonymous substitution rates of RPB2 obtained from pairwise comparisons among Opisthokonta (Table 3.2, Fig. 3.7). (Right): Estimates based on the averaged synonymous substitution rate obtained by Kasuga *et al.* (2002).

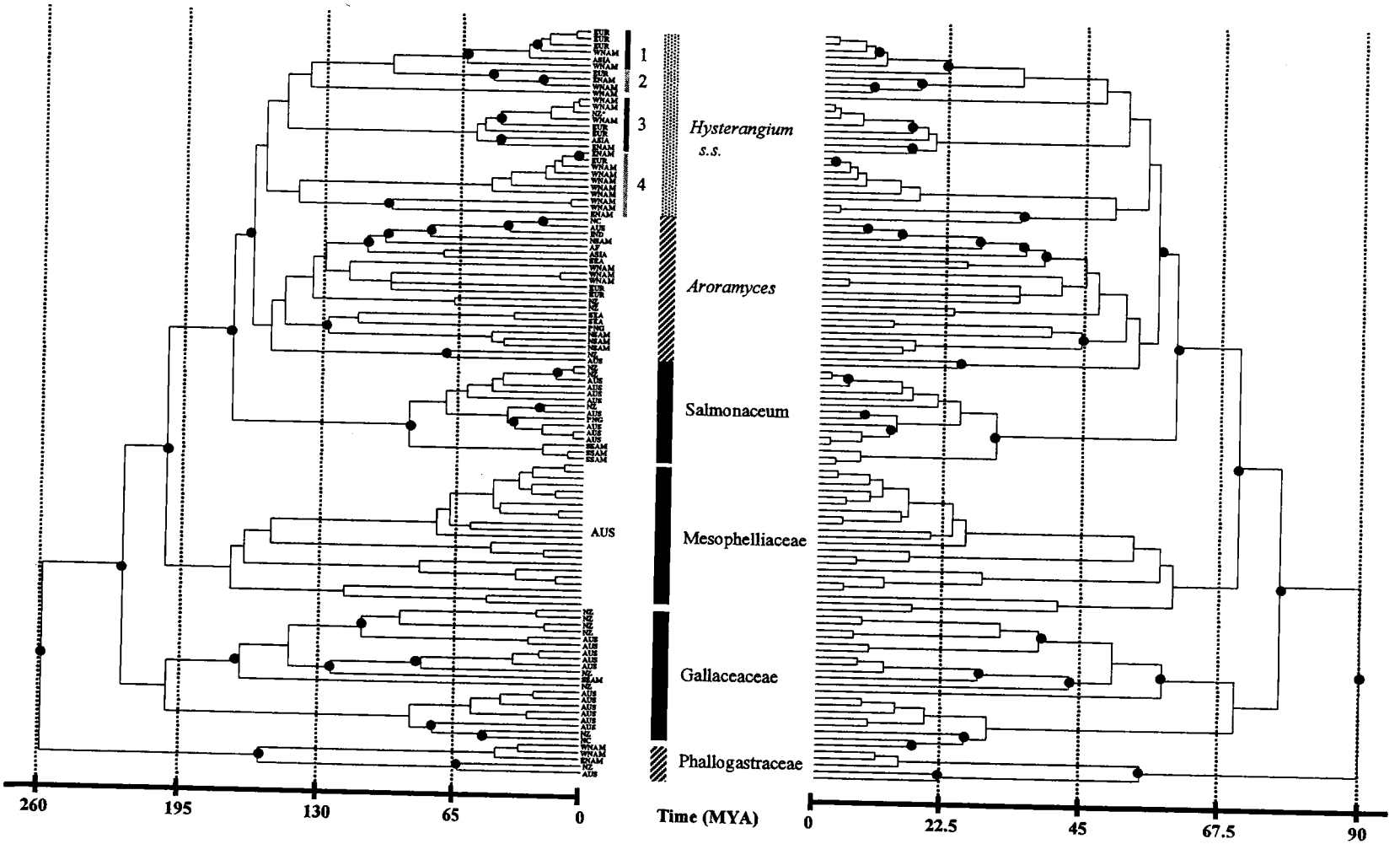

Fig. 3.8. Age estimates for the representative nodes within the Hysterangiales.

North America and Europe split in clade 4 was estimated to be less than 20 MYA (Fig. 3.8, left). The age of the western and eastern North America split was estimated to be ca. 100 MYA in clade 4, but that of clade 2 was estimated to be ca. 30 MYA (Fig. 3.8, left).

Using the average synonymous substitution rate of the Eurotiomycetes (3.0×10^{-9}) obtained by Kasuga *et al.* (2002) resulted in a considerably different age estimate for the Hysterangiales (Table 3.2; Fig. 3.8, right). The origin of the Hysterangiales was estimated to be 89 (± 5) MYA (Table 3.2) and most nodes were demonstrated to be much younger than potential vicariant events (Fig. 3.8, right).

Ancestral ectomycorrhizal host reconstructions

Mapping the ectomycorrhizal hosts on the Hysterangiales phylogeny revealed that many closely related species of the Hysterangiales did not share the same host families. This is especially obvious for many sister species which have different plant families as their ectomycorrhizal hosts (Fig. 3.1, 3.9, 3.10, 3.11). Most major clades within the ECM-Hysterangiales clade were represented by two or more ectomycorrhizal hosts, except the Mesophelliaceae clade, which is strictly associated with *Eucalyptus* (Myrtaceae). These results indicate that frequent host shifts occurred during the evolution of the Hysterangiales.

Ancestral host reconstructions suggested that Myrtaceae is the ectomycorrhizal host for the common ancestor of the ECM-Hysterangiales clade (Fig. 3.9). Furthermore, the common ancestors for the Salmonaceum, Mesophelliaceae and Gallaceaceae were all reconstructed as Myrtaceae (Fig. 3.9). This suggests that the Myrtaceae could serve as important ectomycorrhizal hosts for the initial evolution of the Hysterangiales. Taxa associated with Nothofagaceae were mostly confined to the more terminal clades (Fig. 3.1, 3.9, 3.10). Ancestral host for the common ancestor of the *Hysterangium s.s.* clade and many nodes within it were reconstructed only ambiguously, most of which were equally parsimonious either as Pinaceae or Fagaceae (Fig. 3.11).

The patterns and frequency of host shifts are summarized in Fig. 3.12. Frequent host shifts were observed between the Pinaceae and Fagaceae, and also between Myrtaceae and Nothofagaceae. All other patterns for host shifts, except for the shift from the Myrtaceae to Fagaceae with three possible steps, occurred only once or less. Bidirectional host shifts reconstructed unambiguously were observed between Nothofagaceae and Myrtaceae, and also between Fagaceae and Pinaceae. There were several other possible bidirectional host shifts, but most of them were shown to be the minimum of zero frequency, indicating that most host shifts are probably uni-directional.

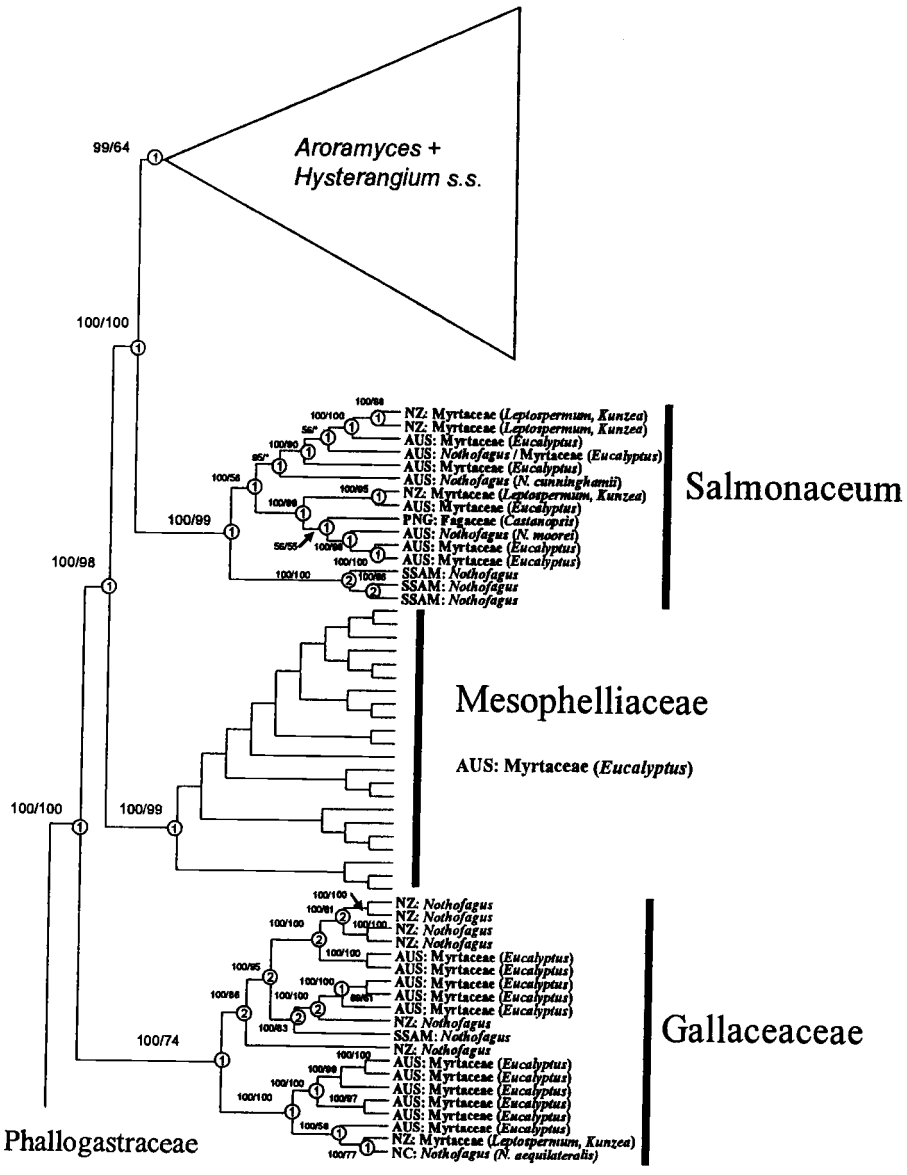

Fig. 3.9. Ancestral ectomycorrhizal host reconstructions for the basal part of the ECM-Hysterangiales. Clade names follow Fig. 3.1. Numbers on branches are nodal support (Bayesian posterior probability/ parsimony bootstrap values; * indicates no bootstrap support). Numbers in circles indicate the ancestral ECM host based on unweighted parsimony reconstructions using MacClade (1=Myrtaceae, 2=Nothofagaceae, 3=Pinaceae, 4=Fagaceae, 5=Dipterocarpaceae, 6=Caesalpinioideae, 7=Ericaceae, 8=Casuarinaceae). Taxon names were replaced by area codes, followed by presumable ectomycorrhizal host families. If known, more specific ectomycorrhizal host names are indicated in parentheses. If ectomycorrhizal hosts can not be identified to a single host family, more than one presumable ECM host family is indicated with a slash.

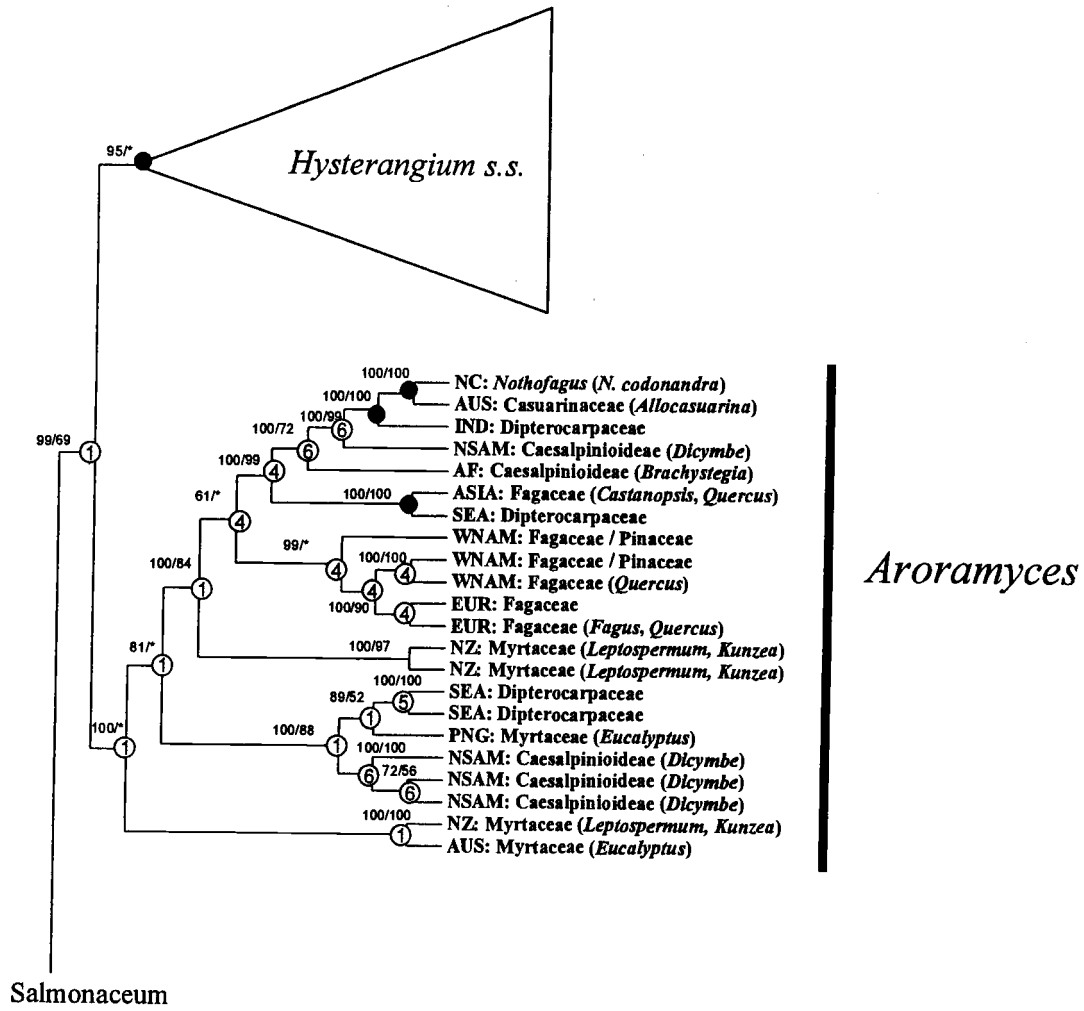

Fig. 3.10. Ancestral ectomycorrhizal host reconstructions for the *Aroramyces* clade. Clade names follow Fig. 3.1. Numbers on branches are nodal support (Bayesian posterior probability/ parsimony bootstrap values; * indicates no bootstrap support). Numbers in circles indicate the ancestral ECM host based on unweighted parsimony reconstructions using MacClade (1=Myrtaceae, 2=Nothofagaceae, 3=Pinaceae, 4=Fagaceae, 5=Dipterocarpaceae, 6=Caesalpinioideae, 7=Ericaceae, 8=Casuarinaceae). Nodes with black circles indicate ambiguous reconstructions. Taxon names were replaced by area codes, followed by presumable ectomycorrhizal host families. If known, more specific ectomycorrhizal host names are indicated in parentheses. If ectomycorrhizal hosts can not be identified to a single host family, more than one presumable ECM host family is indicated separated with a slash.

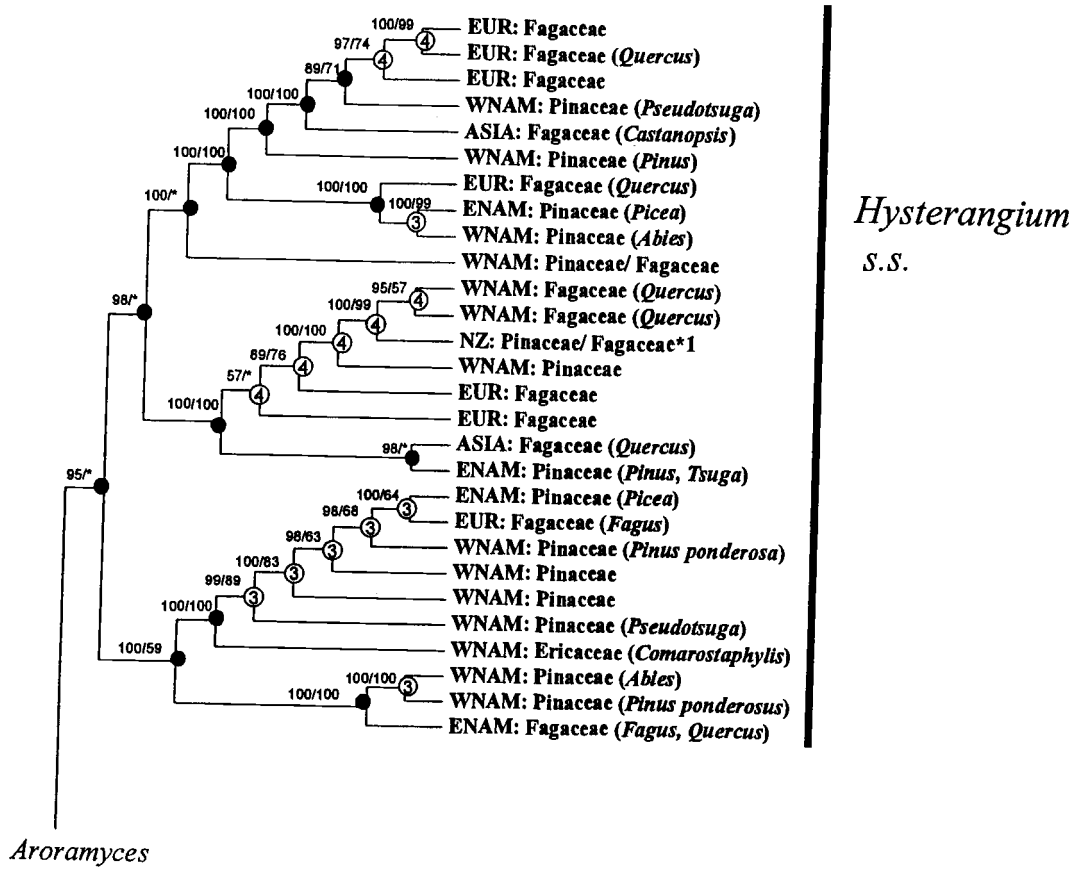

Fig. 3.11. Ancestral ectomycorrhizal host reconstructions for the *Hysterangium s.s.* clade. Clade names follow Fig. 3.1. Numbers on branches are nodal support (Bayesian posterior probability/ parsimony bootstrap values; * indicates no bootstrap support). Numbers in circles indicate the ancestral ECM host based on unweighted parsimony reconstructions using MacClade (1=Myrtaceae, 2=Nothofagaceae, 3=Pinaceae, 4=Fagaceae, 5=Dipterocarpaceae, 6=Caesalpinioideae, 7=Ericaceae, 8=Casuarinaceae). Nodes with black circles indicate ambiguous reconstructions. Taxon names were replaced by area codes, followed by presumable ectomycorrhizal host families. If known, more specific ectomycorrhizal host names are indicated in parentheses. If ectomycorrhizal hosts can not be identified to a single host family, more than one presumable ECM host family is indicated with a slash. *1 = One taxon from New Zealand was coded as polymorphic (Pinaceae or Fagaceae) because its association with introduced Pinaceae or Fagaceae was strongly suspected (see Materials & Methods).

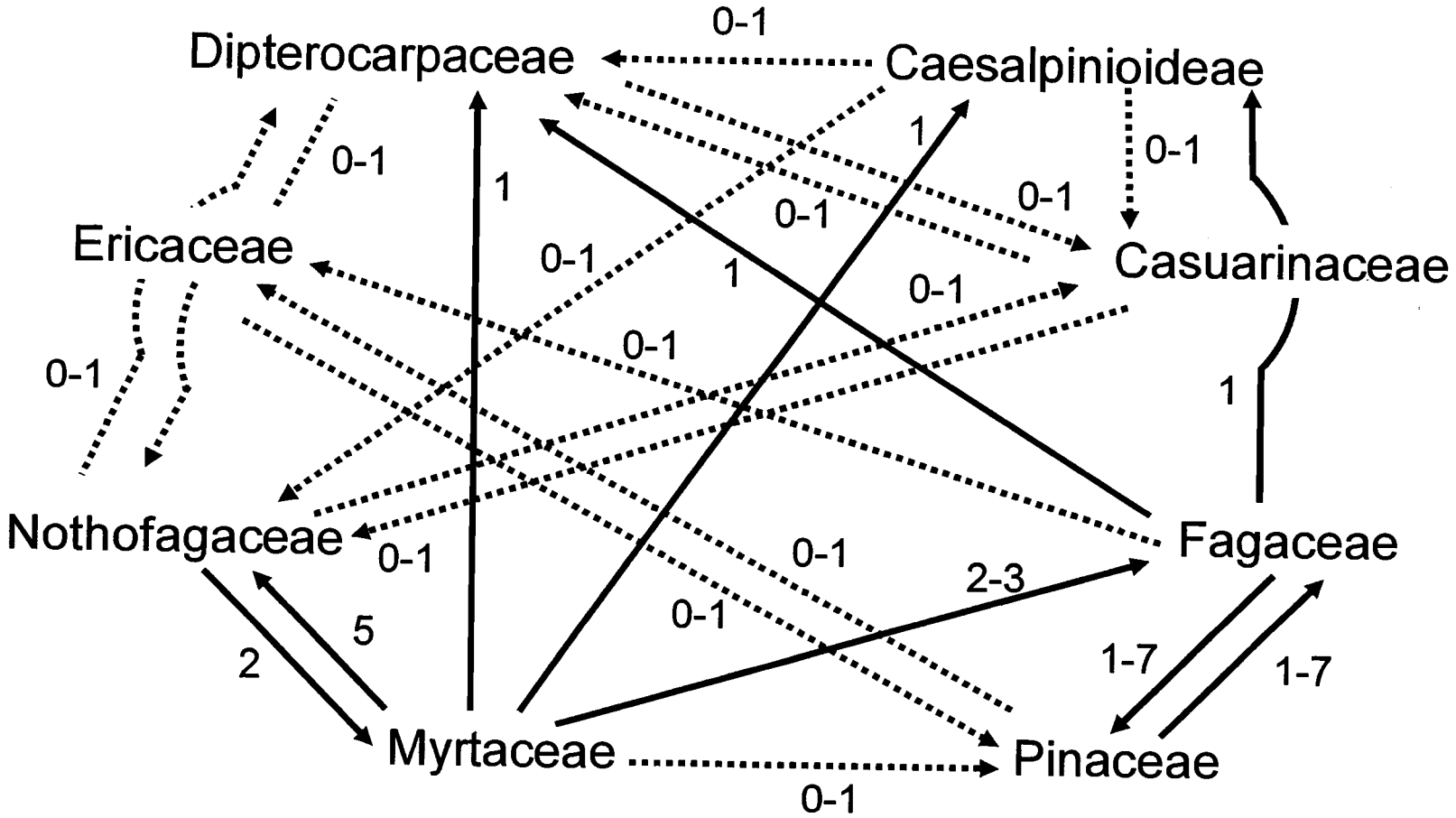

Fig. 3.12. Frequency of ectomycorrhizal host shifts based on unweighted parsimony reconstructions using MacClade. Arrows indicate the direction of host shift. Numbers on arrows indicate the minimum and maximum possible steps. Host shifts with possible 0 occurrences are indicated by dotted arrows. Ectomycorrhizal hosts are coded in 8 states, each state corresponding to host family.

DISCUSSION

Phylogeny and higher-level biogeographical patterns

The tree topology resulting from the phylogenetic analyses (Fig. 3.2) were consistent with the previous analyses, which used 3-gene sequences (nuc-LSU rDNA, mt-SSU rDNA, and *ATP6*) (Hosaka *et al.*, Chapter 2). All non-mycorrhizal taxa belong to the Phallogastraceae clade, and the basal split of the Phallogastraceae and the rest of the Hysterangiales were well-supported (Fig. 3.2). This suggests a single origin of the Hysterangiales ectomycorrhizal habit or though less parsimonious, parallel gains of ectomycorrhizal habit. It is noteworthy that no apparent loss of mycorrhizal habit was observed in the Hysterangiales. Multiple losses of ectomycorrhizal habit have been hypothesized to have occurred during the evolution of Homobasidiomycetes, as well as multiple gains (Hibbett *et al.*, 2000). These results do not support any losses of the ectomycorrhizal habit for the Hysterangiales.

The results of the dispersal-vicariance analyses using DIVA (Fig. 3.3) strongly suggest that the ectomycorrhizal lineages of the Hysterangiales (which is called ECM-Hysterangiales hereafter) originated in the Southern Hemisphere, namely Australia. Unfortunately, taxon sampling from the other Gondwanan regions, including Africa, India, and South America, is not extensive. Most sampling from the Southern Hemisphere was represented by taxa from Australia and New Zealand, which restrict our inferences of the biogeography of the Hysterangiales. Nonetheless, the tree

topology was well-supported showing the basal paraphyly of the Southern Hemisphere taxa. Northern Hemisphere taxa were restricted to more terminal clades, suggesting that range expansion of the Hysterangiales was the result of northward movement from the Southern Hemisphere. Although the results of DIVA analyses were inconclusive in the terminal clades due to ambiguous reconstructions of ancestral areas with numerous possible equally parsimonious combinations (Fig. 3.3), non-monophyly of Northern Hemisphere taxa is a strong indication that range expansion of the Hysterangiales to the Northern Hemisphere happened more than once. According to the DIVA reconstructions, the first range expansion to the Holarctic took place before the split of the *Aroramyces* and *Hysterangium s.s.* clades (Fig. 3.3). At least one more range expansion to the Holarctic took place in the *Aroramyces* clade, but the exact node, and numbers of this range expansion was unclear because of ambiguous reconstructions. Although these results indicate that some terminal clades could be explained by vicariance, the Hysterangiales phylogeny can not be explained strictly by vicariance.

The search for optimal area cladograms using TreeFitter (Fig. 3.6) showed that the optimal area cladograms based on Hysterangiales phylogeny were different from the hypothesized scenarios of Pangaean breakup (Fig. 3.1, 3.6). The analyses produced 13 equally parsimonious area cladograms, and none of them are identical to the geological scenario (Fig. 3.6). Two consistent patterns among 13 cladograms (sister relationships of Australia and New Zealand, and New Caledonia and India) are both incongruent with the geological scenario. The randomization tests showed that these

differences are statistically significant, again indicating the incongruence between strictly vicariant scenario and the Hysterangiales phylogeny. This incongruence could simply be explained by frequent dispersals during the history of the Hysterangiales evolution; however, extinction or the presence of unsampled lineages is also a possibility.

It must be pointed out that the searches for the optimal area cladograms implicitly assume that area relationships are hierarchical whereas in reality, area relationships could be reticulate (Ronquist, 1997). For example, reticulate area relationships could be produced by the disappearance of barriers between previously separated areas, such as establishment of land bridges. Because each node in phylogenetic trees might represent different geological times, the area relationships in different lineages could be the result of very different geological events. Of course, the presence of dispersal and/or extinctions (including unsampled lineages) could also obscure the area relationships. Therefore, apparent incongruence between geological area cladograms and the optimal area cladograms derived from Hysterangiales phylogeny does not mean that vicariance is not the main cause of Hysterangiales biogeography. In this case, examining more terminal clades independently could reveal vicariant patterns more clearly.

Lower-level biogeographical patterns

The incongruence of the Hysterangiales phylogeny and geological history is apparent when an entire Hysterangiales phylogeny is taken into account. The basal paraphyletic patterns of the Southern Hemisphere taxa (Fig. 3.2, 3.3) cannot be explained without implementing dispersal and/or extinctions, assuming that area relationships are hierarchical. Also several inconsistent area relationships observed in the Hysterangiales phylogeny no doubt affected the results of DIVA and TreeFitter analyses. For example, one node showed that a taxon from Papua New Guinea was a sister to Australian taxa (in the Salmonaceum clade; Fig. 3.2, 3.3), which is consistent to geological history, whereas the other node showed that Papua New Guinea and Southeast Asia are the sister areas (in the *Aroramyces* clade; Fig. 3.2, 3.3). Likewise, one node showed the sister relationship of New Caledonia and New Zealand (in the Gallaceaceae clade; Fig. 3.2, 3.3), which is consistent with the geological history, but the other node showed the New Caledonia + Australia sister relationship (in the *Aroramyces* clade; Fig. 3.2, 3.3). Again, limited taxon sampling from several areas restricts our biogeographical inferences. More intensive taxon sampling from those underrepresented areas might reveal that many of these incongruent patterns are simply due to insufficient taxon sampling from these areas.

Despite these inconsistencies, there are several nodes showing congruent patterns to geological history when more terminal nodes were examined. As described above, the sister relationships of Australia and New Guinea (in the Salmonaceum

clade), and New Caledonia and New Zealand (in the Gallaceaceae clade) are both congruent with geological scenario. The sister relationship of Australia and New Guinea is well-documented based on the geological record. Although a large area of New Guinea island has been submerged multiple times, Australia and New Guinea had a direct land connection until 30 MYA (million years ago) or even later (Sanmartín & Ronquist, 2004; McLaughlin, 2001; Hall, 1998). This area relationship is also supported by biogeographical studies of many organisms (summarized in Sanmartín & Ronquist, 2004). The sister relationship of New Zealand and New Caledonia, however, is more controversial. The most commonly cited geological pattern is that New Zealand and New Caledonia had been connected by the Norfolk Ridge until 30 MYA (Sanmartín & Ronquist, 2004; McLaughlin, 2001). However, several evidences suggest that New Zealand and New Caledonia, as well as the Norfolk Ridge, were submerged for a significant period of time from the Late Cretaceous to Mid Tertiary (McLaughlin, 2001; Pole, 1994). For this reason, it is sometimes claimed that the entire New Zealand flora was formed by transoceanic, long distance dispersal (Pole, 1994). Furthermore, not many biogeographical studies showed the sister relationship of New Zealand and New Caledonia. Several alternative hypotheses of geological scenarios have been proposed accordingly. One example includes the potential sister relationship between New Caledonia and New Guinea. It is not a mainstream hypothesis based on geological records, but some biogeographical patterns reflect this hypothesis (Swenson *et al.*, 2001b). As far as we know, area relationships seen in Hysterangiales phylogeny is one of a few examples showing the

sister relationship of New Zealand and New Caledonia, which is congruent to geological history.

The Phallogastraceae clade showed a pattern consistent with the Pangaea breakup (Fig. 3.2, 3.3). Although only a few taxa are included in this clade, the pattern clearly showed the split of the Northern and Southern Hemisphere taxa, which might correspond to the initial split of Pangaea into Gondwana and Laurasia ca. 180 MYA (McLaughlin, 2001; Hallam, 1994; Fig. 3.1). As described above, the Northern Hemisphere taxa in the ECM-Hysterangiales clade (Fig. 3.2) did not form a monophyletic group, so the simple Northern/Southern Hemisphere pattern could not be applied. However, area relationships observed in the *Aroramyces* clade (Fig. 3.3) might be a reflection of this geological event. The *Aroramyces* clade is very intriguing and an important clade for the biogeography of the Hysterangiales. It contains both Southern and Northern Hemisphere taxa, and it is the only clade containing African and Indian taxa. More importantly, area relationships within this clade seem to be congruent to the geological scenario of Pangaean breakup (Fig. 3.1b). Taxa from Africa, northern South America, India, New Caledonia, and Australia form a monophyletic group, which is consistent to the fact that all those areas were once united as a single continent Gondwana. The difference is that Africa and northern South America did not form monophyly, but the general patterns closely resemble the sequence of Pangaean breakup.

During the Permian to Early Jurassic period, Laurasia and Gondwana were connected tightly in the west, but separated in the east by the Tethys Sea (McLaughlin,

2001; Hallam, 1994). This supercontinent Pangaea began its initial split into Laurasia and Gondwana in the Early Jurassic ca. 180 MYA (Hallam, 1994; McLaughlin, 2001). Although a separation between North and South America was initially by only a shallow epicontinental seaway, it later became fully oceanic as the Hispanic Corridor (Hallam, 1994). North and South America became at least partially reconnected later with the formation of the proto-Caribbean in the Mid Cretaceous ca. 100 MYA (Hallam, 1994; Sanmartín & Ronquist, 2004). This connection was broken (in Mid Eocene or 50 MYA) and reestablished (late Tertiary ca. 15 MYA) sometimes later, creating barriers for biotic exchange in different periods of time (Hallam, 1994; Sanmartín & Ronquist, 2004). Therefore, the area relationship between North and South America could be considered one example of a reticulate relationship. For this reason, apparent paraphyly of both Northern and Southern Hemisphere taxa may not be due to dispersal or extinctions, but could correspond to the geological events of Northern and Southern continents split, which took place multiple times.

As discussed above, the area relationships that could correspond to the most ancient vicariant events could be observed in the Phallogastraceae clade and the *Aroramyces* clade, and possibly at the node of the *Aroramyces* and *Hysterangium s.s.* clades split (Fig. 3.3). The patterns are consistent with the initial split of Pangaea into Laurasia and Gondwana. If these range expansions to the Northern Hemisphere occurred through land connections during this time period, the origin of the Hysterangiales must be older than 180 MYA. Because of the reticulate area relationships observed in North and South America, the origin of the Hysterangiales

could be much younger even if the presence of land connections between North and South America is the only explanation for range expansions. However, the area relationships observed in the *Aroramyces* clade (Fig. 3.3) are also congruent with the vicariant events of the East and West Gondwana split, which occurred in the Late Jurassic or 160 MYA (Hallam, 1994; McLaughlin, 2001; Fig. 3.1). Although the connections of Africa, South America, and India to the Northern Hemisphere continents were later reestablished, Australia and New Caledonia remained isolated and have never been reconnected to any other continents. These patterns of area relationships are consistent with the age of the *Aroramyces* clade being at least 160 MYA, and that of the Hysterangiales being even older. Assuming the topology of *Aroramyces* clade is caused by the vicariant event of the East and West Gondwana split, the only geological event that could be responsible for the Northern and Southern Hemisphere splits observed in deeper nodes (in the *Aroramyces* clade and the basal split of the *Hysterangium s.s.* and *Aroramyces* clade; Fig. 3.3) is the initial break up of Pangaea, which took place 180 MYA. The subsequent reconnection and split of Northern and Southern America cannot be responsible for its vicariance because it took place much more recently, 100-50 MYA.

There are also several incongruent patterns to geological history in more terminal nodes. The most frequent pattern is the sister relationship of Australia and New Zealand. Generally, Australia and New Zealand are not considered sister areas. Several geological evidences suggest that New Zealand and New Caledonia were separated from Australia by 80 MYA, while Australia was still connected to South

America via Antarctica (McLaughlin, 2001; Hallam, 1994). If this is the case, we expect to see a sister relationship of Australia and South America, instead of Australia and New Zealand. Although southern South America was represented only in two clades in this study (in the Gallaceaceae and Salmonaceum clades; Fig. 3.3), the patterns in both clades showed that Australia and New Zealand are more closely related to each other than either one of them is to South America. If the phylogeny of the Hysterangiales were at least partially due to vicariance, the sister relationship of Australia and New Zealand, which is apparently incongruent to geological history, must be explained by several independent extinctions in southern South America, presence of unknown lineages still remaining unsampled from South America, or long distance dispersal between Australia and New Zealand. Many independent studies have shown that biogeographical patterns of Australia and New Zealand could only be explained by long distance dispersal between these areas (Pole, 1994; Knapp *et al.*, 2005). The fact that significant areas of New Zealand have been submerged during Late Cretaceous to Mid Tertiary (Pole, 1994; McLaughlin, 2001) also support the ideas that the presence of many if not all terrestrial organisms in New Zealand are due to long distance dispersal, most likely from Australia.

As mentioned above, the area relationships of New Guinea and New Caledonia to the other areas are inconsistent. The geologically inconsistent pattern of New Caledonia with Australia (in the *Aroramyces* clade; Fig. 3.3) is less surprising because neither New Caledonia nor southern South America were well-represented in this study, and the sister relationship of Australia and New Caledonia could be superficial,

simply due to a lack of taxon sampling from South America. The same thing could be true for the other geologically incongruent pattern involving New Guinea. The pattern of ((New Guinea, Southeast Asia), northern South America) is clearly incongruent to geological history. Of course implementing several missing lineages could explain this pattern, but the fact that one of the most intensively sampled areas, i.e. Australia, was not represented in this clade makes this explanation less likely. The origin of Southeast Asia is very complex. It is less controversial that the present Southeast Asia was once located along the northern periphery of Gondwana. The subsequent separations from Gondwana and its northward movement, which occurred multiple times from Devonian through Triassic periods, resulted in the formation of modern Southeast Asia (Metcalfe, 1998). Because the separation between New Guinea and Southeast Asia occurred prior to the initial breakup of Pangaea, it is unlikely that the sister relationship of these two areas are the result of vicariance. Currently New Guinea and Southeast Asia are geographically closely located. Although New Guinea and Southeast Asia currently do not have a direct land connection, numerous islands between those two areas could serve as the dispersal route for the Hysterangiales. The relationship of northern South America with New Guinea/Southeast Asia is of great interest. Long distance dispersal directly between those areas is unlikely considering the vast distance and the absence of an apparent dispersal route between these areas. Again, extinctions or unsampled lineages remain as a possibility, but it could be a result of ancient vicariant events, separation of East and West Gondwana (northern South America and New Guinea), followed by dispersal to Southeast Asia.

Biogeographical patterns for the Holarctic clade

In the *Hysterangium s.s.* clade, western North America appeared to be the important source area for the diversifications of the Holarctic Hysterangiales. Dispersal-vicariance analyses suggest that dispersal occurred from western North America to the other areas for clade 1, 2, and 4, possibly followed by vicariance (Fig. 3.4). In clade 2 and 4, biogeographical patterns are consistent with the scenario that dispersal happened from western North America to eastern North America, and to Europe. If this scenario is true, intercontinental dispersal between North America and Europe might be via the North Atlantic Land Bridge, which was available as a direct land connection until ca. 50 MYA (Sanmartín *et al.*, 2001). However, different geological events must have caused the vicariance for those two clades. In clade 2, eastern and western North America were shown to be sister areas, indicating that the initial dispersal barrier was established between North America and Europe, followed by the establishment of barrier between western and eastern North America (Fig. 3.4, 3.5b). This scenario is consistent with the disappearance of the North Atlantic Land Bridge in 50 MYA, and the secondary formation of the Rocky Mountains in 25 MYA (Sanmartín *et al.*, 2001). In clade 4, however, eastern North America was shown to be a sister clade to Europe, indicating that the initial dispersal barrier was established between western and eastern North America, followed by the establishment of barrier between eastern North America and Europe (Fig. 3.4, 3.5d). While the dispersal barrier between Europe and North America could be easily explained by a

disappearance of the North Atlantic land bridge, there are a couple of possibilities for the establishment of a barrier between western and eastern North America. Although the formation of the current Rocky Mountains occurred in the Late Oligocene or 25 MYA (Sanmartín *et al.*, 2001), the initial formation of the Rocky Mountains began right after the disappearance of the Mid-Continental Seaway, ca. 70 MYA. These early Rocky Mountains were completely eroded by 30 MYA, but could have served as an efficient barrier that caused vicariance of western and eastern North American taxa before the disappearance of the North Atlantic Land Bridge. Another possibility is the opening of the Mid-Continental Seaway that separated western and eastern North America from 100 to 70 MYA (Sanmartín *et al.*, 2001). Before the opening of the Seaway, those areas retained some land connections.

Reconstructions for clades 1 and 3 are more complicated. Both clades showed a sister relationship of western North America and Europe, and clade 3 also showed a sister relationship of eastern North America and Asia (Fig. 3.4), all of which could not be explained by simple vicariant events because these two areas have never been in a direct contact. The eastern North America-Asia disjunction pattern is usually explained by the scenario that taxa once widely distributed throughout the Holarctic went extinct in western North America and/or Europe, leaving the disjunct pattern (Xiang *et al.*, 1998, 2000). While many plant studies showed that this disjunct pattern was caused by dispersal via Beringia, followed by extinction in western North America, there is another possibility, that is, dispersal via the Atlantic land bridge followed by extinction in Europe. Because the most basal node of clade 3 was

reconstructed as widespread, excluding western North America, it is more consistent with the latter scenario.

Although most Holarctic disjunctions are emphasized for the eastern North America-Asia disjunct pattern, the western North America-Europe disjunct pattern was shown to be equally common (Sanmartín *et al.*, 2001). This is consistent in the Hysterangiales phylogeny where two clades showed a sister relationships of western North America and Europe (Fig. 3.4). In clade 1, one of the DIVA reconstructions showed the initial dispersal from western North America to Asia, most likely via Beringia, followed by vicariance. Later dispersal from western North America to Europe happened, which was followed by vicariance. While dispersal from western North America to Europe could be possible either via Beringia or North Atlantic Land Bridge, the former seems to be more likely because the closing of the North Atlantic Land Bridge took place prior to the initial closing of the Beringia, ca. 35 MYA (Sanmartín *et al.*, 2001). Therefore the branching order and DIVA reconstruction of clade 1 are consistent with dispersal from western North America to Palearctic via Beringia in two different times (Fig. 3.4, 3.5a). The first dispersal probably took place before the closing of Beringia 35 MYA, followed by vicariance. The second dispersal took place before the subsequent closing of Beringia, which was less than 10 MYA, followed by extinction in Asia. Paraphyly of western North America in clade 1 also indicates that western North America served as a dispersal source more than once. In clade 3, the direction of dispersal appears to be opposite, from Europe to western North America. Again dispersal to western North America was possible either via

Beringia or Atlantic Land Bridge. In this case, however, neither of these possibilities could be ruled out (Fig. 3.4, 3.5c).

Summary for the biogeographical patterns of the Hysterangiales

These patterns of area relationships suggest that 1) the ECM-Hysterangiales originated in the Southern Hemisphere; 2) Range expansions to the Northern Hemisphere happened multiple times; 3) Higher-level topology was not congruent with a strict vicariant scenario; 4) Some more terminal nodes are congruent with the vicariant scenario whereas others are not; 5) It is likely that several different dispersal routes and vicariant events are responsible for the biogeographical patterns of Holarctic Hysterangiales. Based on those findings, one of the most intriguing questions is whether the Hysterangiales expanded their range through land connections or by ways of transoceanic dispersal. If the range expansion was via land connections, it favors Paleozoic to early Mesozoic origin of the Hysterangiales, when all main continents were united as a supercontinent Pangaea. If transoceanic dispersal is possible, the origin of the Hysterangiales could be much more recent.

Some of recent studies suggest that the gomphoid-phalloid clade, to which the Hysterangiales belongs, could be one of the basal clades of the Homobasidiomycetes, the major taxon of mushroom-forming fungi (Binder & Hibbett, 2002; Hibbett *et al.*, 1997, 2000; Moncalvo *et al.*, 2002; Hibbett & Binder, 2002; Hibbett, 2004; Lutzoni *et*

al., 2004). Although a Precambrian origin of major fungal lineages is postulated by some molecular clock studies (Heckman *et al.*, 2001; Wang *et al.*, 1999; Hedges *et al.*, 2004), the age estimates of the Basidiomycota and the lineages within it, including the Homobasidiomycetes, vary significantly. For example, Heckman *et al.* (2001) estimated that the Basidiomycota originated in ca. 1200 MYA, whereas estimates by Berbee & Taylor (2001) indicated that the origin of the Basidiomycota is ca. 550 MYA. Importantly, both results appear to suggest that the origin of the Homobasidiomycetes predates the initial breakup of Pangaea or 180 MYA (Fig. 3.1). These data are consistent with the potentially ancient and vicariant origin of the Hysterangiales.

Fossil records of Fungi

Fossil records give us important clues for understanding the ancient fungal flora. While there are numerous fossils of fungal spores available, it is very difficult to correlate them to modern taxa. Fossils with more phylogenetically informative characters, such as fruiting bodies, are much rarer. Nonetheless, Paleozoic or an even older origin of the Kingdom Fungi has little controversy. Fungal fossils from the Proterozoic era are well-documented by Butterfield (2005), but the phylogenetic position of these fossils is difficult to evaluate, and some of them may not even belong to fungi. From the Paleozoic era, lichen-like fungi (600 MYA; Yuan *et al.*, 2005),

arbuscular mycorrhizal fungi (460 MYA; Redecker *et al.*, 2000), perithecial ascomycetes (400 MYA; Taylor *et al.*, 1999, 2005) have been documented, but these are all non-Basidiomycota. One controversial Basidiomycota fossil is from 290-million-year-old fungal hyphae (Dennis, 1970). The hyphae have distinct structure known as clamp connections, which are known only from Basidiomycota. However, it cannot be assigned to any specific group of Basidiomycota.

The oldest fossil records for the Homobasidiomycetes are from the Cretaceous period, or ~100 MYA (Hibbett *et al.*, 1997a; Poinar & Brown, 2003). These fossils are preserved in amber, and their morphological characters are exceptionally well preserved. Fossils of *Archaeomarasmius* (Hibbett *et al.*, 1997a) have a well-defined cap, gills, and stalk, all of which can be found in many modern agaric mushrooms. However, it has been shown that morphology of gilled mushrooms evolved multiple times (Hibbett *et al.*, 1997a). Therefore, the exact phylogenetic position of *Archaeomarasmius* remains uncertain. Even in a gomphoid-phalloid clade, to which Hysterangiales belongs, there are taxa forming gills, i.e., *Gloeocantharellus*. Morphologically, however, it is quite different from *Archaeomarasmius*, and it is probably safe to conclude that *Archaeomarasmius* does not belong to a gomphoid-phalloid clade. Phylogenetic position of *Palaeoclavaria* (Poinar & Brown, 2003) is more problematic. Fruiting body morphology of *Palaeoclavaria* is pyriform to club shaped, which is even simpler than gilled mushrooms. Club-shaped morphology can also be seen in several different lineages in Homobasidiomycetes phylogeny (Hibbett *et al.*, 1997b; Hibbett, 2004). One of the extant taxa forming club-shaped morphology

is the genus *Clavariadelphus*, which belongs to a gomphoid-phalloid clade. A possibility of *Palaeoclavaria* being a close relative of *Clavariadelphus* cannot be discarded, but is difficult to evaluate. There are much more recent fossils of Geastraceae (earthstar), which also belongs to the gomphoid-phalloid clade and therefore is closely related to the Hysterangiales, from the Miocene (Megallon-Puebla & Cevallos-Ferriz, 1993).

These fossil records cast no doubt about the existence of fungi in the Paleozoic era. In terms of the Hysterangiales, however, scarce fossil records of mushroom-forming fungi give us little insight into the ancient flora. Based on the fossil records, an Early Cretaceous or older origin of Homobasidiomycetes is likely, but no direct evidence suggests the time of origin for the Hysterangiales.

Age estimates for the Hysterangiales

Because neither biogeographical patterns nor fossil records could distinguish the possibility of ancient (Paleozoic to Early Mesozoic) vs. more recent origin of the Hysterangiales, the age estimate based on synonymous substitution rates could serve as one indication. Even with the fastest rate of synonymous substitution rate, – the most conservative age estimate – the age of the Hysterangiales was estimated as Paleozoic (260 MYA or older; Fig. 3.8). 260 MYA is corresponding to the Permian, when the Pangaea was formed tightly and a direct land connection was available in the

West Pangaea (McLaughlin, 2001; Hallam, 1994). Synonymous substitution rates for the Eurotiomycetes were shown to be $0.9\sim16.7 \times 10^{-9}$ (Kasuga *et al.*, 2002), so substitution rates obtained in this study were generally slower. Although the fastest rate of substitution rates were used to estimate node ages for this study, the range of substitution rates indicate that the origin of the Hysterangiales could be as old as Precambrian. Although this estimate is probably too old, the Paleozoic origin of the Hysterangiales, sometime between the Cambrian and Permian periods, was strongly suggested by this analysis.

One of the weaknesses of this analysis may be insufficient character and taxon sampling. Taxon sampling from the kingdom fungi was focused on Ascomycota and Basidiomycota, two of five major phyla of the kingdom. Even within each phylum, taxon sampling was very sparse, representing only several major clades and perhaps leading to less than desirable estimates. Having more taxa in the dataset would certainly give us more accurate estimates. Character sampling could be even more critical. Although a whole *RPB2* gene is composed of ca. 3000 base pairs, only ca. 800 base pair region was used for this study. Collecting more characters is desirable because having small numbers of characters can lead us to less accurate estimates with low statistical power (Nei *et al.*, 2001). Another issue is the saturation of synonymous sites. Exactly how critical it is for this study not to consider the saturation is unclear.

Despite those potential problems, congruence of the estimated ages and vicariant patterns provide convincing evidence. For example, the age estimates for potential vicariant nodes for the Laurasia/Gondwana breakup were demonstrated to be

ca. 160 MYA for both the Phallogastraceae clade and the split of the *Hysterangium s.s.* and *Aroramyces* clade (Fig. 3.8, left). Given the conservative substitution rate used for this estimate, they could be congruent with the initial Pangaea breakup, which happened ca. 180 MYA (McLaughlin, 2001). The sister relationship of New Zealand and New Caledonia (Gallaceaceae clade) was congruent with geological history, and the node age was estimated to be ca. 50 MYA, which could also be congruent with the geological record of 30 MYA (Sanmartín & Ronquist, 2004; McLaughlin, 2001; Fig. 3.8, right). If these age estimates are accurate, the presence of New Zealand in the Salmonaceum clade must be a result of long distance dispersal because New Zealand was separated from Gondwana before the split of Australia and South America. Alternatively, applying slower substitution rates suggest that the split of Australia and South America could have taken place 100 MYA or even older. Most biogeographical studies refer to the (New Zealand, (Australia, southern South America)) as a sequence of eastern Gondwana breakup. Southern South America is thought to retain a direct land connection to Antarctica until ca. 30 MYA (McLaughlin, 2001). However, Hallam (1994) suggested that there was a seaway separating the southern tip of South America from Antarctica in the Jurassic. Because a land connection between South America and Antarctica is well-documented throughout the Cretaceous until 30 MYA (McLaughlin, 2001), there should not be a dispersal barrier between South America and Australia for the organisms with a Cretaceous origin. However, for the organisms with pre-Jurassic origin, probably including the Hysterangiales, this seaway could

serve as an effective barrier, causing vicariance for taxa from South America and Australia.

Several incongruent patterns were also observed. Varied age estimates for the nodes showing an Australia/New Zealand sister relationship indicate that at least some nodes are not the result of vicariance. New Zealand (and New Caledonia) was separated from Australia ca. 80 MYA (Sanmartín & Ronquist, 2004; McLaughlin, 2001), so the two nodes in the Salmonaceum clade (Fig. 3.8, left) are probably too young to be explained by vicariance even when slower substitution rates are applied, while the nodes in the Gallaceaceae clade was estimated to be older than 100 MYA (Fig. 3.8, left), old enough to be explained by vicariance. A biogeographical pattern observed in the *Aroramyces* clade is very intriguing because it closely resembles the sequence of the Pangaea breakup. The age estimates for this clade, however, were not completely congruent to this scenario. The age estimate of ca. 120 MYA for the Laurasia/Gondwana split appears to be inconsistent to geological history (ca. 180 MYA), and the node consistent to the East and West Gondwana split was estimated to be ca, 100 MYA, seemingly too young for the geological record of ca. 160 MYA. However, the split of India and the rest of East Gondwana (130~80 MYA according to geological records; Fig. 3.1b) appears to be consistent to vicariance. The split of New Caledonia from Australia (ca. 80 MYA according to geological records; Fig. 3.1b) may be too young to be explained by vicariance, even when slower substitution rates are applied. Because Australia and New Caledonia have been isolated from all other continents since separation from Gondwana 80 MYA (for New Caledonia) or 50

MYA (for Australia), the only explanation for the young age for the Australia/New Caledonia split is by long distance, transoceanic dispersal. One caution should be made, however, for the age estimates for the *Aroramyces* clade because this clade is characterized by relatively long branches compared to other lineages (Fig. 3.2), which indicate that substitution rates are higher in this clade. It has been demonstrated that when the numbers of substitutions are large, the method developed by Nei & Gojobori (1986) tend to underestimate the numbers of synonymous substitutions (Nei & Gojobori, 1986), which could lead to the underestimate of the age estimate for the *Aroramyces* clade.

A potential vicariant event caused by the closing of the North Atlantic Land Bridge was observed in clade 2 and 3 (Fig. 3.4, 3.5). The ages for the Europe and North America split in those clades were estimated to be ca. 50 MYA for both clade 2 and 3 (Fig. 3.8, left). This estimate is consistent with the closing of the North Atlantic Land Bridge, ca. 50 MYA (Sanmartín *et al.*, 2001). Therefore, these age estimates are not incongruent to the scenarios in Fig. 3.5. The other intercontinental disjunction between North America and Europe also showed consistent patterns with vicariance. The age estimate for the Europe and western North America split in clade 1 was ca. 30 MYA (Fig. 3.8, left), which could be corresponding to the initial closing of Beringia (35 MYA; Sanmartín *et al.*, 2001). Because the most basal split in clade 1 was estimated to be ca. 65 MYA (Fig. 3.8, left), it could correspond to the closing of the North Atlantic Land Bridge. However, the scenario of multiple dispersals to Asia via Bering Land Bridge depicted in Fig. 3.5a should also be considered.

The age estimate for the intercontinental disjunction between eastern North America and Asia in clade 3 was ca. 50 MYA (Fig. 3.8, left), which is old enough to be explained by the closing of the North Atlantic Land Bridge (Sanmartín *et al.*, 2001). It is very interesting because the same disjunct pattern for plants is usually considered vicariance due to the closing of the Beringia, and the origin for this disjunction lies between the Miocene and Pliocene, less than 20 MYA (Xiang *et al.*, 2000). The same disjunct pattern of animals, on the other hand, appears to have happened during earlier geologic periods, in the Late Cretaceous to Early Tertiary (70-45 MYA), and probably is due to the closing of the North Atlantic Land bridge (Sanmartín *et al.*, 2001). A similar age estimate for animal and the Hysterangiales disjunct pattern as well as the DIVA reconstructions (Fig. 3.4, clade 3) indicate that the disjunction pattern of eastern North America-Asia for the Hysterangiales is probably due to the closing of the North Atlantic Land Bridge, followed by extinction in Europe. The ages for the intercontinental (Europe and eastern North America) and continental (eastern and western North America) disjunction in clade 4 were estimated to be ca. 20 MYA. This pattern could be explained by a vicariance caused by the secondary formation of the Rocky Mountains (25 MYA; Sanmartín *et al.*, 2001), followed by a transoceanic dispersal from eastern North America to Europe (Fig. 3.5d).

These age estimates are somewhat crude and represent initial estimates or hypotheses regarding the origin and diversification of the Hysterangiales. More character and taxon sampling are necessary to obtain more accurate estimates.

Although a Paleozoic origin of the Hysterangiales was consistently demonstrated based on the synonymous substitution rates of *RPB2*, applying the averaged synonymous substitution rate of Kasuga *et al.* (2002) resulted in a Cretaceous origin (Table 3.2; Fig. 3.8, right). The substitution rate of Kasuga *et al.* (2002) is based on different genes and distantly related group of fungi (Eurotiomycetes), so the rate might not be directly applicable for the Hysterangiales. However, the alternative scenario of a Cretaceous origin of the Hysterangiales should not be discarded at this time. For future analyses, it will be important to document if different genes show consistent age estimates for the Hysterangiales and to incorporate additional fossil calibration points.

Ectomycorrhizal habit of the Hysterangiales

Another piece of evidence for inferring the origin of the Hysterangiales is in its ectomycorrhizal association with host plants. A single origin of the ectomycorrhizal habit inferred from the Hysterangiales phylogeny (Fig. 3.2) indicates that the ancestor of the ECM-Hysterangiales was also an ectomycorrhizal fungus. Therefore, the ancestor of the ECM-Hysterangiales must have associated with particular plants for forming ectomycorrhizae. As far as we know, all species of Hysterangiales, except the taxa in the Phallogastraceae clade, are obligate ectomycorrhizal species. As ectomycorrhizal fungi, species of Hysterangiales form symbiotic relationships with host trees. This relationship is considered mutually beneficial. That is, fungi form

ectomycorrhizae on plant roots, and obtain carbon from host plants, and plants obtain water and other important nutrients via fungal hyphae (Halling, 2001; Hacskaylo, 1971). In contrast, saprobic fungi obtain nutrients from dead organic materials, such as woody debris. A generally accepted idea is that ectomycorrhizal fungi have been derived from saprobic fungi (Hacskaylo, 1971; Malloch, 1987). Hibbett *et al.* (2000) concluded based on the phylogeny of Homobasidiomycetes and character state reconstructions that the ectomycorrhizal habit was gained multiple times independently in many different fungal lineages.

The Hysterangiales form ectomycorrhizae with several plant families. Although host range varies, both in conifers and angiosperms, any one species of Hysterangiales only associates with hosts from one plant family and often only one genus or species (Castellano, 1990a, 1999). Major ectomycorrhizal host families for Hysterangiales include Pinaceae, Myrtaceae, Fagaceae, and Nothofagaceae. There are some examples of Hysterangiales associated with Dipterocarpaceae, Ericaceae, Casuarinaceae, and caesalpinioid legumes (Caesalpinioideae) (Castellano, 1990a, 1999; Castellano & Beever, 1996; Castellano *et al.*, 2000). This relative host specificity of species of Hysterangiales ectomycorrhizal systems enables us to assess the historical host-fungus associations, such as host-tracking vs. host-shifting, and gives us clue to the historical biogeography of Hysterangiales.

Frequent host shifts are obvious in Hysterangiales evolution (Fig. 3.9~3.12). Of particular interest is that many of the basal nodes were reconstructed as Myrtaceae (Fig. 3.9). Reconstructions also showed several host shifts from Myrtaceae to other

ectomycorrhizal plant families (Fig. 3.9, 3.10, 3.12). This is in contrast from the traditional view that *Nothofagus* was considered an ancestral host for many ectomycorrhizal fungi in the Southern Hemisphere, and host shifts to Myrtaceae happened more recently as Myrtaceae (especially *Eucalyptus*) expanded its distribution range (Horak, 1983; Moyersoen *et al.*, 2003). In this context it is very interesting to point out that host shifts from *Nothofagus* to Myrtaceae were also observed though with less frequency (Fig. 3.12). Importantly, this and the host shift between Fagaceae and Pinaceae are the only bidirectional host shifts that were reconstructed unambiguously. Another important aspect is that host shifts are not necessarily between two closely related groups of plants. For example, no host shifts between two very closely related plant families, Nothofagaceae and Fagaceae, were observed (Fig. 3.12). On the other hand, host shifts between very distantly related plants, e.g., conifers (Pinaceae) and angiosperms (such as Fagaceae), were frequently observed. This is a good indication that the phylogenies of the Hysterangiales and its ectomycorrhizal host plants do not follow cospeciation patterns.

The host association closely correlates with current geographic distribution. That is, most Northern Hemisphere species associate with hosts from the Fagaceae or Pinaceae and Southern Hemisphere species associate with hosts from the Myrtaceae or Nothofagaceae. This indicates that the Hysterangiales expanded their distribution by frequently changing ectomycorrhizal hosts, and this range expansion was not so confined by host distribution. This might also indicate that there are unobserved host shifts during the evolution of the Hysterangiales. For example, although the ancestral

ectomycorrhizal host for the Hysterangiales was unambiguously reconstructed as the Myrtaceae (Fig. 3.9), it was only based on extant plants, so Myrtaceae may not be the most ancestral host for Hysterangiales. An association with completely different plants, for example extinct conifers or other lineages, remains as a possibility. Martin *et al.* (2001) studied a biogeography and ectomycorrhizal association of the genus *Pisolithus* (Boletales, Homobasidiomycetes), and suggested that the genus originated before the breakup of Pangaea in the Triassic, and the ancestor was a generalist mycorrhizal symbiont. Although no definite evidence supports this hypothesis, the same conclusion may apply to the Hysterangiales.

Parsimony-based reconstruction methods are sometimes criticized because they are dependent on a single tree topology, and do not account for statistical uncertainty involved in ancestral inferences (Huelsenbeck & Bollback, 2001; Huelsenbeck *et al.*, 2001, 2002; Lutzoni *et al.*, 2001). In this study, however, a parsimony based reconstruction was the only possible method. One problem is the numbers of character states necessary for reconstructions. The computer program Multistate can deal with up to 6 states, but the numbers of states (= plant families) necessary for Hysterangiales are 8. This number could be reduced by using ordinal level classification of plants, for example, but 5 or maybe 4 states are minimally required. 4 and 5 states models require 12 and 20 parameters, respectively, which indicate that at least 120 and 200 taxa are required to have enough statistical power (Multistate manual; Pagel, 2002). This was indicated in an initial attempt of Multistate

reconstructions, which showed inconsistent reconstructions for any nodes, and most nodes were reconstructed only ambiguously (data not shown).

Ancestral host reconstructions using the current ectomycorrhizal associations strongly indicated that Myrtaceae as the most ancestral ectomycorrhizal host for the Hysterangiales (Fig. 3.9). Given its obligate association with host plants, the most parsimonious explanation is that the Hysterangiales is as old as or younger than Myrtaceae. However, the age of Myrtaceae is estimated to be younger than 100 MYA (Wikström *et al.*, 2001), more than 150 MYA younger than the conservative age estimate for the Hysterangiales. As discussed below, the age estimates for any ectomycorrhizal plants that are known to be associated with the Hysterangiales are significantly younger than the conservative age estimate of the Hysterangiales. It is important to point out that while each species of the Hysterangiales are associated with specific ectomycorrhizal host trees, host trees can be associated with many other ectomycorrhizal fungi. Therefore, it is an apparent paradox that the Hysterangiales, obligate ectomycorrhizal fungi, originated prior to their host plants. Although errors involved in the age estimates for the Hysterangiales and/or plants could be crucial, this might actually reflect reality. As discussed above, a critical factor is that ancestral host reconstructions could only be based on extant ectomycorrhizal associations, and no information is available for extinct plants. Therefore an alternative scenario is that the Hysterangiales originated prior to its extant hosts and initially associated with different plants, or the Hysterangiales gained its ectomycorrhizal habit independently multiple

times. More detailed biogeography of extant ectomycorrhizal plants as well as extinct plants is discussed below.

Biogeography of Myrtaceae

The Myrtaceae belongs to the order Myrtales. Within the Myrtaceae, ectomycorrhizal hosts for the Hysterangiales are restricted to the subfamily Leptospermoideae, including *Eucalyptus*, *Leptospermum*, and *Kunzea*. All these genera are restricted in Australasia (Sytsma *et al.*, 2004). Species of Hysterangiales associated with Leptospermoideae are mostly restricted to more basal clades, and ancestral host reconstructions strongly suggest that the Myrtaceae to be the most ancestral ectomycorrhizal host for the Hysterangiales.

Eucalyptus is the largest genus for this family, containing more than 600 species (Ladiges *et al.*, 2003). The center of distribution for the *Eucalyptus* lies in Australia, but it is also distributed in Timor, New Guinea, Sulawesi (Indonesia), and Mindanao (Philippines) (Ladiges *et al.*, 2003). No extant species of *Eucalyptus* occurs in New Zealand although fossil records suggest that *Eucalyptus* exist in New Zealand until Pleistocene (Lee *et al.*, 2001). Fossils of *Eucalyptus*-like pollens are also recorded from South America (Rozefelds, 1996). The other genera of Leptospermoideae, *Leptospermum* and *Kunzea*, are also distributed only in Australasia

(Mabberley, 1997), but species of Hysterangiales associated with these genera are known only from New Zealand so far.

The oldest fossil records for Myrtaceae-like pollens are from Santonian or 86 MYA (Herngreen 1975; Muller 1981; Rozefelds, 1996), but the records were from outside of Australia (Borneo). The first appearance of Myrtaceae fossils in Australia is from Mid Paleocene or 60 MYA (Rozefelds, 1996). The oldest fossils for *Eucalyptus*-like pollens are from Late Paleocene or ca. 55 MYA (Rozefelds, 1996). There are also fossil records of *Eucalyptus*-like fruits from the middle Eocene or 48 MYA from central Australia (Greenwood, 1991). All these records indicate that diversifications of myrtaceous plants took place in the Cretaceous and later. Although Leptospermoideae was shown to be paraphyletic, molecular clock estimates showed that the age of the clade containing *Eucalyptus*, *Leptospermum*, and *Kunzea* is not older than 80 MYA (Sytsma *et al.*, 2004). Other workers (Wikström *et al.*, 2001; Schneider *et al.*, 2004) independently showed that the age of Myrtales is 107 MYA or younger. These estimates are not based on the fossil calibration of myrtaceous plants, so the age of Myrtales was not constrained. All those estimates are in agreement with the late Cretaceous to more recent origin of *Eucalyptus*, *Leptospermum*, and *Kunzea*, in apparent conflict with the Paleozoic origin of the Hysterangiales inferred from the synonymous substitution rates.

Biogeography of *Nothofagus*

Most species of Nothofagaceae-associated Hysterangiales belong to one of the Gallaceaceae clade. Although parsimony-based reconstructions strongly showed that Myrtaceae is the ancestral ectomycorrhizal host for the Hysterangiales, one could hypothesize that the Nothofagaceae is the alternative ancestral ectomycorrhizal host for the Hysterangiales. As discussed above, some authors hypothesized that *Nothofagus* is the ancestral host for many ectomycorrhizal fungi, and host shifts to the Myrtaceae took place relatively recently as the Myrtaceae expanded its distribution range (Horak, 1983; Moyersoen *et al.*, 2003).

Nothofagaceae is a monotypic family, containing the genus *Nothofagus*. It belongs to the order Fagales, along with other families such as Fagaceae, Betulaceae, Juglandaceae, Myricaceae, and Casuarinaceae. *Nothofagus* used to be considered a sister genus to *Fagus*, and classified in the family Fagaceae. However, molecular phylogenetic studies showed that *Nothofagus* and Fagaceae are not sister groups (Manos & Steele, 1997; Hilu *et al.*, 2003). Interestingly, host shifts between *Nothofagus* and Fagaceae were not observed despite of their close phylogenetic affinities (Fig. 3.12).

The genus *Nothofagus* (southern beech) has been a prime subject of biogeographical studies (Hill, 2001; Hill & Jordan, 1993; Linder & Crisp, 1995; Manos, 1997; Poole, 2002; Setoguchi *et al.*, 1997; Swenson & Hill, 2001; Swenson *et al.*, 2000, 2001a, b). Its distribution is limited to the Southern Hemisphere: Australia,

New Zealand, New Caledonia, New Guinea, and southern South America, all of which were once united as the supercontinent Gondwana. This distribution pattern strongly suggests that the current distribution of *Nothofagus* is due to vicariance, breakup of Gondwana. The results of the recent studies, however, were at least partially inconsistent with vicariance alone being a sufficient mechanism to explain the current distribution and evolution of *Nothofagus* (Manos, 1997, Swenson & Hill, 2001; Swenson *et al.*, 2001a, b; Knapp *et al.*, 2005). Some long distance dispersal events and many extinction events had to be implemented to explain the pattern of subgeneric relationships and distributions.

Despite of its phylogenetic contradiction to a strict vicariance scenario, there is little doubt about the presence of *Nothofagus* in the Cretaceous period based on numerous fossil records (Dettmann *et al.*, 1990; Hill, 2001; Poole, 2002). Pollen records of *Nothofagidites senectus*, which occurs widely from the present Western Antarctica to Australia, are known from early Campanian (Dettmann *et al.*, 1990). A close relationship between *Nothofagidites* and *Nothofagus* is strongly suggested based on pollen morphology, but no extant *Nothofagus* species has an equivalent pollen type. It is therefore described as an ancestral type (Dettmann *et al.*, 1990). The ancestral type of *Nothofagidites* pollens are known from South America (Maastrichtian~), Western and Southern Antarctica (Campanian~), and New Zealand (Campanian~), but are not known from New Guinea and New Caledonia (Dettmann *et al.*, 1990). The oldest pollen records (brassii type) in New Guinea occur in the Miocene, but no

undisputable fossil records are available so far from New Caledonia (Dettmann *et al.*, 1990).

By the late Cretaceous (Maastrichtian) to early Tertiary, most pollen fossils can comfortably be classified into four pollen types, which are equivalent to extant *Nothofagus* pollen types. Therefore, *Nothofagus* originated in the late Cretaceous, and subgeneric diversification was completed by the early Tertiary (Dettmann *et al.*, 1990; Swenson & Hill, 1990). No fossil records or extant *Nothofagus* species occur in Africa and India (Dettmann *et al.*, 1990; Hill, 2001), indicating that *Nothofagus* originated after the separation of western and eastern Gondwana. Although there are some pollen records from the Northern Hemisphere, most records appear to be misidentified (Dettmann *et al.*, 1990). All those evidences strongly suggest the Cretaceous origin of *Nothofagus*. However, no evidence suggests a Jurassic or older origin of *Nothofagus*, which is again in conflict to the hypothesized origin of the Hysterangiales in the Paleozoic era.

Biogeography of Fagaceae

Most species of Fagaceae-associated Hysterangiales belong to one of the terminal clades (Fig. 3.2, 3.10, 3.11). Therefore it is unlikely that the Fagaceae is the ancestral ectomycorrhizal host for the Hysterangiales. In fact, the Fagaceae was indicated as one of the most derived hosts for the Hysterangiales, having been derived

either from the Myrtaceae or Pinaceae (Fig. 3.12). Some species of the Hysterangiales are associated with Casuarinaceae, the other member of the Fagales, and they are also restricted to more terminal clades within the Hysterangiales (Fig. 3.10). However its close relationship to the Nothofagaceae, abundant fossil records and availability of molecular clock age estimates for the order make it relevant to discuss the biogeography of the Fagaceae.

Currently, this family represents one of the most dominant ectomycorrhizal trees in the Northern Hemisphere. Its distribution extends partially to the Southern Hemisphere, including New Guinea, northern South America (Colombia), and northern Africa, but not to Australia, New Zealand, and southern South America (Mabberley, 1997). *Quercus* is the most diverse genus of the family, and most Fagaceae-associated species of Hysterangiales have been collected from *Quercus* forests. Although records are still limited, *Fagus* and *Castanopsis* are another important ectomycorrhizal host for the Hysterangiales.

The center of diversity for this family lies in Asia and North America. Based on biogeographical analyses coupled with fossil records, Manos & Stanford (2001) suggested an Asian origin of *Fagus*, followed by migrations to Europe and North America. The origin of *Quercus* remained uncertain, but one sub-group, including a currently widely-distributed section *Quercus*, appears to have originated in North America, with subsequent migrations to Asia and Europe (Manos & Stanford, 2001; Manos *et al.*, 1999). The other sub-group including section *Cerris* appears to have originated in Asia with subsequent migrations to North America (Manos & Stanford,

2001; Manos *et al.*, 1999). The origin of extant genera of this family and most intercontinental migrations described above are believed to have occurred during the Tertiary (Wikström *et al.*, 2001; Manos & Stanford, 2001; Manos *et al.*, 1999). No evidence suggests a Southern Hemisphere origin of the Fagaceae, which is also congruent to the results of ancestral host reconstructions showing Fagaceae as the derived state.

The oldest fossil of Fagales is usually attributed to *Protofagacea* from the late Cretaceous or 87 MYA from Georgia, USA (Herendeen *et al.*, 1995). Because of its unique pollen morphology, which is not observed in any extant fagaceous plants, the exact placement of *Protofagacea* in the Fagales phylogeny is uncertain (Wikström *et al.*, 2001; Herendeen *et al.*, 1995). Wikström *et al.* (2001) used the fossil records of *Protofagacea* as a calibration for molecular clock estimates of major angiosperm lineages. The calibration point was treated as a fixed age for the split of Fagales and Cucurbitales. Because of the uncertainty of the phylogenetic position of *Protofagacea*, their calibration could be very conservative. However, Schneider *et al.* (2004) used multiple calibration points outside the Fagales for estimating the ages for major lineages of vascular plants with relaxed molecular clock analyses, and their results still showed the origin of Fagales is 95 MYA or younger, which is consistent with the fossil records. No fossil evidence suggests that Fagaceae ever existed in Australia, New Zealand, or southern South America.

Fossil records of *Nothofagus* and the other Fagales, and molecular clock all agree that the origin of the order is in the Cretaceous, well after the major breakup of

Pangaea, and split of western and eastern Gondwana (McLaughlin, 2001). Again it is apparently in a conflict from the Paleozoic origin of the Hysterangiales inferred from the synonymous substitution rates.

Biogeography of Pinaceae

The Pinaceae are the only conifers associated with the Hysterangiales. It is one of the obligate ectomycorrhizal plant families, and all members of this family are considered to be ectomycorrhizal (Newman & Reddell, 1987). Most Pinaceae-associated species of the Hysterangiales form ectomycorrhizae with either *Pinus* or *Pseudotsuga* although several species are known to be associated with *Picea*, *Tsuga*, or *Abies*. Ectomycorrhizal association with the other genera of Pinaceae, such as *Cedrus* and *Larix* is poorly known. Ancestral host reconstructions suggest that the Pinaceae is the ancestral host for some Fagaceae-associated Hysterangiales although reversal from Fagaceae to Pinaceae were also observed (Fig. 3.11, 3.12). Nonetheless, Pinaceae-associated taxa are restricted in the more terminal clade (Fig. 3.2, 3.11), indicating that it is a derived state.

Like the Fagaceae, the Pinaceae is one of the most dominant ectomycorrhizal trees in the Northern Hemisphere. Over 200 species are known, and its distribution partially extends to the Southern Hemisphere in Central America, West Indies, Sumatra, and Java (Mabberley, 1997). However, no Pinaceae is known from further

south, such as New Guinea, Australia, and South America. A monophyly of conifers and the relative position of Pinaceae are still controversial. Stefanović *et al.* (1998) showed the monophyly of conifers with the basal split of Pinaceae and the rest of Coniferales. On the other hand, several other authors (Burleigh & Mathews, 2004; Chaw *et al.*, 2000; Gugerli *et al.*, 2001) showed the sister relationship of Pinaceae and Gnetales, making Coniferales paraphyletic.

Although fossils of conifers or conifer-like plants are known from the Carboniferous (Miller, 1977), long before the initial breakup of Pangaea, their ecological characters are uncertain. Several fossils from the Triassic period may be early representatives of the Pinaceae (Miller, 1977; Delevoryas & Hope, 1973), however their affinities to the modern Pinaceae is very controversial. The oldest undisputable Pinaceae fossils are known for the genus *Pseudolarix* from the Late Jurrasic to the Early Cretaceous (LePage & Basinger, 1995). There are numerous fossil records of Pinaceae throughout the Northern Hemisphere from the Early Cretaceous, but no unambiguous fossil records exist from the Southern Hemisphere (Florin, 1963). Molecular clock estimates (Wang *et al.*, 2000) showed that the origin of the Pinaceae is Late Jurassic, which is consistent with the fossil records. Significantly, the oldest known fossil of ectomycorrhizae is formed by *Pinus* (LePage *et al.*, 1997). However, the fossil record is a relatively recent one, from middle Eocene or 50 MYA, which set the minimum age for the origin of ectomycorrhizae as 50 MYA (LePage, *et al.*, 1997).

The origin of the Pinaceae is the oldest among known ectomycorrhizal host plants for the Hysterangiales. However, ancestral host reconstructions and lack of Pinaceae from the Southern Hemisphere both agree that the Pinaceae is not the ancestral host for the Hysterangiales. Furthermore, the origin of the Pinaceae is the Late Jurassic, which is again in conflict with the Paleozoic origin of the Hysterangiales.

Biogeography of Dipterocarpaceae

Dipterocarpaceae is a tropical tree family, which belongs to the order Malvales (The Angiosperm Phylogeny Group, 2003; Hilu *et al.*, 2003). It is distributed in Africa, South America, south and Southeast Asia, including New Guinea. It is not distributed in Australia or New Zealand. Several Hysterangiales have been collected from Asian Dipterocarpaceae forests. Asian Dipterocarpaceae belong to the subfamily Dipterocarpoideae, which along with the other two subfamilies, the Monotoideae and Pakaraimoideae, and a closely related family Sarcolaenaceae are all considered to be ectomycorrhizal (Dayanandan *et al.*, 1999; Ducousso *et al.*, 2004; Newman and Reddell, 1987).

Based on the sister relationship of the Dipterocarpoideae and Sarcolaenaceae, Ducousso *et al.* (2004) speculated that ectomycorrhizae of this clade could have originated in 88 MYA, the time of the separation of Madagascar from the India–

Seychelles block. Ducousso *et al.* (2004) further speculated that the origin of ectomycorrhizal symbiosis of Dipterocarpaceae could be about 130 MYA, the time of the separation of both South-America and the India–Madagascar–Seychelles block from Africa, if all members of Dipterocarpaceae are proven to be ectomycorrhizal. However, the recent molecular clock studies (Wikström *et al.*, 2001; Schneider *et al.*, 2004) showed that the origin of Malvales is less than 100 MYA. Fossil records of Dipterocarpaceae are available from Tertiary China (Zhi-Chen *et al.*, 2004) and Borneo (Muller, 1981), but no evidence suggests that the family has ever existed in Australia or New Zealand. In any case, the origin of Dipterocarpaceae ectomycorrhizae appears to be much younger than the age of the Pangaean breakup.

Biogeography of the other ectomycorrhizal hosts

The origin of the other ectomycorrhizal hosts for the Hysterangiales, including the Caesalpinioideae and Ericaceae, are all indicated as Cretaceous or later (Wikström *et al.*, 2001; Schneider *et al.*, 2004), which in turn suggest that the origin of ectomycorrhizae for these plants must be younger than 120 MYA. The age estimates for angiosperms remain uncertain. Molecular clock estimates vary depending on the method used, calibration point, and taxon or character sampling. Several recent studies (Wikström *et al.*, 2001; Sanderson *et al.*, 2004; Sanderson & Doyle, 2001) indicate Jurassic origin of angiosperms, which is in agreement with the oldest fossils of

angiosperms (Sun *et al.*, 1998). However, the age estimates of Schneider *et al.* (2004) showed that the origin of angiosperms could be as old as late Permian. In any case, the radiations of the major lineages of angiosperms, such as rosids, asterids, and monocots, are estimated to be Middle Jurassic to Early Cretaceous, which is in agreement with the post-Jurassic origins of major ectomycorrhizal plant families. Of course the possibility of ancestral angiosperms as ectomycorrhizal plants could not be rejected. However, plant phylogeny strongly indicates that the ancestral lineages of angiosperms are not ectomycorrhizal (Hilu *et al.*, 2003; Newman & Reddell, 1987), and different lineages of plants probably gained the ectomycorrhizal habit independently.

The post-Jurassic origins of major ectomycorrhizal tree families imply that the origin of ectomycorrhizae occurred after the breakup of Pangaea into Laurasia and Gondwana, which began from the early Triassic but was completed by the late Jurassic (Hallam, 1994; McLaughlin, 2001). The present prevalence of ectomycorrhizal trees in all continents except Antarctica indicates that major dispersal events must have occurred for both ectomycorrhizal trees and fungi. Whether these dispersals were through land bridges (or at least chains of islands) or trans-oceanic is uncertain.

Biogeographical patterns and molecular age estimates for extant ectomycorrhizal plants strongly favor the origin of ectomycorrhizal fungi after the major breakup of Pangaea. This means that the Hysterangiales originated in the Mid Mesozoic or later, followed by transoceanic dispersals. This is in sharp contrast to the

Paleozoic origin of the Hysterangiales inferred from the synonymous substitution rates. Furthermore, several biogeographical patterns seemingly congruent to a vicariant scenario must be explained by long distance dispersal. However, as discussed above, the ability of the Hysterangiales to shift its ectomycorrhizal host among distantly related plant families, and incongruent patterns between host and fungal phylogeny both indicate that reconstructions of ancestral host based on extant ectomycorrhizal association may not be the true reflection of the ancient host-fungal association. Although no evidence for the presence of ectomycorrhizae is available from the Paleozoic era, the ancient forests (though composed of completely different plants) might have similar ecological characters to the present forests, and possessed similar fungal flora, including ectomycorrhizal fungi.

Paleozoic to Early Mesozoic forests

Fossil records of land plants are available from the Ordovician (Wellman *et al.*, 2003). It is noteworthy that the fossils of glomalean fungi, which are known as obligate endosymbionts with many land plants, are also available from the Ordovician or about 460 MYA (Redecker *et al.*, 2000). This is consistent with the hypothesis that interactions of fungi with plants facilitated the initial land colonization by plants. This fact suggests that plant-fungal symbiosis has a very ancient origin. Direct fossil evidence of ectomycorrhizae, however, is not available until the Eocene or 50 MYA

(LePage *et al.*, 1997). Fossil evidence of obligate ectomycorrhizal plant taxa from the Cretaceous suggests that the origin of the ectomycorrhizal symbiosis goes back at least to the Cretaceous. The majority of present ectomycorrhizal symbioses are between fungi and tree-forming plants. Tree-forming plants are known long before the Cretaceous, and their floras are probably equivalent to the present day forests. Many members of these ancient forests are now extinct, and their ecological functions are largely unknown. But a possibility exists that the ectomycorrhizal symbiosis originated in those ancient forests long before the Cretaceous.

The first predominant forests were created in the Lower Carboniferous or about 350 MYA. The Lower Carboniferous is characterized by a globally warm climate with generally uniform floras (Chaloner & Meyen, 1973). The "*Lepidodendropsis* flora" was distributed widely across Australia, Africa, South America, and throughout the Northern Hemisphere (Chaloner & Meyen, 1973). They are related to the modern lycopsid plants, but unlike present lycopsids, which are only a minor component for modern floras, they were a dominant component in the Lower Carboniferous (Wikström & Kenrick, 2001; Chaloner & Meyen, 1973). They are characterized by their coal swamp habitat and giant tree forms, and formation of forests. No extant lycopsid plants are known to be ectomycorrhizal. Importantly, conifers or conifer-like plants made their first appearance in the Carboniferous (Miller, 1977).

Toward the end of the Carboniferous, the onset of Gondwanan glaciations caused differentiation in the world floras. And by the Permian, those differentiations

can be clearly seen in the fossil record (Chaloner & Meyen, 1973). Again most taxa of the Permian floras are now extinct, but their vegetation patterns were probably similar to the present. In fact, the biome concept can be readily applied based on the Permian floral and climatic patterns (Ziegler, 1990). Initially, southern Gondwana was represented by a glacial environment, but as the climate warmed up, this region became characterized by a cool temperate climate until the end of the Permian (Ziegler, 1990). The Permian flora of the southern Gondwanan region is characterized by *Glossopteris*, a seed fern distributed widely in this region, including Australia, Antarctica, India, Africa and South America (Plumstead, 1973; Ziegler, 1990). Forests of this region were composed mainly of *Glossopteris*, but associations with conifers became evident (Plumstead, 1973). These conifers include members of the Cordaithales and Voltziales, all extinct lineages (Plumstead, 1973; Ziegler, 1990). By this time, conifers were distributed widely in both the Northern and Southern Hemisphere. Their affinities to the modern conifers are largely unknown.

During the Permian, Pangaea was tightly formed, and the western part of Gondwana (present North and South America, and Africa) had a direct land connection (McLaughlin, 2001; Hallam, 1994). Floral differentiations despite its land connection indicate that climatic differentiations served as effective barriers for many Permian organisms, including *Glossopteris*. Because of the assembly of western Pangaea, the ocean current between the Pacific and Tethys Sea was terminated, and this created a significantly drier climate for most of central Pangaea (Scotese & McKerrow, 1990). For example, central South America and a large part of Europe and

central Asia were characterized by desert climate (Ziegler, 1990), which must have served as effective barriers for organisms adapted for cool temperate forests, including potentially for the Hysterangiales assuming its Paleozoic origin. This is also consistent with the biogeographical patterns of the Hysterangiales, where initial diversifications were restricted in the Southern Hemisphere (Fig. 3.2, 3.3).

The Permian/Triassic boundary experienced one of the largest mass extinction events in Earth's history (McLoughlin *et al.*, 1997). Climatic changes are generally attributed to this mass extinction, but McLoughlin *et al.* (1997) suggested that there must be other reasons besides climatic changes. Additional reasons for this mass extinction are still controversial. A significant increase of fungal spores just after the mass extinction event was reported in the Permian/Triassic boundary (Benton & Twitchett, 2003; Visscher & Brugman, 1986; Eshet *et al.*, 1995). Although the authenticity of the 'fungal spike' is still controversial, this probably corresponds to the availability of a huge amount of dead organic material after the mass extinction event. Accordingly, the Permian/Triassic boundary may correspond to the diversification of many fungal lineages, especially for saprotrophic fungi. A similar fungal spike was reported from the Cretaceous/Tertiary boundary (Vajda & McLaughlin, 2004).

The Triassic experienced another climate warming, which resulted in a more uniform climate throughout the world (Hallam, 1994). Pangaea was still tightly formed, and the land connection in western Pangaea could act as migration route for many organisms. Relatively homogeneous fauna with little evidence of endemism during this period is a strong indication that there are no physical or climatic barriers

for animal migration in Pangaea (Hallam, 1994). This is in sharp contrast to the Permian period, when climatic differentiation was apparent as well as flora and faunal differentiations. The range expansion of the Hysterangiales to the Northern Hemisphere might have happened at this time, following the global warming and diversification of many conifers throughout the world.

This trend of a global warm climate with little endemism for many organisms continues to the Jurassic. During the Jurassic, conifers became a prominent component of the vegetation. Significantly, most families of extant conifers made their first appearance during the Jurassic (Wesley, 1973). However, fossils of Pinaceae have not been found until the Early Cretaceous. Pinaceae is the only extant conifer family known to be obligately ectomycorrhizal. Abundant fossil records of Pinaceae in the Cretaceous and later periods (Florin, 1963) suggest that Pinaceae had never existed in the Southern Hemisphere. There is absolutely no evidence of Pinaceae from Australia.

The appearance of Pinaceae and other ectomycorrhizal host plants in the Cretaceous clearly indicates that forests in this time could support ectomycorrhizal fungi. Forests from older periods lack evidence of ectomycorrhizal fungi, but major components for these ancient forests are now extinct, and their ecological characters, such as a possibility that these forests could support ectomycorrhizal fungi, are largely unknown. There is no doubt that different types of mycorrhizal symbiosis, namely arbuscular mycorrhizae, were already established by the Ordovician (Redecker, 2000). A potential Paleozoic origin of the Hysterangiales suggests that an ancient ectomycorrhizal symbiosis could have been established with a completely different

group of plants, most of which are now extinct. Frequent host shifts between distantly related host plants, and a lack of cospeciation pattern between the Hysterangiales and their host plants both indicate that ancestral ectomycorrhizal hosts for the Hysterangiales could not be reconstructed based solely on the extant host plant information. More palaeobiological studies are needed to explore the presence of ectomycorrhizae and ectomycorrhizal fungi in Paleozoic forests.

Biogeography of animals with emphasis on mycophagy

Because fruiting bodies of the Hysterangiales are truffle-like and mostly produced below-ground, the spores are not disseminated by wind, as is the case in many above-ground mushroom-forming species, which have potential of long distance spore dispersal (Bruns *et al.*, 1989; Thiers, 1985). Instead, they produce a unique aroma to attract small animals, which rely on truffle-like fungi as a large part of their diet (Castellano *et al.*, 1989; Thiers, 1985). Truffle-like fruiting bodies are eaten by small animals and the fungal spores are disseminated with the animal feces (Castellano *et al.*, 1989; Cazares & Trappe, 1994; Claridge & Lindenmayer, 1998; Claridge *et al.*, 1992, 1999; Currah *et al.*, 2000; Fogel & Trappe, 1978; Green *et al.*, 1999; Johnson & McIlwee, 1997; Malajczuk *et al.*, 1997; Maser & Maser, 1988; Maser *et al.*, 1985; Maser *et al.*, 1978; McIlwee & Johnson, 1998; Reddell *et al.*, 1997). Because spore dissemination of truffle-like fungi, including that of the

Hysterangiales, is dependent on such mycophagy (fungal consumption by other organisms), long distance (such as intercontinental) dispersal of spores of truffle-like fungi is arguably less likely.

This association between small animals and truffle-like fungi can be seen in many areas of the world. In the Northern Hemisphere, small mammals especially rodents (such as squirrels, rats, mice, vole), and in the Southern Hemisphere several marsupials (such as potoroo, possum, bettong, bandicoot) eat significant amount of fruiting bodies of truffle-like fungi as part of their diet (Castellano *et al.*, 1989; Cazares & Trappe, 1994; Claridge, 2002; Claridge & Lindenmayer, 1998; Claridge *et al.*, 1992; Currah *et al.*, 2000; Fogel & Trappe, 1978; Green *et al.*, 1999; Johnson & McIlwee, 1997; Malajczuk *et al.*, 1997; Maser & Maser, 1988; Maser *et al.*, 1985; Maser *et al.*, 1978; Reddell *et al.*, 1997). Some small animals are known to eat mainly truffle-like fungi as their diet. Because of the tight interaction between mycophagous animals and truffle-like fungi, some sort of co-evolution scenario is a possibility. For example, one could hypothesize that the origin of mycophagous animals and hypogeous fungi occurred at the same time.

Two major animal groups containing mycophagous animals belong to the Eutheria (placental mammals) and Metatheria (including extant marsupials), and they are probably sister groups (Phillips & Penny (2003); but see Bromham *et al.* (1999) for sister relationships of marsupials and monotremes). Although the age estimates for the origin of the Eutheria and Metatheria vary from Late Triassic to Mid Cretaceous (Kumar & Hedges, 1998; Luo *et al.*, 2003; Bromham *et al.*, 1999), fossil evidence

suggests that both groups originated in Asia (Luo *et al.*, 2003; Huchon & Douzery, 2001). They expanded their distribution to North America and then to South America through land connection to Central America at around the K/T boundary (Nilsson *et al.*, 2003). Metatheria did not reach Australia until late Cretaceous or early Tertiary because the first appearance of marsupial fossils in Australia is from 55 MYA (Bromham *et al.*, 1999). At that time, South America and Australia/New Guinea were still connected through Antarctica, so that marsupials could expand their range via Antarctica. However, New Zealand and New Caledonia were already widely separated by the Tasman Sea. This is consistent with the fact that present-day New Zealand and New Caledonia do not possess any native Eutheria and Metatheria (except a few species of bats). Africa and India were also separated long before marsupials reached Australia.

Although a migration route described above is generally accepted for both the Eutheria and Metatheria, Huchon & Douzery (2001) proposed an alternative route for rodents. They showed that the hystricognath rodents, which are now restricted to the South America, migrated from Asia to Australia, and reached South America through Antarctica during the Eocene. However, it is consistent that both the Eutheria and Metatheria originated in Asia and did not reach Australia until Eocene. This pattern is also consistent with the mammal phylogeny, which shows a more basal position of Asian and North American taxa with terminal Australian taxa (Luo *et al.*, 2003). This means that one of the most important spore vectors for the Hysterangiales and other truffle-like fungi may not be available in Australia until relatively recently.

It must be pointed out that the fossil of *Aukstribosphenos* suggests that primitive placental mammal-like animals were present in Australia in the Early Cretaceous (Rich *et al.*, 1997). *Aukstribosphenos* is probably only distantly related to the modern rodents, and there are some indications that it could be more closely related to the monotremes (Long, 1998; Rich *et al.*, 1997), which diverged from the Metatheria and Eutheria about 180 MYA (Messer *et al.*, 1998). The affinity of *Aukstribosphenos* to the Eutheria is strongly opposed by Archibald (2003). However, a possibility exists that both the Metatheria and Eutheria had already been widespread in both Northern and Southern Hemisphere by the Cretaceous (Murphy *et al.*, 2001), a completely different view from the Tertiary migration scenario. This Pangaean distribution scenario is not supported by fossil records and molecular phylogenetic analyses, making traditional Tertiary migration scenario more likely (Archibald, 2003).

Besides the Metatheria and Eutheria, other mammal-like animals have been present in Australia for a long time ago. A dicynodont, therapsids or mammal-like reptiles, is one of them, distributed worldwide during the Permian and Triassic periods. Although most dicynodonts are believed to have gone extinct by the Late Triassic, fossil evidence suggests that they survived until the Early Cretaceous or 110 MYA in Australia (Thulborn & Turner, 2003). Their long presence in Australia and their herbivorous habit leave a possibility that dicynodonts-like animals served as initial vectors for spore dispersal of the Hysterangiales.

The phylogeny of the Hysterangiales suggests that the initial diversifications occurred in Gondwana, but distribution was still restricted to the southern part of Gondwana, including Australia, New Zealand, and southern South America. Subsequently a great range expansion to the Northern Hemisphere, and to Southeast Asia, Africa, northern South America, and India occurred (Fig. 3.3). If this range expansion was facilitated by the Eutheria/Metatheria range expansions, it might have happened sometimes during the Tertiary, after the arrival of the Metatheria/Eutheria in Australia. If this is true, area relationships seen in the *Aroramyces* clade, which appears to follow the breakup of Gondwana, cannot be explained by vicariance. By the middle Tertiary, positions of most continents were almost identical to the present continental arrangement. Therefore distribution in Africa and India could be explained by dispersal from the Northern continents through land, possibly with small animals as vectors. However, the terminal Australian and New Caledonian taxa in the *Aroramyces* clade must be due to transoceanic dispersal. Age estimates for the nodes corresponding to these range expansions appear to be older than 100 MYA (Fig. 3.8), suggesting that mycophagous animals such as marsupials may not be the most important factor for the initial range expansion of the Hysterangiales.

While mycophagy by small animals, especially rodents and small marsupials, is usually emphasized for truffle-animal interactions, there may be other organisms that could serve as important spore vectors. For example, mycophagy by arthropods is well-documented for many groups of fungi (Martin, 1979). Importantly, the order Phallales (commonly known as stinkhorns), which is closely related to the

Hysterangiales, is one of the prime examples of insect mycophagy (Shorrocks & Charlesworth, 1982; Driessen *et al.*, 1990; Smith, 1956; Driessen & Hemerik, 1991; Stoffolano, *et al.*, 1990; Stoffolano *et al.*, 1989; Fulton *et al.*, 1889). Its phylogenetic affinity with the Hysterangiales leaves a possibility that the ancestor of the Hysterangiales was at least partially dependent on arthropods for spore dispersal. Exactly how arthropods are important for spore dispersal of the Hysterangiales compared to mammals/marsupials is unclear. However, the initially restricted distribution of the Hysterangiales could be explained by its dependency on arthropods for spore dispersal, instead of mammals/marsupials. Some truffle-like fungi are known to emit chemical compounds to attract various insects (Pacioni *et al.*, 1991), which imply that the interactions between the Hysterangiales and arthropods should not be ignored. There are some examples of mycophagy by birds (Simpson, 2000; Claridge, 2002), which imply that fungal spores could potentially be dispersed for a long distance. However, mycophagy by birds is poorly known compared to mammal/marsupial mycophagy, and its potential for long distance dispersal is largely unknown.

Comparative biogeographical patterns of Fungi and plants

Kingdom Fungi is one of the most diverse groups of organisms on earth. Although less than 100,000 species have been described so far, which is less than

insects and vascular plants, the total number of fungal species is estimated to be 1.5 million or even more (Hawksworth, 1991, 2001). Fungi play an important role in ecosystems as saprobes, pathogens, or mutualists, directly affecting the diversity and biogeographical patterns of other organisms. For example, the initial land colonization of plants has traditionally been considered the result of the mutualistic association between fungi and plants. Despite this, biogeography of fungi (or "mycogeography" by Pirozynski, 1983) has not been extensively studied within a phylogenetic framework. The cryptic nature of many fungi makes it difficult to sample, and thus hinders the global scale biogeographical studies.

Many mycogeographic studies have focused on Northern Hemisphere taxa. The genus *Grifola* (maitake) is a mushroom-forming fungus, which causes white rot of trees. Phylogenetic analyses of *Grifola* species revealed a Palearctic vs. Nearctic pattern, with Asian and European taxa being sister groups (Shen *et al.*, 2002). The genus *Amanita* (fly agaric) is another mushroom-forming fungus, but it is an ectomycorrhizal fungus, ecologically different from *Grifola*. The biogeographic pattern of *Amanita* was shown to be similar to *Grifola*, showing a Palearctic vs. Nearctic pattern (Oda *et al.*, 2004). Oda *et al.* (2004) also showed that eastern North American taxa are more closely related to western North American taxa. This pattern is commonly seen in plant biogeographic studies, where a disjunct eastern North America-eastern Asia distribution pattern is not supported by phylogenetic analyses (Xiang *et al.*, 1998).

The other ectomycorrhizal fungi in the Northern Hemisphere showed different patterns. In the genus *Suillus*, some eastern North America and eastern Asia disjunct patterns were reflected by phylogeny (Wu *et al.*, 2000). Western North American taxa were more distantly related to eastern North America + eastern Asian taxa. *Tricholoma matsutake* and its allies (Chapela & Garbelotto, 2004) showed a similar pattern; all Palearctic and eastern North American taxa form a clade. Chapela & Garbelotto (2004) also proposed that the Beringia was a migration route for *T. matsutake* and its allies, not the North Atlantic Land Bridges.

Several studies have dealt with the fungi distributed in both Northern and Southern Hemisphere. Interestingly, mycogeographic patterns frequently showed the New World vs. Old World pattern, not a Laurasia vs. Gondwana pattern, which we expect to see if the present distribution was caused by vicariance. *Pleurotus cystidiosus* and its allies showed that their Old World lineage includes taxa from South Africa, Europe, and Asia including Southeast Asia, and the New World lineage includes taxa from USA and Mexico (Zervakis *et al.*, 2004). While taxa from New Zealand and Australia were only distantly related to the others in this study, and no taxa were represented from South America, the other studies including taxa from South America, Australia and New Zealand still showed the New World vs. Old World pattern. A study by Hibbett (2001) using the genus *Lentinula* showed this pattern, with the New World lineage including Nearctic, Brazil and Venezuela (but not including southern South America), and the Old World lineage including Palearctic, Australia and New Zealand. Furthermore, Asiatic origin of the genus was suggested.

The genus *Schizophyllum* showed a similar pattern, with the New World lineage including Nearctic, and both northern and southern South America, with the Old World lineage including Palearctic, Africa, and Australasia (James *et al.*, 1999, 2001). For both studies, there are some taxa which are exceptions to this rule. These taxa were explained by rare, but recent long distance dispersal. It is worth while to mention that for both *Lentinula* and *Schizophyllum*, a sister relationship between Australian and New Zealand taxa was not shown, in contrast to the biogeographical pattern of the Hysterangiales. All these examples are based on wood rotting fungi, but the genus *Fusarium*, a plant pathogenic fungus, showed a similar pattern, with the Asian and African clade being more closely related to each other than either one of them to the American clade (O'Donnell *et al.*, 1998, 2000). On the other hand, the genus *Cyttaria*, which is parasitic on *Nothofagus* was shown to have (southern South America, (Australia, New Zealand)) relationships (Crisci *et al.*, 1998; Korf, 1983).

Different patterns can also be observed. In the genus *Pleurotus*, basal grades were shown to be represented by both Northern and Southern Hemisphere taxa, whereas more terminal clades were restricted to Northern Hemisphere taxa (Vilgalys & Sun, 1994). While the ancient vicariance or recent dispersal between the Northern and Southern Hemisphere could both explain this pattern, the authors favored the latter explanation because of the presumably young age of the genus. The genus *Panellus* showed a clear Northern (USA, Russia, Sweden, and Switzerland) vs. Southern (Australia and New Zealand) pattern (Jin *et al.*, 2001). While this pattern is consistent

with vicariance, the authors proposed a dispersal scenario from the Northern to Southern Hemisphere through Southeast Asia.

Only a few studies have been conducted on the global biogeography of ectomycorrhizal fungi. The study of the genus *Pisolithus* (Martin *et al.*, 2002) is a very good example, integrating biogeographic patterns with ectomycorrhizal host information. Interestingly, biogeographic patterns of *Pisolithus* are somewhat similar to those of the Hysterangiales. Australia was represented by more than one clade, but most Northern Hemisphere taxa were restricted to one clade. The authors suggested that Australasia could be the center of diversification for *Pisolithus*. Another similarity is the pattern of ectomycorrhizal hosts. Two Australian clades were dominated by *Eucalyptus* associates, and the Northern Hemisphere taxa are mostly associated with either Pinaceae or Fagaceae. Obviously their ectomycorrhizal host associations are correlated with their geographic distribution, just like the example in the Hysterangiales. Whether this similarity of biogeographic pattern between *Pisolithus* and the Hysterangiales is due to their ectomycorrhizal habit, same underlining geological event or simply superficial is hard to evaluate. In this context, it is very interesting that Martin *et al.* (2002) suggested that the ancestor of *Pisolithus* is a generalist mycorrhizal symbiont, and originated before the breakup of Pangaea.

Despite their variety of ecological roles, most fungi described above show certain biogeographical patterns. Most studies concluded that long distance dispersal is a rare event. If this is a general trend, a similar pattern for the Hysterangiales would be expected because there are no reasons to assume that the Hysterangiales has better

dispersal ability than other fungi. Its hypogeous fruiting body habit and relative specificity of ectomycorrhizal host association both support that long distance dispersal would be equally or more difficult compared to the other fungi, such as saprobic mushrooms. Some potential long distance dispersal events observed in the Hysterangiales phylogeny include trans-Tasman dispersal between Australia and New Zealand (especially in the Salmonaceum clade), and between India and Australia + New Caledonia (in the *Aroramyces* clade), which could still be considered rare, especially assuming the Paleozoic origin of the Hysterangiales.

There are numerous examples of plant biogeography involving the Southern Hemisphere. Biogeographical patterns of over 300 plant studies are summarized in Sanmartín & Ronquist (2004). The optimal area cladograms derived from plant datasets showed several incongruent patterns to the geological scenario. These incongruent patterns were further reflected by the fact that the frequency of vicariant events was not significantly different from values expected by chance (Sanmartín & Ronquist, 2004). The optimal area cladograms derived from all plant datasets showed the (southern South America, (Australia, New Zealand)) pattern, similar to the Hysterangiales, but incongruent to the geological history. The plant datasets also showed the sister relationship between New Guinea and New Caledonia, and that Holarctic was nested with Africa and northern South America, which has not been observed in the Hysterangiales.

As discussed by Sanmartín & Ronquist (2004), the young age for many studied plant groups, or extensive extinction events could be some reasons why many plant

phylogenies showed little trace of vicariant patterns. While both possibilities are likely, many studies suggest the young ages of plants, thus dispersal played an important role for plant distribution. For example, the tropical plant family Melastomataceae was shown to have originated in the Late Cretaceous, and its first migration to Africa from the neotropics was less than 20 MYA by long distance dispersal (Renner, 2004; Renner *et al.*, 2001). Likewise, the Lauraceae was shown to have originated in Laurasia, and subsequently dispersed to South America (Chanderbali *et al.*, 2001). The Malpighiaceae also shows a Gondwanan disjunct distribution, but analyses of its biogeography and age estimates supported that it originated in northern South America during Paleocene, with multiple dispersals to Laurasia and Africa (Davis *et al.*, 2002). The importance of intercontinental long distance dispersal was also suggested for many other plant groups, such as Rapateaceae, Bromeliaceae between Africa and South America (Givnish *et al.*, 2000, 2001), Asteraceae and its allies (Bremer & Gustafsson, 1994), Annonaceae and Rhamnaceae (Richardson *et al.*, 2004), Liliales (Vinnersten & Bremer, 2001) and Poales (Bremer, 2002).

The biogeographical pattern of the Asteraceae and its allies (Bremer & Gustafsson, 1994), and the Poales (Bremer, 2002) are of great interest because they both show a Gondwanan origin with subsequent dispersal to the rest of the world, similar to the Hysterangiales. The origins for both groups are estimated to be Mid Cretaceous (for Poales, ca. 110 MYA; Bremer, 2000, 2002) or Eocene (for Asteraceae, ca. 40 MYA; Bremer & Gustafsson, 1994), so long distance dispersal must be

implemented to explain their worldwide distribution. Whether similarities between these plants and the biogeographical pattern of the Hysterangiales are due to the same underlining causes (such as climatic or geological) is unclear. However, two plant groups with different time of origins show very similar patterns, which clearly indicate that the pattern of area relationships alone cannot distinguish the ancient vicariance from recent dispersal. Although the Hysterangiales showed a similar biogeographical pattern to the Poales and Asteraceae, this similarity could possibly be superficial. The age estimate of the Hysterangiales is the other evidence that the origin of the Hysterangiales possibly predated the Jurassic.

Two conflicting biogeographical hypothesis for the Hysterangiales

Hypothesis 1: Late Mesozoic or more recent origin

This hypothesis is compatible with the results of ancestral ectomycorrhizal host reconstructions. The most ancestral host for the Hysterangiales was reconstructed as the Myrtaceae (Fig. 3.9), and the origin of the Myrtaceae was estimated to be Cretaceous or more recent (Sytsma *et al.*, 2004; Wikström *et al.*, 2001; Schneider *et al.*, 2004). Also Cretaceous origins of most ectomycorrhizal host plants support that ectomycorrhizal plants were already available when the Hysterangiales originated. This hypothesis is also compatible with the timing of mammals/marsupials migrations

to Australia. The distribution of the Hysterangiales was initially restricted to the southern Gondwana. In the Early to Mid Tertiary, marsupials reached Australia, and their mycophagous activity could greatly facilitate the range expansion of the Hysterangiales.

Assuming a younger age for the Hysterangiales requires numerous frequent transoceanic dispersals. Because New Zealand and New Caledonia were separated from Gondwana in Cretaceous or 80 MYA, virtually all sister relationships of Australia and New Zealand must be explained by transoceanic dispersal. The split of eastern and western Gondwana took place in the Jurassic, before the origin of most ectomycorrhizal plants. So the intercontinental disjunct patterns observed in the *Aroramyces* clade also must be explained by long distance dispersal. Although a young age estimate does not completely rule out a role of vicariance for the biogeography of the Hysterangiales, it is in apparent conflict with the age estimate based on the synonymous substitution rates. Importantly, however, the age estimate based on the averaged synonymous substitution rate of Kasuga *et al.* (2002) is consistent with a Cretaceous origin of the Hysterangiales (Table 3.2; Fig. 3.8, right). If long distance dispersal is really a rare event, as suggested from a variety of fungal biogeographical studies, the biogeographical patterns of the Hysterangiales must be considered an anomaly.

Hypothesis 2: Paleozoic to Early Mesozoic origin

This is compatible with the age estimate based on the synonymous substitution rates and the southern hemisphere origin of the Hysterangiales. Assuming the ancient origin of the Hysterangiales, many intercontinental disjunct patterns, including some Australia/New Zealand sister relationships, are old enough to be explained by vicariance. The initial breakup of Pangaea into Laurasia and Gondwana could be responsible for vicariance in the Phallogastraceae clade, as well as the *Aroramyces* clade (Fig. 3.3). Also branching order similar to Gondwana breakup observed in the *Aroramyces* clade could be explained by vicariance, possibly without implementing long distance dispersal. Although a possibility of long distance dispersal could not be completely ruled out, only a few intercontinental dispersal events are required to explain the biogeographical patterns of the Hysterangiales. It is uncertain how exactly intercontinental dispersal is possible for hypogeous, ectomycorrhizal fungi, but it could still be considered a rare event considering the 280-million-year or even longer history of the Hysterangiales.

One of the difficulties for this ancient origin hypothesis is that no known ectomycorrhizal host plants existed in the Paleozoic era. It does not affect the presence of Phallogastraceae because they are saprotrophic fungi, but the assumption of a single gain of ectomycorrhizal habit for the Hysterangiales requires that the origin of the Hysterangiales ectomycorrhizal habit predate that of known ectomycorrhizal plants. Although various ancient forests could potentially support ectomycorrhizal fungi,

most major components of such forests are now extinct and their ecological functions remain uncertain. Another difficulty is the range expansion pattern of the Hysterangiales. Climatic differentiations in the Permian could be responsible for the initial diversifications and range restrictions in the southern Gondwana, but subsequent range expansions to the Northern Hemisphere must be facilitated somehow. According to the age estimate, this range expansion took place prior to the arrival of the primary mycophagous animals to Australia. A global warm climate throughout the Triassic and Jurassic could facilitate the range expansion, but no animals during these periods have close affinities to the modern mycophagous animals. For these reasons, it is possible that the four main ectomycorrhizal clades of the Hysterangiales represent independent parallel origins of the ectomycorrhizal symbiosis.

CONCLUSIONS

The phylogenetic hypothesis of the Hysterangiales as well as the results of the ancestral area reconstructions strongly suggest that the ectomycorrhizal lineages of the Hysterangiales originated in the East Gondwana. While multiple range expansions to the Northern Hemisphere could be explained by at least one trans-oceanic dispersal to the Northern Hemisphere, the age estimates based on the synonymous substitution rate indicate a Paleozoic origin of Hysterangiales. This indicates that range expansions of

the Hysterangiales could be possible through land connections before the initial breakup of Pangaea into Laurasia and Gondwana, which took place about 180 million years ago. Although transoceanic dispersal is a more likely scenario for some biogeographical patterns (such as New Zealand-Australia sister relationship in the Salmonaceum clade), many other nodes could be explained by vicariance. Because modern ectomycorrhizal plants were not present during the Paleozoic to Early Mesozoic era, a potential existence of the Hysterangiales during this time must be explained by novel ectomycorrhizal association of the Hysterangiales with unknown plant lineages, most likely with extinct plants. Alternatively, multiple, independent gains of ectomycorrhizal habit must be postulated for the Hysterangiales, which is not the most parsimonious explanation. The Paleozoic origin of the Hysterangiales also indicates that mycophagous animals may not be the most important factor for range expansion. Nonetheless, the alternative hypothesis of a more recent (Cretaceous or later) origin of the Hysterangiales can not be rejected. This hypothesis requires a significant long distance, transoceanic dispersal, which is usually considered a rare event for most fungal groups. Given its hypogeous fruiting body habit, it is very intriguing to know how and if the Hysterangiales could carry out such long distance dispersal. Future research should focus on more sampling from the presently underrepresented areas, such as Africa, India and Asia, to further clarify the biogeographical patterns of the Hysterangiales. Ideally, additional paleontological studies of fossil ectomycorrhizae and mushroom-forming fungi from more ancient geologic periods would be of great benefit. Because no fossils of the Hysterangiales or

closely related lineages are currently known, more robust age estimates will have to be obtained with well-supported, higher-level phylogeny for the kingdom Fungi using the external fossil records.

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CHAPTER 4

Molecular phylogenetics of the gomphoid-phalloid fungi (Homobasidiomycetes, Basidiomycota) with an establishment of the new subclass Phallomycetidae and two new orders

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ABSTRACT

Molecular phylogenetic analyses for the gomphoid-phalloid fungi (Homobasidiomycetes, Basidiomycota) were conducted based on the 5-gene-dataset (nuc-LSU-rDNA, mt-SSU-rDNA, *ATP6*, *RPB2* and *EF1α*) with extensive taxon sampling. The monophyly of the gomphoid-phalloid clade was strongly supported, and four well-supported major subclades were recognized. Three of the four subclades (Geastrales, Hysterangiales and Phallales clades) were entirely represented by gastroid taxa, while only the Gomphales contained both gastroid and non-gastroid taxa. While the gastroid morphology, i.e., *Gautieria*, is derived from epigeous, non-gastroid taxa, i.e., *Ramaria*, in the Gomphales, the topology of the Phallales indicated that truffle-like form is an ancestral morphology of the stinkhorn fruiting bodies. Although basidiospore maturation occurs within the enclosed fruiting bodies of the stinkhorn, the elevation of the mature spore-producing tissue represents an independent origin of the stipe among the Basidiomycota. Comparisons are made between the past and new classification schemes, which are based on the results of phylogenetic analyses. Based on the results of these analyses, a new subclass Phallomycetidae, and two new orders, Hysterangiales and Geastrales, are proposed.

INTRODUCTION

The 'gomphoid-phalloid' fungi were first demonstrated to form a monophyletic group by Hibbett *et al.* (1997) based on ribosomal DNA sequences. Subsequent studies have repeatedly shown strong support for the gomphoid-phalloid clade (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Hibbett *et al.*, 1997; Hibbett & Thorn, 2001; Moncalvo *et al.*, 2002; Humpert *et al.*, 2001; Pine *et al.*, 1999). The relationship of gomphoid-phalloid fungi was not predicted based on previous phylogenetic and taxonomic hypotheses and classifications based on morphology (Reijnders, 2000). In fact, despite its consistent support as a monophyletic group, gomphoid-phalloid fungi share no obvious synapomorphies.

The gomphoid-phalloid clade comprises a group of fungi that exhibits a considerable breadth of both morphological and ecological diversity. The fruiting body morphology includes earthstars, stinkhorns, cannon ball fungi, coral fungi, club fungi, gilled mushrooms, tooth fungi, and false truffles. Both ectomycorrhizal and saprobic taxa are represented, but no pathogenic fungi are known. Because of its diversity, traditional morphology-based taxonomy has classified the fungi belonging to the gomphoid-phalloid clade into several unrelated orders, including the Lycoperdales, Phallales, Nidulariales, Gomphales, Hysterangiales, and Gautieriales (Zeller, 1949; Jülich, 1981; Fig. 4.2), many of which have proven to be polyphyletic in molecular phylogenetic studies (e.g., Hibbett *et al.*, 1997; Krüger *et al.*, 2001).

The goal of this study is to further clarify the phylogenetic relationships within the gomphoid-phalloid fungi with the largest taxon sampling ever conducted and to evaluate the higher-level classification schemes using multigene sequence data.

Review of past classifications

The previous classifications by Zeller (1949) and Donk (1964) and the new classification schemes based on this study are summarized in Fig. 4.2. Traditionally, most taxa in the gomphoid-phalloid clade have been classified in the artificial group, Gasteromycetes (literally means 'stomach fungi'). The Gasteromycetes are characterized by a lack of a forcible spore discharge mechanism with the development and maturation of spores occurring within an enclosed spore-producing tissue or gleba (Miller & Miller, 1988), a morphology often referred to as gastroid. All non-gastroid taxa in the gomphoid-phalloid clade are represented only in the order Gomphales, which has traditionally been classified as the family Gomphaceae in the order Aphyllophorales, along with distantly related taxa such as Cantharellaceae (chanterelles), Ganodermataceae (artist conks), and Polyporaceae (polypores).

The order Phallales was described by Fischer (1900) to accommodate the families Phallaceae (stinkhorns) and Clathraceae (lattice stinkhorns). Cunningham (1931a, b) later added the family Claustulaceae. This ordinal concept was accepted by many subsequent authors, but some authors included members of the Hysterangiales

in the Phallales (Miller & Miller, 1988). The order Hysterangiales was initially treated as a family in the order Hymenogastrales, along with the Hymenogastraceae and Secotiaceae (Fischer, 1900), all of which have been shown to be only distantly related to the gomphoid-phalloid fungi (Peintner *et al.*, 2001; Vellinga *et al.*, 2003). Although this treatment has been widely used by the subsequent authors (such as Fischer, 1933; Cunningham, 1944), some authors (such as Lohwag, 1926; Miller & Miller, 1988) recognized the close affinity of Hysterangiaceae to stinkhorns, and included Hysterangiaceae in the order Phallales. Other authors (Zeller, 1939, 1949; Jülich, 1981) further segregated the Hysterangiales as an independent order from the Phallales although they maintained the view that the Hysterangiales is most closely related to the Phallales. Three families, Hysterangiaceae, Protophallaceae, and Gelopellaceae, are currently recognized in the Hysterangiales. All members of this order are characterized by truffle-like (sequestrate) fruiting bodies, most of which are produced below-ground (hypogeous).

The members of the order Geastrales have been classified into two different orders, Lycoperdales and Nidulariales (Zeller, 1949), both of which have been demonstrated to be polyphyletic (Hibbett *et al.*, 1997; Krüger *et al.*, 2001). Within the Lycoperdales (*sensu* Zeller, 1949), the close relationship between the families Lycoperdaceae (puffballs) and Geastraceae (earthstars) was long assumed. At least one treatment of the group (Kreisel, 1969), however, segregated the Geastraceae from the Lycoperdales, and recognized an independent order, the Geastrales. Molecular phylogenetic studies revealed that the Lycoperdaceae is more closely related to the

Agaricaceae (including the genus *Agaricus*, button mushrooms), and only distantly related to the Geastraceae (Hibbett *et al.*, 1997; Krüger *et al.*, 2001). The other order, the Nidulariales, contains two families, the Nidulariaceae (bird's nest fungi) and Sphaerobolaceae (cannon ball fungi). Although this ordinal concept was accepted by most authors (Zeller, 1949; Jülich, 1981; Miller & Miller, 1988), the monophyly of the order has been rejected with members of the Nidulariaceae demonstrated to be more closely related to the Agaricaceae (Hibbett *et al.*, 1997).

The relationship of the genus *Gautieria* has been unclear until recently. It was initially included in the family Hymenogastraceae (Dodge & Zeller, 1934; Cunningham, 1944) or Hysterangiaceae (Fischer, 1900, 1933), but it has been treated as an independent, monotypic order, Gautieriales, ever since the order was described by Zeller (1948). Although a close relationship of the Gautieriales and Boletales was suggested (Jülich, 1981), molecular phylogenetic studies revealed that it is a member of the Gomphales, nested within *Ramaria* (Humpert *et al.*, 2001), and not closely related to the Boletales. As described above, the other members of the Gomphales are non-gasteromycetous taxa, which were previously classified in the order Aphyllophorales. All members of the family Gomphaceae (*sensu* Donk, 1961) were later divided into several families, and the order Gomphales was described (Jülich, 1981). The genus *Clavariadelphus* was originally included in the family Clavariaceae (Donk, 1964), but the family was shown to be polyphyletic; *Clavariadelphus* is a member of Gomphales, whereas *Clavaria* and *Clavulina* are not members of the gomphoid-phalloid fungi.

MATERIALS AND METHODS

A total of 231 species, 9 outgroup and 222 ingroup taxa, were sampled for this study (Table 4.1). The ingroup taxa were selected based on the phylogeny of previous studies (Humpert *et al.*, 2001; Villegas *et al.*, 1999) and the traditional morphology-based classifications (Dominguez de Toledo & Castellano, 1996; Dring, 1980; Marr & Stuntz, 1973; Zeller, 1949; Jülich, 1981) to cover the diversity of the gomphoid-phalloid fungi.

DNA sequence data were obtained from five independent loci: LR0R-LR3 region for nuclear large subunit ribosomal DNA (nuc-LSU-rDNA); MS1-MS2 region for mitochondrial small subunit ribosomal DNA (mt-SSU-rDNA); ATPase subunit 6 (*ATP6*); bRPB2-6F-bRPB2-7R region for the second largest subunit of RNA polymerase (*RPB2*); EF1-983F and EF1-1567R region for translation elongation factor subunit 1α (*EF1α*). The primers and PCR protocols have been described previously (Vilgalys & Hester, 1990; White *et al.*, 1990; Humpert *et al.*, 2001; Kretzer & Bruns, 1999; Liu *et al.*, 1999; also summarized in Assembling the Fungal Tree of Life website; http://ocid.nacse.org/research/aftol/primers.php). Because sequence data for all five genes were not successfully obtained for all samples, only taxa with at least one protein coding gene sequence, i.e., *ATP6*, *RPB2*, or *EF1α*, were included in phylogenetic analyses.

Dataset combinability was tested using Shimodaira-Hasegawa test (SH-test; Shimodaira & Hasegawa, 1999), implemented in PAUP*4.0b10 (Swofford, 2003).

After detecting no significant conflict among datasets, five-locus datasets were combined and analyzed under maximum parsimony and Bayesian criterion. Maximum parsimony analysis was conducted using PAUP*4.0b10 (Swofford, 2003), with nodal supports tested by bootstrap analysis. Bayesian analysis was conducted using MrBayes ver. 3.0b4 (Huelsenbeck, 2000). More detailed methodologies are mentioned in the figures and references cited.

Ancestral character state reconstructions were performed for fruiting body morphologies (sequestrate vs. nonsequestrate) in the Geastrales and Phallales clade based on the tree topology and branch lengths shown in Fig. 4.1. In this paper, the term 'sequestrate' refers to the truffle-like fruiting body form and the 'nonsequestrate' refers to the other gastroid forms, including stinkhorns, earthstars and cannon ball fungi. Some taxa with uncertain fruiting body types (shown with question mark in Fig. 4.1) were coded as sequestrate, nonsequestrate or polymorphic. Parsimony-based reconstructions were performed using MacClade ver. 4.06 (Maddison & Maddison, 2003) without implementing character weighting. Likelihood-based reconstructions were performed using Multistate ver. 0.8 (Pagel, 1999), and the significance of the difference in likelihood was determined by difference in 2 or more of -ln likelihood of each state, following Pagel (1999).

Table 4.1. Taxon list.

Genus	species	Herbarium	specimen#	GenBank#				
				nucLSU	mtSSU	ATP6	RPB2	EF
OUTGROUP								
Boletellus	*projectellus*	CUW	MB-03-118	AY684158	———	AFTOL*	AY787218	AY879116
Calocera	*cornea*	———	———	AY701526	———	———	AY536286	AY881019
Chamonixia	*sp.*	OSC	Muroi 361	DQ218598	DQ218741	DQ218885	DQ219046	DQ219224
Cortinarius	*iodes*	———	———	AY702013	AF026675	AF388826	AY536285	AY881027
Dacrymyces	*chrysospermus*	———	———	AF287855	AF026642	———	AY218480	———
Rhopalogaster	*transversarium*	OSC	81680	DQ218599	DQ218742	DQ218886	DQ219047	DQ219225
Russula	*compacta*	———	———	AF287888	U27074	———	AY218514	———
Sarcodon	*imbricatus*	———	———	AY586711	———	AF002147	AY218528	———
Suillus	*pictus*	CUW	MB-03-002	AY684154	———	AFTOL*	AY786066	AY883429
Geastrales clade								
Geastrum	*fimbriatum*	OSC	60730	DQ218600	———	DQ218887	DQ219048	DQ219226
Geastrum	*floriforme*	OSC	29328	DQ218485	DQ218660	DQ218769	DQ219049	DQ219227
Geastrum	*fornicatum*	MEL	2087743	DQ218601	DQ218743	DQ218888	DQ219050	DQ219228
Geastrum	*pectinatum*	MEL	2096557	DQ218602	———	DQ218889	DQ219051	DQ219229
Geastrum	*recolligens*	OSC	41996	DQ218486	DQ218661	DQ218770	DQ219052	DQ219230
Geastrum	*rufescens*	OSC	41850	DQ218603	DQ218744	DQ218890	DQ219053	———
Geastrum	*smardae*	OSC	60464	DQ218604	———	DQ218891	DQ219054	———
Geastrum	*sp.*	OSC	T26588	DQ218605	———	DQ218892	———	———
Myriostoma	*coliforme*	OSC	40741	DQ218606	———	DQ218893	DQ219055	DQ219231
Pyrenogaster	*atrogleba*	OSC	60063	DQ218607	———	DQ218894	DQ219056	———
Pyrenogaster	*pityophilus*	OSC	59743	DQ218519	DQ218694	DQ218803	DQ219057	DQ219232
Radiigera	*bushnellii*	OSC	T26259	DQ218608	DQ218745	DQ218895	DQ219058	DQ219233
Radiigera	*fuscogleba*	OSC	58979	DQ218609	———	DQ218896	DQ219059	DQ219234
Radiigera	*fuscogleba*	OSC	59749	DQ218610	———	DQ218897	———	———
Radiigera	*taylorii*	OSC	59760	DQ218520	DQ218695	DQ218804	DQ219060	DQ219235
Sclerogaster	*xerophilus*	OSC	49777	DQ218611	———	———	DQ219061	DQ219236

Table 4.1. (Continued).

Sphaerobolus	*ingoldii*	PSU*	SS19	AY439012	AY488015	———	———	AY487990
Sphaerobolus	*ingoldii*	PSU*	SS26	AY439013	AY488022	———	———	AY487996
Sphaerobolus	*iowensis*	PSU*	SS11	AY439014	AY488008	———	———	AY487984
Sphaerobolus	*iowensis*	PSU*	SS9	AY439010	AY488006	———	———	AY487982
Sphaerobolus	*stellatus*	PSU*	SS12	AF393077	AF026662	AY574789	DQ219062	DQ219237
Gomphales clade								
Beenakia	*fricta*	K	2083	AY574693	AY574766	AY574833	———	DQ219238
Clavariadelphus	*ligula*	OSC	67068	AY574650	AY574723	AY574793	DQ219063	DQ219239
Clavariadelphus	*occidentalis*	OSC	37018	AY574648	AY574721	AY574791	———	———
Clavariadelphus	*truncatus*	OSC	67280	AY574649	AY574722	AY574792	DQ219064	DQ219240
Clavariadelphus	*sp.*	OSC	122861	DQ218612	———	DQ218898	DQ219065	DQ219241
Gautieria	*caudata*	OSC	59201	DQ218483	DQ218658	DQ218767	DQ219066	DQ219242
Gautieria	*coralloides*	OSC	61517	DQ218613	DQ218746	DQ218899	DQ219067	DQ219243
Gautieria	*crispa*	OSC	61308	DQ218484	DQ218659	DQ218768	DQ219068	DQ219244
Gautieria	*otthii*	———	———	AF393058	AF393085	———	AY218486	AY883434
Gautieria	*parksiana*	OSC	58907	AY574652	AY574725	AY574795	———	DQ219245
Gautieria	*pterosperma*	OSC	69649	DQ218614	DQ218747	DQ218900	DQ219069	DQ219246
Gautieria	*rubescens*	OSC	48137	DQ218615	DQ218748	DQ218901	DQ219070	DQ219247
Gautieria	*sp.*	OSC	122685	DQ218616	DQ218749	DQ218902	DQ219071	DQ219248
Gloeocantharellus	*novaezelandiae*	PDD	44960	AY574666	AY574739	AY574809	———	———
Gloeocantharellus	*pallidus*	BPI	54917	AY574673	AY574746	AY574815	———	———
Gloeocantharellus	*purpurascens*	TENN	12793	AY574683	AY574756	AY574823	———	———
Gloeocantharellus	*sp.*	PERTH	06707114	AY574667	AY574740	AY574810	———	———
Gloeocantharellus	*sp.*	OSC	122875	DQ218617	———	DQ218903	DQ219072	DQ219249
Gomphus	*brunneus*	BR	034190-46	AY574680	AY574753	AY574821	———	———
Gomphus	*clavatus*	OSC	97587	DQ218487	DQ218662	DQ218771	———	———
Kavinia	*alboviridis*	O	102140	AY574692	AY574765	AY574832	DQ219073	DQ219250
Kavinia	*himantia*	O	102156	AY574691	AY574764	AY574831	———	———
Lentaria	*pinicola*	SUC	M89	AY574688	AY574761	AY574828	———	DQ219251

Table 4.1. (Continued).

Phaeoclavulina	*africana*	TENN	39621	AY574653	AY574726	AY574796	———	———
Phaeoclavulina	*cokeri*	TENN	36030	AY574701	AY574774	AY574843	———	———
Phaeoclavulina	*curta*	OSC	8711	AY574713	———	AY574858	———	———
Phaeoclavulina	*cyanocephala*	TENN	37827	AY574710	AY574779	AY574854	———	———
Phaeoclavulina	*eumorpha*	TENN	36218	AY574712	AY574781	AY574856	———	———
Phaeoclavulina	*gigantea*	FH	109	AY574703	AY574776	AY574845	———	———
Phaeoclavulina	*grandis*	BR	073158-06	AY574678	AY574751	AY574820	———	———
Phaeoclavulina	*grandis*	OSC	122773	DQ218618	———	DQ218904	DQ219074	DQ219252
Phaeoclavulina	*guyanensis*	FH	84	AY574706	———	AY574848	———	———
Phaeoclavulina	*insignis*	FH	104	AY574704	———	AY574846	———	———
Phaeoclavulina	*longicaulis*	TENN	31836	AY574700	AY574773	AY574842	———	———
Phaeoclavulina	*ochraceovirens*	OSC	23475	AY574714	———	AY574859	———	———
Phaeoclavulina	*pancaribbea*	TENN	31836	AY574707	———	AY574849	———	———
Phaeoclavulina	*sp.*	OSC	122874	———	———	DQ218905	DQ219075	DQ219253
Ramaria	*apiculata*	OSC	23549	AY574695	AY574768	AY574836	———	———
Ramaria	*araiospora*	SUC	M739	AF213068	AF213141	AY574838	DQ219076	DQ219254
Ramaria	*botrytis*	SUC	M457	AY574698	AY574771	AY574840	———	———
Ramaria	*botrytis*	SUC	M803	AF213058	———	DQ218906	———	———
Ramaria	*celerivirescens*	SUC	M460	AF213073	———	DQ218907	DQ219077	DQ219255
Ramaria	*circinans*	SUC	M615	AY574711	AY574780	AY574855	———	———
Ramaria	*flavobrunnescens*	SUC	M7	AF213082	AF213140	DQ220790	DQ219045	DQ219223
Ramaria	*gelatiniaurantia*	SUC	M830	AY574708	AY574777	AY574851	———	———
Ramaria	*moelleriana*	OSC	36422	DQ218619	———	DQ218908	———	———
Ramaria	*rainierensis*	SUC	M231	AF213115	AF213135	AY574834	———	———
Ramaria	*rubella*	———	———	AY645057	———	———	AY786064	AY883435
Ramaria	*rubribrunnescens*	SUC	M844	AF213098	AF213142	AY574852	———	———
Ramaria	*stricta*	SUC	M405	AF213117	AF213138	DQ218805	DQ219078	DQ219256
Ramaria	*stuntzii*	SUC	M214	AF213102	AF213134	AY574850	DQ219079	DQ219257
Ramaria	*suecica*	BPI	s.n.	AY574705	———	AY574847	———	———
Ramaria	*vinosimaculans*	OSC	23287	AY574709	AY574778	AY574853	———	———

Table 4.1. (Continued).

Ramaria	*sp.*	OSC	122865	DQ218620	———	DQ218909	———	———
Ramaria	*sp.*	OSC	122871	DQ218621	———	DQ218910	———	———
Ramaria	*sp.*	OSC	122873	DQ218622	———	DQ218911	DQ219080	———
Ramaria	*sp.*	OSC	122872	DQ218623	———	DQ218912	———	DQ219258
Turbinellus	*flabellatus*	FH	191	AY574674	AY574747	AY574816	———	———
Turbinellus	*flabellatus*	K	1770	AY574681	AY574754	AY574822	———	———
Turbinellus	*floccosus*	OSC	69167	AY574656	AY574729	AY574799	———	DQ219259
Turbinellus	*fujisanensis*	OSA	MY-1842	AY574669	AY574742	AY574811	———	———
Turbinellus	*kauffmanii*	MICH	10069	AY574671	AY574744	AY574813	———	———
Hysterangiales clade								
Andebbia	*pachythrix*	OSC	58809	DQ218523	———	DQ218808	DQ218940	DQ219117
Aroramyces	*gelatinosporus*	OSC	H4010	DQ218524	DQ218698	DQ218809	DQ218941	DQ219118
Aroramyces	*radiatus*	OSC	Verbeken 99-062	DQ218525	DQ218699	DQ218810	DQ218942	DQ219119
Aroramyces	*sp.*	OSC	122858	DQ218528	———	DQ218813	DQ218945	DQ219122
Aroramyces	*sp.*	OSC	122590	DQ218529	DQ218701	DQ218814	DQ218946	DQ219123
Aroramyces	*sp.*	RMS	S. Miller 10030	DQ218530	DQ218702	DQ218815	DQ218947	DQ219124
Aroramyces	*sp.*	OSC	T15013	DQ218526	———	DQ218811	DQ218943	DQ219120
Aroramyces	*sp.*	OSC	T9930	DQ218527	DQ218700	DQ218812	DQ218944	DQ219121
Austrogautieria	*chlorospora*	OSC	46596	DQ218477	———	DQ218761	DQ218948	DQ219125
Austrogautieria	*clelandii*	OSC	62178	DQ218531	———	DQ218816	DQ218949	DQ219126
Austrogautieria	*manjimupana*	OSC	55900	DQ218533	———	DQ218818	DQ218951	DQ219128
Austrogautieria	*sp.*	OSC	122637	DQ218534	———	DQ218819	DQ218955	DQ219132
Austrogautieria	*sp.*	OSC	80139	DQ218479	DQ218654	DQ218763	DQ218953	DQ219130
Austrogautieria	*sp.*	MELU	Beaton 66	DQ218535	———	DQ218820	———	DQ219133
Austrogautieria	*sp.*	OSC	80140	DQ218480	DQ218655	DQ218764	DQ218954	DQ219131
Castoreum	*sp.*	OSC	122814	DQ218536	———	DQ218821	DQ218956	DQ219134
Chondrogaster	*angustisporus*	OSC	62041	DQ218537	DQ218703	DQ218822	DQ218957	DQ219135
Chondrogaster	*pachysporus*	OSC	49298	DQ218538	DQ218704	DQ218823	DQ218958	DQ219136
Gallacea	*dingleyae*	OSC	59606	DQ218539	DQ218705	DQ218824	DQ218959	DQ219137

Table 4.1. (Continued).

Gallacea	*eburnea*	OSC	59601	DQ218482	DQ218657	DQ218766	DQ218960	DQ219138
Gallacea	*scleroderma*	OSC	59621	AY574645	AY574719	AY574787	DQ218961	DQ219139
Gallacea	*sp.*	OSC	122813	DQ218543	DQ218709	DQ218829	DQ218966	DQ219144
Gallacea	*sp.*	PDD	REB2364	DQ218540	DQ218706	DQ218825	DQ218962	DQ219140
Gallacea	*sp.*	OSC	80855	———	DQ218707	DQ218827	DQ218964	DQ219142
Gallacea	*sp.*	OSC	T25038	DQ218541	———	DQ218826	DQ218963	DQ219141
Gummiglobus	*agglutinosporus*	OSC	58784	DQ218544	DQ218710	DQ218830	DQ218967	———
Gummiglobus	*joyceae*	OSC	59485	DQ218488	DQ218663	DQ218772	DQ218968	———
Hallingea	*purpurea*	OSC	Garido 418-A	DQ218545	———	———	DQ218969	DQ219145
Hysterangium	*affine*	OSC	T6884	DQ218546	———	DQ218831	DQ218970	———
Hysterangium	*aggregatum*	OSC	H4262	DQ218489	DQ218664	DQ218773	DQ218971	DQ219146
Hysterangium	*album*	OSC	T15139	DQ218490	DQ218665	DQ218774	DQ218972	DQ219147
Hysterangium	*aureum*	OSC	56988	DQ218491	DQ218666	DQ218775	DQ218973	DQ219148
Hysterangium	*calcareum*	M	Gross 97	DQ218492	DQ218667	DQ218776	DQ218974	DQ219149
Hysterangium	*cistophilum*	OSC	T1088	DQ218493	DQ218668	DQ218777	DQ218975	DQ219150
Hysterangium	*clathroides*	MPU	Szemere 11-SEPT-1955	DQ218547	DQ218711	DQ218832	DQ218976	DQ219151
Hysterangium	*coriaceum*	OSC	64939	AY574686	AY574759	AY574826	DQ218977	DQ219152
Hysterangium	*crassirhachis*	OSC	58056	DQ218494	DQ218669	DQ218778	DQ218978	DQ219153
Hysterangium	*crassum*	OSC	110447	AY574687	AY574760	AY574827	DQ218979	DQ219154
Hysterangium	*epiroticum*	OSC	T6116	DQ218495	DQ218670	DQ218779	DQ218980	DQ219155
Hysterangium	*fragile*	OSC	Kers 3971	DQ218496	DQ218671	DQ218780	DQ218981	DQ219156
Hysterangium	*gardneri*	OSC	T6950	DQ218548	DQ218712	DQ218835	DQ218982	DQ219157
Hysterangium	*hallingii*	OSC	Halling 5741	DQ218497	DQ218672	DQ218781	DQ218983	DQ219158
Hysterangium	*inflatum*	OSC	H4035	DQ218549	———	DQ218836	DQ218984	DQ219159
Hysterangium	*membranaceum*	OSC	T12836	DQ218498	DQ218673	DQ218782	DQ218985	DQ219160
Hysterangium	*neotunicatum*	OSC	T15545	DQ218550	———	DQ218837	DQ218986	DQ219161
Hysterangium	*occidentale*	OSC	47048	AY574685	AY574758	AY574825	DQ218987	DQ219162
Hysterangium	*pompholyx*	OSC	Gross 495	DQ218499	DQ218674	DQ218783	———	DQ219163
Hysterangium	*rugisporum*	OSC	59662	DQ218500	DQ218675	DQ218784	DQ218988	DQ219164
Hysterangium	*rupticutis*	OSC	59667	DQ218551	DQ218713	DQ218838	———	———

Table 4.1. (Continued).

Hysterangium	*salmonaceum*	K	Beaton 33	DQ218501	DQ218676	DQ218785	DQ218989	DQ219165
Hysterangium	*separabile*	OSC	69030	DQ218502	DQ218677	DQ218786	DQ218990	DQ219166
Hysterangium	*setchellii*	OSC	58071	DQ218552	———	DQ218839	DQ218991	DQ219167
Hysterangium	*strobilus*	OSC	T5285	DQ218504	DQ218679	DQ218788	DQ218992	DQ219168
Hysterangium	*youngii*	OSC	59645	DQ218505	DQ218680	DQ218789	DQ218993	DQ219169
Hysterangium	*sp.*	OSC	AHF602	DQ218566	———	DQ218854	DQ219008	DQ219185
Hysterangium	*sp.*	K	K. & G. Beaton	DQ218506	DQ218681	DQ218790	DQ218997	DQ219174
Hysterangium	*sp.*	OSC	Garcia 3779	DQ218559	DQ218721	DQ218847	DQ219001	DQ219178
Hysterangium	*sp.*	OSC	H2022	DQ218568	———	DQ218856	DQ219010	DQ219187
Hysterangium	*sp.*	OSC	H4123	DQ218557	DQ218719	DQ218845	DQ218999	DQ219176
Hysterangium	*sp.*	OSC	H4749	DQ218573	DQ218730	DQ218861	DQ219015	DQ219192
Hysterangium	*sp.*	OSC	H5057	DQ218574	———	DQ218862	DQ219016	DQ219193
Hysterangium	*sp.*	OSC	H5573	DQ218575	DQ218731	DQ218863	DQ219017	DQ219194
Hysterangium	*sp.*	OSC	H6105	DQ218576	DQ218732	DQ218864	DQ219018	DQ219195
Hysterangium	*sp.*	OSC	122859	DQ218571	———	DQ218859	DQ219013	DQ219190
Hysterangium	*sp.*	OSC	122860	DQ218572	DQ218729	DQ218860	DQ219014	DQ219191
Hysterangium	*sp.*	OSC	122836	DQ218577	———	DQ218865	DQ219019	DQ219196
Hysterangium	*sp.*	OSC	122483	DQ218579	———	DQ218867	DQ219021	DQ219198
Hysterangium	*sp.*	PDD	REB-2315	DQ218580	DQ218734	DQ218868	DQ219022	DQ219199
Hysterangium	*sp.*	RMS	S. Miller 10007	DQ218581	———	DQ218869	DQ219023	DQ219200
Hysterangium	*sp.*	RMS	S. Miller 10100	DQ218582	DQ218735	DQ218870	DQ219024	DQ219201
Hysterangium	*sp.*	RMS	S. Miller 10166	DQ218583	DQ218736	DQ218871	DQ219025	DQ219202
Hysterangium	*sp.*	OSC	T13345	DQ218584	DQ218737	DQ218872	DQ219026	DQ219203
Hysterangium	*sp.*	OSC	T17501	DQ218553	DQ218715	DQ218841	———	DQ219171
Hysterangium	*sp.*	OSC	T17856	DQ218569	DQ218727	DQ218857	DQ219011	DQ219188
Hysterangium	*sp.*	OSC	T19263	DQ218561	DQ218723	DQ218849	DQ219003	DQ219180
Hysterangium	*sp.*	OSC	T26367	DQ218586	———	DQ218874	DQ219028	DQ219205
Hysterangium	*sp.*	OSC	T27921	DQ218587	DQ218738	DQ218875	DQ219029	DQ219206
Hysterangium	*sp.*	OSC	T3296	DQ218554	DQ218716	DQ218842	DQ218995	DQ219172
Hysterangium	*sp.*	OSC	T3328	DQ218564	DQ218726	DQ218852	DQ219006	DQ219183

Table 4.1. (Continued).

Hysterangium	*sp.*	OSC	T4794	DQ218558	DQ218720	DQ218846	DQ219000	DQ219177
Hysterangium	*sp.*	OSC	T591	———	DQ218714	DQ218840	DQ218994	DQ219170
Hysterangium	*sp.*	OSC	T6889	DQ218570	DQ218728	DQ218858	DQ219012	DQ219189
Hysterangium	*sp.*	OSC	T6923	DQ218567	———	DQ218855	DQ219009	DQ219186
Hysterangium	*sp.*	OSC	T8997	DQ218588	———	DQ218876	———	DQ219207
Malajczukia	*amicosum*	OSC	59295	DQ218508	DQ218683	DQ218792	DQ219030	DQ219208
Malajczukia	*ingratissima*	OSC	59296	DQ218509	DQ218684	DQ218793	DQ219031	DQ219209
Mesophellia	*arenaria*	OSC	59306	DQ218589	———	DQ218877	DQ219032	DQ219210
Mesophellia	*clelandii*	OSC	59292	DQ218511	DQ218686	DQ218795	DQ219033	DQ219211
Mesophellia	*sabulosa*	OSC	55918	DQ218591	DQ218739	DQ218879	DQ219035	DQ219213
Mesophellia	*trabalis*	OSC	59282	DQ218592	———	DQ218880	DQ219036	DQ219214
Nothocastoreum	*cretaceum*	OSC	79832	DQ218593	———	DQ218881	———	DQ219215
Nothocastoreum	*cretaceum*	OSC	79925	DQ218594	———	———	DQ219037	DQ219216
Phallogaster	*saccatus*	OSC	T13202	DQ218595	DQ218740	DQ218882	DQ219038	DQ219217
Protubera	*hautuensis*	OSC	59673	DQ218517	DQ218692	DQ218801	DQ219039	DQ219218
Protubera	*nothofagi*	OSC	59699	AY574644	AY574718	AY574786	DQ219040	DQ219219
Protubera	*sp.*	OSC	T20068	DQ218596	———	DQ218883	DQ219041	DQ219220
Trappea	*phillipsii*	OSC	56042	DQ218522	DQ218697	DQ218807	DQ219042	———
Trappea	*pinyonensis*	OSC	AHF530	DQ218597	———	DQ218884	DQ219043	DQ219221
Phallales clade								
Anthurus	*archeri*	PDD	REB-2182	DQ218624	DQ218750	DQ218913	DQ219081	DQ219260
Aseroe	*rubra*	OSC	122632	DQ218625	———	DQ218914	DQ219082	DQ219261
Clathrus	*chrysomycelinus*	PDD	75096	DQ218626	———	DQ218915	DQ219083	DQ219262
Clathrus	*ruber*	OSC	79910	———	———	DQ218916	DQ219084	———
Claustula	*fischeri*	OSC	122661	———	———	———	DQ219085	DQ219263
Claustula	*fischeri*	PDD	REB-2326	———	DQ218751	———	DQ219086	DQ219264
Dictyophora	*duplicata*	OSC	38819	DQ218481	DQ218656	DQ218765	DQ219087	DQ219265
Dictyophora	*indusiata*	OSC	36088	DQ218627	DQ218752	DQ218917	DQ219088	DQ219266
Dictyophora	*multicolor*	MEL	1054289	DQ218628	DQ218753	DQ218918	DQ219089	DQ219267

Table 4.1. (Continued).

Gelopellis	*macrospora*	BAFC	30373	DQ218629	———	———	———	DQ219268
Gelopellis	*sp.*	OSC	H4397	DQ218630	DQ218754	DQ218919	DQ219090	DQ219269
Gelopellis	*sp.*	OSC	H4571	DQ218631	DQ218755	DQ218920	DQ219091	DQ219270
Gelopellis	*sp.*	MEL	2063389	DQ218632	———	DQ218921	DQ219092	———
Ileodictyon	*cibarium*	OSC	122734	DQ218633	DQ218756	DQ218922	DQ219093	———
Ileodictyon	*gracile*	MEL	2024221	DQ218634	———	DQ218923	DQ219094	DQ219271
Ileodictyon	*gracile*	MEL	2037639	DQ218635	DQ218757	DQ218924	DQ219095	DQ219272
Ileodictyon	*gracile*	MEL	2054561	DQ218636	———	DQ218925	DQ219096	DQ219273
Kjeldsenia	*aureispora*	OSC	56970	DQ218637	———	———	DQ219097	DQ219274
Kobayasia	*nipponica*	OSC	122862	DQ218638	———	DQ218926	DQ219098	———
Kobayasia	*nipponica*	OSC	122863	DQ218639	———	DQ218927	———	———
Laternea	*triscapa*	OSC	122864	DQ218640	DQ218758	DQ218928	DQ219099	DQ219275
Lysurus	*borealis*	OSC	39531	DQ218641	———	DQ218929	DQ219100	DQ219276
Lysurus	*mokusin*	CUW	MB-02-012	DQ218507	DQ218682	DQ218791	DQ219101	DQ219277
Mutinus	*elegans*	OSC	107657	AY574643	AY574717	AY574785	DQ219102	———
Phallobata	*alba*	PDD	76197	DQ218642	———	DQ218930	DQ219103	DQ219278
Phallus	*costatus*	CUW	MB-02-040	DQ218513	DQ218688	DQ218797	DQ219104	DQ219279
Phallus	*hadriani*	OSC	107658	DQ218514	DQ218689	DQ218798	DQ219044	DQ219222
Phallus	*ravenellii*	CUW	s.n.	DQ218515	DQ218690	DQ218799	DQ219105	DQ219280
Phlebogaster	*laurisylvicola*	OSC	CUP1289	DQ218643	———	———	———	DQ219281
Protubera	*borealis*	OSC	OKM21898	DQ218516	———	DQ218800	DQ219106	DQ219282
Protubera	*canescens*	MEL	2063471	DQ218644	———	DQ218931	DQ219107	DQ219283
Protubera	*canescens*	MEL	2105035	DQ218645	DQ218759	DQ218932	DQ219108	DQ219284
Protubera	*clathroidea*	BPI	s.n.	DQ218646	———	———	DQ219109	DQ219285
Protubera	*jamaicensis*	OSC	T28248	DQ218647	DQ218760	DQ218933	DQ219110	DQ219286
Protubera	*maracuja*	OSC	Garido 2550-A	DQ218518	DQ218693	DQ218802	DQ219111	DQ219287
Protubera	*parvispora*	OSC	59689	DQ218648	———	DQ218934	DQ219112	DQ219288
Protubera	*sabulonensis*	OSC	T12737	DQ218649	———	DQ218935	DQ219113	DQ219289
Protubera	*sp.*	RMS	S. Miller 10143	DQ218650	———	DQ218936	DQ219114	DQ219290
Protubera	*sp.*	DUKE	JM 98/351	AF261555	———	DQ218937	———	DQ219291

Table 4.1. (Continued).

Simblum	*sphaerocephalum*	CUW	MB-02-016	DQ218521	DQ218696	DQ218806	DQ219115	———
Trappea	*darkeri*	OSC	65085	DQ218651	———	DQ218938	DQ219116	DQ219292

Herbarium code: OSC = Oregon State University Herbarium; CUW = Clark University Herbarium; SUC = State University of New York Herbarium; K = Royal Botanic Gardens, Kew, UK; M = Herbarium at Botanische Staatssammlung München, Germany; TENN = University of Tennessee Herbarium; BPI = Herbarium at U.S. National Fungus Collections; DUKE = Duke University Herbarium; MEL = Herbarium at Royal Botanic Gardens, Australia; MELU = University of Melbourne Herbarium; BAFC = Herbarium at Universidad de Buenos Aires, Argentina; PDD = Herbarium at Landcare Research, New Zealand; RMS = University of Wyoming Herbarium; MPU = Herbarium at Université Montpellier II; MICH = University of Michigan Herbarium; FH = Harvard University Herbarium; OSA = Herbarium at Osaka Museum of Natural History, Japan; BR = Herbarium at Jardin Botanique National de Belgique. * PSU = The Pennsylvania State University Mushroom Culture Collection; * AFTOL = see Assembling the Fungal Tree of Life website at http://ocid.nacse.org/research/aftol/primers.php).

RESULTS AND DISCUSSION

Both Bayesian and parsimony analyses showed strong support for the monophyly of the gomphoid-phalloid clade ('Phallomycetidae' in Fig. 4.1). As discussed above, no definitive synapomorphies have been identified for the gomphoid-phalloid fungi; however, some potential synapomorphic characters, including rhizomorph morphology (called 'ampulate hypha', Agerer, 1999), pistillarin content, and structures of septal pore cap, have been proposed (Hibbett & Thorn, 2001; Pine *et al.*, 1999). Four major clades were recognized within the Phallomycetidae; Hysterangiales, Geastrales, Gomphales, and Phallales. All four major clades were supported by 100% posterior probability although bootstrap values for these clades varied from 59 to 98% (Fig. 4.1). The characteristics for each clade are discussed below.

Gomphales clade

This clade corresponds to the order Gomphales (*sensu* Jülich, 1981) with addition of the Gautieriaceae (originally as the order Gautieriales by Zeller, 1948) and Clavariadelphaceae. The monophyly of the Gomphales was consistent with previous studies (Villegas *et al.*, 1999; Giachini *et al.*, 2005; Hosaka, Chapter 2). Fruiting body morphologies include coral fungi (*Ramaria*, *Phaeoclavulina*, and Lentariaceae), club

Fig. 4.1. Phylogeny of the gomphoid-phalloid fungi. Tree topology is based on the Bayesian analyses with 2,000,000 generations of MCMCMC. GTR+G+I model was used for the nuc-LSU-rDNA, mt-SSU-rDNA, and each codon position for the remaining protein coding genes. Maximum parsimony analyses were conducted with 10000 random additions of heuristic search with TBR and Multrees option off, followed by 500 random addition of heuristic search with TBR and Multrees option on, starting with all the trees in memory from the first step. Numbers on branches are Bayesian posterior probability/ maximum parsimony bootstrap values. The provisional taxon names are indicated with a slash (/). Taxon names are followed by symbols indicating fruiting body forms; ■ = sequestrate-gastroid (truffle-like), ♣ = nonsequestrate-gastroid (including stinkhorns, earthstars, and cannon ball fungi), ▼ = non-gasteroid. A few sequestrate taxa in the Phallales clade are marked with question marks (?) because of their uncertainty in fruiting body form (see text for details).

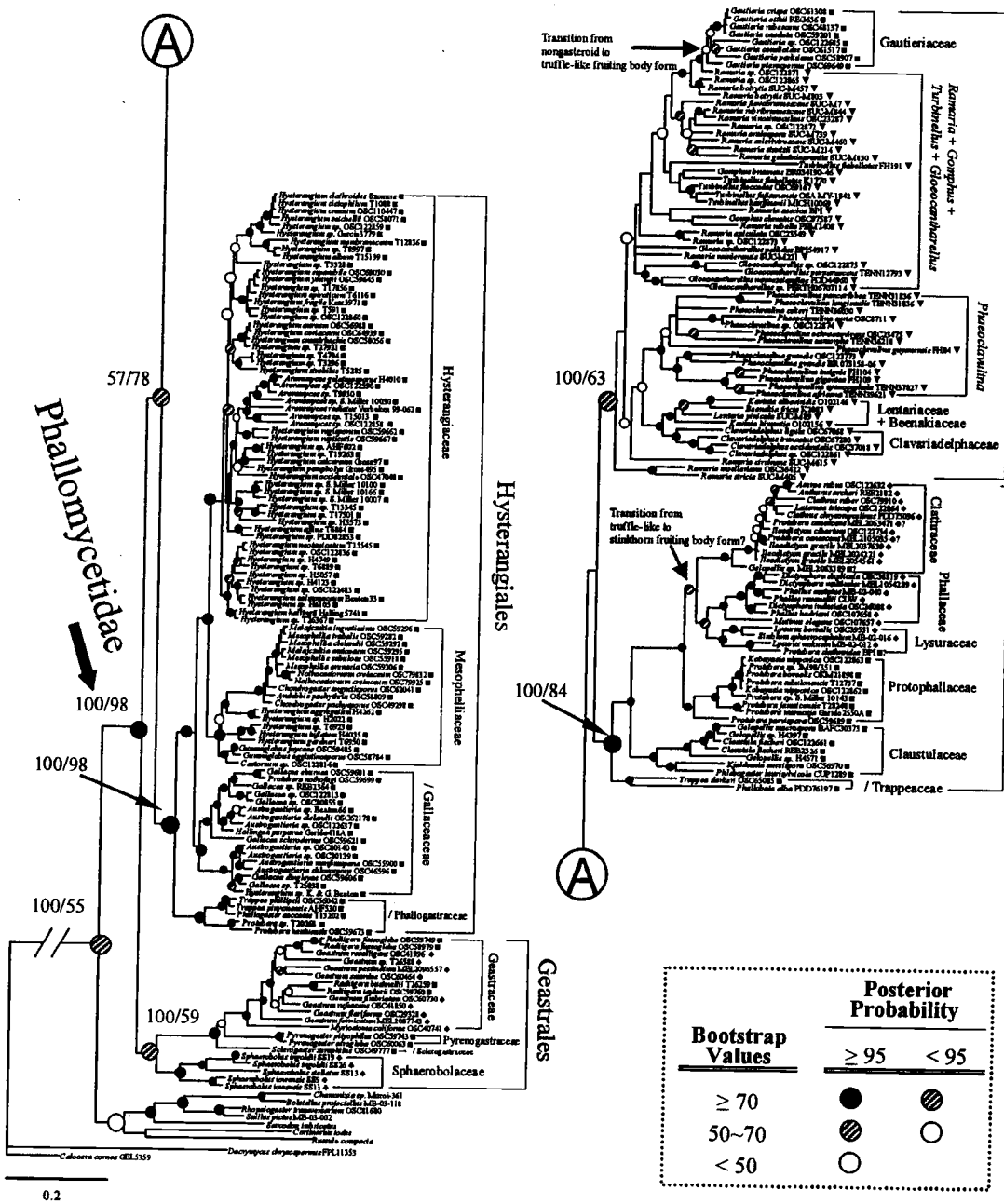

Fig. 4.1. Phylogeny of the gomphoid-phalloid fungi.

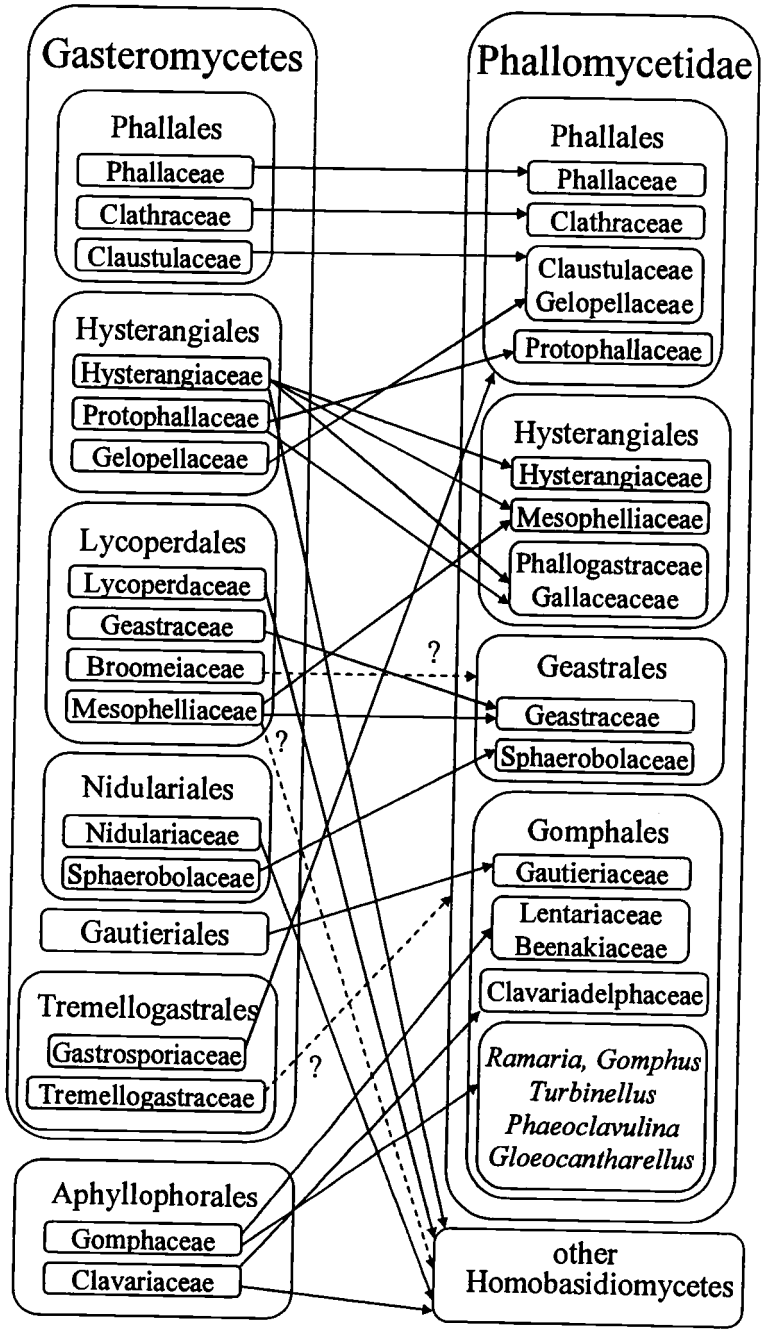

Fig. 4.2. Past and present classifications for the representative gomphoid-phalloid fungi. Left: Classification system by Zeller (1949) for Gasteromycetes; by Donk (1964) for Aphyllophorales. Right: Classification system based on this study. Taxa with dotted arrows and question marks are not included in this study. Multiple arrows arising from the single taxon indicate that the taxon as previously constituted was demonstrated to be polyphyletic.

fungi (Clavariadelphaceae), gilled mushrooms (*Gloeocantharellus*), cantharelloid-gomphoid (*Gomphus*, *Turbinellus,* and *Phaeoclavulina*), tooth fungi (*Kavinia* and *Beenakia*), and false truffles (Gautieriaceae). Despite their macromorphological variations, the members of this clade share a number of microscopic and macrochemical characters, including cyanophilic spore ornamentation, chiastic basidia, hyphal construction, and positive hymenial reaction to ferric sulfate (Donk, 1961, 1964; Villegas *et al.*, 1999).

Humpert *et al.* (2001) and Giachini *et al.* (2005) suggested that branched coral fruiting bodies are ancestral forms for the Gomphales, with multiple derivations of other fruiting body morphologies, such as club, false truffles, cantharelloid, etc. While lack of statistical support for some internal nodes within the Gomphales in this study limits our inferences, the basal positions of some coral fungi, such as *Ramaria moelleriana* and *R. stricta*, are consistent with their conclusions. Tooth fungi (*Kavinia* and *Beenakia*) are restricted to a more terminal clade, indicating that they are derived forms, which is consistent with the hypothesis of Petersen (1971). Gautieriaceae is the only false truffle taxon in the Gomphales and it is restricted to a terminal clade, also indicating their derived form. This is consistent with the conclusions by Humpert *et al.* (2001) and Giachini *et al.* (2005) and the considerable evidence that sequestrate fruiting bodies are derived forms from more complex, epigeous morphology (Thiers, 1984; Miller *et al.*, 2001; Peintner *et al.*, 2001).

The Gomphales also show heterogeneity in their ecological characters. Most species in Lentariaceae, Kaviniaceae, Beenakiaceae, *Phaeoclavulina*, and some

species of *Ramaria* (such as *R. moelleriana, R. stricta* and *R. circinans*) grow and fruit on woody debris, a trait that has led to their general categorization as saprobes. The other taxa of the order are generally considered ectomycorrhizal and while the ectomycorrhizal status of many species of the Gomphales is still unknown (Humpert *et al.*, 2001; Giachini *et al.*, 2005), the formation of ectomycorrhizae by *Turbinellus*, *Gomphus*, and some *Ramaria* species has been confirmed (Agerer, 1996abcd; Agerer *et al.*, 1998). In a study of the evolution of ectomycorrhizae, Hibbett *et al.* (2000) concluded that numerous gains of the ectomycorrhizal symbiosis have occurred in the Homobasidiomycetes. Additionally, they also observed that one unambiguous reversal from ectomycorrhizal to saprobic habit occurred in the lineage leading to *Lentaria*, suggesting that some ectomycorrhizal symbioses are unstable. Although no character state reconstructions were performed in this study, more extensive taxon and character sampling do not support this latter conclusion. In these analyses, *Lentaria* is confidently placed within a group of presumably saprobic species of Gomphales, a topology inconsistent with it being derived from an ectomycorrhizal ancestor through the loss of the ectomycorrhizal symbiosis.

Phallales clade

This clade roughly corresponds to the order Phallales *sensu* Cunningham (1931a, b); with the results of molecular phylogenetic analyses (Fig. 4.1) consistent

with four additional families in this order. Fruiting body morphologies include stinkhorns (Phallaceae), lattice stinkhorns (Clathraceae and Lysuraceae), and false truffles (Protophallaceae, Claustulaceae, and Trappeaceae clade). A few false truffle taxa are also observed in the Clathraceae (*Protubera canescens* and *Gelopellis sp.*) and Lysuraceae (*Protubera clathroidea*) clades. Based on tree topology and original descriptions of these taxa (Beaton & Malajczuk, 1986; Malloch, 1989), it is highly likely that these taxa are immature stinkhorn fruiting bodies and are therefore considered as uncertain fruiting body forms (with question marks in Fig. 4.1). The genus *Gastrosporium* was shown to be the member of the Phallales clade (Hibbett & Binder, 2002), but it was not included in this study due to lack of a protein coding gene sequence. Zeller (1948) included the family Gastrosporiaceae in the order Tremellogastrales, along with the family Tremellogastraceae. The affinity of Tremellogastraceae to the gomphoid-phalloid fungi is, however, still unknown. Most taxa in this order are characterized by having fruiting bodies with a gelatinous layer and a gelatinous to mucilaginous gleba, but *Gastrosporium* has a powdery gleba (Zeller, 1939; Dominguez de Toledo & Castellano, 1996; Miller & Askew, 1982). Spores of most taxa are small, ellipsoid, and smooth without ornamentation, but a few taxa, such as *Kjeldsenia* and *Gastrosporium* have warty spore surfaces (Colgan *et al.*, 1995; Dominguez de Toledo & Castellano, 1997; Miller & Askew, 1982). Most taxa are believed to be saprobic due to their often lignicolous habit, but at least one species (*Protubera canescens*) has been reported to be ectomycorrhizal (Malajczuk, 1988).

The family Lysuraceae was recognized as a separate family from the Clathraceae by Corda (1842) but most subsequent authors treated them as a single family Clathraceae (Zeller, 1949; Dring, 1980; Miller & Miller, 1988; Jülich, 1981). This study shows that Lysuraceae is more closely related to Phallaceae than it is to Clathraceae (Fig. 4.1). Fruiting bodies of the Phallaceae are characterized with a single, unbranched receptacle, and a gleba attached externally on the upper part of receptacle. Fruiting body morphologies of Lysuraceae are similar to Clathraceae in having a gelatinous layer divided by sutures, but differ in having long, stipitate receptacles that are longer than the arms that arise from the receptacle. Also the gleba tends to be attached to the exterior face of the arms, as well as the interior face. Most taxa in Clathraceae have gleba attached only to the interior face of the arms (Dring, 1980).

The family Protophallaceae has been traditionally classified in the Hysterangiales, but this study shows that the family belongs to the Phallales. The genus *Protubera* is polyphyletic in these analyses with species placed in at least three separate clades within the Phallales, including Protophallaceae (including the type species, *P. maracuja*), Lysuraceae, and Clathraceae. In addition, some species of *Protubera* were observed in the Hysterangiales clade. Another member of this clade, the genus *Kjeldsenia* was originally described as a member of Cortinariaceae (Colgan *et al.*, 1995). It is noteworthy that three basal clades within the Phallales (Protophallaceae, Claustulaceae, and Trappeaceae) were all characterized by truffle-like taxa, while taxa with more complex, stinkhorn-like fruiting bodies are restricted to

the more terminal clades, indicating that stinkhorn-like fruiting bodies are derived morphologies in the Phallales. The results of ancestral character state reconstruction were consistent, showing a single transition from sequestrate to stinkhorn fruiting body form (as shown in Fig. 4.1), except when uncertain taxa were coded as sequestrate, which showed ambiguous reconstructions for basal nodes. It is a rare example in the Homobasidiomycetes showing that truffle-like fruiting bodies are ancestral morphologies. Unlike sequestrate taxa in the Agaricales, however, the evolution of truffle-like fruiting bodies in the Phallales does not require gains and/or losses of ballistospory because all taxa in the Phallales are statismosporic.

Mycophagy (the use of fungi as food) by arthropods is well-documented for many groups of fungi (Martin, 1979), and stinkhorn-like fungi are one of the prime examples (Shorrocks & Charlesworth, 1982; Stoffolano, *et al.*, 1989, 1990). On the other hand, major mycophagous animals for truffle-like fungi are rodents and small marsupials, many of which eat significant amounts of fruiting bodies of truffle-like fungi in their diet (Castellano *et al.*, 1989; Claridge, 2002; Malajczuk *et al.*, 1987b). Interestingly, however, spores of truffle-like taxa in the Phallales are not recorded from animal feces in previous studies. Because some truffle-like fungi are known to emit chemical compounds to attract various insects (Pacioni *et al.*, 1991), it is possible that spore dispersal of the Phallales (including sequestrate taxa) is entirely dependent on arthropods.

Hysterangiales clade

This clade contains only sequestrate taxa although fruiting bodies of some taxa, e.g., *Phallogaster* and *Gallacea* occasionally crack open and expose a gleba (Castellano & Beever, 1994; Miller & Miller, 1988). While most taxa possess a gelatinous to cartilaginous gleba, taxa in the Mesophelliaceae clade are characterized by a powdery gleba. Because of their powdery gleba, the Mesophelliaceae has been classified in the Lycoperdales, along with Lycoperdaceae and Geastraceae (Zeller, 1949), and the close relationship of Mesophelliaceae and Hysterangiaceae has never been proposed based on morphological characters. The Mesophelliaceae *sensu* Zeller is, however, polyphyletic. The type genus *Mesophellia* belongs to the Hysterangiales, but the genus *Radiigera* belongs to the Geastrales (Fig. 4.1) and *Abstoma* is most likely related to the Lycoperdaceae. The genus *Rhopalogaster* has been traditionally included in the Hysterangiales (Zeller, 1949), but this study clearly shows that it does not belong to the Phallomycetidae (Fig. 4.1). It is nested within the Boletales, and very closely related to *Suillus*. This indicates that the gelatinous/cartilaginous gleba, one of the defining characters for the Hysterangiales, convergently evolved in the Boletales and Hysterangiales.

Most taxa in the Hysterangiales clade possess ellipsoidal spores that are smooth to minutely warted. One of the exceptions is observed in the Gallaceaceae clade, where the genus *Austrogautieria* possesses longitudinally ridged spores. Within

the Phallomycetidae, *Austrogautieria* and *Gautieria* (in the Gomphales clade) share similar spore morphology and sequestrate habit, but the phylogenetic analysis clearly shows these similarities are due to convergent evolution. Many taxa in the Hysterangiaceae and Mesophelliaceae clade possess spores enveloped in a wrinkled to loose outer membrane (or utricle), whereas taxa in the Gallaceaceae and Phallogastraceae clades do not (Stewart & Trappe, 1985; Castellano & Beever, 1994; Trappe *et al.*, 1996).

Taxa in the Phallogastraceae are most likely saprobic (Miller & Miller, 1988; Castellano, 1990) whereas the rest of the Hysterangiales are all ectomycorrhizal. Although ectomycorrhizal status has not been investigated for all taxa in the Hysterangiales, it was confirmed for some *Hysterangium* spp. (Hysterangiaceae clade; Malajczuk *et al.*, 1987a; Molina & Trappe, 1982; Müller & Agerer, 1996; Raidl & Agerer, 1998), *Mesophellia* and *Castoreum* spp. (Mesophelliaceae clade; Dell *et al.*, 1990), and *Austrogautieria* spp. (Gallaceaceae clade; Lu *et al.*, 1999). In addition, many ectomycorrhizal taxa in the Hysterangiales form dense perennial hyphal mats, which often significantly change the soil chemistry and microorganism biomass (Caldwell *et al.*, 1991; Cromack *et al.*, 1979; Entry *et al.*, 1992; Griffiths *et al.*, 1994; Malajczuk *et al.*, 1987a). Unlike sequestrate taxa in the Phallales, mycophagy by small mammals and marsupials is well-documented for the Hysterangiales, and they often occupy a significant portion of the diet for these animals (Lehmkuhl *et al.*, 2004; Maser & Maser, 1987; Claridge & May, 1994).

Geastrales clade

This clade contains cannon ball fungi (Sphaerobolaceae), earthstars (Geastraceae), and false truffles (Pyrenogastraceae, Sclerogastraceae, and the genus *Radiigera* in Geastraceae clade). The ancestral character reconstruction for fruiting body morphology did not show a clear pattern for the basal nodes of Geastrales clade, but parsimony-based reconstruction indicated that there were at least two independent changes from nonsequestrate to sequestrate (truffle-like) fruiting bodies at the nodes leading to *Radiigera* (*R. fuscogleba* & *R. bushnellii* + *R. taylorii*). This is consistent with the hypothesis that truffle-like fruiting bodies are derived from more complex forms, e.g., agaricoid and boletoid (Peintner *et al.*, 2001).

Zeller (1948) described the family Broomeiaceae in the order Lycoperdales, but it is unclear whether this family is more closely related to the Geastrales or the other Homobasidiomycetes. The genus *Geastrum* and *Myriostoma* in the Geastraceae have fruiting bodies with multiple peridial layers. The outermost peridium (exoperidium) opens in a stellate manner as they mature, exposing the inner peridium (endoperidium) with one (*Geastrum*) to multiple (*Myriostoma*) pores through which spores escape. *Radiigera* and Pyrenogastraceae both have truffle-like fruiting bodies, and their peridium never opens until they are naturally degraded or eaten by mycophagous animals. The gleba of Pyrenogastraceae is divided into multiple peridioles, but this is not the case for *Radiigera*. Both Geastraceae and Pyrenogastraceae have a gleba which becomes black and powdery when mature.

Sclerogastraceae also has truffle-like fruiting bodies, but its gleba never becomes powdery, and it exhibits a yellow to brown color. All taxa described above possess globose spores with a smooth to warty surface.

The structure of the fruiting bodies of the Sphaerobolaceae is unique for the Geastrales. Although the outer peridium opens out stellately, similar to Geastraceae, the gleba is composed of a single peridiole, and never becomes powdery. The peridiole is eventually ejected forcibly by increasing osmotic pressure as the inner layer of the peridium undergoes autolysis. The detailed mechanism of peridiole discharge in *Sphaerobolus* has been extensively studied (e.g., Ingold, 1971, 1972; Fletcher & Cooke, 1984), and despite its small size (fruiting bodies less than 5 mm wide, with peridiole 1 mm wide), the Sphaerobolaceae is capable of ejecting its peridiole upwards of 6 meters (Buller, 1933; Walker, 1927).

Sphaerobolaceae is undoubtedly saprobic because it can easily produce fruiting bodies on artificial media (Flegler, 1984). The nutritional mode for the remaining taxa in the Geastrales, however, remains uncertain. Many species of the genus *Geastrum* grow without obvious ectomycorrhizal plants, and some authors concluded that *Geastrum* is saprobic (Sunhede, 1989; Kreisel, 1969), but at least one species, *G. fimbriatum,* is described as forming ectomycorrhizae (Agerer & Beenken, 1998).

Recently, the genus *Schenella*, which was originally described as myxomycetes (slime molds), was reported to be a synonym of *Pyrenogaster* based on DNA sequence data (Estrada-Torres *et al.*, 2005). Because *Schenella* was described earlier, it has a nomenclatural priority over *Pyrenogaster*. However, the original

descriptions of *Schenella* (MacBride, 1911) show no similarity with the typical *Pyrenogaster* fruiting bodies, and some critical characters, such as basidia and peridial structure, were not observed. Species of *Pyrenogaster* were described from more complete material, and characteristics of fruiting bodies were discussed in detail in Dominguez de Toledo & Castellano (1996) and Malençon & Riousset (1977).

Magallon-Puebla & Cevallos-Ferriz (1993) reported a fossil fruiting body of the Geastraceae from the Late Cenozoic strata of Mexico. Importantly, it is the only undisputable fossil evidence for the Phallomycetidae. While the fossil of *Palaeoclavaria* from 100-million-year-old Burmese amber (Poinar & Brown, 2003) could be related to some taxa in the Phallomycetidae, especially the Clavariadelphaceae, the evidence is inconclusive. The relatively young age of the Geastraceae fossil limits its value as a calibration point for estimating the age of the Geastrales or Phallomycetidae, but it is nonetheless an important and rare record of a well-preserved fossil of a mushroom-forming fungus.

The other fungi

Taylor *et al.* (2003) and Weiss *et al.* (2004) reported that *Geastrum* and Sebacinales are potentially closely related, making the Phallomycetidae polyphyletic. The support for this relationship is rather weak, and it is based solely on nuc-LSU-rDNA sequence data, which has limited resolution power. Sebacinales possesses the

longitudinally septate basidia, but it does not form a clade with the other 'jelly fungi' with the same type of basidia (Weiß & Oberwinkler, 2001). The lack of protein coding gene sequences from representative taxa of the Sebacinales makes it difficult to assess its phylogenetic placement, however including one species of the Sebacinales, *Tremellodendron sp.* (voucher number = PBM 2324) did not change the overall topology of the Phallomycetidae (data not shown). While this might indicate that *Geastrum*-Sebacinales relationship is an artifact of the nuc-LSU-rDNA dataset, future sampling should focus on the multigene sequences from the Sebacinales.

TAXONOMY

Based on the results of phylogenetic analyses discussed above, we propose a new subclass Phallomycetidae to include the Gomphales and Phallales as well as two new orders, Hysterangiales and Geastrales.

Phallomycetidae Hosaka, *subclass. prov.*

Basidiomata hypogeous or epigeous, solitary, gregarious or caespitose, sequestrate or resupinate, effused-reflexed, pileate, turbinate, funnel-shaped, star-shaped, coral-shaped, club-shaped or single to irregularly branched receptacle with a basal volva,

often with basal rhizomorphs which are composed of ampulate hypha. **Hymenium** sometimes turns bluish with ferric sulfate. **Gleba** for gastroid taxa gelatinous, mucilaginous, cartilaginous or powdery at maturity, grey to green, olive, brown, or black, often with well developed columella. **Spores** borne on the exposed hymenium or enclosed gleba, statismosporic or ballistosporic, symmetrical or asymmetrical, globose, subglobose, ellipsoid, elongate, cylindrical to fusiform, smooth, verrucose, echinate or longitudinally ridged, occasionally with a utricle or remnants of an epispore, hyaline to brown in KOH, often cyanophilic.

Type order: Phallales Fischer, in Engler & Prantl, Die Natürlichen Pflanzenfamilien 1: 276, 1900, "Phallineae".

Represented orders: Phallales Fischer, Gomphales Jülich, Hysterangiales Hosaka, *ord. prov.*, Geastrales Hosaka, *ord. prov.*

Discussion- Phallomycetidae: This group is equivalent to the Phallales in the Dictionary of the Fungi 9th edition (Kirk *et al.*, 2001), and the "gomphoid-phalloid clade" *sensu* Hibbett & Thorn (2001). Locquin (1984) used the term 'Phallomycetidae', but did not provide a Latin diagnosis, and therefore it is considered invalid in accordance with Article 36.1 of the International Code of Botanical Nomenclature (ICBN). Furthermore, Phallomycetidae *sensu* Locquin included only stinkhorn-like taxa and a few sequestrate taxa, which is roughly equivalent to the

Phallales clade in this study (Fig. 1). The results of our study strongly support the creation of the subclass Phallomycetidae to accommodate the four distinct but related clades, i.e., Phallales, Hysterangiales, Gomphales, and Geastrales. This organization accommodates the two previously described orders, Phallales and Gomphales with the numerous associated families.

Alternatively, one could treat the entire gomphoid-phalloid clade as an order Phallales, following Kirk *et al.* (2001). However, this treatment would require the creation of four new suborders, or four major clades (Geastrales, Gomphales, Hysterangiales, and Geastrales; Fig. 4.1) would have to be recognized at the family level. This change forces the elimination of several widely recognized families, such as Clathraceae, Gautieriaceae, Mesophelliaceae, Protophallaceae, and Sphaerobolaceae, all of which are supported as monophyletic group in this study (Fig. 4.1). In addition, the gomphoid-phalloid clade is potentially one of the basal clades within the Homobasidiomycetes (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Lutzoni *et al.*, 2004), which further supports the recognition of the subclass status for this group. The use of subclass Phallomycetidae with four orders (Geastrales, Gomphales, Hysterangiales and Phallales) is the best reflection of the higher-level phylogeny, and hence would provide a stable classification system of the Basidiomycota.

Hysterangiales Hosaka, *ord. prov.*

≡ Hysterangiales Zeller, Mycologia 31: 29, 1939, *nom. nud.*

≡ Hysterangiales Locquin, De Taxia Fungorum 1: 48, 1974, *nom. nud.*

Basidiomata hypogeous or epigeous, solitary or gregarious, sequestrate, globose to irregularly shaped, with or without tapering, stem-like sterile base, often covered with adhering sand and soil, or encased in debris and plant roots, often with basal rhizomorphs. **Peridium** sometimes readily separable from gleba, elastic, glutinous or hard and brittle, white to pale yellow, brown, violet, or purple, sometimes staining pink, red, brown, purple, yellow or brown when bruised, 1- to 4-layered, sometimes with a gelatinous subcutis containing sutures that divide the peridium into sections, sometimes incorporating mycorrhizal roots. **Gleba** cartilaginous to gelatinous or becomes powdery at maturity, grey to green, olive or brown, often with labyrinthine to elongated locules, with dendroid, cartilaginous to gelatinous columella, or cork-like central core. **Basidia** 2- to 8-spored. **Spores** statismosporic, mostly symmetrical, ellipsoid, oblong to fusoid, smooth to minutely verrucose, or sometimes ornamented with spines, often with wrinkled to inflated or ephemeral utricle, hyaline, pale green, or brown in KOH, inamyloid, sometimes weakly dextrinoid.

Type family: Hysterangiaceae Fischer, Die Natürlichen Pflanzenfamilien 1: 304, 1900.

Discussion- Hysterangiales: Several authors treated the Hysterangiales as an independent order, segregated from the Phallales (Zeller, 1939, 1949; Jülich, 1981; Locquin, 1974, 1984). However, an extensive literature search revealed that the order had never been published with a Latin diagnosis, and therefore it is considered invalid in accordance with Article 36.1 of the ICBN. Interestingly, Hysterangiales *sensu* Zeller included Protophallaceae and Gelopellaceae, both of which were revealed to be members of the Phallales (Fig. 4.1). Our study revealed several previously unrecognized relationships, i.e., Mesophelliaceae and *Austrogautieria*, which necessitate a redefinition of the Hysterangiales as a new order. There are a few truffle-like genera in the Geastrales and Gomphales but those that do occur in these two orders possess spores that have nonconvergent ridges with rounded margins or are distinctly warted, globose to subglobose with some tint of brown, which distinguish them from all members of the Hysterangiales. For the most part, the Hysterangiales has larger spores than other members of the Phallales. The only exception is the taxa in the Phallogastraceae clade that have similar morphological characteristics to some sequestrate members of the Phallales (Protophallaceae, Claustulaceae, and Trappeaceae clades; Fig. 4.1).

Geastrales Hosaka, *ord. prov.*

≡ Geastrales Kreisel, Grundzüge eines natürlichen Systems der Pilze, 157, 1969,

nom. nud.

≡ Geastrales Locquin, De Taxia Fungorum 1: 57, 1974, *nom. nud.*.

Basidiomata hypogeous or epigeous, solitary, gregarious or caespitose on a common stroma or subiculum, sessile to stipitate, sequestrate or opening with star-shaped to irregular lobes at maturity. **Peridium** two- to five-layered, closed or opening at maturity; if exoperidium opens up at maturity, endoperidium possesses a single to multiple ostioles, or irregularly dehisces, or forcibly discharges a peridiole. **Gleba** with or without divided into a single to multiple peridioles, often powdery at maturity, with or without capillitial threads. **Basidia** globose, clavate, pyriform to tubular, often with a constriction beneath the rounded apex, 4- to 8-spored. **Spores** statismosporic, symmetrical, globose, subglobose to ellipsoid, smooth to ornamented with warts, hyaline to brown in KOH, inamyloid, nondextrinoid.

Type family: Geastraceae Corda, Icones Fungorum 5: 25, 1842, "Geastrideae".

Discussion-Geastrales: Geastrales was published by Kreisel (1969) without a Latin diagnosis and therefore it is considered invalid in accordance with Article 36.1 of the ICBN. Furthermore, Kreisel recognized the order as monotypic, containing a single family Geastraceae, in which only the genera *Geastrum* and *Myriostoma* were recognized. Our study revealed a broader concept of the Geastrales, one that encompasses several previously unrecognized taxa in the order, i.e., Pyrenogastraceae,

Sphaerobolaceae, and *Sclerogaster*. For the most part, the order Geastrales differs from other members of the Phallomycetidae in having basidiomata which open stellately or irregularly, exposing the endoperidium with one to multiple ostioles through which spores are released, or forcibly discharging the peridiole. The truffle-like taxa of the Geastrales, i.e., Pyrenogastraceae, *Radiigera* and *Sclerogaster* differ from the similar taxa in other orders of the Phallomycetidae by the combination of their spore characters including globose to subglobose shape and verrucose to warty ornamentation. The order differs from Gastrosporiaceae and *Calvarula* by having a membranous endoperidium versus a gelatinous one (Dominguez de Toledo & Castellano, 1996; Miller & Askew, 1997). This is the least sampled group in Phallomycetidae. Future study is required to further clarify the familial and generic concept within the order.

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CHAPTER 5

Taxonomic revisions of the Phallomycetidae (Homobasidiomycetes, Basidiomycota) with emphasis on the Hysterangiales

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ABSTRACT

Based on the results of previous phylogenetic analyses for the subclass Phallomycetidae (gomphoid-phalloid fungi), four new families, 7 new genera and 22 new combinations are proposed. The order Hysterangiales is divided into four families, including two new families, Gallaceaceae and Phallogastraceae. All seven new genera are proposed from the order Hysterangiales, including *Austrogautieria, Beeveromyces, Cazomyces, Cribbangium, Insulomyces, Rodwayomyces* and *Viridigautieria.* The order Phallales is divided into six families, including one new family, Trappeaceae. The order Geastrales is divided into four families and a new family Sclerogastraceae is proposed. The analyses revealed that significant changes of the circumscription are necessary for many taxa, and emended taxon descriptions are provided accordingly.

INTRODUCTION

The Phallomycetidae (Homobasidiomycetes, Basidiomycota) is commonly known as the gomphoid-phalloid fungi (Hibbett & Thorn, 2001). Because of its morphological diversity, traditional taxonomy failed to recognize gomphoid-phalloid fungi as a single entity. Hibbett *et al.* (1997) first demonstrated the monophyly of the gomphoid-phalloid fungi, which were repeatedly supported by subsequent studies (Binder & Hibbett, 2002; Hibbett & Binder, 2002; Hibbett *et al.*, 1997; Hibbett & Thorn, 2001; Moncalvo *et al.*, 2002; Humpert *et al.*, 2001; Pine *et al.*, 1999). An initial attempt was made by Kirk *et al.* (2001) to incorporate these results into a formal classification scheme by treating the gomphoid-phalloid fungi as a single order Phallales. Later Hosaka *et al.* (Chapter 4) analyzed a 5-gene dataset of gomphoid-phalloid fungi. The results clearly showed that there are four major clades within the gomphoid-phalloid clade, and each of them was well-supported and in two cases, i.e., Phallales and Gomphales, with a long history of ordinal status. Hosaka *et al.* (Chapter 4) elevated the Phallales *sensu* Kirk *et al.* to subclass status, and proposed a 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 in the Gomphales were made by Giachini (2005) accordingly. However, the taxonomic revisions for the remaining orders (Hysterangiales, Phallales, and Geastrales) have not been conducted yet. Some taxa,

such as *Protubera* and *Trappea* are especially problematic because they were demonstrated to be polyphyletic, and placed in both the Phallales and Hysterangiales clade (Hosaka *et al.*, Chapter 2, 4).

The goal of this study is to incorporate the previous results of the phylogenetic analyses (Hosaka *et al.*, Chapter 3 & 4) into a formal classification scheme. Taxonomic revisions are made for the Geastrales, Phallales, and Hysterangiales. The emphasis is on the familial-level revisions for the Geastrales and Phallales, while familial- and generic-level revisions are made for the Hysterangiales.

MATERIALS & METHODS

For the most part, macroscopic and microscopic characters were based on the literature. Macroscopic characters for undescribed taxa were obtained from fresh material if available. Chemical tests involved observing color reaction of fresh tissues to 10% $FeSO_4$ and Melzer's reagent. Microscopic characters for the undescribed taxa were observed from dried materials mounted in 5% KOH, Melzer's reagent, or $FeSO_4$, and examined by light microscopy. Habitat and distribution are based on collections examined by the author, herbarium records, or from the literature.

Classification schemes were based on the phylogenetic trees from the previous multi-gene studies and only recognized monophyletic taxa. Taxonomic revisions of

the Phallales and Geastrales were based on Hosaka *et al.* (Fig. 3.1, Chapter 3), and those of the Hysterangiales were based on Hosaka *et al.* (Fig. 2.1, Chapter 2). For Phallales, DNA sequences from three taxa (*ATP6* for *Gelopellis purpurascens*, GenBank accession# = DQ218939; nuc-LSU-rDNA for *Gastrosporium simplex,* GenBank accession# = AF518618; *EF1α* for *Calvarula excavata,* GenBank accession# = DQ219293) were added and analyzed under maximum parsimony and Bayesian analyses (See methods described in Chapter 4 and references cited.). Those taxa successfully produced only single-gene sequences. Taxa not included in these analyses were characterized based on the morphological and ecological characters to determine their appropriate taxonomic placement. Some taxa that could not be assigned confidently to any particular group were treated as *incertae sedis.*

The descriptions for all recognized families are provided for the Hysterangiales, Phallales, and Geastrales. New genera and combinations are proposed only for the taxa in the Hysterangiales. Accordingly, descriptions and the list of recognized species are provided for all genera in the Hysterangiales, except for several genera in the family Mesophelliaceae (see discussion under Mesophelliaceae). No changes of the circumscription of genera are made for the taxa in the Phallales and Geastrales. The species lists are not provided for these two orders, but the type species are listed under each genus.

RESULTS AND DISCUSSION

An initial attempt of phylogenetic analysis revealed that three newly added taxa all belong to the order Phallales (data not shown). Because these three taxa had only single-gene sequences, and the amount of missing data could adversely affect the phylogenetic inferences of the Phallomycetidae, subsequent analyses were conducted using only the Phallales taxa. The overall topology of the Phallales was identical with and without the addition of the three taxa in question. Two taxa (*Gastrosporium simplex* and *Calvarula excavata*) were consistently placed in the Lysuraceae clade (Fig. 5.2a, b) by both parsimony and Bayesian analyses, although the Lysuraceae clade received lower nodal support with addition of those taxa. *Gelopellis purpurascens* was confidently placed as a sister taxon to *Dictyophora multicolor* (Fig. 5.2c). *Gelopellis* and *Protubera* were both demonstrated to be polyphyletic, and are discussed under several families in the order Phallales and Hysterangiales.

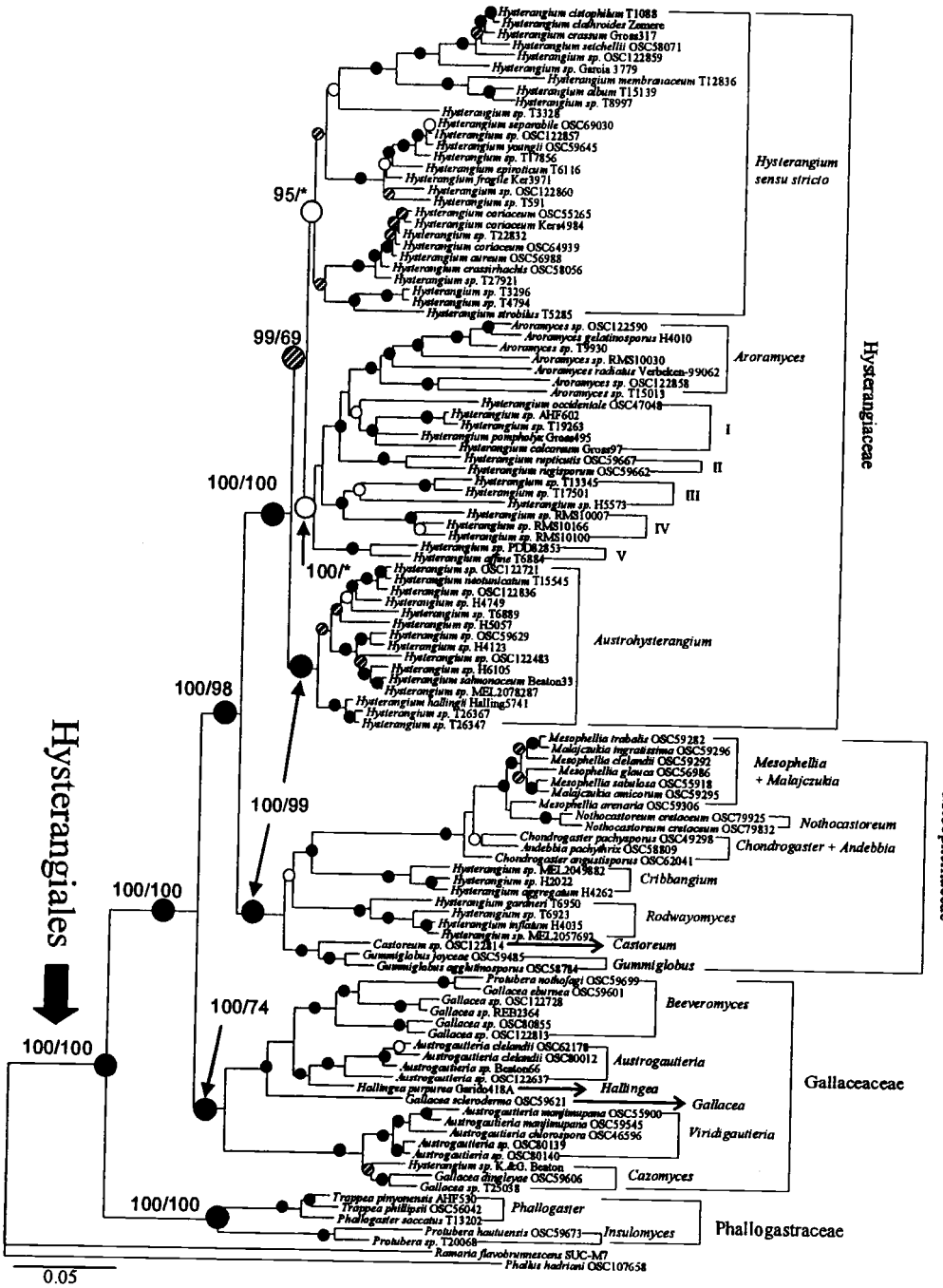

Fig. 5.1. Phylogeny of the Hysterangiales. Tree topology was taken from Hosaka *et al.* (Chapter 3). Numbers on branches are Bayesian posterior probability (BP)/ Maximum parsimony bootstrap values (PB). * indicates no support. Supports for the remaining nodes are indicated by symbols: black circles = BP≥95 & PB≥70; hatched circles = BP<95 & PB≥70 or BP≥95 & 50≤PB<70; white circles = BP≥95 & PB<50 or BP<95 & 50≤PB<70.

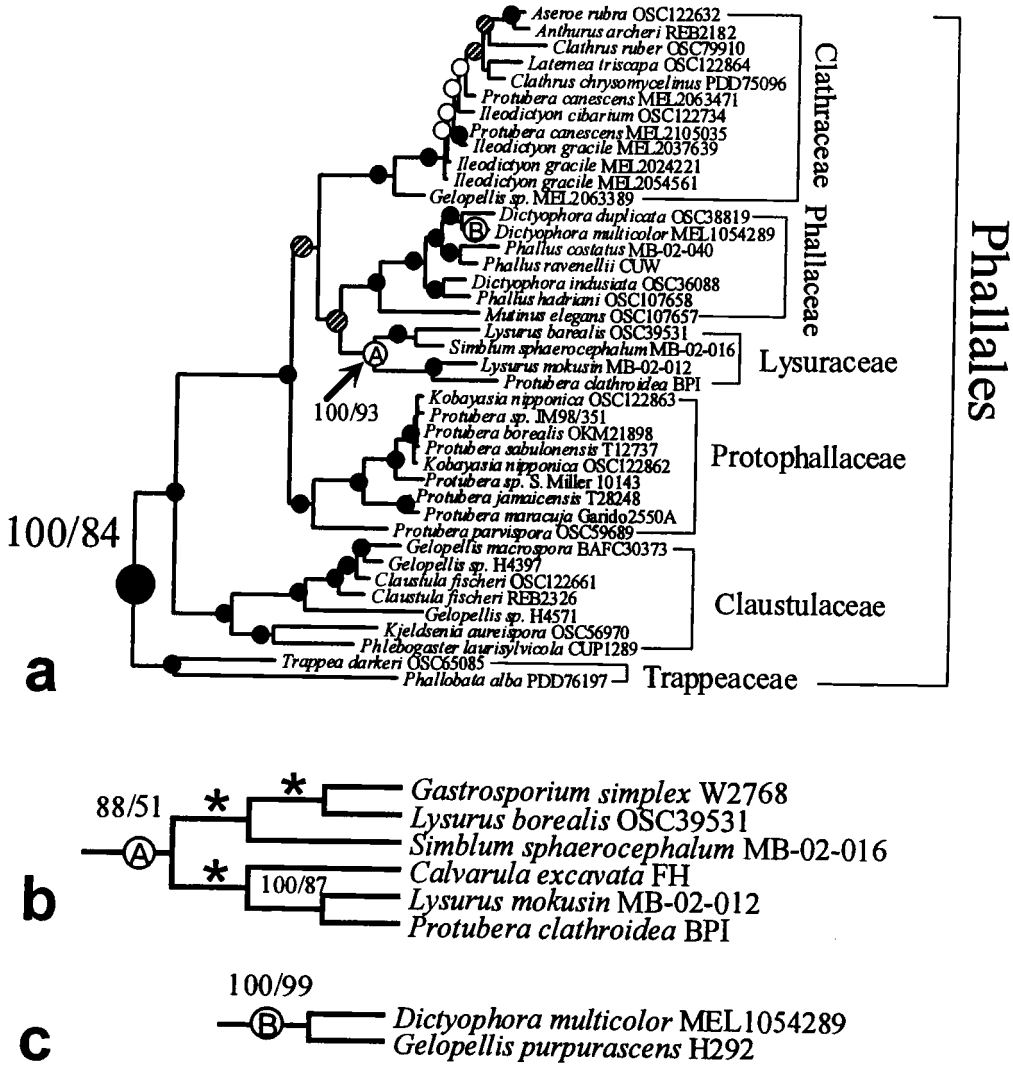

Fig. 5.2. Phylogeny of the Phallales. a) Tree topology was taken from Hosaka *et al.* (Chapter 4). Numbers on the basal node are Bayesian posterior probability (BP)/ Maximum parsimony bootstrap values (PB). Supports for the remaining nodes are indicated by symbols: black circles = BP≥95 & PB≥70; hatched circles = BP<95 & PB≥70 or BP≥95 & 50≤PB<70; white circles = BP≥95 & PB<50 or BP<95 & 50≤PB<70. b) Strict consensus of the Lysuraceae (node A in Fig. 5.2a) based on parsimony analysis with the addition of two taxa with only a single-gene sequence (*Gastrosporium simplex* & *Calvarula excavata*). Numbers on branches indicate Bayesian posterior probability/parsimony bootstrap value. Asterisks indicate the nodes collapsed in 50% majority consensus of 190,000 MCMCMC sampled trees. c) Strict consensus of the node B in Fig.5.2a based on parsimony analysis with addition of a single-gene sequence from *Gelopellis purpurascens*. Numbers on branches indicate Bayesian posterior probability/parsimony bootstrap value.

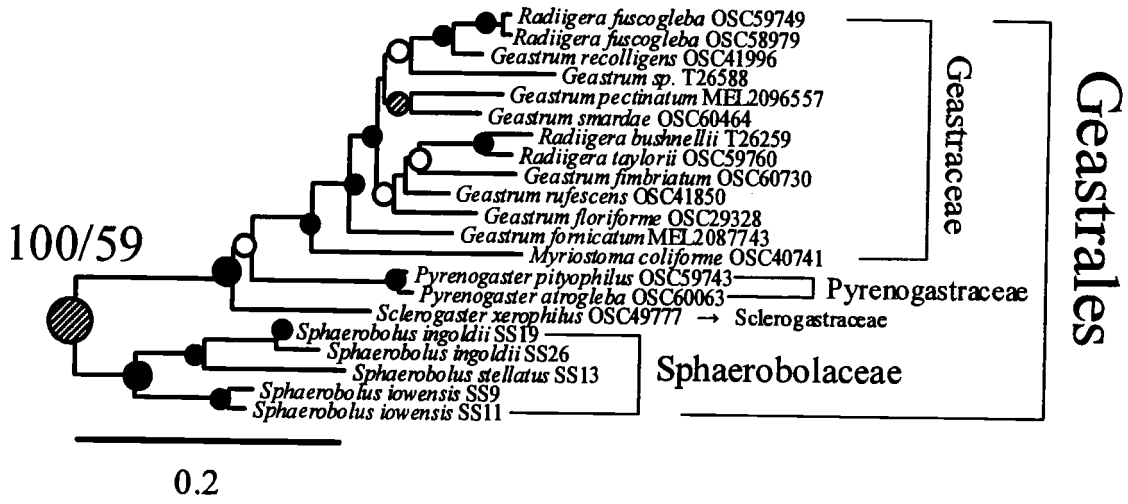

Fig. 5.3. Phylogeny of the Geastrales. Tree topology was taken from Hosaka *et al.* (Chapter 4). Numbers on the basal node are Bayesian posterior probability (BP)/ Maximum parsimony bootstrap values (PB). Supports for the remaining nodes are indicated by symbols: black circles = BP≥95 & PB≥70; hatched circles = BP<95 & PB≥70 or BP≥95 & 50≤PB<70; white circles = BP≥95 & PB<50 or BP<95 & 50≤PB<70.

TAXONOMY

Phallomycetidae Hosaka, *subclass. prov.*

Hysterangiales Hosaka, *ord. prov.*

≡ Hysterangiales Zeller, Mycologia 31: 29, 1939, *nom. nud.*

≡ Hysterangiales Locquin, De Taxia Fungorum 1: 48, 1974, *nom. nud.*

Hysterangiaceae Fischer, Die Natürlichen Pflanzenfamilien 1: 304, 1900, Hosaka, *emend. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or somewhat irregularly lobed, with single to numerous basal rhizomorphs or adherent rhizomorphs along sides and top of basidiomata. **Peridium** 1- to 3-layered, white at first, often staining pink to red, or brown when dried or bruised. **Gleba** gelatinous to cartilaginous, green to olive, or brown. **Columella** often distinct, dendroid, gelatinous to cartilaginous, translucent or opaque. **Basidia** 2- to 6-spored. **Spores** statismosporic, symmetrical, ellipsoid to oblong or fusiform, smooth to minutely verrucose, or ornamented with spines, hyaline to pale green or brown in KOH, often covered by wrinkled to uniformly inflated utricle.

Type genus: *Hysterangium* Vittadini, Monogr. Tuberac. p.13, 1831.

Discussion- Hysterangiaceae: The combination of gelatinous-cartilaginous gleba, spores with distinct utricle, and ectomycorrhizal habit distinguish Hysterangiaceae from most other members of the Hysterangiales. Several Southern Hemisphere species formerly placed in *Hysterangium* are transferred to new genera, and are discussed under *Cribbangium* and *Rodwayomyces* (Mesophelliaceae).

The molecular analyses reveal three distinct clades within the Hysterangiaceae (Fig. 5.1). The most basal clade is well-supported and characterized by a Southern Hemisphere distribution and association with Myrtaceae or Nothofagaceae as ectomycorrhizal hosts. The taxa in this clade are transferred to the new genus *Austrohysterangium.* Two remaining clades are not strongly supported, but the taxa in the *Hysterangium sensu stricto* clade are characterized by a Northern Hemisphere distribution, a gleba with a green tint, spores usually with minute ornamentation and association with Pinaceae or Fagaceae as ectomycorrhizal hosts.

The other clade includes morphologically and ecologically heterogeneous taxa. The genus *Aroramyces* is characterized by a brown gleba and distinctly ornamented spores consisting of spines within an inflated utricle. The taxa in clade I are characterized by a Northern Hemisphere distribution with pink or brown tints to the gleba. Clade II is characterized by taxa with minute ornamentation of the spores, green tint to the gleba, occurrence in the Southern Hemisphere and association with Myrtaceae. Clade III is characterized by taxa from Southeast Asia with a green tint to the gleba. Clade IV is characterized by taxa from Guyana (South America) with a green tint to the gleba and association with Caesalpinioideae (Leguminosae). Clade V

is characterized by taxa from Australia and New Zealand with smooth spores, green gleba and association with Myrtaceae.

At this time, we hesitate to synonymize the taxa in clades I~V with *Aroramyces*. A monophyly of *Aroramyces* + clade I~V is supported only by the Bayesian analysis, and lumping all the taxa in these clades into *Aroramyces* obscures the circumscription of the genus. Likewise we hesitate to synonymize *Aroramyces* with *Hysterangium* because of its distinct morphological characteristics. We do not create new genera for taxa in these clades because several nodes are only poorly supported and the interrelationships are uncertain. We are aware that *Hysterangium* is paraphyletic, but have decided to leave taxa in clades I~V as *Hysterangium sensu lato* until a more robust phylogenetic hypothesis becomes available. The following taxa are recognized in this family:

Aroramyces Castellano & Verbeken, Karstenia 40: 12, 2000.

Description after Castellano *et al.* (2000):

Basidiomata hypogeous to subepigeous, solitary to gregarious, sequestrate, subglobose to irregularly shaped, surface tomentose, with single to numerous rhizomorphs, often with adhering soil particles. **Peridium** up to 3-layered. **Gleba** brown, with irregular locules. **Columella** distinct, gelatinous. **Basidia** 2- to 4-spored. **Spores** statismosporic, symmetrical, ellipsoid, ornamented with spines embedded

within a nearly uniformly inflated utricle, brown in KOH, nondextrinoid, inamyloid. **Utricle** distinct, saccate, inflated, attached only at base.

Type species: *Aroramyces radiatus* (Lloyd) Castellano, Verbeken & Walleyn

Discussion- ***Aroramyces***: The combination of brown gleba, spores ornamented with spines, and the uniformly inflated, saccate utricle distinguish *Aroramyces* from all other Hysterangiaceae. The following species are currently recognized.

Aroramyces gelatinosporus (Cribb) Castellano, Karstenia 40: 13, 2000.

= *Hysterangium gelatinosporum* Cribb, Paps. Dept. Bot. Univ. Queensland 3: 156, 1958.

Aroramyces radiatus (Lloyd) Castellano, Verbeken & Walleyn, Karstenia 40: 12, 2000.

≡ *Hymenogaster radiatus* Lloyd, Mycol. Writings 7, Mycol. Notes 73: 1304, 1925.

≡ *Gymnoglossum radiatum* (Lloyd) Bottomley, Bothalia 4: 499, 1948.

≡ *Dendrogaster radiatus* (Lloyd) Zeller & Dodge, Ann. Mo. Bot. Gard. 21: 688, 1934.

Hysterangium Vittadini, Monogr. Tuberac. p.13, 1831, Hosaka, *emend. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or somewhat irregularly lobed, with a single to numerous basal rhizomorphs or adherent rhizomorphs along sides and top of basidiomata. **Peridium** 1- to 3-layered, white at first, often staining pink to red, or brown when dried or bruised. **Gleba** gelatinous to cartilaginous, green to olive. **Columella** often distinct, dendroid, gelatinous to cartilaginous, translucent or opaque. **Basidia** 2- to 6-spored. **Spores** statismosporic, symmetrical, ellipsoid to oblong or fusiform, usually minutely verrucose, hyaline to pale green in KOH, inamyloid or occasionally weakly dextrinoid. **Utricle** distinct and wrinkled or occasionally absent.

Type species: *Hysterangium clathroides* Vittadini

Discussion-*Hysterangium*: *Hysterangium sensu stricto* is distinguished from other members of the Hysterangiaceae by the combination of green tints to the gleba, spores usually with minute ornamentation, association with Pinaceae or Fagaceae as ectomycorrhizal hosts, and occurrence in the Northern Hemisphere. As discussed above, we hesitate to synonymize *Aroramyces* with *Hysterangium* because of its distinct morphological characters. We have decided to leave *Hysterangium sensu lato* (including taxa in the clades I~V) as paraphyletic group until more robust

phylogenetic hypothesis becomes available. The following species are currently recognized in *Hysterangium sensu stricto*:

Hysterangium album Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 87, 1929.

Hysterangium aureum Zeller, Mycologia 33: 201, 1941.

= *Hysterangium stoloniferum* var. *brevisporum* Zeller, Mycologia 39: 288, 1947.

= *Hysterangium affine* var. *oreades* Zeller, Mycologia 31: 18, 1939.

Hysterangium cinereum Harkness, Cal. Acad. Sci. Proc. III. 1: 254, 1899.

Hysterangium cistophilum (Tulasne & Tulasne) Zeller & Dodge sensu Tulasne & Tulasne, non sensu Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 107, 1929.

≡ *Hysterangium clathroides* var. *cistophilum* Tulasne & Tulasne in Durieu de Masion-Neuve, Expl. Sci. de l'Algérie, Bot. 1: 395, 1846-1849.

Hysterangium clathroides Vittadini, Monogr. Tuberac. p.13, 1831.

= *Hysterangium siculum* Mattirolo, Malpighia, 14: 86, 1900.

Hysterangium coriaceum Hesse, Hypog. Deutschl. 1: 101, 1891.

= *Hysterangium graveolens* Velenovsky, Novitates Mycologicae, p.170, 1939.

= *Hysterangium fuscum* Harkness, Cal. Acad. Sci. Proc. III. 1: 257, 1899.

= *Hysterangium hessei* Soehner, Zeitschr. f. Pilzk. 3: 29-32, 1949.

= *Hysterangium knappii* Soehner, Sydowia 6: 253-254, 1952.

= *Rhizopogon virens* Karsten, Finska Vet.-Soc. Bidrag Natur och Folk 3: 354-355, 1876.

= *Rhizopogon virescens* Karsten in Saccardo, Syll. Fung. 9: 280, 1891.

Hysterangium crassirhachis Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 101, 1929.

Hysterangium crassum (Tulasne & Tulasne) Fischer, Schweiz. Zeitschr. Pilzk. 16: 104, 1938.

≡ *Hysterangium clathroides* var. *crassum* Tulasne & Tulasne, Fung. Hypog. pp. 81-82, 1851.

Hysterangium epiroticum Pacioni, Nova Hedwigia 40: 80-83, 1984.

Hysterangium fragile Vittadini, Monogr. Tuberac. p. 14, 1831.

= *Hysterangium stoloniferum* Tulasne, Ann. Sci. Nat. Bot. II. 19: 376, 1843.

= *Hysterangium stoloniferum* var. *mutabile* Bucholtz, Soc. Imp. Nat. Moscou Bull. 4: 467-470, 1908.

= *Hysterangium stoloniferum* var. *rubescens* (Quelet) Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 112, 1929.

= *Hysterangium rubescens* Patouillard, Bull. Soc. Mycol. Fr. 30: 351, 1914, non Tulasne, Ann. Sci. Nat. II. 19: 375, 1843.

Hysterangium membranaceum Vittadini, Monogr. Tuberac. p. 14, 1831.

≡ *Splanchnomyces membranaceus* (Vittadini) Corda, Icones Fungorum 6: 41, 1854.

= *Hysterangium harknesii* Zeller & Dodge, Ann. Mo. Bot. Gard.16: 102, 1929.

Hysterangium nephriticum Berkley, Ann. Mag. Nat. Hist. 13: 350, 1844.

≡ *Splanchnomyces nephriticum* (Berkeley) Corda, Icones Fungorum 6: 79, 1854.

Hysterangium petri Mattirolo, Malpighia 14: 262, 1900.

Hysterangium pseudostoloniferum Svrček in Pilát, Flora CSR, p. 100, 1958.

Hysterangium separabile Zeller, Mycologia 33: 203, 1941.

≡ *Hysterangium clathroides* Vittadini sensu Zeller & Dodge pro parte, Ann. Mo. Bot. Gard. 16: 95-96, 1929.

Hysterangium setchellii Fischer, Ber. Schweiz. Bot. Ges. 48: 33, 1938.

≡ *Hysterangium clathroides* var. *crassum* Tulasne & Tulasne sensu Zeller & Dodge, non sensu Tulasne, Ann. Mo. Bot. Gard. 16: 96, 1929.

Hysterangium simlense K.S. Thind & I. P.S. Thind in Thind, Thind & Sharma, Indian Phytopath. 35: 615, 1982, "*simlensis*".

Hysterangium strobilus Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 90, 1929.

≡ *Hysterangium clathroides* Vittadini sensu Coker & Couch, non Vittadini, Gast. Eastern United States, pp. 17-19, 1928.

Hysterangium thwaitesii Berkley & Broome, Ann. Mag. Nat. Hist. II. 2: 267, 1848.

≡ *Splanchnomyces thwaitesii* (Berkeley & Broome) Corda, Icones Fungorum, p.42, 1854.

= *Hysterangium rickenii* Soehner, Pilz- und Kräuterfreund 4: 190-192, 1921.

= *Hysterangium rickenii* var. *pinetorum* Soehner, Pilz- und Kräuterfreund 4: 191, 1921.

Hysterangium youngii Castellano & Beever, N.Z. J. Bot. 32: 318, 1994.

Excluded taxa from *Hysterangium*: The following species are transferred to new genera and are discussed under *Austrohysterangium* (Hysterangiaceae), *Cribbangium* and *Rodwayomyces* (Mesophelliaceae).

Hysterangium aggregatum Cribb, Paps. Dept. Bot. Univ. Queensland 3: 156, 1958.

Hysterangium crassipariete Castellano & Muchovej, Mycotaxon 57: 331, 1996.

Hysterangium gardneri Fischer, Bot. Zeit. 66: 164, 1908.

Hysterangium hallingii Castellano & Muchovej, Mycotaxon 57: 333, 1996.

Hysterangium incognitum Castellano & Muchovej, Mycotaxon 57: 334, 1996.

Hysterangium inflatum Rodway, Paps. & Proc. Roy. Soc. Tasmania 1717: 108, 1918.

Hysterangium neocaledonicum Patouillard, Soc. Mycol. France Bull. 31: 34, 1915.

Hysterangium neotunicatum Castellano & Beever, N.Z. J. Bot. 32: 314, 1994.

Hysterangium salmonaceum Beaton, Pegler & Young, Kew Bull. 40: 440, 1985.

Hysterangium spegazzinii Castellano & Muchovej, Mycotaxon 57: 336, 1996.

Hysterangiaceae *incertae sedis*: The following taxa are placed in clades I~V and are excluded from *Hysterangium sensu stricto*. As discussed above, we hesitate to synonymize the taxa in these clades with *Aroramyces*, and have decided to leave them as *Hysterangium sensu lato* until more robust phylogenetic hypothesis becomes available. Future research may in fact recognize multiple genera for these taxa.

Hysterangium affine Massee & Rodway in Massee, Kew Bull. Misc. Info. 1898: 127, 1898.

= *Hysterangium affine* var. *irregulare* Massee, Kew Bull. Misc. Info. 1901: 158, 1901.

= *Hysterangium affine* var. *tenuispora* Rodway, Paps. & Proc. Roy. Soc. Tasmania 1911: 27, 1912.

Hysterangium calcareum Hesse, Hypog. Deutschl. 1: 97, 1891.

Hysterangium occidentale Harkness, Cal. Acad. Sci. Proc. III. 1: 255, 1899.

Hysterangium pompholyx Tulasne & Tulasne, Ann. Sci. Nat. Bot. II. 19: 375, 1843.

= *Hysterangium rubricatum* Hesse, Jahrb. f. wiss. Bot. 15: 631, 1884.

Hysterangium rugisporum Castellano & Beever, N.Z. J. Bot. 32: 316, 1994.

Hysterangium rupticutis Castellano & Beever, N.Z. J. Bot. 32: 318, 1994.

Doubtful taxa in *Hysterangium*: The following taxa have uncertain affinities. Their morphological characters clearly separate them from *Hysterangium sensu stricto*. Many of them probably do not belong to the Phallomycetidae. However, their generic placement is uncertain at this time.

Hysterangium hokkaidoense Kobayasi, J. Jap. Bot. 61: 146, 1986.

Hysterangium moselei (Berkley & Broome) Zeller & Dodge in Dodge & Zeller, Ann. Mo. Bot. Gard. 21: 682, 1934.

≡ *Hymenogaster moselei* Berkeley & Broome, J. Linn. Soc. 16: 40, 1840.

≡ *Hymenogaster moselei* (Berkeley & Broome) De Toni in Saccardo, Syll. Fung. 7: 172, 1888.

Hysterangium neglectum Massee & Rodway in Massee, Kew Bull. Misc. Info. 1899: 181, 1899.

Hysterangium subglobosum Cribb, Paps. Dept. Bot. Univ. Queensland 3: 157, 1958.

Austrohysterangium Hosaka, *gen. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or somewhat irregularly lobed, often with numerous basal rhizomorphs. **Peridium** 1- to 3-layered, white at first, staining pink to red or brown when dried or bruised. **Gleba** gelatinous to cartilaginous, pale gray, green-yellow, olive, olive-brown, or dark green. **Columella** often distinct, dendroid, gelatinous to cartilaginous, translucent, tends to become brown to nearly black when dried. **Basidia** 4- to 6-spored. **Spores** statismosporic, symmetrical, ellipsoid to oblong or fusiform, smooth, hyaline to pale green in KOH. **Utricle** distinct, wrinkled and irregularly inflated. **Habitat** under Myrtaceae (*Eucalyptus* spp., *Leptospermum,* and *Kunzea*) or *Nothofagus* spp. **Distribution** only in the Southern Hemisphere (Australia, New Caledonia, New Zealand, Papua New Guinea, South America).

Etymology: Latin, "southern *Hysterangium*," referring to its distribution in the Southern Hemisphere.

Type species: *Hysterangium hallingii* Castellano & Muchovej, Mycotaxon 57: 333, 1996.

Discussion-*Austrohysterangium*: The genus is segregated from *Hysterangium s.s.* based on strong support from the molecular analyses (Fig. 5.1). Morphologically, the genus is not readily distinguishable from the rest of Hysterangiaceae. However, the combination of smooth spores and the Southern Hemisphere distribution distinguish this genus from most other members of the Hysterangiaceae. The genus is currently known only from the Southern Hemisphere and is always associated with Myrtaceae or *Nothofagus*. The following species are currently recognized:

Austrohysterangium crassipariete Hosaka, *comb. prov.*

≡ *Hysterangium crassipariete* Castellano & Muchovej, Mycotaxon 57: 331, 1996.

Austrohysterangium hallingii Hosaka, *comb. prov.*

≡ *Hysterangium hallingii* Castellano & Muchovej, Mycotaxon 57: 333, 1996.

Austrohysterangium incognitum Hosaka, *comb. prov.*

≡ *Hysterangium incognitum* Castellano & Muchovej, Mycotaxon 57: 334, 1996.

Austrohysterangium neocaledonicum Hosaka, *comb. prov.*

≡ *Hysterangium neocaledonicum* Patouillard, Soc. Mycol. France Bull. 31: 34, 1915.

Austrohysterangium neotunicatum Hosaka, *comb. prov.*

≡ *Hysterangium neotunicatum* Castellano & Beever, N.Z. J. Bot. 32: 314, 1994.

= *Hysterangium tunicatum* Cunningham, Trans. Roy. Soc. New Zealand 67: 409, 1938, *nom. nud.*

Austrohysterangium salmonaceum Hosaka, *comb. prov.*

≡ *Hysterangium salmonaceum* Beaton, Pegler & Young, Kew Bull. 40: 440, 1985.

Austrohysterangium spegazzinii Hosaka, *comb. prov.*

≡ *Hysterangium spegazzinii* Castellano & Muchovej, Mycotaxon 57: 336, 1996.

Mesophelliaceae (Cunningham) Jülich, Bibl. Mycol. 85: 379, 1981, Hosaka, *emend. prov.*

≡ Lycoperdaceae tribus Mesophellieae Cunningham, Proc. Linn. Soc. New South Wales 57: 315, 1932.

= Chondrogastraceae Locquin, De Taxia Fungorum 1: 48, 1974, *nom. nud.*

Basidiomata hypogeous or subepigeous, sequestrate, globose to subglobose or irregularly lobed, often covered with sand, soil, plant roots, and the other organic matter, often formed in clusters. **Peridium** elastic, glutinous or hard and brittle, white to brown, 1- to 4-layered, often incorporating the surrounding plant root into the peridial structure. **Gleba** often deliquescent or powdery when mature, grey to green, dark olive or nearly black, often with gelatinous, dendroid columella, or cork-like central core. **Spores** statismosporic, symmetrical, ellipsoid to fusiform, smooth, often with wrinkled to inflated utricle or with remnants of utricle adhering to spore. **Habitat** under Myrtaceae, especially *Eucalyptus* spp.

Type genus: *Mesophellia* Berkley, Trans. Linn. Soc. London 22: 131, 1857.

Discussion- Mesophelliaceae: This family is unique in the Hysterangiales in having a powdery gleba at maturity. A few taxa with gelatinous gleba are also included in this family, i.e., *Chondrogaster*, *Rodwayomyces, and Cribbangium*. Many members of the family tend to form basidiomata in clusters, and often incorporate the surrounding organic matter into the peridial structure. The affinity of *Chondrogaster* to other members of this family has been unclear, but the molecular evidence clearly supports its placement in the Mesophelliaceae (Fig. 5.1). The family Chondrogastraceae was published by Locquin (1974) without a Latin diagnosis and is considered invalid in accordance with Article 36.1 of the ICBN. At this time, we have not modified the generic concept of the following genera nor made any recombinations within them so

we do not provide a generic description and refer readers to the literature cited for each genus. The unchanged genera include *Andebbia, Castoreum, Chondrogaster, Gummiglobus, Gummivena, Malajczukia, Mesophellia,* and *Nothocastoreum*. The generic concept of some taxa, especially the relationship between *Mesophellia* and *Malajczukia*, will need critical reevaluation. The following taxa including two new genera are recognized in this family:

Andebbia Trappe, Castellano & Amaranthus, Aust. Syst. Bot. 9: 808, 1996.

Type species: *Andebbia pachythrix* (Cooke & Massee) Trappe, Castellano & Amaranthus

≡ *Diploderma pachythrix* Cooke & Massee in Cooke, Grevillea 18: 50, 1890.

≡ *Mesophellia pachythrix* (Cooke & Massee in Cooke) Lloyd, Lycoperdaceae in Australia and New Zealand, 1905.

Castoreum Cooke & Massee in Cooke, Grevillea 15: 100, 1887.

Type species: *Castoreum radicatum* Cooke & Massee

Chondrogaster Maire, Bull. Soc. Mycol. Fr. 40: 312, 1925.

Type species: *Chondrogaster pachysporus* Maire

Gummiglobus Trappe, Castellano & Amaranthus, Aust. Syst. Bot. 9: 804, 1996.

Type species: *Gummiglobus joyceae* Trappe, Castellano & Amaranthus.

Gummivena Trappe & Bougher, Australasian Mycologist 21: 9, 2002.

Type species: *Gummivena potorooi* Trappe & Bougher

Malajczukia Trappe & Castellano in Trappe, Castellano & M. J. Trappe, Aust. Syst. Bot. 5: 618, 1992.

Type species: *Malajczukia viridigleba* Trappe & Castellano

Mesophellia Berkley, Trans. Linn. Soc. London 22: 131, 1857.

= *Inoderma* Berkeley p.p., J. Linn. Soc. London 18: 386, 1881.

= *Potoromyces* Hollós, Növen. Közl. 1: 155-156, 1902.

Type species: *Mesophellia arenaria* Berkeley

Nothocastoreum Beaton, in Beaton & Weste, Trans. Br. Mycol. Soc. 82: 666, 1984.

Type species: *Nothocastoreum cretaceum* (Lloyd) Beaton

≡ *Diploderma cretaceum* Lloyd, Mycol. Notes: 1057, 1920.

≡ *Castoreum cretaceum* (Lloyd) Cunningham, Proc. Linn. Soc. New South Wales 57: 320, 1932.

Rodwayomyces Hosaka, *gen. prov.*

Basidiomata hypogeous or subepigeous, sequestrate, globose to subglobose or irregularly lobed, with rhizomorphs attached at base or along sides, surface tomentose to felt-like, with adhering soil and organic matter. **Peridium** white with brown patches when fresh, often staining pink when exposed or bruised, a single layer composed of irregularly shaped, somewhat inflated hyphae; peridial hyphae thinner near the gleba, more inflated towards the surface; occasionally numerous crystalline particles adhering to outer hyphae. **Gleba** gelatinous, olive-grey to bright green when young, dark olive to nearly black and often deliquescent when mature. **Columella** gelatinous, dendroid, translucent. **Basidia** 4- to 6-spored. **Spores** statismosporic, symmetrical, ellipsoid, smooth, hyaline to pale green in KOH. **Utricle** often very distinct and inflated up to 2.5 µm, forming a cylinder around spore, or sometimes inconspicuous. **Habitat** under *Eucalyptus* spp.

Etymology: Latin, "Rodway's fungus," in honor of Leonard Rodway, a prodigious collector of sequestrate fungi from Tasmania, Australia and the author of the type species of the genus.

Type species: *Hysterangium inflatum* Rodway, Paps. & Proc. Roy. Soc. Tasmania 1717: 108, 1918.

Discussion- ***Rodwayomyces***: The taxa in this genus have originally been included in *Hysterangium* because of their green, gelatinous gleba and spores with a distinct utricle. However, molecular evidence (Fig. 5.1) clearly suggests that they are more closely related to *Mesophellia* and *Nothocastoreum*. *Rodwayomyces* is similar to members of the Hysterangiaceae in many characters but is distinct in a combination of the blackening of the gleba at maturity, the relatively small spore size, the usually inflated utricle, and the unique peridial structure. At present, two species are recognized in the genus, and they have been collected from many parts of the world (including Australia, Brazil, Ecuador, France, New Zealand), but both are known only from natural or planted *Eucalyptus* stands (Castellano & Beever, 1994). The ectomycorrhizal formation of *Rodwayomyces inflatum* with *Eucalyptus* spp. has been experimentally confirmed (Malajczuk *et al.*, 1987). The following species are recognized:

Rodwayomyces inflatum (Rodway) Hosaka, *comb. prov.*

≡ *Hysterangium inflatum* Rodway, Paps. & Proc. Roy. Soc. Tasmania 1717: 108, 1918.

= *Hysterangium eucalyptorum* Lloyd, Mycol. Notes 65: 1031, 1921.

= *Hysterangium pterosporum* Donadini & Riousset, Trav. Sci. Parc Nation. Port-Cros 5: 12, 1979.

Rodwayomyces gardneri (Fischer) Hosaka, *comb. prov.*

≡ *Hysterangium gardneri* Fischer, Bot. Zeit. 66: 164, 1908.

= *Hysterangium fischeri* Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 109, 1929.

Cribbangium Hosaka, *gen. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or irregularly shaped, tiny (mostly ≤ 3 mm in diameter), surface tomentose, clustered and imbedded in soil, roots, and profuse white mycelium, lacking rhizomorphs. **Peridium** not separable from gleba, white when fresh, not staining when exposed or bruised, a single layer composed of thin-walled, compactly interwoven hyphae. **Gleba** gelatinous, green to gray-green. **Columella** absent. **Basidia** 2-spored, constricted near the base. **Spores** statismosporic, symmetrical, ellipsoid to fusiform, smooth, hyaline to pale green in KOH. **Utricle** inconspicuous, adhering closely to spore wall. **Habitat** under *Eucalyptus* spp. or *Tristania* spp.

Etymology: Latin, "Cribb's vessel," named for J.W. Cribb of Queensland, Australia, an accomplished mycologist and the author of the type species of the genus.

Type species: *Hysterangium aggregatum* Cribb, Paps. Dept. Bot. Univ. Queensland 3: 156, 1958.

Discussion- *Cribbangium*: The species of *Cribbangium* were originally included in *Hysterangium* because of the green, gelatinous gleba and spores with an inconspicuous utricle. However, molecular evidence (Fig. 5.1) clearly suggests that they are more closely related to *Mesophellia* and *Nothocastoreum*. *Cribbangium* differs from any other members of the Hysterangiales in having unusually small basidiomata, which are formed in clusters and imbedded in soil, organic matter, and profuse mycelium. The following species are recognized:

Cribbangium aggregatum (Cribb) Hosaka, *comb. prov.*

≡ *Hysterangium aggregatum* Cribb, Paps. Dept. Bot. Univ. Queensland 3: 156, 1958.

Cribbangium pumilum (Rodway) Hosaka, *comb. prov.*

≡ *Hysterangium pumilum* Rodway, Paps. & Proc. Roy. Soc. Tasmania 1917: 109, 1919.

≡ *Hysterangium pumilum* Rodway in Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 115-116, 1929.

Gallaceaceae Hosaka, *fam. prov.*

≡ Gallaceaceae Locquin, De Taxia Fungorum 1: 52, 1974, *nom. nud.*

Basidiomata hypogeous to subepigeous, sequestrate, globose to broadly obpyriform or irregularly shaped, often with a basal rhizomorph. **Peridium** persistent or sometimes cracking to expose gleba, white, pale yellow, pale pink, brown, violet, or purple, often staining pink, red, or brown when exposed or bruised, 1- to 2-layered. **Gleba** gelatinous or somewhat friable, sometimes deliquescent at maturity, gray, orange-yellow, yellow-green, olive, brown, or purple-brown, with rounded to labyrinthiform locules. **Columella** distinct and dendroid or rudimentary, translucent to white or pale orange-yellow. **Basidia** 2- to 6- spored. **Spores** statismosporic, symmetrical or sometimes asymmetrical, smooth or ornamented with longitudinal ridges, hyaline to pale green or brown in KOH. **Utricle** mostly absent.

Type genus: *Gallacea* Lloyd, Mycol. Writings 1. Lycoperd. Australia: 37, 1905.

Discussion-Gallaceaceae: Gallaceaceae was published by Locquin (1974) without a Latin diagnosis and therefore regarded as invalid in accordance with Article 36.1 of the ICBN. Gallaceaceae differs from the Phallogastraceae in having larger spores ($\geq$ 6 µm long) and an ectomycorrhizal habit. The gelatinous, rarely cartilaginous gleba and the spores without a utricle distinguish it from the remaining Hysterangiales. The family is currently known only from the Southern Hemisphere (Australia, New Caledonia, New Zealand, and South America), and is associated either with *Nothofagus* or Myrtaceae (*Eucalyptus*, *Leptospermum*, *Kunzea*) spp. The following taxa are recognized:

Gallacea Lloyd, Mycol. Writings 1. Lycoperd. Australia: 37, 1905, Hosaka, *emend. prov.*

Basidiomata hypogeous to subepigeous, sequestrate, globose to irregularly shaped, surface tomentose to scaly, often with a basal rhizomorph. **Peridium** not separable from gleba, persistent, purple to violet when fresh, often becomes brown when exposed or dried, 1-layered. **Gleba** gelatinous, olive-brown, deep yellow-brown to dark gray-brown, with elongate to irregular locules; often developing large schizogenous cavities. **Columella** often poorly developed, dendroid, translucent. **Basidia** 4- to 6- spored. **Spores** statismosporic, symmetrical, ellipsoid, smooth, yellow-olive to brown in KOH. **Utricle** absent.

Type species: *Gallacea scleroderma* (Cooke) Lloyd

Discussion- *Gallacea*: *Gallacea* as previously constituted was polyphyletic, and was placed in our study in at least three separate clades within the Gallaceaceae (Fig. 5.1). Accordingly, all species except the type species, *G. scleroderma*, are transferred to new genera. *Gallacea* is readily distinguishable from the remaining Hysterangiales by a combination of distinct purple pigmentation in the peridium and the size and shape of the spores. It is currently known only from New Zealand (Castellano & Beever, 1994). The following species is the sole member of this genus at this time:

Gallacea scleroderma (Cooke) Lloyd, Mycol. Writings 1. Lycoperd. Australia: 38, 1905.

≡ *Mesophellia scleroderma* Cooke, Grevillea 14: 11, 1885.

≡ *Hysterangium sclerodermum* (Cooke) Cunningham, Proc. Linn. Soc. New S. Wales 59: 165, 1934.

= *Rhizopogon violaceus* Cooke & Massee in Cooke, Grevillea 21: 1, 1892.

= *Gallacea violacea* (Cooke & Massee) Lloyd, Mycol. Writings 7: 1201, 1923.

Excluded taxa from *Gallacea*: The following taxa are transferred to new genera and are discussed below.

Gallacea avellanea Patouillard, Bull. Soc. Mycol. Fr. 27: 38, 1910.

Gallacea dinglyae Castellano & Beever, N.Z. J. Bot. 32: 307, 1994.

Gallacea eburnea Castellano & Beever, N.Z. J. Bot. 32: 308, 1994.

Cazomyces Hosaka *gen. prov.*

Basidiomata hypogeous to subepigeous, sequestrate, globose to broadly obpyriform, surface glabrous or faintly fibrillose, sometimes cracking to expose gleba. **Peridium** white to yellow, often mottled with brown patches, often staining brown when exposed or bruised but lacking pink tint, 2-layered with epicutis composed of

periclinal hyphae. **Gleba** gelatinous, often deliquescent at maturity, red-brown to dark gray-brown, or olive, occasionally with white flecks; locules more or less radially elongate. **Columella** distinct, dendroid, gelatinous, often with cottony, white region near base. **Basidia** 4- to 6- spored, cylindrical to subclavate. **Spores** statismosporic, symmetrical or sometimes slightly asymmetrical, ellipsoid, smooth, sometimes with a sterigmal attachment, pale green to yellow-brown in KOH. **Utricle** absent.

Etymology: Latin, "Castellano (Caz)'s fungus," in honor of Dr. Michael A. Castellano for his studies of sequestrate fungi and his particular contribution to our understanding of the Hysterangiales.

Type species: *Gallacea dinglyae* Castellano & Beever, N.Z. J. Bot. 32: 307, 1994.

Discussion- ***Cazomyces***: The combination of smooth, ellipsoid spores without a utricle, peridial epicutis composed of periclinal hyphae, and tendency of peridium to stain brown without a pink tint distinguish it from the remaining Hysterangiales. The genus is currently known only from the Southern Hemisphere (Australia, New Caledonia and New Zealand), and is associated either with *Nothofagus* or Myrtaceae (*Eucalyptus, Leptospermum, Kunzea*) spp. The following species are recognized:

Cazomyces avellanea (Patouillard) Hosaka, *comb. prov.*

≡ *Gallacea avellanea* Patouillard, Bull. Soc. Mycol. Fr. 27: 38, 1910.

Cazomyces dinglyae (Castellano & Beever) Hosaka, *comb. prov.*

≡ *Gallacea dinglyae* Castellano & Beever, N.Z J. Bot. 32: 307, 1994.

Beeveromyces Hosaka *gen. prov.*

Basidiomata hypogeous to subepigeous, sequestrate, globose to subglobose or irregularly shaped, often irregularly grooved, surface glabrous, sometimes cracking to expose gleba. **Peridium** white to pale yellow or pale pink when fresh, often staining pink-brown when exposed or bruised, 2-layered; epicutis mostly composed of interwoven hyphae; subcutis sometimes divided by sutures. **Gleba** gelatinous, often deliquescent at maturity, dark olive to brown, occasionally with white flecks; locules radially elongate or irregularly shaped, sometimes developing schizogenous cavities. **Columella** distinct, dendroid, gelatinous, translucent to opaque, sometimes with cottony, white tissue arising from peridium. **Spores** statismosporic, symmetrical or sometimes slightly asymmetrical, ellipsoid to oblong, smooth, sometimes with a sterigmal attachment, pale green to pale olive in KOH. **Utricle** absent or rarely present.

Etymology: Latin, "Beever's fungus," named for Dr. Ross E. Beever of New Zealand, an accomplished mycologist and the collector of the type species of the genus.

Type species: *Gallacea eburnea* Castellano & Beever, N.Z. J. Bot. 32: 308, 1994.

Discussion- ***Beeveromyces***: The combination of ellipsoid spores without a utricle, peridial epicutis composed of interwoven hyphae, and tendency of the peridium to stain with pink tints distinguish it from the remaining Hysterangiales. The genus is currently known only from the Southern Hemisphere (New Zealand and Australia), and is associated either with *Nothofagus* or *Eucalyptus* spp. The following species are recognized:

Beeveromyces eburnea (Castellano & Beever) Hosaka, *comb. prov.*

≡ *Gallacea eburnea* Castellano & Beever, N.Z. J. Bot. 32: 308, 1994.

Beeveromyces nothofagi (Castellano & Beever) Hosaka, *comb. prov.*

≡ *Protubera nothofagi* Castellano & Beever, N.Z. J. Bot. 32: 324, 1994.

Hallingea Castellano in Castellano & Muchovej, Mycotaxon 57: 339, 1996.

Description after Castellano & Muchovej (1996):

Basidiomata hypogeous to subepigeous, sequestrate, globose to irregularly shaped. **Peridium** red, pale purple to lavender or violet, staining dark red, vinaceous or purple when bruised, 2-layered. **Gleba** gray, gray-olive, olive-brown to purple-brown; locules irregular to elongate. **Columella** translucent, often arising from a sterile base. **Spores** statismosporic, mostly asymmetrical, fusiform to narrowly ellipsoid, smooth, hyaline to pale green in KOH. **Utricle** mostly absent.

Type species: *Hallingea purpurea* (Zeller & Dodge) Castellano

Discussion- ***Hallingea***: All recognized species of *Hallingea* were originally described as *Hysterangium* spp., but Castellano & Muchovej (1996) transferred them to *Hallingea*, ascribing them as a member of the Boletaceae. Although only one species was included in the phylogenetic analyses (Fig. 5.1), the result clearly places *Hallingea* in the Hysterangiales. Its relatively large sized (≥ 15 μm long), and fusiform to narrowly ellipsoid and often asymmetric shaped spores distinguish it from other members of the Gallaceaceae. It is associated with *Nothofagus* spp. and currently known only from South America (Argentina and Chile). The following species are recognized from this genus:

Hallingea carneorosea (Horak) Castellano in Castellano & Muchovej, Mycotaxon 57: 340, 1996, "*carneoruseus*".

≡ *Hysterangium carneoroseum* Horak, Sydowia 17: 200, 1963.

Hallingea purpurea (Zeller & Dodge) Castellano in Castellano & Muchovej, Mycotaxon 57: 341, 1996, "*purpureus*".

≡ *Hysterangium purpureum* Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 110, 1929.

Hallingea violacea (Horak) Castellano in Castellano & Muchovej, Mycotaxon 57: 343, 1996, "*violaceus*".

≡ *Hysterangium violaceum* Horak, Sydowia 17: 198, 1963.

Austrogautieria E.L. Stewart & Trappe, Mycologia 77: 675, 1985, Hosaka, *emend. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or somewhat irregularly shaped, usually with single rhizomorph at base. **Peridium** persistent, white to pale yellow or brown. **Gleba** somewhat friable, orange-yellow to brown, without green or olive tints, with rounded to elongate or labyrinthiform locules. **Columella** often poorly developed, rudimentary and sparsely branched to dendroid, gelatinous, hyaline to pale orange-yellow. **Basidia** 2-spored. **Spores** statismosporic, symmetrical, ellipsoid to ovoid with a mammalate apex, surface ornamented with often forking and

anastomosing longitudinal ridges that converge at the spore apex, pale yellow to orange or brown in KOH.

Type species: *Austrogautieria macrospora* E.L. Stewart & Trappe

Discussion-*Austrogautieria*: Our phylogenetic analyses clearly showed that *Austrogautieria* is only distantly related to *Gautieria* (Gomphales). These two genera share longitudinally ridged basidiospores, but *Austrogautieria* differs from *Gautieria* in having longitudinal ridges that have acute margins that converge at the spore apex versus the nonconvergent ridges with rounded margins of *Gautieria* (Stewart & Trappe, 1985). Two species originally described as *Austrogautieria* are transferred to a new genus, *Viridigautieria*, based on the molecular evidence (Fig. 5.1). *Austrogautieria* and *Viridigautieria* are the only taxa in the Hysterangiales having longitudinally ridged spores. *Austrogautieria* differs from *Viridigautieria* in having a brown gleba and mammalate spore apices versus green to olive gleba and obtuse spore apices. *Austrogautieria* is currently known only from the Southern Hemisphere (Australia and New Zealand), and is associated either with *Nothofagus* or *Eucalyptus* spp. The following species are recognized:

Austrogautieria albida (Massee & Rodway) Hosaka, *comb. prov.*

≡ *Hymenogaster albidus* Massee & Rodway in Masse, Bull. Misc. Inf. Kew 1901: 158, 1901.

≡ *Gautieria albida* (Massee & Rodway) Cunningham, Proc. Linn. Soc. New South Wales 59: 172, 1934.

≡ *Gautieria albida* (Massee & Rodway) Zeller & Dodge, Ann. Mo. Bot. Gard. 21: 704, 1934.

Austrogautieria clelandii Stewart & Trappe, Mycologia 77: 681, 1985.

Austrogautieria costata Stewart & Trappe, Mycologia 77: 679, 1985.

≡ *Gautieria costata* Cunningham, Trans. Roy. Soc. New Zealand 67: 410, 1938, *nom. nud.*

Austrogautieria macrospora Stewart & Trappe, Mycologia 77: 684, 1985.

≡ *Gautieria macrospora* Cunningham, *nom. nud.*, Proc. Linn. Soc. New South Wales 60: 120, 1935.

Austrogautieria rodwayi (Massee) Stewart & Trappe, Mycologia 77: 684, 1985.

≡ *Hymenogaster rodwayi* Massee, Kew Bull. Misc. Inf. 1898: 126, 1898.

≡ *Gautieria rodwayi* (Massee) Zeller & Dodge in Cunningham, Proc. Linn. Soc. New South Wales 59: 172, 1934.

≡ *Gautieria rodwayi* (Massee) Zeller & Dodge in Dodge & Zeller, Ann. Mo. Bot. Gard. 21: 702, 1934.

Excluded taxa from *Austrogautieria*: The following species are transferred to a new genus, *Viridigautieria* and discussed below.

Austrogautieria chlorospora Stewart & Trappe, Mycologia 77: 678, 1985.

Austrogautieria manjimupana Trappe & Stewart in Trappe & Stewart, Mycologia 77: 676, 1985.

Viridigautieria Hosaka, *gen. prov.*

Basidiomata hypogeous, sequestrate, globose to subglobose, or somewhat irregularly shaped, usually with a single rhizomorph at base. **Peridium** persistent, white to pale yellow or brown. **Gleba** somewhat friable, yellow-green to dark green, or dark olive-brown, with rounded to elongate or labyrinthiform locules. **Columella** rudimentary to narrowly dendroid, gelatinous, gray to pale orange-yellow. **Basidia** 2-spored. **Spores** statismosporic, symmetrical, ellipsoid to ovoid with an obtuse apex, surface ornamented with longitudinal, straight to slightly sinuate or forked ridges that converge at the spore apex, green-yellow to yellow-green in KOH.

Etymology: Latin, "green *Gautieria*", referring to gleba which always has a green to olive tint.

Type species: *Austrogautieria manjimupana* Trappe & Stewart in Trappe & Stewart, Mycologia 77: 676, 1985.

Discussion-*Viridigautieria*: Two species are segregated from *Austrogautieria* based on molecular evidence (Fig. 5.1). *Viridigautieria* and *Austrogautieria* are the only taxa in the Hysterangiales having longitudinally ridged basidiospores. *Viridigautieria* differs from *Austrogautieria* in having a green to olive gleba and obtuse spore apices versus the brown gleba and mammalate spore apices in *Austrogautieria*. *Viridigautieria* is currently known only from Australia, and associated with *Eucalyptus* spp. The following species are recognized:

Viridigautieria chlorospora (Stewart & Trappe) Hosaka, *comb. prov.*

≡ *Austrogautieria chlorospora* Stewart & Trappe, Mycologia 77: 678, 1985.

Viridigautieria manjimupana (Trappe & Stewart) Hosaka, *comb. prov.*

≡ *Austrogautieria manjimupana* Trappe & Stewart in Trappe & Stewart, Mycologia 77: 676, 1985.

Phallogastraceae Hosaka, *fam. prov.*

≡ Phallogastraceae Locquin, De Taxia Fungorum 1: 56, 1974, *nom. nud.*

Basidiomata epigeous or hypogeous, sequestrate, globose to irregularly shaped, with or without tapering, stem-like sterile base; rhizomorphs attached at base. **Peridium** white at first, sometimes staining pink to red when bruised, sometimes with gelatinous subcutis containing sutures that divide the peridium into sections. **Gleba** gelatinous,

green to olive, sometimes with a layer of sterile, white locules directly beneath the peridium. **Columella** usually distinct, dendroid, translucent to opaque, sometimes dividing the gleba into sharply delimited sections. **Basidia** 6-8 spored. **Spores** statismosporic, symmetrical, ellipsoid, oblong to cylindrical, small (≤ 6 μm long), smooth, hyaline to pale green in KOH, nondextrinoid, inamyloid. **Utricle** absent.

Type genus: *Phallogaster* Morgan, J. Cincinnati Soc. Nat. Hist. 15: 171, 1893.

Discussion-Phallogastraceae: Locquin (1974) published the family Phallogastraceae without a Latin diagnosis and it is considered invalid in accordance with Article 36.1 of the ICBN. The taxa in this family are presumably saprobic (Miller & Miller, 1988; States, 1991), as opposed to an ectomycorrhizal habit of the remaining members of the Hysterangiales. Fruiting bodies of the Phallogastraceae usually grow within the humus layer or on rotten wood and they are often found without a confirmed mycorrhizal host in the vicinity. Their nutritional status deserves further study. The family is also unique in having a stem-like base, sterile locules in the outer gleba, or infrequent sutures segregating the peridium. The relatively small spore size (≤ 6 μm long) and elongate to cylindrical spore shape also distinguish this family from the rest of the Hysterangiales. The following taxa are recognized:

Phallogaster Morgan, J. Cincinnati Soc. Nat. Hist. 15: 171, 1893, Hosaka, *emend. prov.*

Basidiomata hypogeous or epigeous, sequestrate, globose, pyriform, clavate, or somewhat irregularly shaped, with or without tapering, stem-like sterile base, usually with basal rhizomorphs. **Peridium** white at first, often staining pink when exposed or bruised, often turns green-blue with iron sulfate solution. **Gleba** gelatinous, green to olive, sometimes with a layer of sterile, white locules directly beneath the peridium. **Columella** distinct, translucent to white, dendroid, sometimes dividing the gleba into sharply delimited sections. **Basidia** 6- to 8-spored. **Spores** statismosporic, symmetrical, ellipsoid, oblong to cylindrical, smooth, hyaline to pale green in KOH, nondextrinoid, inamyloid. **Utricle** absent.

Type species: *Phallogaster saccatus* Morgan

Discussion-*Phallogaster*: Our study strongly suggests that two species of *Trappea* (*T. phillipsii* and *T. pinyonensis*) belong to the Hysterangiales, and are only distantly related to the type species of *Trappea* (*T. darkeri)*, which is nested within the Phallales (Fig. 5.2). Accordingly, those two species of *Trappea* are transferred to the genus *Phallogaster* and new combinations are proposed here. The genus is distinct in having a somewhat irregular shaped to club-shaped basidiomata, a pink staining of peridium, a layer of sterile locules beneath the peridium, and oblong spores. One species,

Phallogaster globosus, does not fit comfortably in this genus (Castellano & Beever, 1994). While this species undoubtedly belongs to the Phallomycetidae, its generic placement is uncertain at this time. The following taxa are recognized:

Phallogaster phillipsii (Harkness) Hosaka, *comb. prov.*

≡ *Hysterangium phillipsii* Harkness, Cal. Acad. Sci. Proc. III. 1: 255, 1899

≡ *Trappea phillipsii* (Harkness) Castellano, Mycotaxon 38: 7. 1990.

Phallogaster pinyonensis (States) Hosaka, *comb. prov.*

≡ *Trappea pinyonensis* States, Mycotaxon 41: 128, 1991.

Phallogaster saccatus Morgan, J. Cincinnati Soc. Nat. Hist. 15: 172, 1893.

Phallogaster whitei Peck, Bull. N.Y. State Museum 116: 31, 1907.

Doubtful taxon in *Phallogaster*: See Castellano & Beever (1994) for the discussion on the following species.

Phallogaster globosus Lloyd, Mycol. Writings 5: 739, 1917.

Insulomyces Hosaka, *gen. prov.*

Basidiomata epigeous on decaying leaves and branches, sequestrate, globose to depressed, slightly ridged, surface finely tomentose, with basal rhizomorphs.

Peridium not easily separable from gleba, white when fresh, bruising pale pink to pale red, with a gelatinous subcutis which is divided into sections by infrequent sutures. **Gleba** gelatinous, pale olive to gray-olive. **Columella** distinct, dendroid, gelatinous, translucent. **Spores** statismosporic, symmetrical, ellipsoid, with obtuse apex, small (up to 5.5 × 2 μm), smooth, hyaline to pale green in KOH, nondextrinoid, inamyloid. **Utricle** absent.

Etymology: Latin, "island fungus," referring to its distribution that is so far restricted to the islands of Tasmania and New Zealand.

Type species: *Protubera hautuensis* Castellano & Beever, N.Z. J. Bot. 32: 322, 1994.

Discussion-*Insulomyces*: The occurrence of sutures through the gelatinized subcutis, small, smooth and ellipsoid spores without a utricle, and saprobic habit distinguish this genus from the other Hysterangiales. The genus is only known from Tasmania, Australia and New Zealand. The following taxa are recognized in this genus:

Insulomyces burburianus (Rodway) Hosaka, *comb. prov.*

≡ *Hysterangium burburianum* Rodway, Proc. & Paps. Roy. Soc. Tasmania 1917: 109, 1918.

Insulomyces hautuensis (Castellano & Beever) Hosaka, *comb. prov.*

≡ *Hysterangium hautu* Cunningham, Trans. Roy. Soc. New Zeal. 67: 409,

1938, *nom. nud.*

≡ *Protubera hautuensis* Castellano & Beever, N.Z. J. Bot. 32: 322, 1994.

Phallales Fischer in Engler & Prantl, Die Natürlichen Pflanzenfamilien 1: 276, 1900, "Phallineae", Hosaka, *emend. prov.*

≡ Phallales Cunningham, Proc. Linn. Soc. N.S.W. 56: 3, 1931.

Basidiomata hypogeous or epigeous, sequestrate or gymnocarpic, globose to irregularly lobed, sometimes opens up irregularly with or without forming a receptacle at maturity; receptacle branched or unbranched, subspherical to ovoid, latticed or stem-like, pseudoparenchymatous or composed of tubes, with or without a campanulate pileus. **Rhizomorphs** often well-developed and usually attached at base. **Peridium** white at first, sometimes staining yellow to red or brown when exposed or bruised; inner layer often gelatinous, with or without peridial sutures. **Gleba** gelatinous, often becomes mucilaginous or powdery at maturity, gray to brown, dark brown, or deep olivaceous, with or without paracapillitium, often with fetid odor. **Basidia** 4- to 8-spored. **Spores** statismosporic, symmetrical, globose to ellipsoid or oblong, smooth or ornamented with minute to irregularly shaped warts, sometimes covered by smooth to wrinkled or inflated utricle, hyaline to brown in KOH, sometimes strongly cyanophilic, nondextrinoid, inamyloid.

Type family: Phallaceae Fischer, in Engler & Prantl, Die Natürlichen Pflanzenfamilien 1: 289, 1900.

Discussion-Phallales: The order traditionally has contained three families, Phallaceae, Clathraceae and Claustulaceae (Cunningham, 1931; Zeller, 1949). The results of our molecular phylogenetic analyses suggest there are three additional families in this order. The family Lysuraceae was recognized as a separate family from the Clathraceae by Corda (1842) but most subsequent authors treat them as a single family Clathraceae. The family Protophallaceae has traditionally been ascribed to the Hysterangiales (Zeller, 1939, 1949). The family Trappeaceae is newly described here. Taxa forming 'stinkhorn' type basidiomata are easily distinguishable from any other fungi by their pseudoparenchymatous (or tubular) receptacle with mucilaginous and often foul-smelling gleba. Most taxa in this order are characterized by having basidiomata with a thick gelatinous layer directly beneath the thin outer peridium and a gelatinous to mucilaginous gleba, but a few genera, such as *Calvarula* and *Gastrosporium* have powdery gleba at maturity (Zeller, 1939; Dominguez de Toledo & Castellano, 1997; Miller & Askew, 1982). Spores of most taxa are small, ellipsoid, and smooth without ornamentation, but a few taxa, such as *Kjeldsenia* and *Gastrosporium* have a warty spore surface (Colgan *et al.*, 1995; Dominguez de Toledo & Castellano, 1997; Miller & Askew, 1982).

Phallaceae Fries, Systema Mycologicum 2: 281, 1823, "Phalloideae".

≡ Phalloideae Chevallier, Flore Générale des Environs de Paris 1: 120, 1826.

≡ Phallaceae Fischer, in Engler & Prantl, Die Natürlichen Pflanzenfamilien 1: 289, 1900.

Description after Jülich (1981):

Basidiomata hypogeous and globose to ellipsoid when young, epigeous with an unbranched receptacle when mature. **Peridium** white, red to purple, 2- to 3-layered; outer layer thin, membranous and elastic, inner layer thick, gelatinous and continuous; the peridium opens up at maturity and remains as volva at the base of the receptacle. **Receptacle** stipitate, unbranched, cylindrical or fusiform, hollow, with one or several layers of chambers, pseudoparenchymatous, with or without a campanulate pileus. **Gleba** formed only on the exterior face of the cap or the upper part of the receptacle, mucilaginous at maturity, olive-brown, fetid. **Basidia** narrowly clavate to fusiform, 4- to 8-spored. **Spores** statismosporic, symmetrical, cylindrical to narrowly ellipsoid, smooth, hyaline, pale yellow or olivaceous in KOH.

Type genus: *Phallus* L.: Pers., Synopsis Methodica Fungorum, p. 242, 1801.

Discussion-Phallaceae: The characteristic basidiomata with a single, unbranched receptacle, and a gleba attached externally on the upper part of the receptacle distinguish the Phallaceae from other families in the Phallales. One sequestrate taxon,

Gelopellis purpurascens, was revealed as a member of this family. Based on its close relationship to *Dictyophora* and the original description of this species (Beaton & Malajczuk, 1986), we consider *G. purpurascens* to be an immature stage of a *Dictyophora* spp. The holotype specimen of *G. purpurascens* deserves further examination. The following taxa are recognized in this family:

Aporophallus A. Möller, Bot. Mitt. Trop. 7: 68, 1895.

Type species: *Aporophallus subtilis* A. Möller

Dictyophora Desvaux, J. Bot., Paris 2: 92, 1809.

≡ *Hymenophallus* Nees, Syst. Pilz. Schw., p. 251, 1817.

≡ *Phallus* sect. *Hymenophallus* Fries, Syst. Mycol. 2: 282, 1822.

= *Sophronia* Persoon in Gaud., Voyage aut. Monde 178, 1836.

= *Retigerus* Raddi, Mem. Soc. Ital. Moden. 20: 46, 1829.

= *Clautriavia* Patouillard, Bull. Soc. Mycol. Fr. 14: 190, 1898.

≡ *Clautriavia* (Patouillard) Lloyd, Mycol. Writ. 3: 24, 1909.

Type species: *Dictyophora indusiata* Desvaux

Echinophallus Hennings, Bot. Jahrb. Syst. 25: 505, 1898.

Type species: *Echinophallus lauterbachii* Hennings

Endophallus Zang & Petersen, Mycologia 81: 488, 1989.

Type species: *Endophallus yunnanensis* Zang & Petersen

Itajahya A. Möller, Bot. Mitt. Trop. 7: 79, 1895.

= *Alboffiella* Speg., An. Mus. Nac. Hist. Nat. B. Aires 6: 183, 1898.

Type species: *Itajahya galericulata* A. Möller

Mutinus Fries, Summa Vegetabilium Scandinaviae 2: 434, 1849, nom. cons.

≡ *Phallus* sect. *Cynophallus* Fries, Syst. Mycol. 2: 284, 1822.

≡ *Cynophallus* (Fries) Corda, Icones Fungorum 6: 19, 1854.

= *Aedycia* Rafinesque in Desvaux, J. Bot. 1: 222, 1808.

= *Ithyphallus* Gray, Nat. Arr. Brit. Pl. 1: 675, 1821.

= *Corynites* Berkley & Curtis, Trans. Linn. Soc., 21: 149, 1855.

= *Caromyxa* Montagne, Syll. Cryptogam., 281,1856.

= *Jansia* Penzig, Ann. Jard. Bot. Buitenzorg 16: 139, 1899.

= *Floccomutinus* Hennings, in Engler, Am. J. Trop. Med. 22: 109, 1895.

Type species: *Mutinus caninus* (Huds.) Fries

Phallus L.: Persoon, Synopsis Methodica Fungorum, p. 242, 1801.

≡ *Morellus* Eaton, Manual of Botany for the Northern and Middle States 2: 118, 324, 1818.

≡ *Phallus* sect. *Ithyphallus* Fries, Syst. Mycol. 2: 283, 1822.

≡ *Ithyphallus* (Fries) Fischer, Jahrb. Bot. Gart. Berlin 4: 41, 1886.

= *Satyrus* Bosc, Magazin Ges. naturf. Freunde, Berlin 5: 86, 1811.

= *Lejophallus* Fries, Syst. Mycol. 2: 283, 1822.

= *Dictyopeplos* Kuhl & Hasselt, Konst en Letter Bode: 327, 1824.

= *Dictyophallus* Corda, Ann. Stud. Mycol., p. 190, 1842.

= *Kirchbaumia* Schulzer, Verh. k. Zool.-bot. Gesell. Wien, 16: 798, 1866.

= *Omphallophallus* Kalchbrenner, Flora, 46: 95, 1883.

= *Cryptophallus* Peck, Bull. Torrey Bot. Club 24: 147, 1897.

= *Jaczewskia* Mattirolo, Mem. R. Accad. Torino. Ser. 2, 63: 214, 1912.

Type species: *Phallus impudicus* L.: Persoon

Staheliomyces Fischer, Mitth. Naturf. Ges. Bern, 1920: 142, 1921.

Type species: *Staheliomyces cinctus* Fischer

Xylophallus (Schlechtendal) Fischer in Engler & Prantl, Nat. Pflanzenfam. 7A: 96, 1933.

Type species: *Xylophallus xylogenus* (Montagne) Fischer

Phallaceae *incertae sedis*: As discussed above, we consider the following species to be an immature *Dictyophora* spp.

Gelopellis purpurascens Beaton & Malajczuk, Trans. Br. Mycol. Soc. 87: 479, 1986.

Clathraceae Chevallier, Flore Générale des Environs de Paris 1: 120, 1826.

≡ Clathraceae Corda, Icones Fungorum 5: 28, 1842.

≡ Clathraceae Fischer in Engler & Prantl, Nat. Pflanzenfam. 1: 280, 1900.

Description after Jülich (1981) with the exclusion of *Lysurus* from the family: **Basidiomata** hypogeous and globose to ellipsoid when young, epigeous with a branched receptacle when mature. **Peridium** white, 2- to 3-layered; outer layer thin, membranous and elastic, inner layer thick and gelatinous which is divided by radially arranged sutures connecting the peridium and receptacle; the peridium opens up at maturity and remains as volva at the base of the receptacle. **Receptacle** subspherical to ovoid, latticed or with apically fused or finally divergent receptacular arms which are usually longer than receptacle, pseudoparenchymatous or composed of tubes. **Gleba** formed only on the interior face of the receptacle, mucilaginous at maturity, olive-brown, fetid. **Basidia** narrowly clavate, 4- to 8-spored. **Spores** statismosporic, symmetrical, cylindrical to narrowly ellipsoid, smooth, hyaline, pale olive or yellow-brown in KOH.

Type genus: *Clathrus* Micheli: Pers., Synopsis Methodica Fungorum 2: 241, 1801.

Discussion-Clathraceae: We remove the family Lysuraceae from the Clathraceae based on the results of the molecular phylogenetic analyses (Fig. 5.2). The Clathraceae differs from the Lysuraceae in having basidiomata with the arms which are typically

longer than the receptacles. In addition, the gleba in the Clathraceae tends to be attached only to the interior face of the arms. The epigeous taxa in the Lysuraceae have gleba attached both to the interior and exterior face of the arms (Dring, 1980). One species formerly placed in *Protubera*, *P. canescens*, is now included in this family. Based on its close relationship to *Ileodictyon* and the original description of this species (Beaton & Malajczuk, 1986), we consider *P. canescens* to be an immature stage of an *Ileodictyon* spp. The holotype specimen of *P. canescens* deserves further examination. One undescribed species of *Gelopellis* (MEL2063389) was also placed in the Clathraceae (Fig. 5.2a). Whether this is truly a sequestrate taxon or an immature fruiting body of epigeous Clathraceae is uncertain at this time. The following genera are recognized in this family:

Anthurus Kalchbrenner in Kalchbrenner & MacOwan, Grevillea 9: 2, 1880.

= *Neocolus* Liou & Hwang, Contr. Inst. Bot., Nat. Acad. Peiping 4: 341, 1936.

Type species: *Anthurus muellerianus* Kalchbrenner

Aseroë Labillardière: Fries, Systema Mycologicum 2: 289, 1823.

= *Clathiscus* Mont., Ann. Sci. Nat., Ser. 2, 16: 278, 1841.

Type species: *Aseroë rubra* Labillardière: Fries

Blumenavia A. Möller in Schimper, Bot. Mitt. a. d. Tropen 7: 57, 1895.

Type species: *Blumenavia rhachodes* A. Möller

Clathrus Micheli: Persoon, Synopsis Methodica Fungorum 2: 241, 1801.

= *Colonnaria* Rafinesque, Med. Repos. Hex. 2, 5: 355, 1808.

= *Clathrus* sect. *Clethria* Browne: Fries, Systema Mycologicum 2: 287, 1823.

≡ *Clethria* (Browne: Fries) Berkeley, London J. Bot. 4: 48, 1845.

= *Clathrella* Fischer in Engler & Prantl, Nat. Planzenfam. 1: 284, 1900.

Type species: *Clathrus ruber* Micheli: Persoon

Colus Cavalier & Sechier, Ann. Sci. Nat. Bot. Ser. 2, 3: 253, 1835.

Type species: *Colus hirudinosus* Cavalier & Sechier

Ileodictyon Tulasne in Raol, Ann. Sci. Nat. Bot. Ser. 3, 2: 114, 1844.

Type species: *Ileodictyon cibarium* Tulasne

Laternea Turpin in Cuvier, Dict. Sci. Nat. 25: 248, 1822.

≡ *Clathrus* sect. *Laternea* (Turpin) Fries, Syst. Mycol. 2: 287, 1823.

Type species: *Laternea triscapa* Turpin

Ligiella J. A. Sáenz, Mycologia 72: 338, 1980.

Type species: *Ligiella rodrigueziana* J. A. Sáenz

Linderiella Cunningham, N.Z. J. Sci. Tech. 23: 1713, 1942.

≡ *Linderia* Cunningham, Proc. Linn. Soc. N.S.W. 56: 192, 1931, *nom. rej.*

Type species: *Linderiella columnata* (Bosc.) Cunningham

Pseudoclathrus B. Liu & Y. S. Bau, Mycotaxon 10: 293, 1980.

Type species: *Pseudoclathrus cylindrosporus* Liu & Bau

Pseudocolus Lloyd, The Phalloids of Australasia, p.18, 1907.

Type species: *Pseudocolus fusiformis* (Fischer) Lloyd

Clathraceae *incertae sedis*: As discussed above, we consider the following species to be an immature *Ileodictyon* spp.

Protubera canescens Beaton & Malajczuk, Trans. Br. Mycol. Soc. 87: 481, 1986.

Lysuraceae Corda, Icones Fungorum 5: 28, 1842, "Lysuroideae", Hosaka, *emend. prov.*

= Gastrosporiaceae Pilát, Bull. Soc. Mycol. Fr. 50: 46, 1934.

Basidiomata hypogeous or epigeous, sequestrate or opens up irregularly with or without forming a single receptacle branching into several arms; receptacle always longer than the arms. **Rhizomorphs** often well-developed and attached at base. **Peridium** white at first, sometimes staining brown when exposed or bruised;

endoperidium gelatinous, often divided by radially arranged sutures. **Gleba** gelatinous to mucilaginous or becomes dry or powdery at maturity, with or without paracapillitium; if receptacle is formed, gleba tends to be attached to the exterior face of the arms, as well as the interior face. **Basidia** 4- to 8-spored. **Spores** statismosporic, symmetrical, globose to ellipsoid, sometimes somewhat angular, smooth to verrucose, hyaline to pale yellow in KOH, nondextrinoid, inamyloid.

Type genus: *Lysurus* Fries, Systema Mycologicum 2: 285, 1823.

Discussion-Lysuraceae: The genus *Lysurus* and *Simblum* have traditionally been classified as members of the Clathraceae (Zeller, 1949; Dring, 1980; Miller & Miller, 1988), but our molecular phylogenetic analyses suggest that they are more closely related to the Phallaceae (Fig. 5.2). The analyses also suggest that *Gastrosporium* is closely related to the other members of the Lysuraceae. Our molecular analyses also reveal that the monotypic genus *Calvarula* is a member of Lysuraceae. *Calvarula* and *Gastrosporium* have sequestrate basidiomata and no receptacle is formed (Zeller, 1939), unlike *Lysurus* and *Simblum*. The family is similar to Clathraceae and Protophallaceae in having a gelatinous layer divided by sutures beneath the peridium but the epigeous taxa differ from Clathraceae in having long, stipitate receptacles which are longer than the arms that arise from the receptacle. In addition, the gleba tends to be attached to the exterior face of the arms, as well as the interior face. The latter character is shared by the taxa in the "Lysuroid series" which include *Colus,*

Pseudocolus, Lysurus, and *Aseroë* (Dring, 1980). However, the molecular analyses clearly show that the "Lysuroid series" is not monophyletic (Fig. 5.2). Therefore for the epigeous taxa, we only include *Lysurus, Neolysurus,* and *Simblum* in the Lysuraceae. *Calvarula* and *Gastrosporium* are the only taxa in the Phallales having a powdery gleba at maturity, but they share a characteristic of a gelatinous endoperidium with other members of the Lysuraceae. One species formerly placed in the genus *Protubera* (*P. clathroidea*) was revealed to be a member of this family. Although its generic placement is uncertain at this time, it is possible that *P. clathroidea* is an immature *Lysurus* spp. The holotype specimen of *P. clathroidea* deserves further examination. The following genera are recognized:

Calvarula Zeller, Mycologia 31: 23, 1939.

Type species: *Calvarula excavata* Zeller

Gastrosporium Mattirolo, Mem. R. Accad. Torino Ser. 2. 53: 361, 1903.

Type species: *Gastrosporium simplex* Mattirolo

= *Calvatia defodiodis* Lloyd, Mycol. Writ. 4 (Letter 44): 8, 1913.

≡ *Disciseda defodioides* (Lloyd) Zeller, Mycologia 39: 308, 1947.

= *Gastrosporium beccarianum* Lloyd, Mycol. Writ. 7: 1265, 1924.

= *Leucorhizon nidificum* Velenovsky, Mycologia 2: 50, 1925.

Lysurus Fries, Systema Mycologicum 2: 285, 1823.

= *Foetidaria* St Hilaire, Ann. Sci. Nat. Bot. Ser. 2, 3: 191, 1835.

= *Aseroephallus* Leprieur & Montagne, Ann. Sci. Nat. Bot. Ser. 3, 4: 360, 1845.

= *Lysurus* sect. *Schizmaturus* Corda, Icones Fungorum 6: 22, 1854.

≡ *Schizmaturus* (Corda) Kalchbrenner, Phall. Nov., p. 15, 1880.

= *Lysurus* sect. *Desmaturus* Schlechtendal, Linnaea 31: 180, 1861.

≡ *Desmaturus* (Schlecht.) Kalchbrenner, Phall. Nov., p. 15, 1880.

= *Kalchbrennera* Berkeley, Gardener's Chronicle n.s. 5: 785, 1876.

= *Pharus* Petch, Ann. R. Bot. Gard. Peradeniya 7: 59, 1919, non *Pharus* Browne, Hist. Jam. 344, 1756.

= *Mycopharus* Petch, Trans. Brit. Mycol. Soc. 10: 281, 1926.

= *Lloydia* Chow, Bull. Fan Memor. Inst. Biol. Bot. Ser. 6: 27, 1935, non *Lloydia* Salisb. Trans. Hort. Soc. 1: 328, 1812.

≡ *Sinolloydia* Chow, Bull. Fan Memor. Inst. Biol. Bot. Ser. 7: 165, 1936.

Type species: *Lysurus mokusin* (Cibot: Pers.) Fries

Neolysurus O.K. Miller, Ovrebo & Burk, Mycol. Res. 95: 1230, 1991.

Type species: *Neolysurus arcipulvinus* O.K. Miller, Ovrebo & Burk

Simblum Klotzsch, Botanical Miscellany 2: 164, 1831.

= *Dictyobole* Atkinson & Long, Bot. Gaz. 34: 42, 1902.

Type species: *Simblum periphragmoides* Klotzsch

Lysuraceae *incertae sedis*: As discussed above, the future study may reveal the following species to be an immature *Lysurus* spp.

Protubera clathroidea Dring, Mycol. Pap. CMI, 98: 3, 1964.

Protophallaceae Zeller, Mycologia 31: 22, 1939.

Description after Zeller (1939):

Basidiomata hypogeous or epigeous, sequestrate, subglobose. **Peridium** with a thin and membranous outer layer, covering a thick gelatinous inner layer which is divided by radially arranged sutures connecting the peridium and gleba. **Gleba** gelatinous to cartilaginous, olive to brown, usually sectored by gelatinous plates radiating from the base or from a columella. **Spores** statismosporic, symmetrical, ellipsoid to cylindrical, small, olivaceous to brown.

Type genus: *Protophallus* Murrill Mycologia 2: 25, 1910. (syn.: *Protubera* A. Möller, in Schimper, Bot. Mitt. Trop. 7: 10, 1895.)

Discussion-Protophallaceae: This is a monotypic family, containing a single genus *Protubera*. Although a few other genera have been proposed, such as *Kobayasia, Protophallus, Protuberella,* we follow the treatment of Malloch (1989) by

synonymizing all these genera. The family differs from Phallaceae, Clathraceae and Lysuraceae in having strictly sequestrate basidiomata with a gelatinous gleba. It differs from Claustulaceae and Trappeaceae in having basidiomata with a gelatinous layer directly beneath the peridium that is divided by sutures. The molecular analyses show that two species of *Protubera*, *P. canescens* and *P. clathroidea*, are placed in the Clathraceae and Lysuraceae, respectively, and excluded from the family. Likewise, *P. nothofagi* and *P. hautuensis* belong to the Hysterangiales, and are excluded from the family. The following taxa are recognized:

Protubera A. Möller, in Schimper, Bot. Mitt. Trop. 7: 10, 1895.

= *Protophallus* Murrill Mycologia 2: 25, 1910.

= *Protuberella* S. Imai & A. Kawamura, Science Rep. Yokohama Nat. Univ., Section 2, 7: 4, 1958.

= *Kobayasia* S. Imai & A. Kawamura, Science Rep. Yokohama Nat. Univ., Section 2, 7: 5, 1958.

Type species: *Protubera maracuja* A. Möller

Recognized species for *Protubera*:

Protubera africana Lloyd, Mycol. Notes 64: 987, 1920.

Protubera brunnea (Zeller) Zeller, Mycologia 40: 644, 1948.

≡ *Protophallus brunneus* Zeller, Mycologia 31: 28, 1939)

Protubera borealis S. Imai, Bot. Mag., Tokyo 1: 223, 1936.

≡ *Protuberella borealis* (S. Imai) S. Imai & A. Kawamura, Science Rep. Yokohama Nat. Univ., Section 2, 7: 4, 1958.

Protubera jamaicensis (Murrill) Zeller, Mycologia 40: 644, 1948.

≡ *Protophallus jamaicensis* Murrill, Mycologia 2: 25, 1910.

Protubera maracuja A. Möller, Bot. Mitt. Trop. 7: 10, 1895.

Protubera nipponica Y. Kobayasi in Nakai & Honda, Novae Fl. Japan 2: 25, 1938.

≡ *Kobayasia nipponica* (Y. Kobayasi) S. Imai & A. Kawamura, Science Rep. Yokohama Nat. Univ., Section 2, 7: 5, 1958.

Protubera parvispora Castellano & Beever, N.Z. J. Bot. 32: 326, 1994.

Protubera sabulonensis Malloch, Mycotaxon 34: 144, 1989.

Protubera termitum R. Heim, Termites et Champignons. p.178, 1977.

Excluded taxa from *Protubera*: The following species are transferred to other genera or families. See discussion under Clathraceae, Lysuraceae (order Phallales), *Insulomyces* (Phallogastraceae, Hysterangiales), and *Beeveromyces* (Gallaceaceae, Hysterangiales).

Protubera canescens Beaton & Malajczuk, Trans. Br. Mycol. Soc. 87: 481, 1986.

Protubera clathroidea Dring, Mycol. Pap. CMI, 98: 3, 1964.

Protubera hautuensis Castellano & Beever, N.Z. J. Bot. 32: 322, 1994.

Protubera nothofagi Castellano & Beever, N.Z. J. Bot. 32: 324, 1994.

Claustulaceae Cunningham, Proc. Linn. Soc. N. S. W. 56: 198, 1931, Hosaka, *emend. prov.*

= Gelopellaceae Zeller, Mycologia 31: 20, 1939.

Basidiomata hypogeous or epigeous, sequestrate, globose to irregularly shaped, often with one to several basal rhizomorphs, sometimes covered with adhering debris. **Peridium** white at first, becomes yellow, red, or brown when exposed or bruised, 1- to 2-layered, without peridial sutures. **Gleba** gelatinous to cartilaginous, gray to brown, dark brown, or deep olivaceous, sometimes surrounded by a thick, continuous gelatinous layer. **Columella** gelatinous, simple or branched, sometimes inconspicuous. **Basidia** mostly 4-spored. **Spores** statismosporic, symmetrical, ellipsoid to oblong, mostly longer than 5 μm, smooth or ornamented with irregularly shaped warts, sometimes with smooth to wrinkled utricle, hyaline to brown in KOH, sometimes strongly cyanophilic, nondextrinoid, inamyloid.

Type genus: *Claustula* Curtis, Ann. Bot., Lond. 40: 476, 1926.

Discussion-Claustulaceae: The family Claustulaceae was originally described by Cunningham (1931) as a monotypic family, containing a single genus *Claustula*. The molecular phylogenetic analyses (Fig. 5.2) strongly support that *Gelopellis, Kjeldsenia* and *Phlebogaster* are closely related. The family Claustulaceae (Cunningham, 1931) has nomenclatural priority over Gelopellaceae (Zeller, 1939). The genus *Kjeldsenia*

was originally described as a member of the Cortinariaceae (Colgan *et al.*, 1995), but the analyses strongly suggest that correct placement is in the Phallales. The family differs from the Clathraceae, Lysuraceae and Protophallaceae in having basidiomata with a gelatinous peridial layer without sutures. It differs from the Phallaceae in having strictly sequestrate basidiomata, and larger, often ornamented spores with mostly 4-spored basidia. One species of *Gelopellis, G. purpurascens,* is nested within the Phallaceae, and excluded from the family. The following taxa are recognized in this family:

Claustula Curtis, Ann. Bot., Lond. 40: 476, 1926.

Type species: *Claustula fischeri* Curtis

Phlebogaster Fogel, Contr. Univ. Mich. Herb. 14: 79, 1980.

Type species: *Phlebogaster laurisylvicola* Fogel

Kjeldsenia Colgan, Castellano & Bougher, Mycotaxon 55: 175, 1995.

Type species: *Kjeldsenia aureispora* Colgan, Castellano & Bougher

Gelopellis Zeller, Mycologia 31: 20, 1939.

Type species: *Gelopellis macrospora* Zeller

Recognized species for *Gelopellis*:

Gelopellis macrospora Zeller, Mycologia 31: 21, 1939.

Gelopellis rufus Dring, Kew Bull., Addit. Ser. 31: 741, 1977.

Gelopellis shanxiensis B. Liu & K. Tao, Acta Mycol. Sin. 7: 72, 1988.

Gelopellis thaxteri (Zeller & Dodge) Zeller, Mycologia 31: 22, 1939.

≡ *Hysterangium thaxteri* Zeller & Dodge, Ann. Mo. Bot. Gard. 16: 114, 1929.

Excluded taxon from *Gelopellis*: We consider the following species to be an immature *Dictyophora*. See discussion under Phallaceae (Phallales).

Gelopellis purpurascens Beaton & Malajczuk, Trans. Br. Mycol. Soc. 87: 479, 1986.

Trappeaceae Hosaka, *fam. prov.*

Basidiomata hypogeous to epigeous, sequestrate, globose to irregularly shaped, often distinctly convoluted or lobed. **Peridium** not easily separable from gleba, often evanescent, white when fresh, brown when bruised or exposed. **Rhizomorphs** attached at base, or emergent from base of columella, white, single or clustered. **Gleba** gelatinous, green to olive, located under sterile, gelatinized lobes or locules. **Columella** distinct, dendroid, opaque. **Basidia** cylindrical to clavate, 4- to 8-spored.

Spores statismosporic, symmetrical, ellipsoid to oblong, small (mostly ≤ 5 µm long), smooth, hyaline to pale green in KOH, nondextrinoid, inamyloid. **Utricle** absent.

Type genus: *Trappea* Castellano, Mycotaxon 38: 2, 1990.

Discussion-Trappeaceae: Two monotypic genera, *Phallobata* and *Trappea*, are included in this family. Both genera have been classified as members of Hysterangiaceae (Cunningham, 1926; Castellano, 1990), but molecular phylogenetic analysis strongly suggests that they are more closely related to the stinkhorn families, Phallaceae and Clathraceae (Fig. 5.2). Trappeaceae differs from Clathraceae, Phallaceae, and Lysuraceae, in having strictly sequestrate basidiomata with gelatinous gleba. Trappeaceae differs from Claustulaceae and Protophallaceae in having irregularly lobed basidiomata with gleba located under sterile lobes or locules. Two species originally described as *Trappea* (*T. phillipsii* and *T. pinyonensis*) are now transferred to the genus *Phallogaster* (Hysterangiales). The following genera are recognized in this family:

Phallobata Cunningham, Trans. N.Z. Inst. 56: 73, 1926.

Type species: *Phallobata alba* Cunningham

≡ *Hysterangium lobatum* Cunningham, Trans. Roy. Soc. N.Z. 67: 408, 1938.

Trappea Castellano, Mycotaxon 38: 2, 1990.

Type species: *Trappea darkeri* (Zeller) Castellano

≡ *Hysterangium darkeri* Zeller, Mycologia 39: 17, 1939.

Excluded taxa from *Trappea*: The following species are transferred to *Phallogaster*. See discussion under *Phallogaster*.

Trappea phillipsii (Harkness) Castellano, Mycotaxon 38: 7. 1990.

≡ *Hysterangium phillipsii* Harkness, Cal. Acad. Sci. Proc. III. 1: 255, 1899

Trappea pinyonensis States, Mycotaxon 41: 128, 1991.

Geastrales Hosaka, *ord. prov.*

≡ Geastrales Kreisel, Grundzüge eines natürlichen Systems der Pilze, 157, 1969, *nom. nud.*

≡ Geastrales Locquin, De Taxia Fungorum 1: 57, 1974, *nom. nud.*

Geastraceae Corda, Icones Fungorum 5: 25, 1842, "Geastrideae".

≡ Lycoperdaceae tribus Geastreae Cunningham, Proc. Linn. New South Wales 52: 251, 1927.

≡ Geastraceae Fischer in Engl. & Prantl, Nat. Pflanzenf. 7A: 72, 1933.

Description after Jülich (1981):

Basidiomata hypogeous or epigeous, solitary, gregarious or caespitose on a common stroma or subiculum, sessile to stipitate, sequestrate or opening with star-shaped to irregular lobes at maturity. **Peridium** two- to four-layered, closed or the exoperidium splitting at maturity often from the apex downward into several stellate rays; endoperidium usually with single or multiple ostioles or irregularly dehisces. **Gleba** never divided into peridioles, at first soft, fleshy, white becoming powdery, dark brown to black at maturity, with or without capillitial threads. **Basidia** globose, clavate or pyriform, often with a constriction beneath the rounded apex, 4- to 8-spored. **Spores** statismosporic, symmetrical, globose to ellipsoid, ornamented with warts, brown in KOH, nondextrinoid, inamyloid.

Type genus: *Geastrum* Pers.: Pers., Syn. Meth. Fung., 131, 1801.

Discussion-Geastraceae: The family has traditionally been classified as a member of the Lycoperdales (Zeller, 1949; Jülich, 1981), but molecular phylogenetic studies revealed that the Geastraceae is a member of the gomphoid-phalloid clade (Hibbett *et al.*, 1997). Some authors suggested the potential close affinity of the Geastraceae to the Sebacinales (Taylor *et al.*, 2003; Weiss *et al.*, 2004), but the analyses were based only on nuclear ribosomal RNA gene, and the monophyly of the Geastraceae and Sebacinales was not strongly supported. The Geastraceae differs from other members of the Geastrales by having fruiting bodies with a powdery gleba that is not divided

into peridioles. Only *Geastrum, Radiigera,* and *Myriostoma* are represented in the current study and more detailed phylogenetic study with more taxon sampling is necessary to further clarify the generic concepts within the family. The following genera are recognized in this family:

Geastrum Pers.: Pers., Syn. Meth. Fung., 131, 1801.

= *Plecostoma* Desvaux., J. Bot. Paris 2: 99-100, 1809.

= *Geaster* Micheli: Fries, Syst. Mycol. 3: 8, 1832.

= *Cycloderma* Klotzsch., Linnaea 7: 203, 1832.

= *Coilomyces* Berkley & Curtis, J. Acad. Nat. Sci. Philadelphia 2: 279, 1853.

Type species: *G. coronatum* Persoon

Radiigera Zeller, Mycologia 36: 628, 1944.

Type species: *Radiigera fuscogleba* Zeller

Phialastrum Sunhede, Syn. Fung. 1: 66, 1989

Type species: *Phialastrum barbatum* (Dissing & Lange) Sunhede

≡ *Geastrum barbatum* Dissing & Lange, Bull. Jard. État Brux. 32: 382-384, 1962.

Geasteroides Long, Mycologia 9: 271, 1917.

= *Terrostella* Long, Mycologia 37: 605, 1945.

Type species: *Geasteroides texensis* Long

≡ *Geasteropsis texensis* (Long) Fischer in Engler & Prantl, Nat. Pflanzenf. 7A: 75, 1933.

≡ *Terrostella texensis* (Long) Long, Mycologia 37: 605-607, 1945.

Geasteropsis Hóllos, Növényt. Közlem. 2: 72, 1903.

Type species: *Geasteropsis conrathii* Hóllos

≡ *Trichaster conrathii* (Hóllos) Long, Mycologia 37: 603-605, 1945.

≡ *Geastrum conrathii* (Hóllos) Ponce de Leon, Fieldiana 31: 312-313, 1968.

Myriostoma Desvaux, J. Bot., Paris 2: 103, 1809.

Type species: *Myriostoma coliforme* (Dickson) Corda, Anleitung zum Studium der Mycologie f. 16-17, 1842.

≡ *Lycoperdon coliforme* Dickson, Pl. Crypt. Brit. p. 24, 1785.

Trichaster Czerniaiev, Bull. Soc. Nat. Moscou 18: 149, 1945.

Type species: *Trichaster melanocephalus* Czerniaiev

Pyrenogastraceae Jülich, Bibl. Mycol. 85: 387, 1981, Hosaka, *emend. prov.*

Basidiomata hypogeous to subepigeous, sequestrate or occasionally irregularly splitting at the apex at maturity, globose to subglobose, often covered with cottony mycelial layer. **Peridium** white, yellow-white to pale yellow-brown, often staining pink when bruised or exposed; 2- to 3-layered. **Gleba** soft, fleshy, white when young, becoming dark yellow-brown or lack and powdery at maturity; immature gleba with long, radiating locules that develop into well-developed to poorly developed peridioles at maturity, usually with elastic, nonseptate, smooth, dark red-brown capillitial threads. **Columella** distinct, of interwoven hyphae, spherical to subspherical. **Basidia** tubular to filiform, 4- to 8-spored. **Spores** statismosporic, symmetrical, globose to ellipsoid, apiculate, verrucose, brown in KOH, nondextrinoid, inamyloid.

Type genus: *Pyrenogaster* Malençon & Riousset, Bull. Soc. Mycol. Fr. 93: 289, 1977.

= *Schenella* T. MacBride sensu Estrada-Torres *et al.*, Mycologia 97: 139-149, 2005, non sensu T. MacBride, Mycologia 3: 39. 1911

Type species: *Pyrenogaster pityophilus* Malençon & Riousset

≡ *Schenella pityophilus* (Malençon & Riousset) Estrada & Lado, Mycologia 97: 147, 2005.

Discussion-Pyrenogastraceae: Pyrenogastraceae differs from the other members of Geastrales in having the gleba divided into peridioles (Dominguez de Toledo & Castellano, 1996). A recent molecular study suggested the genus *Schenella,* which was originally described as a myxomycete, is a synonym of *Pyrenogaster* (Estrada-Torres *et al.*, 2005). Because *Schenella* was described in 1911, it would have nomenclatural priority over *Pyrenogaster*. However, *Schenella* was described as "fructification aethalioid, depressed, flat, covered by a fragile but continuous crust... (MacBride, 1911)," which is not consistent with any aspect of the morphology of *Pyrenogaster* basidiomata. Furthermore, no other characters, such as basidia and peridial structures were observed in the type material of *Schenella* (MacBride, 1911). *Pyrenogaster* has been fully described and studied (Dominguez de Toledo & Castellano, 1996; Malençon & Riousset, 1977), and its characteristics are inconsistent with the circumscription of the genus *Schenella*. We therefore reject the recombination of *Pyrenogaster pityophilus* into *Schenella pityophilus*.

Sclerogastraceae Hosaka, *fam. prov.*

≡ Sclerogastraceae Locquin, De Taxia Fungorum 1: 48, 1974, *nom. nud.*

Basidiomata hypogeous, sequestrate, globose to irregular, surface smooth to floccose, often embedded in mycelial mats in organic soil layer, often in close clusters. **Peridium** often easily separable from gleba, white, pale yellow to brown, sometimes

staining red. **Gleba** pale yellow, deep yellow, orange or brown, with small locules filled with spores at maturity. **Columella** absent to moderately developed. **Basidia** cylindrical to clavate, with short sterigma. **Spores** statismosporic, symmetrical, globose, mostly minutely echinulate or verrucose, hyaline to brown in KOH, nondextrinoid, inamyloid.

Type genus: *Sclerogaster* R. Hesse, Hypog. Deutschl. 1: 84, 1891.

Type species: *Sclerogaster lanatus* R. Hesse

Discussion-Sclerogastraceae: Sclerogastraceae was published by Locquin (1974) without a Latin diagnosis and therefore regarded as invalid in accordance with Article 36.1 of the ICBN. The family is monotypic, containing the genus *Sclerogaster* Hesse. Traditionally, *Sclerogaster* has been considered to be closely related to *Octaviania* (Boletales) (Jülich, 1981; Castellano *et al.*, 1989) or *Hydnangium* (Dodge & Zeller, 1936; Zeller & Dodge, 1935), but molecular phylogenetic analysis strongly suggests placement in the Geastrales with close affinity to the Geastraceae and Pyrenogastraceae (Fig. 5.3). Sclerogastraceae, Geastraceae and Pyrenogastraceae all possess globose spores with a surface ornamented with spines or warts. The Sclerogastraceae differs from the Pyrenogastraceae and Geastraceae, in having a yellow to orange gleba versus brown to black gleba that turns powdery at maturity. Only one species (*Sclerogaster xerophilus*) was sampled from approximately 15

described species. More taxon sampling is necessary for a more definitive placement of these taxa.

Sphaerobolaceae Schröter, in Cohn, Kryptogamen-Flora von Schlesien 3: 688, 1889, "Sphaerobolacei".

Description after Jülich (1981):

Basidiomata epigeous, globose to subglobose, opens up stellately at maturity, sessile, ca. 2 mm wide, whit to yellow. **Peridium** 4- to 5-layered; exoperidium ruptures at maturity and forcibly discharge the peridiole with evagination of the inner peridial layers. **Gleba** with single peridiole, gelatinous to cartilaginous. **Basidia** narrowly clavate, 4- to 8-spored. **Spores** statismosporic, symmetrical, globose to ellipsoid, smooth, hyaline in KOH.

Type genus: *Sphaerobolus* Tode: Persoon, Synopsis Methodica Fungorum 115, 1801.
Type species: *Sphaerobolus stellatus* Tode: Persoon

Discussion-Sphaerobolaceae: Sphaerobolaceae is a monotypic family, containing a single genus *Sphaerobolus*. The most extensive phylogenetic study of the genus was done by Geml *et al.* (2005), revealing the three major lineages within the genus. The family has traditionally been classified in the order Nidulariales (Zeller, 1949; Jülich,

1981), but the molecular data strongly suggest its affinity to the Geastrales (Hibbett *et al.*, 1997; Fig. 5.3). The family can easily be distinguished from any other members of Geastrales by having tiny basidiomata which forcibly eject a single peridiole. The detailed mechanism for gleba discharge was discussed by Ingold (1971, 1972) and Walker (1927).

Phallomycetidae *incertae sedis*: The following taxa may have affinities with the Phallomycetidae, but their taxonomic placement is uncertain. We have decided to leave them as *incertae sedis* at this time.

Broomeia Berkley, London Journal of Botany 3: 193, 1844.

Circulocolumella S. Ito & S. Imai, Sci. Rep. Yokohama Nat. Univ., Section 2, 6: 3, 1957.

Clathrogaster Petri, Malpighia 14: 125-126, 1900.

Diplocystis Berkley & Curtis, J. Linn. Soc., Bot. 10: 344, 1869.

Hoehneliogaster Lohwag, Beih. Z. Bot. Centralblatt 42: 299, 1926.

Lycogalopsis Fischer, Berich. Deut. Bot. Ges. 4: 192, 1886.

Maccagnia Mattirolo, Mem. Roy. Acad. Nazionale dei Lincei Ser. 5, 13: 17, 1922.

Saprogaster Fogel & States, Mycotaxon 80: 317, 2001.

Tremellogaster Fischer, in Mitteil., Naturf. Ges. Bern 1923: 49-56, 1924.

Vandasia Valenovsky, České houby IV-V, p. 805, 1922.

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Castellano, M. A., and R. E. Beever. 1994. Truffle-like Basidiomycotina of New Zealand: *Gallacea*, *Hysterangium*, *Phallobata*, and *Protubera*. N.Z. J. Bot. 32: 305-328.

Castellano, M. A., and J. J. Muchovej. 1996. Truffle-like fungi from South America: *Hysterangium sensu lato*. Mycotaxon 57: 329-345.

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Colgan III, W., M. A. Castellano, and N. L. Bougher. 1995. NATS truffle and truffle-like fungi 2: *Kjeldsenia aureispora* gen. et sp. nov., a truffle-like fungus in the Cortinariaceae. Mycotaxon 55: 175-178.

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Cunningham, G. H. 1926. A new genus of the Hysterangiaceae. Trans. New Zealand. Inst. 56: 71-73.

Cunningham, G. H. 1931. The Gasteromycetes of Australasia. XI. Linn. Soc. New South Wales. 56: 182-200.

Curtis, K. M. 1926. The morphology of *Claustula fischeri*, gen. et sp. nov. A new genus of phalloid affinity. Ann. Bot. 15: 471-477.

Dodge, C. W., and S. M. Zeller. 1936. *Hydnangium* and related genera. Ann. Mo. Bot. Gard. 23: 565-598.

Dominguez de Toledo, L. S., and M. A. Castellano. 1996. A revision of the genera *Radiigera* and *Pyrenogaster*. Mycologia 88: 863-884.

Dring, D. M. 1980. Contributions towards a rational arrangement of the Clathraceae. Kew Bull. 35: 1-96.

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

Fogel, R. 1980. Additions to the hypogeous mycoflora of the Canary Islands and Madeira. Contr. Univ. Mich. Herb. 14: 75-82.

Geml, J., D. D. Davis, and D. M. Geiser. 2005. Phylogenetic analyses reveal deeply divergent species lineages in the genus *Sphaerobolus* (Phallales: Basidiomycota). Mol. Phyl. Evol. 35: 313-322.

Hibbett, D. S., E. M. Pine, E. Langer, G. Langer, and M. J. Donoghue. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc. Natl. Acad. Sci. USA 94: 12002-12006.

Ingold, C. T. 1971. The glebal mass of *Sphaerobolus*. Trans. Br. Mycol. Soc. 56: 105-113.

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Jülich, W. 1981. Higher taxa of basidiomycetes. J. Cramer, Vaduz.

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

MacBride, T. H. 1911. A new genus of Myxomycetes? Mycologia 3: 39.

Malajczuk, N., B. Dell, and N. Bougher. 1987. Ectomycorrhiza formation in *Eucalyptus*. III. Superficial ectomycorrhizas initiated by *Hysterangium* and *Cortinarius* species. New Phytol. 105: 421–428.

Malençon, G., and L. Riousset. 1977. *Pyrenogaster pityophilus* G. Malençon et L. Riousset, nouveau genre et nouvelle espèce de Gastéromycète (Geastraceae). Bull. Soc. Myc. Fr. 93: 289-311.

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Stewart, E. L., and J. M. Trappe. 1985. The new genus *Austrogautieria* (Basidiomycotina), segregate from *Gautieria*. Mycologia 77: 674-687.

Taylor, D. L., T. D. Bruns, T. M. Szaro, and S. A. Hodges. 2003. Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. Am. J. Bot. 90: 1168–1179.

Walker, L. B. 1927. Development and mechanism of discharge in *Sphaerobolus iowensis* n. sp. and *S. stellatus* Tode. Elisha Mitchell Sci. Soc. 42: 151-178.

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Zeller, S. M. 1949. Keys to the orders, families, and genera of the Gasteromycetes. Mycologia 41: 36-58.

Zeller, S. M., and C. W. Dodge. 1935. New species of Hydnangiaceae. Ann. Mo. Bot. Gard. 22: 365-373.

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CHAPTER 6

Conclusions

In this dissertation, an initial taxon sampling was emphasized for the Hysterangiales. Many truffle-like taxa, however, unexpectedly showed closer affinities to the Phallales than to the Hysterangiales. These taxa include *Calvarula, Phallobata, Protubera* and *Trappea*. The genus *Rhopalogaster* has been traditionally placed in the family Hysterangiaceae, but the analyses clearly showed its affinity to the Boletales (Fig. 4.1, Chapter 4). Even more surprising was inclusion of several taxa, e.g., *Austrogautieria* and Mesophelliaceae in the Hysterangiales. *Austrogautieria* is the only taxon in the Hysterangiales possessing longitudinally ridged spores, and Mesophelliaceae has never been proposed as a member of the Hysterangiales because of its powdery gleba at maturity. These new findings necessitated that taxon sampling be expanded to understand the evolutionary relationships within the Hysterangiales. Through collaboration with Drs. Admir Giachini and Eduardo Nouhra, who generated a large dataset for the Gomphales, our understanding for a higher-level phylogeny of the gomphoid-phalloid fungi (subclass Phallomycetidae) has significantly increased.

In Chapter 2, a monophyly of the four major clades (Hysterangiales, Gomphales, Geastrales, and Phallales clades) was demonstrated based on a 3-gene dataset (nuc-LSU-rDNA, mt-SSU-rDNA and *ATP6*). Although the interrelationships among those major clades remained unresolved, the sister relationship of the

Hysterangiales and Gomphales clades was suggested both by parsimony and Bayesian analyses. The alternative topologies, however, could not be rejected statistically, which clearly demonstrated that more taxon and/or character sampling is necessary to clarify the higher-level phylogeny of the gomphoid-phalloid fungi. This is the first study showing the polyphyly of the genus *Protubera*, close relationships between *Hysterangium* and Mesophelliaceae, and homoplastic origin of *Gautieria*-like spore morphology. The ancestral character state of spore discharge mechanism was reconstructed using both parsimony and likelihood methods. Although parsimony-based reconstructions appeared to favor the independent gain of ballistospory in Gomphales, the results varied across different tree topologies and gain: loss cost ratios. Bayesian-Multistate analyses showed ambiguous reconstructions for the basal nodes, but also indicated that the average rate of losses of ballistospory is 4.7 times higher than gains. This fact as well as the polyphyletic origins of gastroid taxa and the complex mechanism of ballistospory favors the multiple, parallel losses of ballistospory within the gomphoid-phalloid clade.

In Chapter 3, the phylogeny of the Hysterangiales as well as the results of the ancestral area reconstruction strongly suggests that the ectomycorrhizal lineages within the Hysterangiales originated in the East Gondwana. While multiple range expansions to the Northern Hemisphere could be explained by at least one trans-oceanic dispersal to the Northern Hemisphere, the age estimates based on the synonymous substitution rate indicated a Paleozoic origin of the Hysterangiales. This indicates that range expansions of the Hysterangiales could be possible through land

connections before the initial breakup of Pangaea into Laurasia and Gondwana, which took place about 180 million years ago. Because modern ectomycorrhizal plants were not present during the Paleozoic to Early Mesozoic era, the potential existence of the Hysterangiales during this time must be explained either by novel ectomycorrhizal association of the Hysterangiales with unknown plant lineages, most likely with extinct plants, or alternatively, multiple, independent gains of the ectomycorrhizal habit must be postulated for the Hysterangiales, which is not the most parsimonious explanation. The Paleozoic origin of the Hysterangiales also indicates that mycophagous animals may not be the most important factor for range expansion. Nonetheless, the alternative hypothesis of more recent (Cretaceous or later) origin of the Hysterangiales remains as a possible explanation. This hypothesis requires significant long distance, transoceanic dispersal, which is usually considered a rare event for most fungal groups. Given its hypogeous fruiting body habit, it is intriguing to know how and if the Hysterangiales could accomplish such frequent, long distance dispersal.

Chapter 4 focused on the multigene phylogeny of the gomphoid-phalloid fungi with the most extensive taxon sampling ever made for this group. The monophyly of the gomphoid-phalloid clade was further confirmed. Four major subclades (Hysterangiales, Geastrales, Gomphales and Phallales clades) within the gomphoid-phalloid clade were also demonstrated to be monophyletic. The interrelationships among those subclades were, however, not resolved despite the intensive character sampling using five loci (nuc-LSU-rDNA, mt-SSU-rDNA, *ATP6*, *RPB2* and *EF1α*).

Based on the results of these analyses, a new subclass Phallomycetidae, and two orders, the Hysterangiales and Geastrales, are proposed. The four major subclades each have unique characteristics, and the evolutionary patterns of their fruiting body morphology are intriguing. All species of the Hysterangiales are characterized by truffle-like fruiting bodies. On the other hand, most species of the Gomphales are characterized by nongastroid fruiting bodies except for *Gautieria*, which is characterized by truffle-like fruiting bodies. It is clear that truffle-like fruiting bodies of *Gautieria* are derived morphology from nongastroid forms. The Geastrales and Phallales are both characterized by gastroid fruiting bodies. The Geastrales included numerous gastroid fruiting body morphologies including earthstars, false-truffles, and cannonball fungi, but the polarity of fruiting body evolution could not be determined. The Phallales included both false-truffle and stinkhorn fruiting body morphologies with the truffle-like morphologies supported as ancestral for the order and the stinkhorn morphology reconstructed as a derived character state, representing independent origin of the stipe.

In Chapter 5, taxonomic revisions were made based on the results of previous chapters. Four new families (Gallaceaceae, Phallogastraceae, Trappeaceae, Sclerogastraceae), 7 new genera (*Austrohysterangium, Cribbangium, Rodwayomyces, Beeveromyces, Cazomyces, Insulomyces, Viridigautieria*), and 22 new combinations were proposed. The descriptions of the order Phallales, five families (Hysterangiaceae, Mesophelliaceae, Lysuraceae, Claustulaceae, Pyrenogastraceae), and four genera (*Hysterangium, Gallacea, Austrogautieria, Phallogaster*) were emended. Species of

Hysterangium were reassigned to *Hysterangium, Austrohysterangium, Cribbangium* and *Rodwayomyces,* but some uncertainties remained for the taxonomic placement for some species of *Hysterangium sensu lato*. Species of *Gallacea* were reassigned to *Gallacea sensu stricto, Cazomyces* and *Rodwayomyces*. Species of *Austrogautieria* were reassigned to *Austrogautieria sensu stricto* and *Viridigautieria*. The type species of *Trappea* (*T. darkeri*) was placed in the Phallales, but the remaining species (*T. phillipsii* & *T. pynionensis*) were demonstrated to be the members of the Hysterangiales, and were synonymized with *Phallogaster*. Some species of the truffle-like genus *Protubera* were demonstrated to be the members of the Hysterangiales, and *P. hautuensis* and *P. nothofagi* were transferred to the genera *Insulomyces* and *Beeveromyces*, respectively. The remaining species of *Protubera* were all shown to belong to the family Protophallaceae of the order Phallales, except for two species, *Protubera canescens* and *P. clathroidea*, which were nested within the Clathraceae and Lysuraceae, respectively, and were considered to represent immature fruiting bodies of stinkhorns. Likewise, a truffle-like taxon *Gelopellis purpurascens* was demonstrated to be very closely related to *Dictyophora* spp., and was considered immature stage of *Dictyophora*. Several genera were treated as Phallomycetidae *incertae sedis*, including *Broomeia, Circulocolumella, Clathrogaster, Diplocystis, Hoehneliogaster, Lycogalopsis, Maccagnia, Saprogaster, Tremellogaster* and *Vandasia*. Future research is necessary to determine the appropriate taxonomic placement for these taxa.

Taxon sampling for the major clades (Hysterangiales, Phallales, Geastrales and Gomphales) within the Phallomycetidae has not been done with a balanced effort. For example, very intensive taxon sampling was conducted for the Hysterangiales, but much less taxa have been sampled for the Geastrales. Likewise, taxon sampling for the Phallales was biased toward truffle-like taxa. To understand the diversity and phylogenetic relationships of the Phallomycetidae, future research should emphasize the Geastrales and epigeous Phallales. On the other hand, biogeographical study of the Hysterangiales (Chapter 4) revealed that some discrepancies between the Hysterangiales phylogeny and geological records may be due to insufficient taxon sampling. It is apparent that taxon sampling for the Hysterangiales has not been completed to fully understand the lower-level phylogeny of the order. To understand the biogeographical patterns of the Hysterangiales, future study should focus on presently underrepresented areas, e.g., Africa and Asia.

Finally, another unresolved question is on the evolution of the nutritional mode for the Phallomycetidae. A general pattern seems to suggest that ectomycorrhizal lineages have been derived from saprobic ancestors, but the nutritional mode for many taxa is still unknown. Although they are generally considered saprobic, some taxa in the Geastrales and Phallales are reported to be ectomycorrhizal. Their nutritional status definitely deserves further study. A molecular approach would facilitate answering this question, and DNA sequences generated throughout this dissertation work would become a foundation for future study.

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