

Molecular phylogenetics of the gomphoid-phalloid fungi 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 were conducted based on

the five gene dataset 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 were represented entirely by gastroid taxa, and only Gomphales contained both gastroid and non-gastroid taxa. While the gastroid morphology is derived from epigeous, nongastroid taxa in Gomphales, the topology of 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 Basidiomycota. Comparisons are made between previous 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.

Key words: *atp6*, *EF-1 α* , homobasidiomycetes, rDNA, *RPB2*, systematics

INTRODUCTION

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 (FIG. 1u), stink-horns (FIGS. 1f–i), cannonball fungi (FIG. 1w), coral fungi (FIG. 1m), club fungi, gilled mushrooms, tooth fungi, resupinate fungi (FIG. 1p) and false truffles (FIGS. 1a–c, j, k, o, v). Both ectomycorrhizal and saprobic taxa are represented. Because of its diversity traditional morphology-based taxonomy has classified the fungi of the gomphoid-phalloid clade into several distantly related orders, including Lycoperdales, Phallales, Nidulariales and Gomphales (Zeller 1949, Jülich 1981).

Review of previous classifications.—The traditional classifications (e.g. Zeller 1949, Donk 1964) differ dramatically with the new classification scheme proposed herein (SUPPLEMENTARY FIG. 1). Many members of the gomphoid-phalloid clade can be broadly classified as gastroid fungi (e.g. *Hysterangium*, *Geastrum* and *Phallus*). Gastroid fungi traditionally were assigned to Gasteromycetes, which are now known to be an artificial assemblage (Hibbett et al 1997). The spores of these fungi develop and mature within an

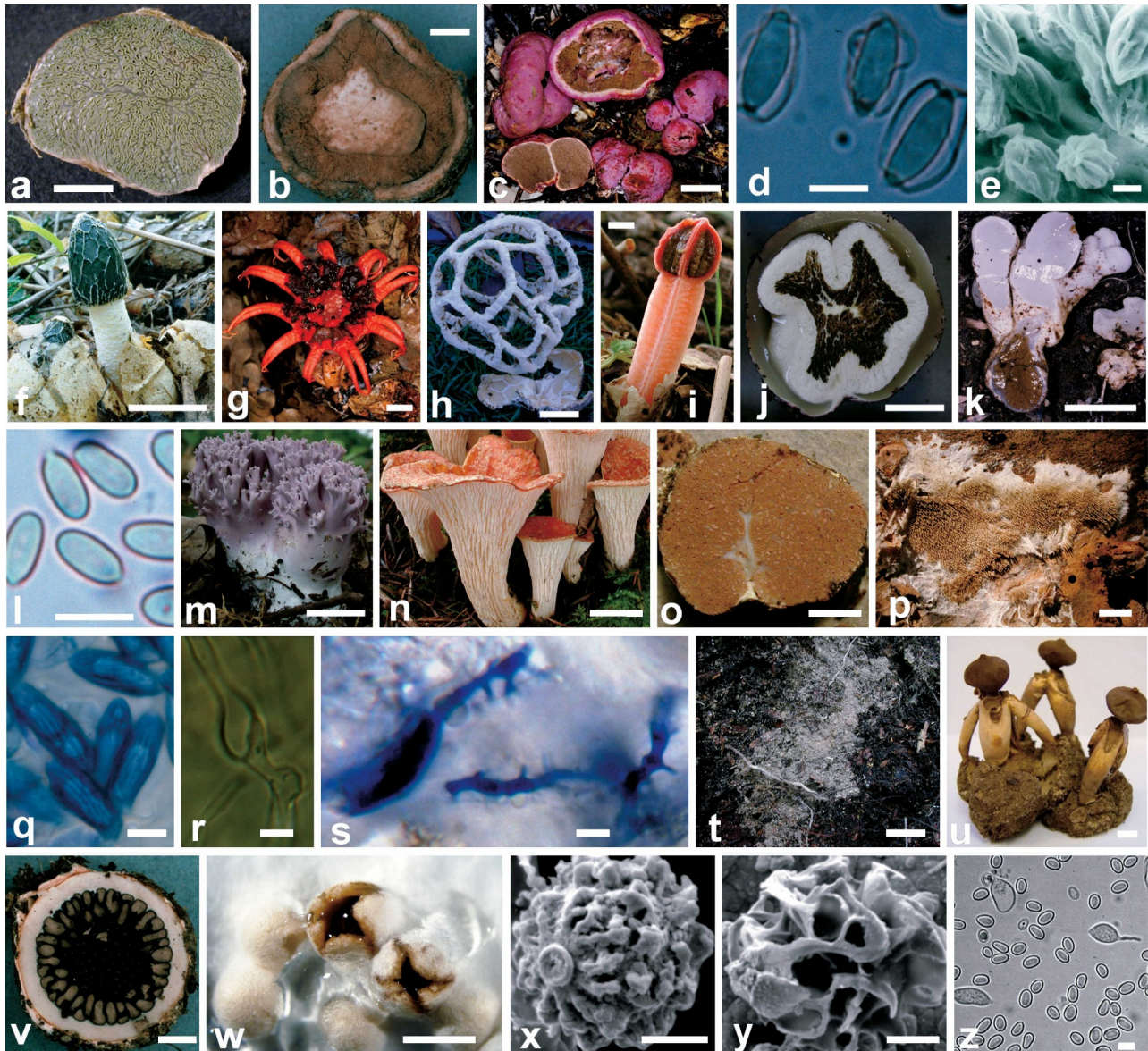


FIG. 1. Macro- and microscopic characters of the gomphoid-phalloid fungi. a–e. Hysterangiales clade. a. *Hysterangium setchellii* (OSC122929). b. *Mesophellia castanea* (H1340). c. *Gallacea scleroderma* (PDD55140). d. Basidiospores of *H. inflatum* (H349). Note spores enclosed in utricle. e. Basidiospores of *Austrogautieria rodwayi* (L. Rodway 116). f–l. Phallales clade. f. *Phallus impudicus* (photo courtesy of Koukichi Maruyama). g. *Aseroë rubra* (PDD60341). h. *Ileodictyon cibarium* (PDD64844). i. *Lysurus mokusin* (photo courtesy of Ikuo Asai). j. *Claustula fischeri* (PDD87966). k. *Phallobata alba* (PDD56745, photo courtesy Peter Johnston). l. Basidiospores of *I. cibarium* (OSC122730). m–t. Gomphales clade. m. *Ramaria fennica* (photo courtesy Ikuo Asai). n. *Turbinellus* (*Gomphus*) *floccosus* (photo courtesy Ikuo Asai). o. *Gautieria* sp. (OSC122685). p. *Kavinia* sp. (RPL69050, photo courtesy Patrick Leacock). q. Basidiospores of *R. botrytis* (photo courtesy Koukichi Maruyama). Note cyanophilic ornamentation. r. Ampullate hypha of *R. eumorpha* (T25800, photo courtesy Efren Cazares). s. Acanthohypha of *R. cystidiophora* (Giachini 03) stained with cotton blue (photo courtesy Efren Cazares). t. Hyphal mat formed by *Ramaria* sp. Note sharp contrast in color between mat-soil (white) and non-mat-soil (black). u–z. Geastrales clade. u. *Geastrum fornicatum* (H.D. Thiers 24491). v. *Pyrenogaster pityophilus* (T11761). w. *Sphaerobolus stellatus* (SS13). x. Basidiospore of *G. coronatum* (AEF1443). y. Basidiospore of *Myriostoma coliforme* (AN014674). z. Basidiospores and germinating gemmae of *S. stellatus* (SS13). Bars: a, b, v = 5 mm; c, g, i–k, o, p, u = 1 cm; d, e, l, q–s, z = 5 μ m; f, h, m, n = 5 cm; t = 10 cm; w = 1 mm; x, y = 1 μ m.

enclosed spore-producing tissue or gleba; furthermore they lack a forcible spore discharge mechanism, termed statismosporic (Miller and Miller 1988). Nongastroid members of the gomphoid-phalloid clade (e.g. *Gomphus*, *Ramaria*) are found only within Gomphales. These fungi traditionally were classified in the family Gomphaceae within Aphyllophorales, along with distantly related taxa such as Cantharellaceae, Ganodermataceae and Polyporaceae (Donk 1964).

Phallales was described by Fischer (1898) to accommodate Phallaceae and Clathraceae. Cunningham (1931a, b) later added Claustulaceae. Hysterangiales initially was treated as a family of Hymenogastrales, along with Hymenogastraceae, which is distantly related to the gomphoid-phalloid fungi (Peintner et al 2001). Although this treatment has been widely used by subsequent authors (e.g. Cunningham 1944), some authors (e.g. Miller and Miller 1988) recognized the affinity of Hysterangiaceae to stinkhorns and included Hysterangiaceae in Phallales. Other authors (Zeller 1939, Jülich 1981) segregated Hysterangiales as an independent order from Phallales although they maintained the view that Hysterangiales is most closely related to Phallales. Three families, Hysterangiaceae, Protophallaceae and Gelopellaceae, were recognized in Hysterangiales (Zeller 1949, Jülich 1981). All members of this order are characterized by truffle-like (sequestrate) fruiting bodies, most of which are produced below ground.

The members of Geastrales have been classified into two orders, Lycoperdales and Nidulariales (Zeller 1949), both of which have been demonstrated to be polyphyletic (Hibbett et al 1997, Krüger et al 2001). Within Lycoperdales (*sensu* Zeller 1949) the close relationship between Lycoperdaceae and Geastraceae was long assumed. Kreisel (1969), however, segregated Geastraceae from Lycoperdales and recognized an independent order, Geastrales. Molecular phylogenetic studies revealed that Lycoperdaceae is nested within the euagarics clade and is related only distantly to Geastraceae (Hibbett et al 1997, Krüger et al 2001). Nidulariales contains two families, Nidulariaceae and Sphaerobolaceae. Although this ordinal concept was accepted by many authors (Zeller 1949, Jülich 1981, Miller and Miller 1988) the monophyly of the order has been rejected because it has been demonstrated that members of Nidulariaceae are nested within the euagarics clade while Sphaerobolaceae is allied with Geastraceae (Hibbett et al 1997).

Gautieria previously had been included in Hymenogastraceae, Hysterangiaceae (Cunningham 1944) and as an independent, monotypic order Gautieriales (Zeller 1948). Although its close relationship with Boletales also was suggested (Jülich 1981), molecular

phylogenetic studies revealed that *Gautieria* is nested within Gomphales (Humpert et al 2001). As mentioned above other members of Gomphales are nongastroid taxa, which previously were classified in Aphyllophorales (Donk 1964). Members of Gomphaceae (*sensu* Donk) later were divided into several smaller families and placed in a new order Gomphales (Jülich 1981). *Clavariadelphus* originally was included in Clavariaceae (Donk 1964), but the family was shown to be polyphyletic; *Clavariadelphus* is a member of Gomphales, whereas *Clavaria* and *Clavulina* are nested respectively within the euagarics and cantharelloid clade (Pine et al 1999).

MATERIALS AND METHODS

Taxa sampled, along with GenBank accession numbers are provided (SUPPLEMENTARY TABLE I). 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–EF1-1567R region for translation elongation factor subunit 1 α (*EF1 α*). The primers and PCR protocols have been described previously (summarized in Assembling the Fungal Tree of Life Website <http://aftol.org/primers.php>).

Phylogenetic analyses were conducted for the concatenated five locus dataset under Bayesian and parsimony criteria. Maximum parsimony analyses were conducted with PAUP*4.0b10 (Swofford 2002) with the heuristic search option (TBR and MULTTREES on) and 1000 replicates of random addition sequence. Nodal supports were tested by bootstrapping of 500 replicates with the heuristic search option (TBR and MULTTREES off) with five random addition sequences. Bayesian analysis was conducted with MrBayes ver. 3.0b4 (Huelsenbeck and Ronquist 2001) with 2 000 000 generations of MCMCMC by sampling every 100th tree. The GTR+G+I model was used for the nuc-LSU-rDNA, mt-SSU-rDNA, and each codon position for the remaining protein coding genes. The support of nodes was tested by posterior probabilities obtained from majority rule consensus after deleting the trees during burn-in.

Ancestral character state reconstructions of fruiting body morphologies under binary coding (sequestrate vs. non-sequestrate) were performed in the Geastrales, Gomphales and Phallales clades based on the tree topology and branch lengths are provided (SUPPLEMENTARY FIG. 2). 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 cannonball fungi. Some taxa with uncertain fruiting body types (question marks in FIG. 2, SUPPLEMENTARY FIG. 2) were coded in alternative analyses as sequestrate, nonsequestrate or polymorphic (SUPPLEMENTARY TABLE II). Parsimony-based reconstructions were performed with MacClade ver. 4.06 (Maddison and Maddison 2003) without implementing

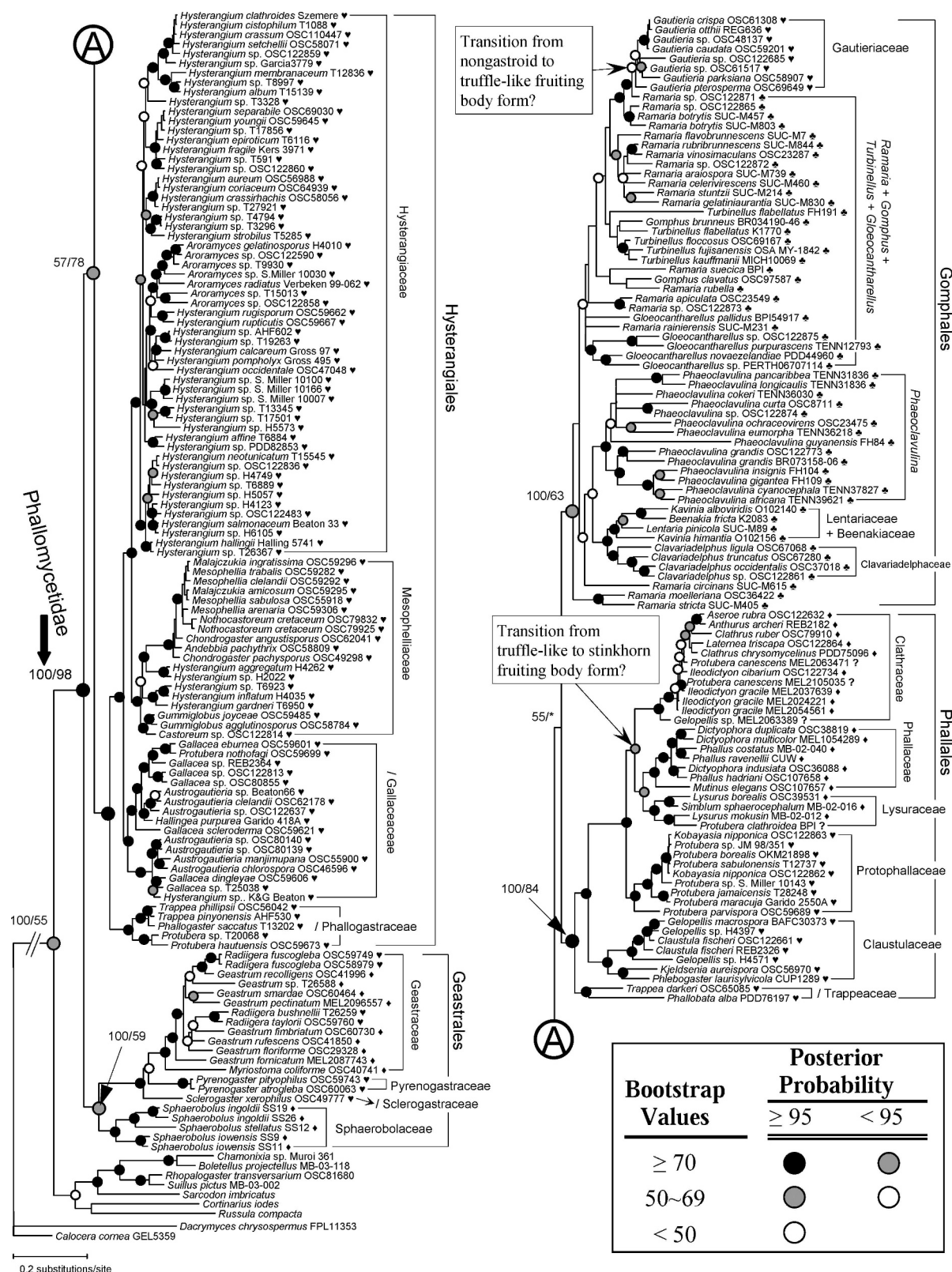


FIG. 2. Phylogeny of the gomphoid-phalloid fungi. Tree topology is based on the Bayesian analyses. Numbers on branches are Bayesian posterior probability/ maximum parsimony bootstrap values (shown as percentage). The provisional taxon names are indicated with a slash (/). Taxon names are followed by symbols indicating fruiting body forms: ♥ = sequestrate-gastroid (truffle-like), ♦ = nonsequestrate-gastroid (including stinkhorns, earthstars and cannonball fungi), ♣ = nongastroid.

character weighting. Likelihood-based reconstructions were performed with Multistate ver. 0.8 (Pagel 2003), 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, also see Hibbett 2004 for analytical details).

RESULTS AND DISCUSSION

Both Bayesian and parsimony analyses showed strong support for the monophyly of the gomphoid-phalloid clade ("Phallomycetidae" in FIG. 2). No definitive synapomorphies have been identified for the gomphoid-phalloid fungi; however, some potential synapomorphic characters, including rhizomorph morphology (presence of ampullate hyphae and acanthohypha, Agerer and Iosifidou 2004; FIG. 1r, s), pistillarin content and structures of septal pore cap, have been proposed (Hibbett and Thorn 2001). In addition some members of the gomphoid-phalloid clade, such as *Gautieria*, *Hysterangium*, *Ramaria* and *Geastrum*, are known to produce thick hyphal mats in soil (Agerer and Iosifidou 2004, Nohra et al 2005, Sunhede 1989; FIG. 1t). Although most of these characters are not exclusive to the gomphoid-phalloid fungi the yellowish filled acanthocystidia and associated "exuded drops of pigments" have been reported only from the gomphoid-phalloid fungi (e.g. *Geastrum*, *Gomphus*, *Phalloogaster* and *Ramaria* [Agerer and Iosifidou 2004]). Four major clades were recognized within 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. 2).

Gomphales clade.—This clade corresponds to the order Gomphales (*sensu* Jülich 1981) with addition of Gautieriaceae and Clavariadelphaceae. The monophyly of Gomphales was consistent with previous studies (Villegas et al 1999). Fruiting body morphologies include coral fungi (e.g. *Ramaria* and Lentariaceae; FIG. 1m), club fungi (Clavariadelphaceae), gilled mushrooms (*Gloeocantharellus*), cantharelloid-gomphoid (e.g. *Gomphus* and *Turbinellus*, Giachini 2007; FIG. 1n), tooth fungi (*Beenakia*), resupinate fungi (*Kavinia*; FIG. 1p) and false truffles (Gautieriaceae; FIG. 1o). Despite their macromorphological variations the members of this clade share a number of microscopic and macrochemical characters, including cyanophilic spore ornamentation (FIG. 1q), hyphal

construction and positive hymenial reaction to ferric sulfate (Donk 1964, Villegas et al 1999).

Humpert et al (2001) suggested that branched coral fruiting bodies are ancestral forms for Gomphales. While lack of statistical support for some internal nodes within 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 (SUPPLEMENTARY TABLE VI). Gautieriaceae is the only false truffle taxon in Gomphales and it is restricted to a terminal clade, also indicating their derived form. This is consistent with the hypothesis that sequestrate fruiting bodies are derived forms from more complex, epigeous morphology (Thiers 1984, Peintner et al 2001). Gomphales also show heterogeneity in their ecological characters. Most species in Lentariaceae, Kaviniaaceae, 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 generally are considered ectomycorrhizal, and while the ectomycorrhizal status of many species of Gomphales is still unknown, the formation of ectomycorrhizae by *Turbinellus*, *Gomphus* and some *Ramaria* species has been confirmed (Agerer and Iosifidou 2001, Nohra et al 2005).

Phallales clade.—It roughly corresponds to the order Phallales *sensu* Cunningham (1931a, b), with the results of molecular phylogenetic analyses (FIG. 2) suggesting the inclusion of more members to this order. Fruiting body morphologies include stinkhorns (Phallaceae, FIG. 1f), lattice stinkhorns (Clathraceae and Lysuraceae, FIGS. 1g–i) and false truffles (Protophallaceae, Claustulaceae and Trappeaceae; FIGS. 1j, k). A few false truffle taxa also are observed in Clathraceae (*Protuberula canescens* and *Gelopellis* sp.) and Lysuraceae (*Protuberula clathroidea*). Based on tree topology and original descriptions of these taxa (Beaton and Malajczuk 1986, Malloch 1989) it is likely that these taxa are unopened stinkhorn fruiting bodies and therefore are considered as uncertain fruiting body forms (indicated by question marks in FIG. 2). *Gastrosporium* was shown to be the member of Phallales (Hibbett and Binder 2002) but it was not included in this study due to lack of a protein coding gene sequence. Zeller (1948) included Gastrosporaceae in Tremellogastrales, but *Tremellogaster* in Tremellogastraceae was demonstrated to be a member

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A few sequestrate taxa in the Phallales clade are indicated by question marks because of their uncertainty in fruiting body form (see text for details).

of Boletales (Binder and Hibbett 2006 this issue). The affinity of the remaining genus (*Clathrogaster*) to the gomphoid-phalloid fungi, however, is still unclear. 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 (Domínguez de Toledo and Castellano 1997). Spores of most taxa are small, ellipsoid and smooth without ornamentation (FIG. 1l) but a few taxa, such as *Kjeldsenia* and *Gastrosporium*, have warty spore surfaces (Colgan et al 1995, Domínguez de Toledo and Castellano 1997). Most taxa are believed to be saprobic due to their lignicolous habit, but at least one species (*Protuberia canescens*) has been reported to be ectomycorrhizal (Malajczuk 1988).

Lysuraceae was recognized as a separate family from Clathraceae by Corda (1842) but most subsequent authors treated them as a single family Clathraceae (e.g. Dring 1980, Jülich 1981). This study shows that Lysuraceae is more closely related to Phallaceae than it is to Clathraceae (FIG. 2). Fruiting bodies of Phallaceae are characterized by a single, unbranched receptacle and a gleba attached externally on the upper part of receptacle (FIG. 1f). 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 (Dring 1980; FIG. 1i). Also the gleba of Lysuraceae tends to migrate to the exterior face of the arms (Dring 1980; FIG. 1i). Species in Clathraceae have gleba attached only to the interior face of the arms (Dring 1980; FIGS. 1g, h).

Protophallaceae traditionally has been classified in Hysterangiales, but this study shows that the family belongs to Phallales. *Protuberia* is polyphyletic in our analyses with species placed in at least three separate clades within Phallales, including Protophallaceae (and the type species, *P. maracuja*), Lysuraceae and Clathraceae. In addition some species of *Protuberia* were observed in Hysterangiales clade. Another member of this clade, *Kjeldsenia*, originally was described as a member of Cortinariaceae (Colgan et al 1995). It is noteworthy that three basal clades within Phallales (Protophallaceae, Claustulaceae and Trappeaceae) all were characterized by truffle-like taxa, while taxa with more complex, stinkhorn-like fruiting bodies are restricted to more terminal clades, indicating that stinkhorn-like fruiting bodies are derived morphologies in Phallales. The results of ancestral character state reconstruction were consistent, showing a single transition from sequestrate to stinkhorn fruiting body form (FIG. 2, also see SUPPLEMENTARY TABLE II-i), 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 Agaricales, however, the evolution of truffle-like fruiting bodies in Phallales does not require gains and/or losses of ballistospory because all taxa in 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 (Nouhra and Domínguez 1994). On the other hand major mycophagous animals for truffle-like fungi are rodents and small marsupials, many of which eat significant amounts of truffle-like fungi in their diet (Lehmkuhl et al 2004). Spores of truffle-like taxa in Phallales, however, are not well documented from animal feces. Because many truffle-like fungi are known to emit chemical compounds to attract insects (Pacioni et al 1991) it is possible that spore dispersal of Phallales is entirely dependent on arthropods.

Hysterangiales clade.—It contains only sequestrate taxa although fruiting bodies of some taxa (e.g. *Phallogaster* and *Gallacea*) occasionally crack open and expose the gleba (Castellano and Beever 1994). While most taxa possess a gelatinous to cartilaginous gleba (FIGS. 1a, c) species in Mesophelliaceae clade are characterized by a powdery gleba (FIG. 1b). This feature is the reason why Mesophelliaceae has been classified in Lycoperdales, along with Lycoperdaceae and Geastraceae (Zeller 1949), and previous workers were not able to infer the close relationship of Mesophelliaceae and Hysterangiaceae. Mesophelliaceae *sensu* Zeller, however, is polyphyletic. The type genus *Mesophellia* belongs to Hysterangiales, but *Radiigera* belongs to Geastrales (FIG. 2) and *Abstoma* is most likely related to Lycoperdaceae. *Rhopalogaster* traditionally has been included in Hysterangiales (Zeller 1949), but this study clearly shows that it is nested within Boletales (FIG. 2).

Most taxa in Hysterangiales clade possess ellipsoidal spores that are smooth to minutely warted. One of the exceptions is observed in Gallaceaceae clade, where *Austrogautieria* possesses longitudinally ridged spores (FIG. 1e). Within Phallomycetidae *Austrogautieria* (FIG. 1e) and *Gautieria* (in Gomphales) share similar spore morphology and sequestrate habit, but the phylogenetic analysis shows these similarities are due to convergent evolution. Many taxa in Hysterangiaceae and Mesophelliaceae clade possess spores enveloped in a wrinkled to loose outer membrane (or utricle, FIG. 1d) whereas taxa in Gallaceaceae and Phallogastraceae clades do not (Stewart and Trappe 1985, Castellano and Beever 1994, Trappe et al 1996).

Taxa in Phallogastraceae clade are most likely saprobic (Miller and Miller 1988, Castellano 1990) whereas the rest of Hysterangiales all are ectomycorrhizal. Although ectomycorrhizal status has not been investigated for all taxa in Hysterangiales it was confirmed for some *Hysterangium* spp. (Hysterangiaceae clade, Agerer and Iosifidou 2004), *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 Hysterangiales form dense perennial hyphal mats, which often significantly change the soil chemistry and microorganism biomass (Griffiths et al 1994). Unlike sequestrate taxa in Phallales, mycophagy by small mammals and marsupials is well documented for Hysterangiales and they often occupy a significant portion of the diet for these animals (Lehmkuhl et al 2004).

Geastrales clade.—It contains cannonball fungi (Sphaerobolaceae, FIG. 1w), earthstars (Geastraceae, FIG. 1u) and false truffles (Pyrenogastraceae, Sclerogastraceae and *Radiigera*; FIG. 1v). The ancestral character reconstruction for fruiting body morphology did not show a clear pattern for the basal nodes of the Geastrales clade, but parsimony-based reconstruction indicated that there were at least two independent changes from nonsequestrate to sequestrate fruiting bodies at the nodes leading to *Radiigera* (TABLE III, SUPPLEMENTARY INFORMATION). This again is consistent with the hypothesis that truffle-like fruiting bodies are derived forms (Thiers 1984, Peintner et al 2001).

Zeller (1948) described Broomeiaceae in Lycoperdales, but it is unclear whether this family is more closely related to Geastrales than to other homobasidiomycetes. Likewise some genera such as *Phialastrum* and *Trichaster* were not included in this study although they traditionally have been placed in Geastraceae (Sunhede 1989). *Geastrum* and *Myriostoma* have fruiting bodies with multiple peridial layers. The exoperidium opens in a stellate manner as it matures, exposing the endoperidium with one (*Geastrum*) to multiple (*Myriostoma*) stomata, through which spores escape (FIG. 1u). *Radiigera* and Pyrenogastraceae both have truffle-like fruiting bodies, and their peridia do not open until they are naturally degraded or eaten by mycophagous animals. The gleba of Pyrenogastraceae is divided into multiple peridioles (FIG. 1v). Species within Geastraceae and Pyrenogastraceae have glebae that become brownish to blackish and powdery at maturity. Sclerogastraceae also have truffle-like fruiting bodies, but the mature gleba never becomes powdery and exhibits a yellowish to brownish color. Most taxa

described above possess globose spores with a warty surface (FIG. 1x), but *Myriostoma* possesses ornamentation that is fused into ramified ridge-like projections (Sunhede 1989; FIG. 1y).

The structure of the fruiting bodies of Sphaerobolaceae is unique for Geastrales. Although the outer peridium opens out stellately, similar to Geastraceae, the gleba is composed of a single peridiole (FIG. 1w), which never becomes powdery and contains numerous hyaline, smooth, subglobose to ellipsoid spores (FIG. 1z). The peridiole eventually is ejected forcibly (Ingold 1972). Members of Sphaerobolaceae are undoubtedly saprobic because they can easily produce fruiting bodies on artificial media (Flegler 1984), but the nutritional mode for the remaining taxa in Geastrales remains uncertain. Many species of *Geastrum* grow without obvious ectomycorrhizal plants, and some authors concluded that *Geastrum* is saprobic (Sunhede 1989, Kreisel 1969); however, at least one species, *G. fimbriatum*, is described as forming ectomycorrhizae (Agerer and Beenken 1998).

TAXONOMY

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

Phallomycetidae Hosaka, Castellano et Spatafora, subclass. nov.

Basidiomata hypogaea vel epigaea, solitaria, gregaria vel caespitosa, sequestrata, resupinata, effuso-reflexa, pileata, turbinata, infundibuliformia, coralliformia, clavata, stellata vel receptacula singula vel irregulariter ramosa a volva basali exorienti. Rhizomorphae saepe conspicuae ad basim vel latera basidiomatum, tegetem densam hyphalem interdum producentes, interdum hyphis ampullaribus acanthocystidiis contentis luteolis. Hymenium interdum cum FeSO_4 coerulescens. Gleba taxorum gastroidum maturitate gelatinosa, mucilagina, cartilaginea vel pulverea, grisea, viridis, olivacea, brunnea, cinnamomea vel nigra, saepe columella bene evoluta. Sporae in hymenio exposito enatae vel in gleba inclusae, ballistosporicae vel statismosporicae, symmetricae vel asymmetricae, globosae, subglobosae, ellipsoideae, elongatae, cylindricae vel fusiformes, laeves, verrucosae, echinatae vel porcatae, interdum utriculo vel vestigeis episporae, in KOH hyalinae vel brunneae, saepe cyanophilae. Ordo typus: Phallales. Ordines inclusi: Phallales, Gomphales, Hysterangiales, Geastrales.

Basidiomata hypogeous or epigeous, solitary, gregarious or caespitose, sequestrate or resupinate, effused-reflexed, pileate, turbinate, infundibuliform, coralliform, clavate, stellate or with a single to irregularly branched receptacle arising from a basal volva. Rhizomorphs often conspicuous at base or sides of basidiomata, occasionally producing dense hyphal mats in soil, sometimes with ampullate hyphae and acanthocystidia with yellowish contents. Hymenium sometimes turning blue with ferric sulfate. Gleba of gastroid taxa gelatinous, mucilaginous, cartilaginous or powdery at maturity, gray to green, olive, brown, cinnamon or black, often with a well developed columella. Spores borne on an exposed hymenium or enclosed within a gleba, statismosporic or ballistosporic, symmetrical or asymmetrical, globose, subglobose, ellipsoid, elongate, cylindrical to fusiform, smooth, verrucose, echinate or ridged, occasionally with a utricle or remnants of an epispore, hyaline to brown in KOH, often cyanophilic.

Type order. Phallales E. Fischer, in Engler and Prantl, *Die Natürlichen Pflanzenfamilien* 1 (1**):276, 1898, "Phallineae".

Orders included. Phallales E. Fischer, Gomphales Jülich, Hysterangiales Hosaka et Castellano, ord. nov., Geastrales Hosaka et Castellano, ord. nov.

Remarks. This group is equivalent to Phallales in the Dictionary of the Fungi 9th edition (Kirk et al 2001) and the "gomphoid-phalloid clade" *sensu* Hibbett and 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 Phallales clade in this study (FIG. 2). 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.

On the other hand 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 Phallales; FIG. 2) would have to be recognized at the family level. This change forces the elimination of several widely recognized families, such as Clathraceae, Mesophelliaceae, Protophalla-ceae and Sphaerobolaceae, all of which are supported as monophyletic groups in this study (FIG. 2). In

addition the gomphoid-phalloid clade is potentially one of the basal clades within the homobasidiomycetes (Binder and Hibbett 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 is the best reflection of the higher-level phylogeny and hence would provide a stable classification system of Basidiomycota.

Hysterangiales Hosaka et Castellano, ord. nov.

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

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

Basidiomata hypogaea vel epigaea, solitaria vel gregaria, sequestrata, globosa vel irregulare, saepe basi contracta, cauloide, sterili, saepe arenaque solo adhaerenti tecta vel radicellis inclusa. Rhizomorphae ad basem vel latera basidiomatum conspicuae, tegetem densam hyphalem in solo saepe producentes, interdum crystallis calciis oxylatis affixis. Peridium e gleba interdum facile separabile, elasticum, glutinum vel durumque fragile, album, luteolum, brunneum, violaceum vel purpureum, interdum rubellenscens, rubescens, brunnescens, purpurescens, lutescens ubi contusum, stratis 1–4, interdum subcute gelatinosa vel mycorrhizas includens. Gleba maturitate cartilaginea, gelatinosa vel pulvereae, griseae, viridis, olivaceae vel brunneae, saepe loculis labyrinthinis vel elongatis, saepe columella dendroidea, cartilaginea vel gelatinosa, vel nucleo molli vel cartilagineo vel structuris huius modi deficiens. Basidia 1–8-spora. Sporae statismosporicae, pro parte maxima symmetricae, ellipsoideae, oblongae vel fusioideae, laeves vel minute verrucosae vel interdum spinosae, saepe utriculo rugoso, inflato, vel ephemero, hyalinae, pallide virides vel brunneae in KOH, inamyloideae, interdum dilute dextrinoideae. Familia typica: Hysterangiaceae.

Basidiomata hypogeous or epigeous, solitary or gregarious, sequestrate, globose to irregular, with or without a tapering, stem-like sterile base, often covered with adhering sand and soil or encased in debris and rootlets. Rhizomorphs conspicuous at base or sides of basidiomata, often producing dense hyphal mats in the soil, occasionally with attached calcium oxalate crystals. 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, 1–4-layered, sometimes with a gelatinous subcutis containing sutures that divide the peridium into sections, sometimes incorporating mycorrhizae. Gleba at maturity cartilaginous to gelatinous or powdery, gray to green, olive or brown, often with labyrinthine to elongated locules, with

a dendroid, cartilaginous to gelatinous columella or a soft to rubbery central core or lacking such structures. Basidia 2–8-spored. Spores statismosporic, mostly symmetrical, ellipsoid, oblong to fusoid, smooth to minutely verrucose, or sometimes spinose, often with a wrinkled to inflated or ephemeral utricle, hyaline, pale green, or brown in KOH, inamyloid, sometimes weakly dextrinoid.

Type family. Hysterangiaceae E. Fischer, Die natürlichen Pflanzenfamilien 1(1**):304, 1899.

Remarks. Several authors treated Hysterangiales as an independent order, segregated from 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. Of interest, Hysterangiales *sensu* Zeller included Protophallaceae and Gelopellaceae, both of which were revealed to be members of Phallales (FIG. 2). Our study revealed several previously unrecognized relationships (i.e. Mesophelliaceae and *Austrogautieria*, which necessitate a redefinition of Hysterangiales as a new order). There are a few truffle-like genera in Gomphales and Geastrales 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 Hysterangiales.

Geastrales Hosaka et Castellano, ord. nov.

- ≡ Geastrales Kreisel, Grundzüge eines natürlichen Systems der Pilze, 157, 1969, nom. nud.
- ≡ Geastrales Locquin, De Taxia Fungorum 1:57, 1974, nom. nud.

Basidiomata hypogaea vel epigaea, solitaria, gregaria vel caespitosa in stromate vel subiculo communi, maturitate sequestrata vel pro radiis stellatis vel irregularibus dehiscencia; corpus endoperidialis sessilis vel stipitatus. Rhizomorphae saepe conspicuae basem basidiomatum, interdum hyphis ampullaribus et acanthocystidiorum contentis luteolis. Peridium stratis 2–5. Si maturitate exoperidium aperit, tum corpus endoperidialis stoma unum vel stomata aliquot possedit vel irregulariter dehiscat vel peridiolum per vim mittit. Gleba patellis tramalibus a columella centrali radians vel in peridiolum unum vel peridiola aliquot dividens vel loculis sphaericis vel elongatis, lutea, aurantiaca, brunnea vel nigra, maturitate saepe pulveracea; capillitium praesens vel absens. Basidia globosa, clavata, pyriformes vel tubulares, saepe sub apice rotundato constricta, sporis 4–8. Sporae statismosporicae, symmetricae, globosae, subglobosae vel ellipsoideae, laeves, verrucosae, inamyloideae, nondextrinoideae. Familia typica: Geastraceae.

Basidiomata hypogaeus or epigeus, solitary, gregarious or caespitose on a common stroma or subiculum, sequestrate or opening as stellate to irregular rays at maturity; endoperidial body sessile to stipitate. Rhizomorphs often conspicuous at base of basidiomata, sometimes of ampullate hyphae and acanthocystidia with yellowish contents. Peridium 2–5-layered; if the exoperidium opens at maturity, the endoperidial body possesses one to multiple stomata, or dehisces irregularly, or forcibly discharges a peridiole. Gleba of tramal plates radiating out from a central columella, or divided into one to multiple peridioles, or with spherical to elongate locules, yellow to orange, brown, or black, often powdery at maturity; capillitium present or absent. Basidia globose, clavate, pyriform to tubular, often constricted below the rounded apex, 4–8-spored. Spores statismosporic, symmetrical, globose, subglobose to ellipsoid, smooth to verrucose, echinulate or with ramified ridges, hyaline to brown in KOH, inamyloid, nondextrinoid.

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

Remarks. 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 (1969) recognized the order as monotypic, containing a single family Geastraceae, in which only *Geastrum* and *Myriostoma* were recognized. Our study revealed a broader concept of Geastrales, one that encompasses several previously unrecognized taxa in the order, such as Pyrenogastraceae, Sphaerobolaceae and *Sclerogaster*. For the most part Geastrales differs from other members of Phallomycetidae in having basidiomata that open stellately or irregularly and forcibly discharge a peridiole, or expose the endoperidial body with one to multiple stomata through which spores are released. The truffle-like taxa in Geastrales (e.g. Pyrenogastraceae, *Radiigera* and *Sclerogaster*) generally can be distinguished from the similar taxa in other orders of Phallomycetidae by the combination of their spore characters, including a globose to subglobose shape and verrucose to echinulate ornamentation. The order differs from Gastrosporiaceae by having a membranous endoperidium rather than a gelatinous one (Domínguez de Toledo and Castellano 1997).

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LITERATURE CITED

- Agerer R, Beenken L. 1998. *Geastrum fimbriatum* Fr. + *Fagus sylvatica* L. Descriptions of ectomycorrhizae 3:13–18.
- , Iosifidou P. 2004. Rhizomorph structures of Hymenomycetes: a possibility to test DNA-based phylogenetic hypotheses? In: Agerer R, Piepenbring M, Blanz P, eds. *Frontiers in Basidiomycote Mycology*. Eching, Germany: IHW-Verlag. p 249–302.
- Beaton G, Malajczuk N. 1986. New species of *Gelopellis* and *Protuberia* from Western Australia. *Trans Br Mycol Soc* 87:478–482.
- Binder M, Hibbett DS. 2002. Higher-level phylogenetic relationships of homobasidiomycetes (mushroom-forming fungi) inferred from four rDNA regions. *Mol Phylogenet Evol* 22:76–90.
- , ———. 2006. Molecular systematics and biological diversification of Boletales. *Mycologia* 98:971–983.
- Castellano MA. 1990. The new genus *Trappea* (Basidiomycotina, Hysterangiaceae), a segregate from *Hysterangium*. *Mycotaxon* 38:1–9.
- , Beever RE. 1994. Truffle-like Basidiomycotina of New Zealand: *Gallacea*, *Hysterangium*, *Phallobatia* and *Protuberia*. *NZ J Bot* 32:305–328.
- Colgan W, Castellano MA, Bougher NL. 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.
- Corda ACJ. 1842. *Icones Fungorum hucusque cognitorum* V. Prague: JG Calve. 92 p.
- Cunningham GH. 1931a. The gasteromycetes of Australasia. X. The Phallales, part I. *Proc Linn Soc NSW* 56:1–15.
- . 1931b. The gasteromycetes of Australasia. XI. The Phallales, part II. *Proc Linn Soc NSW* 56:182–200.
- . 1944. The gasteromycetes of Australia and New Zealand. Dunedin, New Zealand: John McIndoe. 236 p.
- Dell B, Malajczuk N, Grove TS, Thomson G. 1990. Ectomycorrhiza formation in *Eucalyptus*. IV. Ectomycorrhizas in the sporocarps of the hypogeous fungi *Mesophellia* and *Castorium* in Eucalypt forests of Western Australia. *New Phytol* 114:449–456.
- Domínguez de Toledo LS, Castellano MA. 1997. First report of *Gastrosporium simplex* (gasteromycetes) from South America. *Mycotaxon* 64:443–448.
- Donk MA. 1964. A conspectus of the families of Aphyllophorales. *Persoonia* 3:199–324.
- Dring DM. 1980. Contributions towards a rational arrangement of the Clathraceae. *Kew Bull* 35:1–96.
- Fischer E. 1898. Phallineae. In: Engler HGA, Prantl KAE, eds. *Die Natürlichen Pflanzenfamilien* 1(1**). Leipzig: Wilhelm Engelmann. p 276–288.
- Flegler SL. 1984. An improved method for production of *Sphaerobolus* fruit bodies in culture. *Mycologia* 76:944–946.
- Giachini AJ. 2007. Systematics of the Gomphales: the genus *Gomphus* s. lat. *Mycol Res.* (In press).
- Griffiths RP, Baham JE, Caldwell BA. 1994. Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biol Biochem* 26:331–337.
- Hibbett DS. 2004. Trends in morphological evolution in homobasidiomycetes inferred using maximum likelihood: a comparison of binary and multistate approaches. *Syst Biol* 53:889–903.
- , Binder M. 2002. Evolution of complex fruiting-body morphologies in homobasidiomycetes. *Proc R Soc Lond B* 269:1963–1969.
- , Pine EM, Langer E, Langer G, Donoghue MJ. 1997. Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. *Proc Natl Acad Sci USA* 94:12002–12006.
- , Thorn RG. 2001. Basidiomycota: homobasidiomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. *The Mycota VII part B, Systematics and evolution*. Berlin: Springer-Verlag. p 121–168.
- Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755.
- Humpert AJ, Muench EL, Giachini AJ, Castellano MA, Spatafora JW. 2001. Molecular phylogenetics of *Ramaria* and related genera: evidence from nuclear large subunit and mitochondrial small subunit rDNA sequences. *Mycologia* 93:465–477.
- Ingold CT. 1972. *Sphaerobolus*: the story of a fungus. *Trans Br Mycol Soc* 58:179–195.
- Jülich W. 1981. Higher taxa of basidiomycetes. *Biblioth Mycol* 85:1–485.
- Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. *Ainsworth and Bisby's Dictionary of the Fungi*. 9th ed. Wallingford, Oxon: CABI Bioscience. 624 p.
- Kreisel H. 1969. *Grundzüge eines natürlichen Systems der Pilze*. Jena, Germany: Gustav Fischer Verlag/Cramer. 245 p.
- Krüger D, Binder M, Fischer M, Kreisel H. 2001. The Lycoperdales. A molecular approach to the systematics of some gasteroid mushrooms. *Mycologia* 93:947–957.
- Lehmkuhl JF, Gould LE, Cazares E, Hosford DR. 2004. Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests. *For Ecol Manag* 200:49–65.
- Locquin MV. 1974. *De Taxia Fungorum I*. Paris: UAE Mondedition. 64 p.
- . 1984. *Mycologie générale et structurale*. Paris: Masson. 551 p.
- Lu X, Malajczuk N, Brundrett M, Dell B. 1999. Fruiting of putative ectomycorrhizal fungi under blue gum (*Euca-*

- lyptus globules*) plantations of different ages in Western Australia. *Mycorrhiza* 8:255–261.
- Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett D, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Shoemaker R, Sung GH, Lücking R, Lumbsch T, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hansen K, Harris RC, Hosaka K, Lim YW, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R. 2004. Assembling the Fungal Tree of Life: progress, classification and evolution of subcellular traits. *Am J Bot* 91:1446–1480.
- Maddison DR, Maddison WP. 2003. MacClade ver. 4.06: analysis of phylogeny and character evolution. Sunderland, Massachusetts: Sinauer Associates.
- Malajczuk N. 1988. Ecology and management of ectomycorrhiza in regenerated ecosystems in Australia. In: Sylvia DM, Hung LL, Graham JH, eds. *Mycorrhizae in the next decade*. Gainesville, Florida: University of Florida. p 290–292.
- Malloch D. 1989. Notes on the genus *Protuberia* (Phallales) *Mycotaxon*. 34:133–151.
- Martin MM. 1979. Biochemical implications of insect mycophagy. *Biol Rev* 54:1–21.
- Miller OK, Miller HH. 1988. *Gasteromycetes. Morphological and development features with keys to the orders, families and genera*. Eureka, California: Mad River Press. 157 p.
- Nouhra ER, Domínguez de Toledo LS. 1994. Interacción entre Phallales (Basidiomycotina) e insectos (Coleópteros y Dípteros). *Bol Soc Arg Bot* 30:21–24.
- , Horton TR, Cazares E, Castellano MA. 2005. Morphological and molecular characterization of selected *Ramaria* mycorrhizae. *Mycorrhiza* 15:55–59.
- Pacioni G, Bologna MA, Laurenzi M. 1991. Insect attraction by *Tuber*: a chemical explanation. *Mycol Res* 95:1359–1363.
- Pagel M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst Biol* 48:612–622.
- . 2003. Multistate v0.8. Division of Zoology, School of Animal and Microbial Sciences, University of Reading, UK.
- Peintner U, Bougher NL, Castellano MA, Moncalvo JM, Moser MM, Trappe JM, Vilgalys R. 2001. Multiple origins of sequestrate fungi related to *Cortinarius* (Cortinariaceae). *Am J Bot* 88:2168–2179.
- Pine EM, Hibbett DS, Donoghue MJ. 1999. Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mitochondrial and nuclear rDNA sequences. *Mycologia* 91:944–963.
- Stewart EL, Trappe JM. 1985. The new genus *Austrogautieria* (Basidiomycotina), segregate from *Gautieria*. *Mycologia* 77:674–687.
- Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sunderland, Massachusetts: Sinauer Associates.
- Thiers HD. 1984. The secotoid syndrome. *Mycologia* 76:1–8.
- Trappe JM, Castellano MA, Malajczuk N. 1996. Australasian truffle-like fungi. VII. *Mesophellia* (Basidiomycotina, Mesophelliaceae). *Aust Syst Bot* 9:773–802.
- Villegas M, de Luna E, Cifuentes J, Torres AE. 1999. Phylogenetic studies in Gomphaceae *sensu lato* (Basidiomycetes). *Mycotaxon* 70:127–147.
- Zeller SM. 1939. New and noteworthy Gasteromycetes. *Mycologia* 31:1–32.
- . 1948. Notes on certain gasteromycetes, including two new orders. *Mycologia* 40:639–668.
- . 1949. Keys to the orders, families, and genera of the Gasteromycetes. *Mycologia* 41:36–58.