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Aurantiopileus mayanensis a new genus and species of polypore (Polyporales, Basidiomycota) from Belize with connections to existing Asian species

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Abstract: A new genus and species of polypore, *Aurantiopileus mayanensis*, is described from Central America based on a visually striking collection made in the cloud forests of the Maya Mountains in Belize. Phylogenetic analysis using nLSU sequences and distinctive morphological characters support the erection of a new genus to accommodate this species. Two Asian species formerly placed in *Gloeoporus* are also considered to be members of this new genus and the new combinations are provided, along with a key to the three taxa now placed in *Aurantiopileus*.

Key words: *Aurantiopileus mayanensis*, *Gloeoporus*, *Phlebia,* brightly colored polypores, key to species of *Aurantiopileus*, Doyle's Delight, Maya Mountains.

Introduction: During the second expedition to the highest peak in the Maya Mountains of Belize (Doyle's Delight), 20-30 new taxa of macrofungi were discovered. One of these collections is presented here as a new genus and species of polypore based on morphological and molecular evidence. The remote cloud forest habitat is not easily accessible by normal forms of transportation (automobile/walking), but does lend itself to helicopter assisted drop-off expeditions and two expeditions have now been completed at this remote site (August 2004 and August 2007). The remoteness of the area appears to play a role in the discovery of large numbers of new and yet to be described taxa of macrofungi.

Baroni et al. (2007 and 2008) and Ryvarden et al. (2009) have published on new taxa from this remote site (one new genus and three new species). Baroni et al. (2009) presented data on three more new genera from Doyle's Delight that are now in preparation for publication. As we and other experts in various groups sort through the 1,000 or so collections acquired during those two trips, more new taxa and reports will be forthcoming.

The Doyle's Delight site is considered a lower montane (1100 meters amsl) subtropical wet forest (Holdridge et al. 1971). The forest is composed of mixed tropical Angiosperm trees with some cloud forest elements rich with epiphytes. Some of the trees were *Euterpe precatoria* Mart., *Colpothrinax cookii* Read, *Cyrilla racemiflora* L., *Sloanea floribunda* Benth., *Magnolia sp.*, *Clusia sp.*, *Neea sp.*, *Calatola sp.*, *Quercus spp.*, etc. The downed and decaying decorticate log from which our sample came was not readily identifiable. The rot caused by *Aurantiopileus mayanensis* appeared to be a white stringy rot typical of hardwood angiosperm substrates.

The following description of this new genus and species is accompanied by a key to species we include in this new genus. A full color image of this new taxon is presented here and can also be seen at: http://news.nationalgeographic.com/news/2008/10/081031-belize-mushroom-missions.html.

Materials and Methods: The color codes cited are from Kornerup and Wanscher (1978). The abbreviations for the herbaria where specimens are preserved follow Holmgren et al. (1990). The standard mounting media for examination of specimens of the Polyporaceae and allied groups were used, i.e., Melzer's Reagent, 2% potassium hydroxide (KOH), and cotton blue in lactic acid. The formulae for these can be found in Kirk et al. (2001). Morphological data are based upon examination of basidiomata of *Aurantiopileus mayanensis* (T.J. Baroni 10228), *Gloeoporus dolosus* Corner (E.J.H. Corner, 4.X66, E00159719) and *G. pendens* Corner (RSNB 5200, E00159705).

Molecular data are based on basidiomata of *Aurantiopileus mayanensis* (T.J. Baroni 10228) and additional sequences from GenBank. Tissues for DNA extraction were fixed in the field using fine mesh silica gel in Corning 2.0 ml Self Standing MicroCentrifuge tubes with o-ring seal screw caps. DNA extraction from basidiomata and PCR procedures followed Palmer et al. (2008). In brief, small fragments of basidiomata were excised with a sterile scalpel and placed in 1.5 mL microcentrifuge tubes. DNA was extracted using GeneClean III kits (Qbiogene) following manufacturer's protocols. The internal transcribed spacer (ITS) region of rDNA was PCR amplified using the fungal-specific primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and a section of the nuclear large subunit (nLSU) rDNA was amplified using primers LR16 and LR0R (Hopple and Vilgalys

1994, Klonowska et al. 2003). PCR products were purified and sequenced following Lindner and Banik (2009). Sequences were deposited in GenBank with the following accession numbers: HM772139 (partial nLSU) and HM772140 (ITS region).

Sequences were aligned manually using Sequence Alignment Editor (Se-Al) v2.0a9. The nLSU sequence obtained from *Aurantiopileus mayanensis* was aligned with 18 additional sequences from Genbank (*Bjerkandera adusta* (Willd.) P. Karst. AF287848, *Climacodon septentrionalis* (Fr.) P. Karst. AY684165, *Crustodontia chrysocreas* (Berk. & M.A. Curtis) Hjortstam & Ryvarden AY293199, *Gloeoporus dichrous* (Fr.) Bres. EU118627, *G. taxicola* (Pers.) Gilb. & Ryvarden AY586656, *Laetiporus sulphureus* (Bull.) Murrill EU402532, *Lilaceophlebia livida* (Pers.) Spirin & Zmitr. AF141624, *L. subserialis* (Bourdot & Galzin) Spirin & Zmitr. AF141631, *Mycoacia aurea* (Fr.) J. Erikss. & Ryvarden AY586691, *M. uda* (Fr.) Donk AF141614, *Phanerochaete chrysorhiza* (Torr.) Budington & Gilb. AF139967, *Phlebia acerina* Peck AF141615, *P. centrifuga* P. Karst. AF141618, *P. radiata* Fr. AF287885, *P. rufa* (Pers.) M.P. Christ. AF141628, *P. tremellosa* (Schrad.) Nakasone & Burds. AF141632, *Polyporus squamosus* (Huds.) Fr. AF393069, *Scopuloides hydnoides* (Cooke & Massee) Hjortstam & Ryvarden EU118665) and two sequences from Karl-Henrik Larsson (*Mycoacia fuscoatra* (Fr.) Donk and *Mycoaciella bispora* (Stalpers) J. Erikss. & Ryvarden), indicated with collection numbers beginning KHL or EL (pers. comm.). Sequences were selected for alignment based on nLSU and ITS BLAST searches in GenBank, which indicated that *Phlebia radiata* and other members of the phlebioid clade within the polyporoid clade (*sensu* Binder et al. 2005) were the closest matches to *Aurantiopileus mayanensis*. Phylogenetic analyses were performed only on nLSU data because ITS data could not be unambiguously aligned.

Maximum parsimony was implemented in PAUP 4.0b10 (Swofford 2002). Heuristic searches were conducted with characters unordered and of equal weight and gaps treated as missing data. Default settings were used with the following exceptions: stepwise-addition option was set to random with 100 replicates, steepest descent was used with the TBR branch swapping option and the number of trees saved was set to automatically increase. Bootstrap support for clades (Felsenstein 1985) was estimated from 1000 heuristic searches with the same settings described above, with the exception that the stepwise-addition option was set to random with 50 replicates. Bayesian inference was implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using default settings with the GTR model. One million generations were performed with samples taken in increments of 100. The first 2500 trees (25%) were considered the "burnin" and were excluded from construction of the consensus tree. After running the analysis the standard deviation of the split frequencies was examined to confirm it was below 0.01, the potential scale reduction factor was examined to confirm all parameters were close to 1.0, and the plot of the generations versus the log probability of the data (the log likelihood values) was examined to confirm it had reached the stationary phase.

Results

Aurantiopileus Ginns, D. L. Lindner & T. J. Baroni, *gen. nov.*

Basidiomata comparate parva, quum recentia textura succulenta vel gelatinoss, hymenophoro poroideo, contextu gelatinoso, cystidia tenui-tunicatis in trama gelatinosa inclusis, systemate hyphali monomitico, fibulis praedita, basidiosporis parvis late ellipsoideis (4-6 x 2.8 -4.6 um), inamyloideis; data molecularia, e nLSU collecta, Aurantiopileum e generibus aliis proxime affinibus in clado phlebioideo inclusis separantia.

MycoBank#: MB 518879
Typus: *Aurantiopileus mayanensis* Ginns, D. L. Lindner & T. J. Baroni

Basidiomata relatively small, with a fleshy/gelatinous texture when fresh, a poroid hymenophore, gelatinous context and trama with thin walled cystidia embedded in the gelatinous trama, hyphal system monomitic, with clamp connections, basidiospores small and broadly ellipsoid (4-6 x 2.8-4.6 µm), inamyloid, and molecular data from nLSU separate *Aurantiopileus* from other genera in the phlebioid clade with which it is most closely allied.

Aurantiopileus mayanensis Ginns, D. L. Lindner & T. J. Baroni *sp. nov.* (Figures 1-5)

Etymology: genus name after the orange pileus and the species name commemorates the locality, Maya Mountains.

Basidiocarpus sessilis, in vivo omnino carneus et aurantiacus. Pileus in sicco albus vel pallidus brunneus, margo sterilis. Hymenophorus in sicco aurantiacus, poris, angularis, 2-4 per mm, tubulis concoloribulus usque 1 mm long. Systema hypharum monomiticum, hyphis fibulatis, 3-6 µm diam., tenu-tunicatis, indextrinoideis. Cystidius in trama, clavatus, 35-100 x 5-15 µm, paries tenuis, hyalinin, levibus. Basidiosporis ellipsoideus vel latus ellipsoideus, (4.6-) 5.0-6.0 x (3.4-) 3.6-4.2 (-4.6) µm, paries tenuis, hyalinin, levibus, inamyloides.

MycoBank #: MB 518880

Collection data**:** Belize: Cayo District: Maya Mountains: Doyle's Delight Peak: North Ridge Trail above Creek/Waterhole on north side, N 16 30 14.3, W 89 03 04.3 (WGS84), elevation 1100 meters, 28 Aug. 2007, coll. T. J. Baroni 10228 (HOLOTYPE: CORT; ISOTYPES: BRH and CFMR).

Habitat**:** Associated with a white, stringy rot of a 1 ft (30 cm) diameter downed decorticated hardwood log in mixed tropical cloud forest.

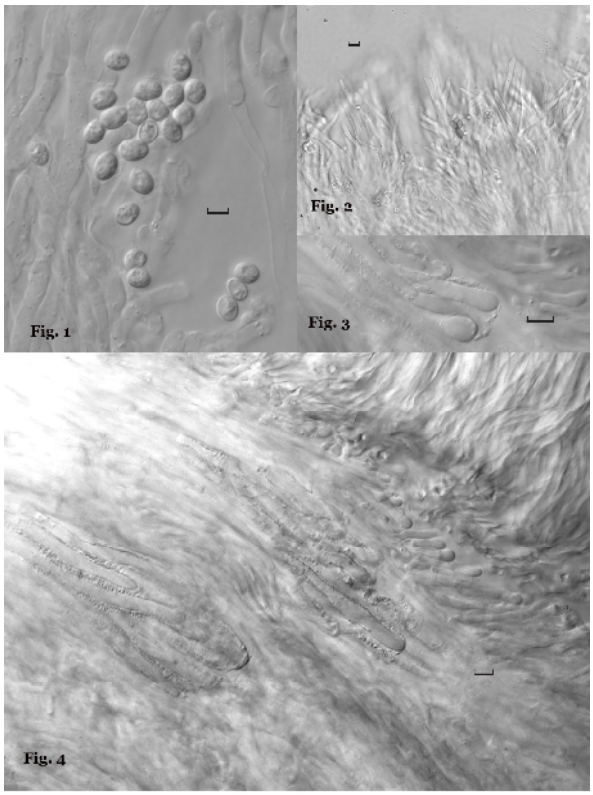
Distribution: Belize, known only from the type locality.

Basidioma when fresh orange (7A-B7-8), soft, gel-like, deeper orange over margin (8B8), effused-reflexed or substipitate, 20-60 mm broad, circular when substipitate or dimidiate when lacking a stipe. **Pilei** imbricate, confluent and attached together, intergrading. **Pileus surface** glabrous, rugulose, expallent hairs near attachment white, slightly zonate. **Pore surface** orange; pores round or angular, very fine (2-4 per mm) and shallow (approximately 1 mm deep) or appearing somewhat rugulose-pitted or fluted. **Pore edges** fimbriate to dentate. **Context** orange, watery, gel-like, 2 mm thick.

When dried **pileus surface** white to pale brown, radially striate, striae fine, slightly glossy. **Margin** thin, hard, orange, plane or inrolled. **Context** hard (horny), waxy, orange with pale brown, radiating striate, 1.0-2.5 mm thick. **Pores** orange, round to angular, (2-) 3 (-4) per mm.

Hyphal system monomitic. **Hyphae** with clamp connections, walls hyaline, negative in Melzer's, acyanophilous. Pore edges with hyphae in fascicles, 3.6-4.0 µm diameter, thin-walled. Tramal and context hyphae with thickened, gelatinous walls that merge into structureless mucilage and the hyphae appear to be imbedded in a gelatinous matrix. Pileus surface hyphae sparse, in fascicles, forming a discontinuous, pallid layer composed of hyphae (3-) 4 (-5) µm diameter, walls thin to 0.5 µm thick. **Gloeopleurous hyphae** scattered in trama and context, contents homogeneous, pale yellow in KOH, red in phloxine (unlike other hyphae), 3-6 µm diameter, branched. **Cystidia** confined to tramal core, imbedded, typically in vertically oriented fascicles, clavate, narrowly clavate or fusoid, 35-100 x 5-15 µm, walls thin, smooth,

negative in Melzer's, some with a thick, gelatinous coating, contents homogeneous, slightly refractive in KOH, dark blue in Cotton Blue. **Basidia** clavate, 18-26 x 6-7 µm, four sterigmate, each slender, 5 µm long. **Basidiospores** ellipsoid to broadly ellipsoid, (4.6-) 5.0-6.0 x (3.4-) 3.6-4.2 (-4.6) µm (n = 20), walls smooth, thin, hyaline, negative in Melzer's, most containing one large oil drop.



Figures 1-4. *Aurantiopileus mayanensis*. T. J. Baroni 10228 : **Fig. 1.** Basidiospores at 1000x, DIC. Scale bar = 5 µm. **Fig. 2.** Sterile cells on pore edges at 400x, DIC. Scale bar = 10 µm. **Fig. 3.** Embedded tramal cystidia at 1000x, DIC. Scale bar = 20 µm. **Fig. 4.** Embedded tramal cystidia at 400x, DIC. Scale bar = 10 µm.

Discussion

Morphological Considerations: The distinctive macroscopic features of fresh specimens of *Aurantiopileus* are a fleshy/ gelatinous texture, a poroid hymenophore and relatively small (less than 60 mm broad) basidiomata. Microscopically the combination of gelatinous context and trama, cystidia imbedded in the trama, monomitic hyphal system, hyphae with clamp connections, and small (4.0-6.0 x 2.8-4.6 µm), mostly broadly ellipsoid basidiospores distinguish the genus. Molecular data from the nLSU of *A. mayanensis* (Fig. 6) also help to distinguish this genus, and indicate *Aurantiopileus* is distinct from other similar genera (e.g. *Bjerkandera* P. Karst.*, Gloeoporus* Mont.*, Phlebia* Fr.) and falls in the phlebioid clade within the polyporoid clade (*sensu* Binder et al. 2005).

Figure 5. *Aurantiopileus mayanensis*. T. J. Baroni 10228. Basidiomata, *in situ*.

Similar embedded cystidia occur in several other species of the Polyporales. Two from the North Temperate zone and microscopically similar to *A. mayanensis* are *Phlebia radiata* and *P. rufa*. In tropical Asia two species of *Gloeoporus*, in addition to similar cystidia have partly gelatinized basidiomata and several microscopic features similar to those of *A. mayanensis*. These Asian species were described by Corner (1989) when he proposed thirteen new species of *Gloeoporus* from Malaysia and Japan. At the same time he transferred the type species of *Bjerkandera* and *Skeletocutis* Kotl. & Pouzar to

Gloeoporus. One result was the broadening of the circumscription of the genus *Gloeoporus*.
***New combinations proposed*:** We prefer a narrower generic concept and, based on their endotramal cystidia and predominately broadly ellipsoid basidiospores, transfer two species described in *Gloeoporus* by Corner (1989) to *Aurantiopileus*:

1. ***Aurantiopileus dolosus*** (Corner) Ginns & D. L. Lindner *comb. nov.*
(Basionym: *Gloeoporus dolosus* Corner, 1989, Nova Hedwigia, Beihefte 96: 50)
2. ***Aurantiopileus pendens*** (Corner) Ginns & D. L. Lindner *comb. nov.*
(Basionym: *Gloeoporus pendens* Corner, 1989, Nova Hedwigia, Beihefte 96: 54).

Corner stated that *Aurantiopileus dolosus* and *A. pendens* have "the same kind of spores, hyphae and extrahymenial setae [cystidia]," but differ in the form of the fruit-body, the strigose pileus and the pink tubes with agglutinated tramal hyphae. The type specimens of *A. dolosus* from Malaya and *A. pendens* from Borneo both have the same basidiomata form (i.e., podoporoid (dorsally substipitate)) that Corner (1989) described only in *A. pendens*. The reflexed habit of *A. dolosus* reported by Corner is not obvious in the dried collection. Both have fine, radiating ridges on the pileus surface but in *A. dolosus* some terminate as projecting spines or extend beyond the margin to give a strigose appearance that is lacking in *A. pendens*.

Both *A. dolosus* and *A. pendens* were described by Corner as primarily white but in *A. dolosus* the pileus, tubes and pores were "drab white with a pinkish tinge;" the coloration was attributed to the encrustations on the cystidia. The cystidia of *A. pendens* "appear to have been colourless in the living state but, in material preserved in alcohol-formalin, the exudate was turned claret red" and the encrustations "turning pale pinkish ... in potash." Thus the pink/red tints occur in both *A. dolosus* and *A. pendens* but under different circumstances. All the dried basidiomata of *A. dolosus* are brownish black; a color that may be a result of their having been in alcohol-formalin. Although the brownish black coloration is distinct from the pale orange brown basidiomata of *A. pendens*, it may not be taxonomically significant. We did not find any microscopic features to clearly distinguish *A. dolosus* from *A. pendens*. The three *Aurantiopileus* species apparently occupy a similar niche (i.e., the fragments of decomposed, hardly recognizable wood and humus are attached to the basidiomata). The basidiomata of *A. mayanensis* shrunk somewhat on drying and become hard and waxy but the margin and pores retained the distinctive orange color.

Key to the species of Aurantiopileus:

1a	Pileus orange, basidiospores ellipsoid to broadly ellipsoid, (4.6-) 5.0-6.0 x (3.4-) 3.6-4.2 (-4.6) µm.....***A. mayanensis***
1b	Pileus white, basidiospores broadly ellipsoid to subglobose, 3.5-4.8 x 3-4 µm.....2
2a	Tubes pale pink .....***A. dolosus***
2b	Tubes white .....***A. pendens***

Molecular considerations: While DNA-based data from *A. dolosus* and *A. pendens* would be desirable to confirm the coherence of *Aurantiopileus*, molecular data are not currently available for either of these species. The age of the type collections of *A. dolosus* and *A. pendens* and the way in which they were stored make it likely that DNA-based data will be difficult to obtain from these specimens, although DNA extraction has not yet been attempted. Further collecting in tropical Asia may turn up additional specimens of these species, thus affording an opportunity to obtain DNA sequence data.

At the current time, all of our molecular data are based on *A. mayanensis*, the type of the genus. These data (Fig. 6) place *A. mayanensis* firmly in the phlebioid clade within the polyporoid clade

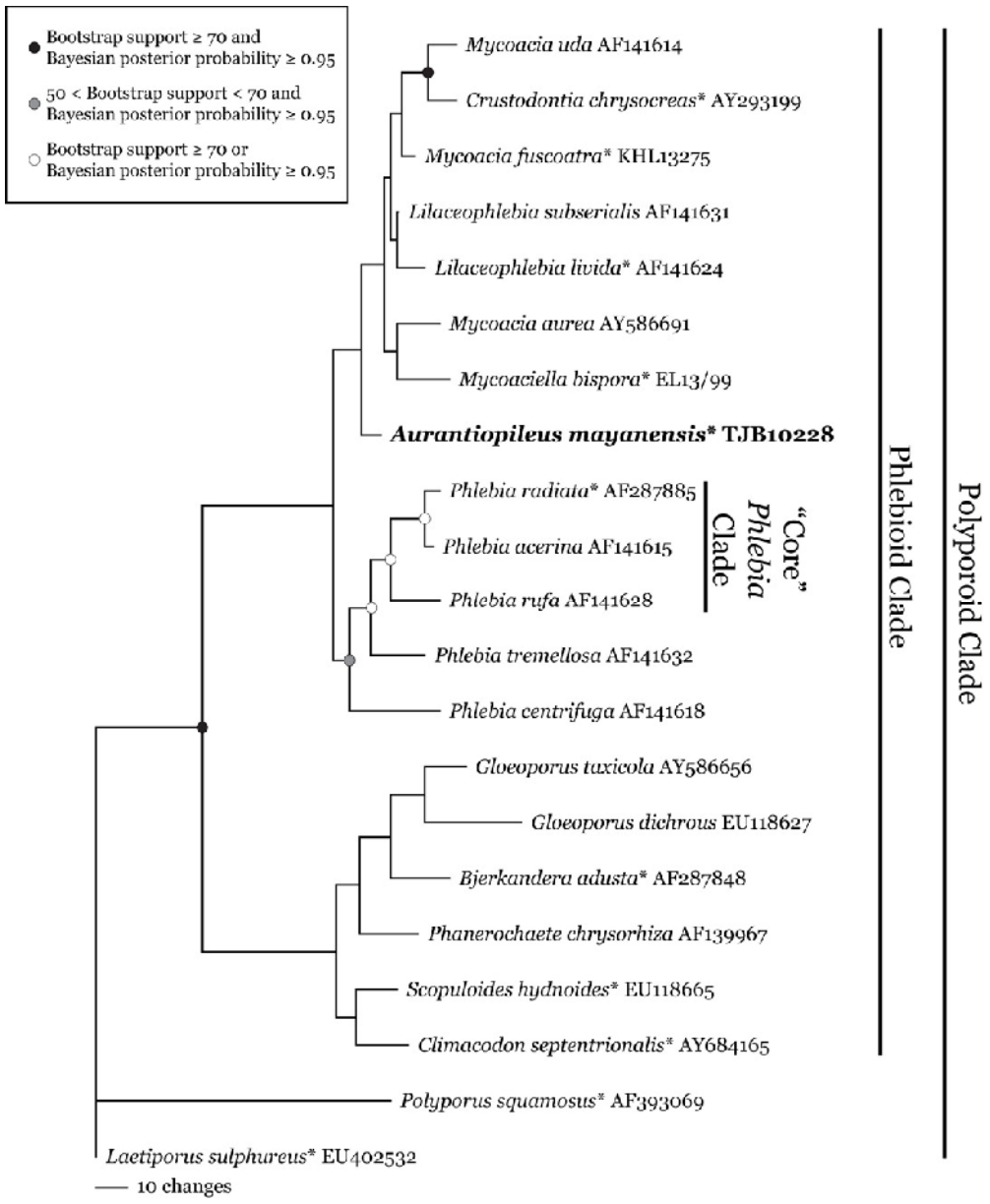


Figure 6. Inferred phylogeny based on nLSU. The tree shown is one of 58 equally parsimonious trees (Length = 461, CI = 0.60, RI = 0.49). Statistical support for nodes is based on parsimony and Bayesian analyses. The asterisk after a name indicates that it is the type species of that genus.

(*sensu* Binder et al. 2005). The nLSU analysis places *Aurantiopileus* outside of the "core" clade of *Phlebia* species, one of the genera to which *Aurantiopileus* is most closely related. In addition, the ITS data from *A. mayanensis*, which could not be easily aligned to any species in the phlebioid clade, help to confirm that *A. mayanensis* is an un-described species and unallied with any existing genus. The only species in this analysis with ITS regions that could be unambiguously aligned were *Phlebia acerina*, *P. radiata* (the type of genus *Phlebia*), and *P. rufa*. As these three species are clearly closely related, we consider them the "core" of genus *Phlebia*. Sequencing of additional DNA regions will be necessary to determine whether taxa such as *P. tremellosa* and *P. centrifuga* should be included within the genus *Phlebia*. Many of the species within this broader group, including *Crustodontia chrysocreas*, *Lilaceophlebia livida*, *L. subserialis*, *Mycoacia aurea*, *M. fuscoatra*, and *M. uda*, have been included in genus *Phlebia* by some authors (e.g. Lombard et al. 1975, Nakasone 1997). However, the inability to align ITS sequences across these taxa, coupled with the divergence of nLSU sequences, make it untenable at the present time to include all of these species in one genus.

Unfortunately many relationships within the phlebioid clade remain unresolved. The analysis in this work and in other studies (Binder et al. 2005, Hallenberg et al. 2008) indicates that *Bjerkandera adusta* (type species of the genus) and two *Gloeoporus* species (*G. conchoides* Mont., the type species, has not been included in published molecular phylogenic studies) are within the phlebioid clade but not closely related to *Phlebia* or *Aurantiopileus*. Other macroscopically similar genera, such as *Skeletocutis*, have yet to be fully examined. *Skeletocutis amorpha* (Fr.) Kotl. & Pouzar (type species of the genus) was examined by Binder et al. (2005) but could not be placed with certainty in any subclades of the polyporoid clade. A great deal of additional morphological and molecular work will be needed to resolve relationships within the phlebioid clade, a diverse and under-sampled lineage of fungi.

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Young, and Dr. Bruce Holst. Ms. Patricia Eckel produced the Latin descriptions.

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