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A new species of *Pleurocollybia* (*Tricholomataceae*; *Agaricales*; *Basidiomycetes*) from Belize

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Abstract—A new species, *Pleurocollybia imbricata*, is described from the Maya Mountains of Belize and a new combination in *Pleurocollybia* is proposed. A key to the known species of *Pleurocollybia* is also provided.

Keywords—agarics, Doyle's Delight, siderophilous inclusions, taxonomy

Introduction

Pleurocollybia Singer was proposed as a new monotypic genus to accommodate *Gymnopus praemultifolius* Murrill (Murrill 1945) based on several features: eccentric stipe, clampless hyphae, minute basidiospores (very small for an agaric, e.g. "2.7-3.5 × 2.5-3.2 μm"), and lack of necropigments (Singer 1947). Singer (1947) compared *Pleurocollybia* to two morphologically similar genera, *Callistosporium* Singer and *Podabrella* Singer (now considered a synonym of *Termitomyces*, Frøslev et al. 2003), but separated *Pleurocollybia* from them by the eccentric stipe and very small basidiospores. *Podabrella* (= *Termitomyces*)

produces a reddish/pinkish colored spore deposit while those of *Pleurocollybia* and *Callistosporium* are white. *Podabrella* (= *Termitomyces*) also produces siderophilous bodies in the basidia, while siderophilous bodies are not present in *Pleurocollybia*. *Callistosporium* has abundant brightly colored necropigments in the basidiospores, basidia and tramal hyphae, while these pigments are not present in *Pleurocollybia*. A BLAST search based on ITS sequences from *Pleurocollybia imbricata* match closely to several ITS sequences of exemplars of *Callistosporium*. Although not definitive, this similarity in ITS sequences may indicate a close relationship with *Callistosporium* as Singer originally suspected (Singer 1947) and seems to be indicated by a study of the nlsu rDNA region as well since *Callistosporium* and *Pleurocollybia* are found in the same clade, the /callistosporioid clade (Moncalvo et al. 2002).

Since 1947, eight more species have been added to *Pleurocollybia*, including four new species described from the neotropics by Singer (Singer 1955, 1963, 1969, 1978), and 2 new combinations into *Pleurocollybia* from existing neotropical taxa (Singer 1970, 1986). Two additional species were placed in the genus by recombinations of existing taxa, one from Sri Lanka/Africa (Pegler 1977) and one from New Zealand (Horak 1971). With the addition of this newest taxon, and the new combination proposed in this paper, we now know of 11 species that belong in the genus, with the greatest number being New World neotropical taxa (9 species).

Pleurocollybia is characterized by a combination of distinctive features: the stipe is typically eccentric or lateral or lacking, only a truly central stipe is known for two species, thus the habit is pleurotoid or collybioid-pleurotoid, the lamellae are crowded and very narrow, adnate or adnexed or emarginate-adnexed, the lamellulae are truncate, the basidiospores are small and white in deposits, smooth, inamyloid, the basidia lack siderophilous/cyanophilous bodies, the basidiospores, basidia and hyphae of the trama lack of necropigments, and the habitat for the basidiomata is typically lignicolous on rotting wood. Clamps may be present or absent.

A key to all known species is provided.

Materials and methods

Color notations in the macroscopic descriptions are from Kornerup & Wanscher (1978). Methods used in preparation of microscopic structures were those of Baroni (1981). Testing for cyanophilic reactions of spore walls and for cyanophilic bodies in basidia was carried out as follows: un-revived dry lamella fragments were gently heated over a flame in a drop of cotton blue/lactic acid (Singer 1986) on a clean glass slide; when the mountant began to release vapor (not boiled), the fragment was removed and placed in a clear drop of lactic acid at room temperature and washed to remove excess dye. This fragment was finally

transferred to a fresh drop of clear lactic acid at room temperature on a clean slide to make a squash mount. It has previously been shown that siderophilous inclusions in basidia can be determined by using the cotton blue/lactic acid test described above (Baroni 1981), since siderophilous inclusions stain in a similar fashion in cotton blue. All measurements of anatomical features were made in mounts of 3% KOH under an oil immersion lens. The designations used for basidiospore measurements are those of Baroni & Horak (1994) where n = number of spores measured, Q = range of length/width of individual spores and Q_m = mean of those Q values. All measurements were made with an Olympus BHS light microscope under Hoffman interference optics using an ocular micrometer or by using a semi-automated image analysis system (a GTCO digitizer pad and Metrics5 software written by Dr. David Malloch). Descriptive statistical analysis of the measurements was obtained using EXCEL 5.0 and SigmaStat 1.0. All illustrations of microscopic features were made with the aid of a drawing tube and the final plates were prepared using a WACOM pen drawing tablet and Adobe Illustrator 10. All longitude/latitude readings listed were made by hand held GPS (GARMIN Etrex Vista) set on the WGS84 Datum standard or the UTM standard.

Taxonomy

New species

Pleurocollybia imbricata T.J. Baroni, Lodge & D. L. Lindner, sp. nov.

Mycobank MB510698

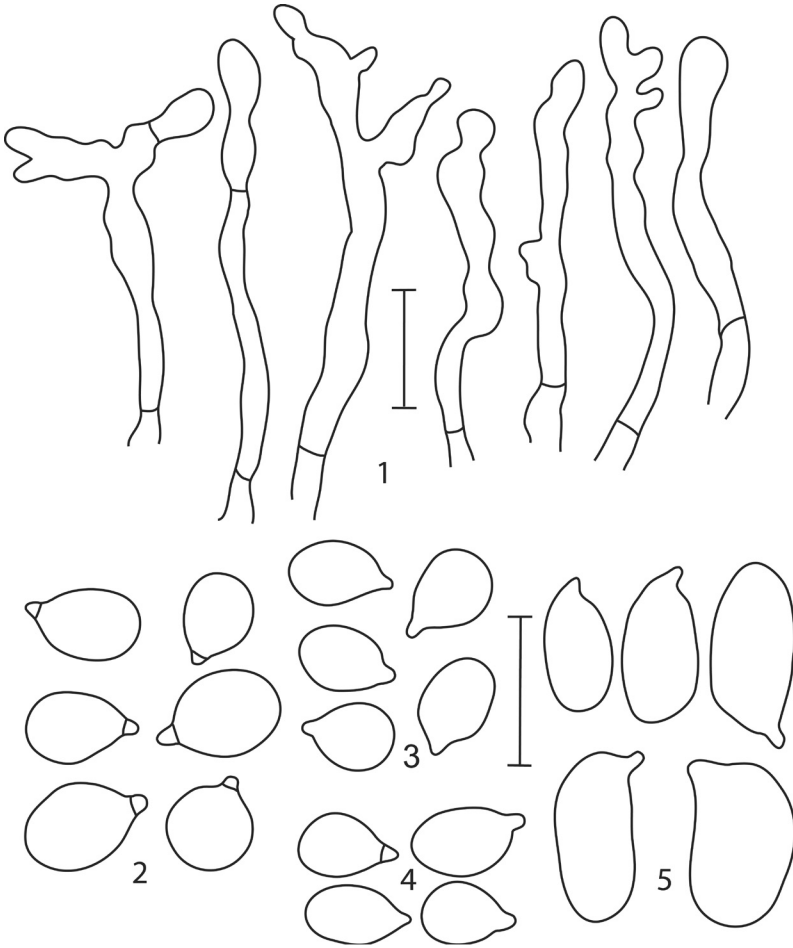
FIGS. 1-2, 6

Basidiomata pleurotoidea, imbricata, in subiculo crasso albo implicito, in ligno putrido. Ab congeneribus differt basidiomatibus dense imbricato-compaginatibus ut videtur stipite carentibus, marginibus pilei profunde incisus lobatisque, pileo incano pallide canido-brunneo, odore farinaceo, sapore amaro farinaceo, basidiosporis parvis subglobosis, 3-4 × 2-3.2 μm, cheilocystidiis cylindricis contortis.

HOLOTYPE T. J. Baroni 9847, Maya Mountains, Belize (BRH; ISOTYPES, CORT, NY)

ETYMOLOGY — From the Latin *imbricatus*. The species is named for its overlapping pilei, like roof tiles.

BASIDIOMATA pleurotoid, densely imbricate and produced from a thick white matted subiculum on decaying woody substrate. **PILEUS** pale grayish buff or grayish orange or grayish brown (5–6B2–3, Alabaster, Birch Bark, Flesh or 5C2–3, Birch Grey, Brownish Orange), 15–60 mm broad, plano-convex becoming plane (or when young and on top of the woody substrate infundibuliform! – only one basidioma out of 30 or more), irregularly dimidiate, subspathulate, deeply incised, lobed, lobes overlapping, producing small conchate “caps” from margins of lobes, densely imbricate-shelving, hoary canescent over most of surface with darker brown colors below canescence, canescence often grown over or around small bits of woody debris trapping particles on the surface, margin inrolled.



Figs. 1-2: *Pleurocollybia imbricata* (9847 TJ Baroni, Isotype):
Fig. 1, cheilocystidia, scale bar = 10 μ m. Fig. 2, basidiospores.
Fig. 3 *Pleurocollybia densifolia* (F.S. Earle 578, Holotype) — basidiospores.
Fig. 4 *Pleurocollybia praemultifolia* (F17468, Holotype) — basidiospores.
Fig. 5. *Pleurocollybia amara* (F17336, Holotype) — basidiospores.
Scale bar for all basidiospores = 5 μ m.

Context watery grayish brown, approx. 1 mm thick. LAMELLAE grayish brown (\pm concolorous with pileus) but also with flesh pinkish hues, adnexed or adnate, extremely crowded with numerous irregular tiers of truncate lamellulae, some so tightly crowded they have grown together, frequently producing ball-like clumps of white pubescent outgrowths on the fused lamella edges, moderately



Fig. 6. *Pleurocollybia imbricata* basidiomata in situ. TYPE (9847 T. J. Baroni). 1×.

broad (± 1 mm), edges \pm even, concolorous with faces. STIPE central at first but quickly strongly eccentric or lateral and highly reduced, approx. 1 mm \times 1 mm, densely white fibrillose appressed; all stipes arising from a dense, thick whitish subiculum covering the woody substrate. ODOR fruity at first or when cut, farinaceous when flesh is crushed. TASTE bitter!! and farinaceous.

BASIDIOSPORES 3–4 \times 2–3.2 μm , ($n=41$, $L_m = 3.3 \pm 0.19$, $W_m = 2.6 \pm 0.32$, $Q = 1.07\text{--}1.3$ (-1.6), $Q_m = 1.26 \pm 0.14$), subglobose or broadly ellipsoid, round in polar view, smooth, with a very small apiculus ($< 0.5 \mu\text{m}$), hyaline, inamyloid, weakly cyanophilic or acyanophilic. BASIDIA 8–12.1 \times 4–5.6 μm , 4-sterigmate, broadly clavate, hyaline, lacking cyanophilic bodies. CHEILOCYSTIDIA hyaline, scattered or abundant, cylindrical, frequently contorted, often branched, some subclavate, some septate, (14–) 22–36 \times 2.4–3.2 μm . PLEUROCYSTIDIA absent. LAMELLAR TRAMA composed of parallel hyphae, cylindrical or slightly inflated, 4–16 μm in diam, cells mostly short. PILEUS CONTEXT hyaline, a compact layer of short cylindrical hyphae, 4.8–16.2 μm in diam. PILEIPELLIS hyaline, 60–120 μm deep, of loosely entangled, erect, cylindrical hyphae, 1.6–4.8 μm in diam., mostly trichodermial in aspect, hyphal ends frequently contorted, and often branched. CLAMP CONNECTIONS absent.

HABIT: Lignicolous on downed decaying large (40-60 cm diam) dicotyledenous log, over mosses on the side and underside of the log. August.

MATERIAL EXAMINED: Belize; Cayo District, Maya Mountains, Doyle's Delight UTM 81843W 24593W, 1035 m alt., on north trail to creek from summit, in creek bed area, 13 August 2004, T. J. Baroni 9847 (with Dan Lindner), (HOLOTYPE: BRH; ISOTYPES: NY, CORT).

ADDITIONAL MATERIAL EXAMINED: *Gymnopus praemultifolius*, USA, Florida, Alachua County, Gainesville area, on decaying hardwood log, shade, 7 July 1938, collected and determined W. A. Murrill, F17468 (HOLOTYPE: FLAS). *Gymnopus densifolius* Murrill, Jamaica, Port Antonio, on a much decayed stump, 23 November 1902, F. S. Earle 578 (HOLOTYPE: NY)

COMMENTS: *Pleurocollybia imbricata* is distinguished from other species in this genus by the lateral obscure stipes arising from a thick whitish subiculum, the deeply incised and frequently lobed margins on the imbricate pilei, the hoary-canescens grayish pileus surface and the conspicuous cheilocystidia (FIGS. 1 & 6). Macroscopically, this taxon may be confused with *P. apoda*, *P. paradoxa*, or *P. praemultifolia*. *P. imbricata* is similar to *P. apoda* and *P. paradoxa* due to the absence or obscurity of stipes at maturity. *P. imbricata* is different from both of these taxa, because it produces at maturity imbricate shelving basidiomata. *P. apoda* and *P. paradoxa* do not produce imbricate shelving basidiomata. In addition, *P. imbricata* has cheilocystidia. *P. apoda* and *P. paradoxa* lack cheilocystidia. *P. imbricata* has a hoary-canescens pileus surface, while *P. apoda* and *P. paradoxa* have glabrous pileus surfaces.

Pleurocollybia imbricata is similar to *P. praemultifolia*, in that, they both have imbricate basidiomata and they produce conspicuous cheilocystidia. However, *P. imbricata* has a pale grayish or grayish brown colored hoary-canescens pileus and the individual basidiomata are produced from a dense whitish subiculum on highly reduced, lateral obscure stipes, while *P. praemultifolia* has a chestnut colored glabrous pileus and the conspicuous stipes are merely eccentric and not arising from a subiculum. The basidiospores of *P. imbricata* are subglobose or broadly ellipsoid (FIG. 2) and are larger than the spores of *P. praemultifolia* (FIG. 4).

New Combinations

Pleurocollybia amara (Murrill) Singer ex T. J. Baroni & Bocsusis, **comb. nov.**

MYCOBANK MB510957

FIG. 5

BASIONYM: *Gymnopus amarus* Murrill, Proc. Fla. Acad. Sci. 7:109. 1945

= *Collybia amara* Murrill, Proc. Fla. Acad. Sci. 7:127. 1945, nom. alt.

Singer (1975, 1986) listed *Pleurocollybia amara* as one of the seven, and then eight, species he accepted in the genus. However, after searching the literature (Mueller & Wu 1997; <http://www.speciesfungorum.org/Names/Names.asp>), it became obvious to us that Singer never validly published the new combination

he had originally discussed (Singer 1970). We borrowed the type and the information we obtained is described below. *Gymnopus amarus* belongs in *Pleurocollybia* and we validate that new combination here.

The following description of macroscopic features is from Murrill (1945).

“Pileus conic-convex, not expanding, cespitose, 2 cm broad; surface dry, smooth, glabrous, avellaneous, the disk tinged with isabelline, margin even, entire, inflexed; context thin, gray, opaque, bitter at once, odorless; lamellae adnate, inserted, narrow, close, white, unchanging, entire to eroded; spores ellipsoid, smooth, hyaline, uniguttulate, $5-6 \times 3-4 \mu$; stipe tapering upward, smooth, glabrous, white, $3 \times 0.3-0.6$ cm. Type collected by West and Murrill on a much-decayed pine log in Sugarfoot Hammock, near Gainesville, Fla., August 4, 1938 (F17336). Gray above and white below, with bitter flesh.”

Microscopic features were obtained from our examination of the holotype:

BASIDIOSPORES $5.6-7.2 \times 3-4 \mu\text{m}$ ($n = 10$, $L_m = 6.27 \pm 0.57$, $W_m = 3.37 \pm 0.35$, $Q = 1.75-2$, $Q_m = 1.86 \pm 0.10$), ellipsoid in face and profile, round in polar view, smooth, hyaline, inamyloid, acyanophilous. BASIDIA $19.4-24.3 \times 5.6-6.4 \mu\text{m}$, 4-sterigmate, clavate, hyaline, lacking cyanophilous bodies. HYMENIAL CYSTIDIA absent. CLAMPS present on hyphae in hymenium.

HABITAT: Lignicolous on well decayed pine log. August.

Material examined: USA, Florida, Alachua County, Gainesville area, Sugarfoot Hammock. 4 August 1938, West and Murrill F17336 (HOLOTYPE: FLAS)

COMMENTS: The small, inamyloid, acyanophilous, smooth basidiospores, crowded, narrow lamellae, truncate lamellulae (?), lack of necropigments, lack of cyanophilous bodies in the basidia, bitter taste and habitat on decaying wood indicate this species belongs in *Pleurocollybia* even though the stipe is not conspicuously and consistently eccentric (Singer 1970). Singer (1986) clearly accepted this species in *Pleurocollybia* but never formally made the combination he considered some time earlier (Singer 1970). He did not make the combination earlier because this species was one of just a few taxa of *Pleurocollybia* that possessed clamp connections on its hyphae and did not show clearly eccentric stipe attachments. Since this taxon is not commonly collected (we only know of the type collection), it has not been the subject of a molecular phylogenetic analysis as yet.

Unfortunately the holotype of *G. amarus* consists of only broken fragments and these are not plentiful nor in very good condition. One can determine that the lamellae are crowded and narrow, but that is all. Most of the remaining lamellae and lamellulae on the pileus fragments have been shattered at the pileus connection so that few whole structures are left. It is not possible to determine if the lamellulae were truncate on the few pileus fragments remaining. However, Singer (1970) indicates that *Gymnopus amarus* has the typical truncate lamellulae.

Table 1 – Abbreviated bibliographic and distribution information for species of *Pleurocollybia*.

TAXON	PUBLISHED AS <i>PLEUROCOLLYBIA</i>	DISTRIBUTION
<i>P. amara</i>	(2008) This publication	Florida
<i>P. apoda</i> Singer	1955 Mycologia 47: 769	Panama
<i>P. brunescens</i>	1973 Beih. Sydowia 7: 17	Cuba
<i>P. cibaria</i> Singer	1963 Bol. Soc. Argent. 10: 207	Peru
<i>P. cremea</i> (G. Stev.) E. Horak	1971 N.Z. J. Bot. 9: 415	New Zealand
<i>P. densifolia</i> (Murrill) Singer	1986 Agar. In Mod. Tax., Ed.4, p. 280	Jamaica
<i>P. imbricata</i>	(2008) This publication	Florida
<i>P. paradoxa</i> Singer	1969 Beih. Nova Hedwigia 29: 55	Chile
<i>P. praemultifolia</i> (Murrill) Singer	1947. Mycologia 39(1): 80	Florida
<i>P. pulcherrima</i> Singer	1978 Nova Hedwigia 29: 12	Colombia
<i>P. versiformis</i> (Berk.) Pegler	1977 Kew Bull. Add. Ser. VI: 96	Sri Lanka (Type) & Tanzania

Clarifications

Pleurocollybia brunescens (Earle) Singer,

[as *brunescens*] *Beih. Sydowia* 7: 17 (1973)

= *Geopetalum brunescens* Earle, 1906, In. An. Est. Cent. Agr. de Cuba 1:235, non

[= '*Micromphale brunescens* Earle', sensu Dennis 1953, lapsus calami].

In the process of obtaining information on all known taxa of *Pleurocollybia*, we discovered misinformation in the published literature that had been followed by several authors and was cited in Index Fungorum incorrectly. [The information in Index Fungorum has since been corrected after we pointed out the problems.] The basionym for *Geopetalum brunescens* Earle was cited incorrectly in the literature as a *Micromphale brunescens* (sic) Earle by Dennis (1953), Pegler (1977, 1983) and Singer (1973) apparently based on an error in citation by Dennis (1953). There is no *Micromphale brunescens* described in the literature, by Earle or any other author. Pegler (1987) obviously recognized the error by correctly listing the synonyms of *Pleurocollybia brunescens* (sic) as *Geopetalum brunescens* (sic) but incorrectly maintaining the spelling of the specific epithet as *brunescens* instead of the original and perfectly correct *brunescens*.

Key to the known species of *Pleurocollybia*

1. Stipe present and typically eccentric or lateral at maturity, rarely central 2
1. Stipe absent or obscure at maturity 9
2. Pileus \pm 4 mm broad when dried; stipe 11×0.4 mm; pileus, stipe and basal mycelium violet colored; spores $3\text{--}4.5 \times 2.5\text{--}3$ μm (Singer 1978) *P. pulcherrima*
2. Pileus larger; stipe broader; violet colors absent 3
3. Fruit bodies white or cream color 4
3. Fruit bodies tan or cinnamon-brown or chestnut brown or grayish 5
4. Pileus 20–100 mm broad, white, spathulate, translucent, non-striate; spores $5.5\text{--}8 \times 3.5\text{--}4.5$ μm (Pegler 1977) *P. versiformis*
4. Pileus 15–35 mm broad, cream-white or deep cream color, orbicular or reniform; spores $2.5\text{--}3.5 \times 2.5$ μm , weakly amyloid (Segedin 1996!) *P. cremea*
5. Pileus 10–15 mm broad, cinnamon-brown; stipe pallid, 10 mm \times 2 mm, eccentric with maturity, pruinose especially above; spores globose or subglobose, (4–5 μm Murrill 1916; $2.3\text{--}2.8 \times 1.7\text{--}2.2$ μm short ellipsoid, Singer 1970; $3.2\text{--}4 \times 2.4\text{--}3.2$ μm , ! from Holotype, Fig. 3) *P. densifolia*
5. Pileus larger than 15 mm 6
6. Pileus spathulate, pale grayish brown; stipe lateral, white, silky; spores globose 3–4 μm (Earle 1906) *P. brunescens*
6. Pileus orbicular; stipe eccentric or central 7
7. Gregarious on soil among moss; stipe central; taste mild *P. cibaria*
7. Lignicolous; taste bitter 8
8. Basidiomata densely imbricate; pileus livid chestnut, 35–45 mm broad, odor of anise, taste bitter; stipe eccentric, pruinose, pale yellowish-white; cheilocystidia present, cylindrical-contorted, narrowly clavate, some branched; spores $1.5\text{--}2.0$ μm (Murrill 1945), but $2.8\text{--}3.2 \times 2.4\text{--}2.8$ μm (! from Holotype) *P. praemultifolia*
8. Basidiomata not imbricate; pileus avellaneous or disc isabelline, 20 mm broad, odorless, taste bitter; stipe central, glabrous, white; cheilocystidia absent; spores ellipsoid, $5\text{--}6 \times 3\text{--}4$ μm (Murrill 1945; $5.6\text{--}7.2 \times 3\text{--}4$ μm TYPE!) *P. amara*
9. Basidiomata imbricate shelving; pileus 15–60 mm broad, margins deeply incised and frequently lobed, pale-grayish buff or grayish-brown, surface hoary-canescens; cheilocystidia present; spores $3\text{--}4 \times 2\text{--}3.2$ μm , subglobose or ellipsoid *P. imbricata*
9. Basidiomata not imbricate; pileus margin not incised or lobed, surface glabrous; cheilocystidia absent 10
10. Pileus up to 42 mm broad, pale ochraceous; spores $2.7\text{--}3.0 \times 1.8\text{--}2.0$ μm , ellipsoid *P. apoda*
10. Pileus up to 11 mm broad, pale cinnamon brown; spores $3\text{--}5 \times 2\text{--}2.5$ μm , ellipsoid *P. paradoxa*

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