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The wild edible mushroom *Pleurocollybia cibaria* from Peru is a species of *Gerhardtia* in the Lyophyllaceae (Agaricales)

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Abstract – *Pleurocollybia cibaria* is a wild edible mushroom sold in markets in Peru. The genus *Pleurocollybia* shares a phylogenetic alliance with the Tricholomatineae in the family Biannulariaceae (= Catathelasmataceae), however, no taxonomic investigations of *P. cibaria* have been performed since it was originally described over 50 years ago. Here we employ a molecular phylogenetic analysis including *P. cibaria* and compare a modern extant collection with that of the holotype to confirm its systematic placement and provide an updated taxonomic description of the species. Fresh material of *P. cibaria* was compared with that of the holotype collection using light microscopy and scanning electron microscope methods. Molecular phylogenetic analyses of the internal transcribed spacers (ITS) of the nuclear ribosomal RNA tandem repeat were performed. Microscopic analyses of fresh and type material of *P. cibaria* support their conspecificity. For the first time, we reveal the presence of minutely verruculose basidiospores and cyanophilic and siderophilous bodies in the basidia of *P. cibaria*, which support a morphological alliance with the genus *Gerhardtia* (family Lyophyllaceae), not *Pleurocollybia* (family Biannulariaceae). Morphological and molecular data support the transfer of *P. cibaria* to the genus *Gerhardtia*.

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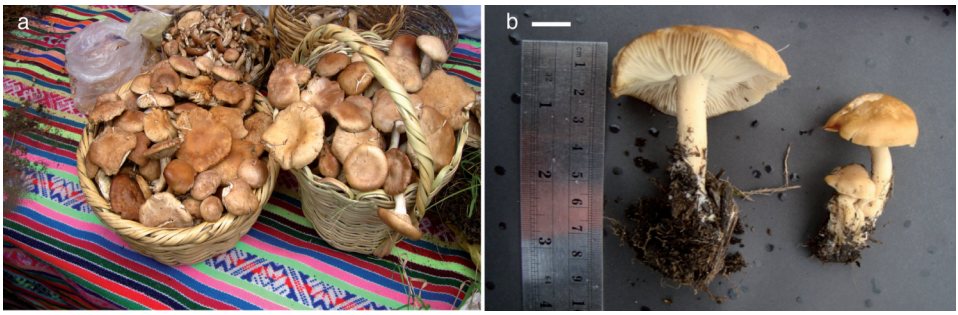

Fig. 1. Fruit bodies of *Pleurocollybia cibaria*. **a.** Fruit bodies as sold in a Peruvian marketplace. **b.** Individual fruit bodies. Scale bar equals 1 cm. Photos by A. Simoni.

INTRODUCTION

Pleurocollybia cibaria Singer is a commonly collected wild edible mushroom from high elevations in Peru sold in Peruvian markets (Singer, 1963; Fig. 1a). The fungus has been the subject of recent ecological and physiological studies in a graduate dissertation work (Fig. 1b) (Simoni, 2015). However, no modern description of *P. cibaria* has been produced since it was first described more than fifty years ago and placed in the family Tricholomataceae R. Heim ex Pouzar, a family of Agaricales (Singer, 1986). Investigation into the taxonomic composition of the Agaricales has revealed that the family is a monophyletic group of at least eight clades interpreted as genera (Sánchez-García *et al.*, 2014). To date three species ascribed to *Pleurocollybia* Singer have been sequenced and found to be polyphyletic within the family Biannulariaceae Jülich, an earlier name for Catathelasmataceae Wasser (Sánchez-García *et al.*, 2016), which is now placed in the suborder Tricholomatineae (Dentinger *et al.*, 2016). Eleven species of *Pleurocollybia* have been described with nine species known from the Neotropics (Baroni *et al.*, 2008). Most of these have a pleurotoid habit, occur on wood and have smooth basidiospores features at odds with *P. cibaria*, which has a central stipe, occurs on soil, and possesses obscurely verruculose-punctate basidiospores (the latter in contrast to the protologue). Here we investigate the systematic status of *P. cibaria* and compare fresh material ascribed to this species with that of the holotype using morphological analyses. A molecular analysis from recently collected material from Peru is also performed.

MATERIALS AND METHODS

Taxonomy, light and SEM microscopy – The taxonomic description is produced from the Latin protologue (Singer, 1963) and supplemented with updated microscopic data observed from fresh material (*Simoni s.n.*; TENN 071148) and the holotype (BAFC) [Thiers, continuously updated]. Light microscopy was done on an Olympus BX50 with DIC optics. All measurements were made at 1000×. Number of spores measured per collection is indicated by “n”. Mean length and width of

spores are reported as Lm and Wm, respectively. Q values refer to the quotient of spore length divided by width. Qm refers to the mean Q value. Cyanophilic reactions on the basidiospore wall surface and in basidia were determined by using Cotton Blue in lactic acid (Baroni *et al.*, 2008; Singer, 1986).

Basidia were also examined for siderophilous granulation in an acetocarmine acetic solution following 'method A' described by Kühner (1938) and Grund & Marr (1965) with the following modification: small fragments of hymenia were soaked in a drop of acetocarmine acetic acid solution on a glass slide for 60-90 sec. The tissue in the drop of solution was then gently heated over an alcohol lamp, not allowing the fluid to boil but constantly stirring the tissue in the solution with a moderately rusty dissecting needle in order to add the necessary iron to the reaction. A standardized mordanting solution and heating procedure as described by Grund & Marr (1965) was not used. When the solution turned dark or almost black, the tissue was removed and placed into a drop of acetocarmine on a clean separate slide. The tissue was gently splayed out and examined under the compound light microscope.

Scanning electron micrographs were made on a JEOL JSM 6010PLUS/LA, at 20kV with a working distance of 10 mm and a spot size of 30 from rehydrated and critical point dried basidiospores (Kluting *et al.*, 2014).

DNA isolation, sequencing and phylogenetic analysis – DNA isolation and sequencing of the internal transcribed spacers (ITS) from the nuclear ribosomal RNA repeat and the most variable region of the gene *rpb2* (between domains 6 and 7) was performed following previously published protocols (Sánchez-García *et al.*, 2014). Based on blastn results, the most similar ITS sequences were downloaded from NCBI and aligned using ClustalX 2.0.9 (Larkin *et al.*, 2007) and saved as a nexus file. Manual minor adjustments to the alignment were performed in MacClade 4.08 (Maddison & Maddison, 2005). The resulting nexus file was then saved as a phylip file using SEAVIEW v3.2 (Galtier *et al.*, 1996). RAxML versions 7.2.8 (Stamatakis, 2006) and 8.0.0 (Stamatakis, 2014) were used to perform a Maximum Likelihood (ML) phylogenetic analysis including 250 bootstrap replicates and a GTRGAMMA model of molecular evolution. One analysis included all sites, and a second analysis excluded potentially ambiguously aligned sites. Sequences of *Sagaranella* V. Hofst. *et al.* and *Myochromella* V. Hofst. were used for outgroup purposes based on Vizzini *et al.* (2015). The resulting phylogram was rooted with *Sagaranella* and *Myochromella* (Vizzini *et al.*, 2015) as *Calocybella* and *Gerhardtia* are sister taxa (Vizzini *et al.*, 2017). GenBank sequences employed in this study are indicated in Fig. 2. Datasets and tree files produced by this work are available directly from the senior author. ITS and *rpb2* sequences of *P. cibaria* have been submitted to GenBank (accession no. KX981985-KX981986).

RESULTS

Light microscopic analyses revealed the presence of weakly verruculose or faintly punctate basidiospores in both fresh and holotype material. The punctae on the spores were cyanophilic. Likewise, the presence of cyanophilous and siderophilous bodies was observed in basidia and basidioles of the holotype specimen, less so in the fresh material. However, amorphous globules were observed in the latter using acetocarmine, consistent with the presence of siderophilous granulation. These features support placement of *P. cibaria* in the family Lyophyllaceae Jülich.

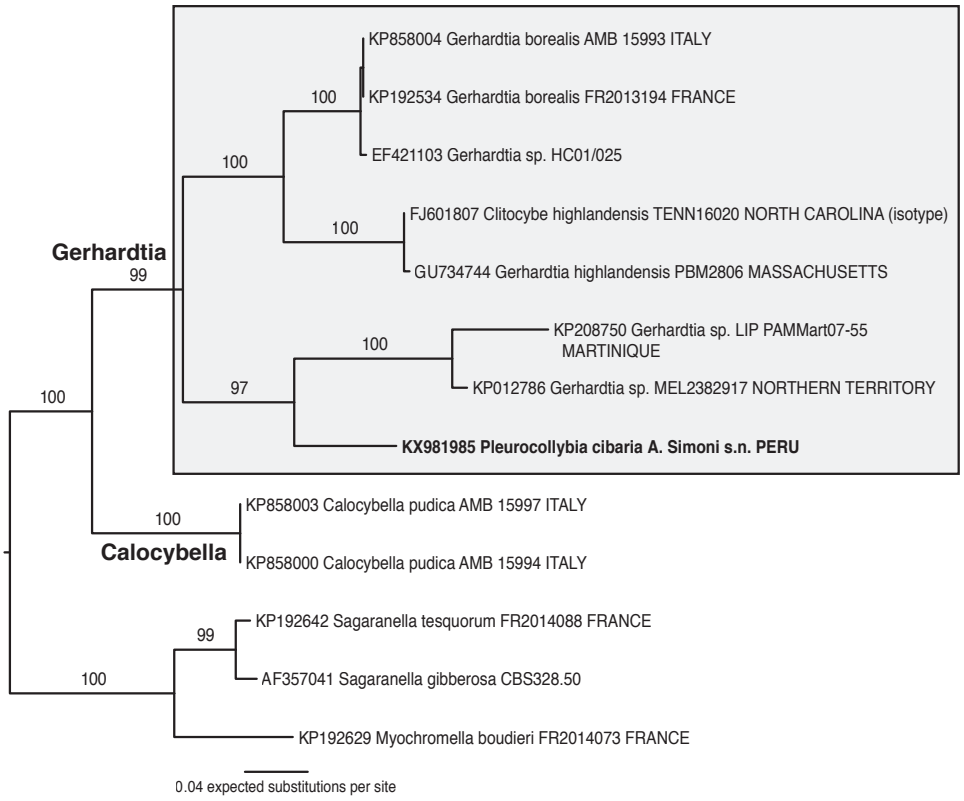

Fig. 2. ML phylogenetic tree of ITS sequences showing the nested placement of *Pleurocollybia cibaria* within the genus *Gerhardtia* (shaded in gray). ITS accession numbers on GenBank are indicated before species names. Numbers above branches are bootstrap proportions.

PCR amplification of the ITS region from a DNA extract derived from a fragment of the holotype resulted in contamination. Much of the holotype appears to be infected with mold.

An ITS sequence from recent material (A.S. *s.n.*) proved to be 87-90% similar to seven ITS sequences of the genus *Gerhardtia* Bon and 87-90% similar to five sequences of *Calocybella* Vizzini, Consiglio & Setti, two genera of the family Lyophyllaceae. Twelve ITS sequences of *Gerhardtia* and *Calocybella*, including three outgroups of former *Tephrocybe* Donk species (now *Sagaranella* and *Myrochromella*), were analyzed phylogenetically (707 sites in total across the alignment) together with the *P. cibaria* ITS sequence. *Pleurocollybia cibaria* was found to be nested within the genus *Gerhardtia* (Fig. 2) and most closely related to undetermined but tropical samples of *Gerhardtia* from Martinique and the Northern Territory of Australia. Every internode in this topology receives > 97% bootstrap support. Removal of up to 62 potentially ambiguously aligned sites resulted in the same topology and measure of bootstrap support (data not shown).

Blastn searches of the *rpb2* sequence of *P. cibaria* indicate highest similarities with samples of Lyophyllaceae as well (*Gerhardtia*, *Tephrocybe*, *Lyophyllum*). The

rpb2 amino acid sequence of *P. cibaria* is most similar (99%) to that of *Gerhardtia* sp. HC01/025 (accession no. ABR10043.1) and 97% similar to amino acid sequences of *Tephrocybe* and *Lyophyllum*. Taken together, the blast results of ITS and *rpb2*, the strongly supported ITS phylogeny, and morphological data, strongly support *P. cibaria* as a species of *Gerhardtia.* Transfer of *P. cibaria* to *Gerhardtia* is necessary to maintain the monophyly of *Gerhardtia*.

TAXONOMY

Gerhardtia cibaria (Singer) Matheny, Sánchez-García & T.J. Baroni, **comb. nov**.

Basionym: *Pleurocollybia cibaria* Singer, *Boletín de la Sociedad Argentina de Botaníca* 10: 207 (1963).

Mycobank: MB 819729

Pileus 8-40 mm wide, brownish-fuscous to fuscous; surface smooth, moist but not viscid; margin entire, at first incurved and smooth; convex or depressed or umbilicate. **Lamellae** yellowish white, very crowded or rarely close, moderately broad at maturity, mostly irregularly adnexed, at times sinuate or adnate, with age separating from the stipe apex, lamellulae abruptly truncate. **Stipe** ± 45 × 2-5 mm wide, white at the apex, concolorous with the pileus below, irregularly cylindric, smooth, solid, at times weakly curved to excentric, not oblique. **Context** white, fleshy in the pileus, fleshy-fibrous in the stipe, taste sharp, odor and taste farinaceous.

Basidiospores (4.5-) 4.8-6.4 (-8.0) × 3.2-4.8 µm (n = 30; Lm = 5.8 ± 0.86; Wm = 3.5 ± 0.38; Q = 1.4-2.3, Qm = 1.68 ± 0.22) [HOLOTYPE: (4.5-) 4.8-6.4 (-8.8) × (3.0-) 3.2-4.0 (-4.8) µm (n = 20; Lm = 5.8 ± 1.22; Wm = 3.6 ± 0.51; Q = 1.4-2.0, Qm = 1.62 ± 0.18)] elliptical or short cylindrical, some with a tapered and slightly angled-distal end (Fig. 3a); surface weakly or minutely verruculose (Fig. 3a, b), the small punctae or bumps cyanophilic (Fig. 3c). **Basidia** 20-28 × 6.4-8 µm, 4-sterigmate, clavate, with cyanophilus and siderophilous granules present (Fig. 3d) or with amorphous dark pigment globules in acetocarmine. **Pleurocystidia** and cheilocystidia absent. **Lamellar trama** a hyaline layer of parallel cylindric hyphae, these 2.4-6.4 µm wide. **Pileipellis** a hyaline repent layer of cylindric hyphae, these 1.6-3.2 µm wide, not encrusted; pileus context composed of a hyaline layer of interwoven hyphae, these 4.8-12 µm wide, hardly differentiated from the pellis hyphae (Fig. 3e). **Stipitipellis** a hyaline repent layer of cylindric hyphae, 2.4-4.0 µm wide, similar to pileipellis, caulocystidia absent. **Clamp connections** absent from hyphal septa in all tissues.

Specimens examined: PERU: Cusco, Úrubamba, Chicón, *Horacio Zamalloa* 201, 6 February 1963 (BAFC, holotype); Cusco, in grassland near *Stipa ichu* and Puna grass, *A. Simoni s.n.*, 21-Mar-2015 (TENN 071148).

DISCUSSION

The macroscopic description is a translation of the original Latin diagnosis (Singer, 1963). The pileus colors are quite dark fuscous or brownish fuscous in dried specimens, but as shown in Fig. 1 fresh specimens have yellowish-brown or

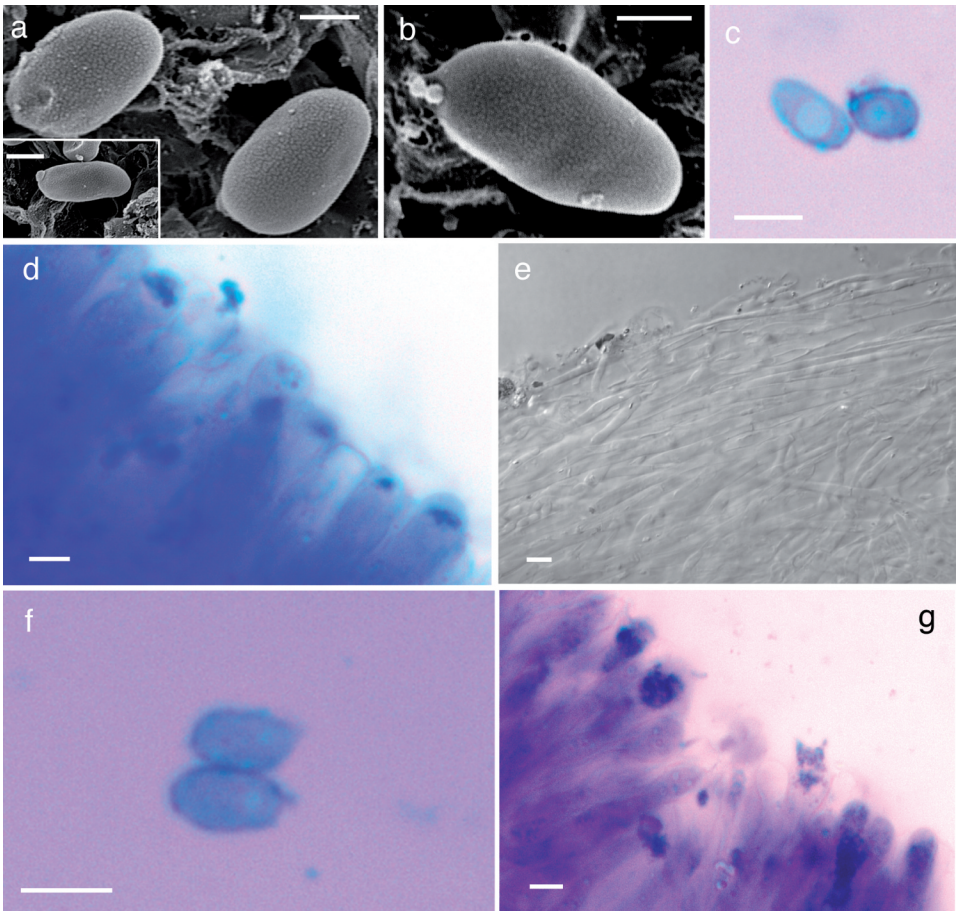

Fig. 3. Microscopic features of *Gerhardtia cibaria* and *G. highlandensis*: **a.** SEM of *G. cibaria* basidiospores demonstrating a minutely verruculose spore wall surface (A.S. s.n.) and the tapered and slightly angled distal end found on some spores (see insert). Scale bars = 2 µm. **b.** SEM of *G. cibaria* basidiospores demonstrating a faint verruculose spore wall surface and tapered distal end (*H. Zamalloa* 201, holotype of *Pleurocollybia cibaria*). Scale bar = 2 µm. **c.** Cyanophilic reaction of punctae on basidiospores of *G. cibaria* (A.S. s.n.). Scale bar = 5 µm. **d.** Cyanophilous bodies in basidia of *G. cibaria* (A.S. s.n.). Scale bar = 5 µm. **e.** Pileilpellis of *G. cibaria* (A.S. s.n.). Scale bar = 5 µm. **f.** Cyanophilic basidiospores of *G. highlandensis* (TJB6595, CORT). **g.** Cyanophilous bodies in basidia of *G. highlandensis* (TJB6595, CORT).

moderately brown colored pilei with the stipes much paler than the pileus or more sordid cream color than fuscous or brownish-fuscous. It appears the original macroscopic description was taken from at least partially dried specimens. The microscopic description presented here is our own. The basidospores of the holotype material are similar to those of *A. Simoni s.n.* and in both collections are elliptic with the walls cyanophilic and odd, minute, irregularly scattered blue pigmented bumps in Cotton Blue. In the presence of this dye, the verruculose ornamentation is more evident. In their dried state, both collections closely resemble each other given the

dark colored pilei and yellowish lamellae. The cyanophilous and siderophilous bodies in the basidia are more evident in the type, but numerous amorphous dark pigment globules were observed in acetocarmine in the more freshly collected material. A portion of basidiospores of both collections features a tapered and slightly angled distal end (Fig. 3a), an odd and distinctive character shared with another species of *Gerhardtia*, *G. highlandensis* (Hesler & A.H. Sm.) Consiglio & Contu, which also shares the cyanophilous spore ornamentation and globules in the basidia (Fig. 3f-g).

Pleurocollybia cibaria deviates from the genus *Pleurocollybia* by the minutely verruculose or punctate basidiospores, the presence of cyanophilous and siderophilous bodies in basidia, the non-pleurotoid habit, and occurrence on soil (not wood). An ITS sequence of recently collected *P. cibaria*, which we find sufficiently similar to the type, is dissimilar to other species of *Pleurocollybia*. Species of *Pleurocollybia* possess smooth basidiospores, lack cyanophilous and siderophilous bodies in the basidia, and typically are lignicolous (Baroni *et al.,* 2008). The presence of minutely verruculose or punctate spores, cyanophilous and siderophilous bodies in the basidia, absence of clamp connections, non-lignicolous habit, and general overall aspect are consistent with species of *Gerhardtia* (family Lyophyllaceae), with which the authors are familiar. A phylogenetic analysis of *P. cibaria* strongly places this species within *Gerhardtia* (Fig. 2). Of note, *Gerhardtia* was recently emended (Bon, 1994), to include at least one species from New Zealand with smooth basidiospores (Vizzini *et al.,* 2015), *G. pseudosaponacea* J.A. Cooper & P. Leonard (Cooper, 2014).

The genus *Calocybella*, which is closely related to *Gerhardtia*, differs by the flesh that reddens or turns violaceous red in alkaline solutions and by the presence of clamp connections (Vizzine *et al.*, 2015). *Sagaranella* is distinguished from *Gerhardtia* by the fruit bodies with a mycenoid habit, presence of clamp connections, and nitrophilous ecology. *Myrochromella* differs from *Gerhardtia* by the smallish fruit bodies, free to nearly free lamellae, smooth basidiospores, and presence of clamp connections (Hofstetter *et al.,* 2014).

The disposition of *Pleurocollybia* as a genus is known best from morphological data (Baroni *et al.,* 2008). Sequences of the type of the genus, *P. praemultifolia* (Murr.) Singer, are not available. The type species, however, appears to be common in northern Florida (U.S.A.) having been repeatedly collected in Gainesville, Florida between 1938 and 1943 (herbarium records from MycoPortal). Recent results (Sánchez-García *et al.,* 2016) suggest at least two species of *Pleurocollybia*, *P. brunnescens* (Earle) Singer and *P. imbricata* T.J. Baroni *et al.* form a monophyletic group in the family Biannulariaceae (= Cathalasmataceae).

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