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Lactocollybia subvariicystis, a new species of little known genus *Lactocollybia* from subtropical south China

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Abstract

Lactocollybia is poorly known in China. Prior to this report, only a single species of this genus was reported from tropical Yunnan Province in the country. In this study, *Lactocollybia subvariicystis* is reported as a new species. It is characterized by its mycenoid habit with white basidiomata, white, adnate to sinuate lamellae, numerous gloeocystidia in pileus-stipe context, commonly present clamp connections, and association with living trunks of *Acacia confusa*. Although the phylogenetic relationships of *Lactocollybia* with other genera remain unclear to science, the taxonomic position of the new species within the genus is clear with morphological and molecular evidence. It is a member of genus *Lactocollybia* sect. *Albae*. Description, color photographs, comparison with phenotypically similar taxa and line drawings are presented.

Key words – Marasmiaceae – molecular phylogeny – subtropical fungus – taxonomy

Introduction

Lactocollybia Singer is a little known genus in the family Marasmiaceae. The genus has five sections: i. sect. *Bertrandiella* (Heim) Singer, ii. sect. *Lactocollybia* Singer, iii. sect. *Albae* Singer, iv. sect. *Aurantiacae* Singer and v. sect. *Graminicolae* Singer. These are classified based on their various characters such as habit of the carpophores (collyboid, pleurotoid, mycenoid and omphalioid), presence or absence of latex, subhymenium structure, clamp connections, gloeocystidia, pigmentation of the basidiomata, and living (on living cortex of trees, on cycads, fallen fruits, etc.) or lignicolous habitat (Singer 1986).

Nineteen *Lactocollybia* species have been reported worldwide up to date (www.indexfungorum.com, Nov. 2016). They are mainly distributed in tropical to subtropical regions, and few of the species can be found in the warm temperate zones in North America and South America (Singer 1986). In China, only a single species *L. epia* (Berk. & Br.) Pegler has been reported from tropical area of Yunnan Province before (Yang 2000). In the present study, another species of the genus was found to be a new species to science, which is mycenoid in habit and found on living trunks of *Acacia confusa* Merr.

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Molecular study for the genus *Lactocollybia* had not yet been studied. Only few sequences of the nuclear ribosomal internal transcribed spacer (ITS) including partial sequences of nuclear ribosomal large subunit (nrLSU) are available for this genus. In this study, three nuclear ribosomal DNA sequences were generated from the newly collected specimens of *Lactocollybia*. The species is delimitated with morphological data and molecular evidence of ITS sequence analysis.

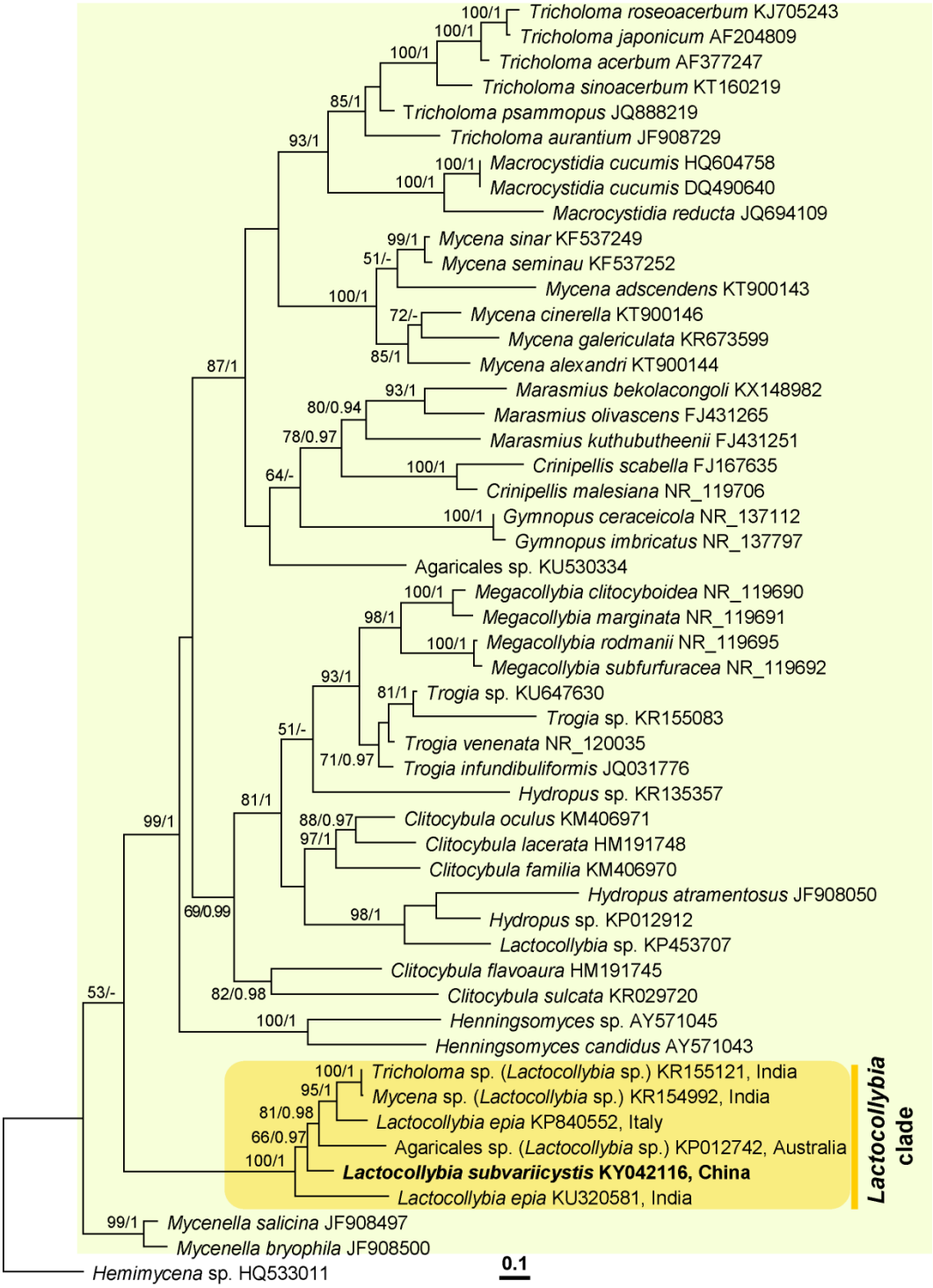

Fig. 1 – RAxML tree and phylogenetic relationships of *Lactocollybia* inferred from ITS sequences analysis. RAxML bootstrap supports values (>50%) and Bayesian posterior probabilities (>0.90) are indicated on branches. *Hemimycena* sp. is rooted as outgroup. Newly generated sequence for *L. subvariicystis* is highlighted in bold. GenBank accession numbers are provided after the species name.

Materials & methods

Morphological methods

Macro-morphological descriptions were based on the field notes and photos of basidiomata. Color codes mentioned in the description are from Kornerup & Wanscher (1978). The examined materials were collected along the road sides close to Huanghuagang Commemoration Park, Guangzhou, China, and deposited in the Fungal Herbarium of Guangdong Institute of Microbiology (GDGM), Guangzhou, China. Micro-morphological observations were done from the dried specimens and methodology followed those in Hosen et al. (2016a, b).

Molecular methods

DNA extraction and PCR protocols are as those used in Hosen et al. (2013). ITS1/ ITS4 (White et al. 1990) and LROR/LR5 (Vilgalys & Hester 1990) primer pairs were used to amplify the nuclear ribosomal internal transcribed (ITS) region. PCR products were purified with PCR Purification Kit (Sangon Biotech Co., Shanghai). Purified PCR products were sent to commercial sequencing company for sequencing (Majorbio, Guangzhou, China). Sequences were blasted against NCBI nucleotide database and closest sequences were downloaded together with other taxa of Hydropoid clade (*Clitocybula* (Singer) Singer ex Métrod, *Henningsomyces* Kuntze, *Hydropus* Kühner ex Singer and *Megacollybia* Kotl. & Pouzar and) of Matheny et al. (2006). Additionally, some representative taxa of *Crinipellis* Pat., *Gymnopus* (Pers.) Gray, *Hemimycena* Singer, *Macrocystidia* Joss., *Marasmius* Fr., *Mycenella* (J.E. Lange) Singer, *Tricholoma* (Fr.) Staude and *Trogia* Fr., were retrieved from GenBank in order to see the close relativeness of *Lactocollybia* in molecular phylogenetic analysis. *Hemimycena* was selected as outgroup for molecular phylogenetic analysis. The matrix was aligned with MAFFT (Katoh et al. 2005). Maximum likelihood phylogenetic tree was generated using RAxML v.7.2.6 (Stamatakis 2006) with 100 bootstrap replications, and supported with posterior probabilities using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003).

Results

Molecular phylogenetic results

In this study, three sequences (ITS: KY042116, nrLSU: KY042117 and KY042118) were generated from the newly collected materials of *Lactocollybia* and deposited in the GenBank. Only the ITS sequence was used for the reconstruction of a molecular phylogenetic tree, and nrLSU sequences were deposited to GenBank for future reference. Percent identity between *L. subvariicystis* and *L. epia* is 87–88 based on blast search results of ITS sequence; while nrLSU sequence from the Chinese collection *L. subvariicystis* is 97% identical to those of Italian *L. epia* (KP840552). The ITS dataset includes 51 fungal sequences and consisting of 981 nucleotide sites (gaps included) of which 604 are parsimony informative characters. The aligned ITS dataset is deposited in TreeBASE (S119966). ITS sequence of the Chinese *L. subvariicystis* nests in a clade with those of *L. epia* and supported by both ML and BI analyses (100% ML BS, 1.0 PP). The summarized result of this analysis is presented in Fig. 1.

Taxonomy

Lactocollybia subvariicystis Iqbal Hosen & T.H. Li, **sp. nov.** Figs 2–3
MycoBank MB 819173

Diagnosis – Differs from *L. variicystis* in having obviously longer but narrower basidiospores (8–)8.5–9.5(–10.5) × 4.5–5.3 µm, not or hardly plicate margin and habitat with *A. confusa*.

Etymology – The epithet "*subvariicystis*" (Lat.) refers to the morphology that is similar to *L. variicystis*.

Fig. 2 – Basidiomata of *Lactocollybia subvariicystis*. a. Iqbal 911 (GDGM 46535). b–c. Iqbal 912 (GDGM 46536, holotype). d. Iqbal 913 (GDGM 46537, paratype).

Typification – CHINA. Guangdong Province, Guangzhou, Huanghuagang Commemoration Park, 10 Sep 2016, Iqbal 912 (GDGM 46536, holotype).

Basidiomata small-sized, mycenoid. *Pileus* 10–20 mm broad, hemispherical then expanding, becoming convex or flattened with age, smooth, dry, hygrophanous, slightly depressed at disc in some pilei, milk white (1A1), white to creamy-white (1A1), without striation or hardly plicate at the margin in some pilei; context 1.2 mm thick at the center of pileus, white, unchanged when cut or injured. *Lamellae* 12–17 × 1.5–2 mm, adnexed to sinuate, subdistant, up to 1 mm wide, not or occasionally forked or not anastomosing, white to creamy white (1A1); lamellulae common, with several ranks and various widths. *Stipe* 20–30 × 1–1.5 mm, cylindrical, slightly thickening towards base, central to slightly eccentric, decurved, smooth but very fine pruinose under a lens, white to creamy-white (1A1), fistulose, unchanged when cut or injured; basal mycelium white.

Basidiospores [60/3/3] (8–)8.5–9.5(–10.5) × 4.5–5.3 µm, [Q = 1.65–1.81 (–1.92), Q_m = 1.74 ± 0.10], amygdaliform to broadly fusoid, thin-walled, pale yellow when observed in H_2O and 5% KOH, inamyloid, smooth, apiculus conspicuous up to 0.7 µm long. *Basidia* 22–27 × 6–7 µm, clavate, hyaline and pale yellow in H_2O, thin-walled, mostly 4-spored, occasionally 2- or 3-spored, with sterigmata up to 5 µm long; subhymenium thin, with subcellular to ramose hyphae. *Hymenophoral trama* regular, densely arranged parallel hyphae 4–10 µm wide. *Pileal tramma* interwoven, composed of 5–12 µm wide hyphae, mixed with abundant fusoid gloeocystidia which are sharply attenuated towards ends. *Cheilo-* and *pleurocystidia* 30–50 × 9–13 µm, crowded, variable in shape, cylindrical to subcylindrical, fusoid to subfusoid with mucronate apex, lageniform with long necked up to 30 µm. *Pileipellis* a cutis, composed of hyaline hyphae and refractive gloeocystidia; hyphae 3–8 µm wide, hyaline, smooth, thin-walled; gloeocystidia numerous, fusoid 10–18 µm wide, abruptly tapering at both ends, often forked, refractive, with vacuolar pigmentation. *Stipe trama* composed of parallel hyphae 6–12 µm wide, colorless or pale yellow, mixed with abundant fusoid gloeocystidia. *Caulocystidia* present similar to cheilocystidia but smaller. *Clamp connections* common.

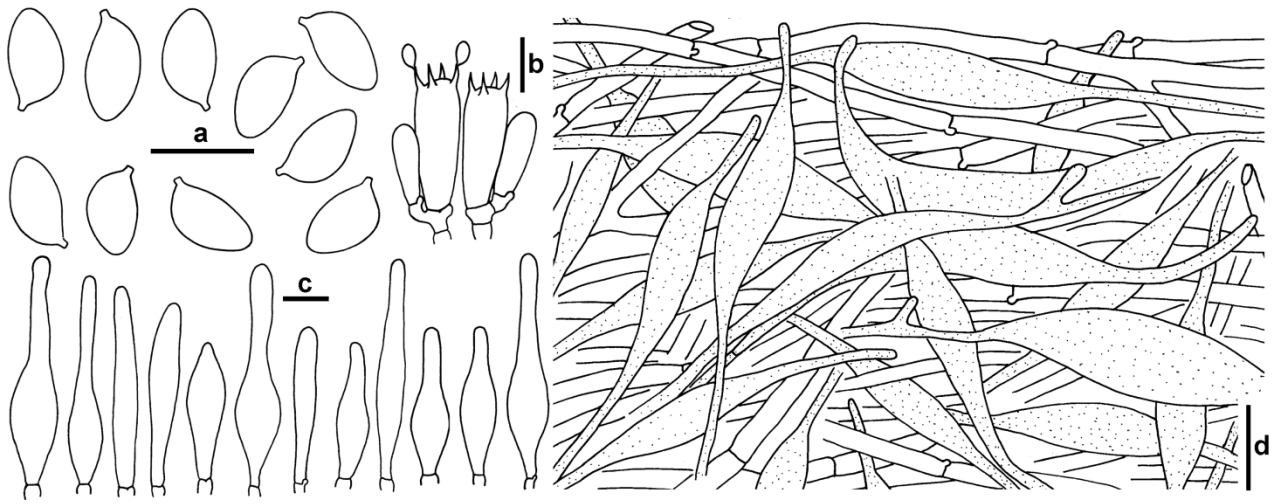

Fig. 3 – Microscopic features of *Lactocollybia subvariicystis* (Iqbal 912, GDGM 46536, holotype). a. Basidiospores. b. Basidia with basidioles. c. Cheilo- and pleurocystidia. d. Pileipellis. Scale bars: a–c 10 µm, d 20 µm.

Known distribution – Known only from the type locality in China.

Habit and habitat – Solitary, caespitose or in small groups on the standing living trunks of *A. confusa*.

Materials examined – China. Guangdong Province, Guangzhou, along the roadsides around a bus stop station at Huanghuagang Commemoration Park, 9 Sep 2016, Iqbal 911 (GDGM 46535); same location; same location, 12 Sep 2016, Iqbal 913 (GDGM 46537, paratype).

Discussion

Lactocollybia subvariicystis is characterized by its small, white, hygrophanous basidiomata, sinuate to adnexed lamellae, common presence of fusoid gloeocystidia in the context of pileus, lamellae and stipe which is sharply tapering at both ends, and associated with the living tree of *A. confusa*. This species is a member of sect. *Albae* because of having subcellular to ramose elements in subhymenium, abundant clamps connection, numerous gloeocystidia, and habitat on living trees (Singer 1986).

The phylogenetic relationships of *Lactocolybia* remained unknown despite modern species delimitation are based on molecular approaches. Most reports for *Lactocollybia* were from morphological based studies. For instance, only sequences for *L. epia* are available in GenBank out of 19 species of this genus. In the phylogenetic analysis, the Chinese *L. subvariicystis* grouped with *L. epia* from Italy and India, *Tricholoma* sp. (KR155221)/*Mycena* sp. (KR154992) from India, and one Agaricales sp. (KP012742) from Australia with maximum BS support (Fig. 1). It should be mentioned that other *Tricholoma* or *Mycena* species are nested in other clades, quite far from *Lactocollybia* clade with strong BS supports (Fig. 1). Furthermore, *Tricholoma* has no clamp connections and cystidia (Singer 1986), while *Mycena* has amyloid basidiospores (Singer 1986) which are not fitted with the taxonomic concept of *Lactocollybia*. The substantial taxonomic treatments for the Indian and Australian collections are not available to compare with *Lactocollybia*. Based on the present molecular results, it may conclude that the uploaded ITS sequences to GenBank for *Mycena* sp. (KR154992), *Tricholoma* sp. (KR155221) and Agaricales sp. (KP012742) might be the members of *Lactocollybia*. Previously *Lactocollybia* was supposed to be a close relative to *Macrocystidia* (Singer 1986) because of the numerous gloeocystidia, abundant cystidia and inamyloid basidiospores (Singer 1986). However, in the present study, they have not significantly close relativeness to each other, being nested in two different clades (Fig. 1). Interestingly, one collection from Mexico (TENN 055867) is deposited in GenBank (KP453707) as *Lactocollybia* sp. is grouped with *Hydropus* species with strong BS support (98% ML BS, 1.0 PP; Fig. 1), which may not be a true *Lactocollybia* (Marisol Sanchez-Garcia, pers. comm.). Besides the

sequence of '*L. epia* KP840552' from Italy and that of '*L. epia* KU320581' from India should represent two different species of *Lactocollybia*, since the sequences have only 83% identity. The phylogenetic relationships of *Lactocollybia* spp. within the genus or other genera remain unresolved because only limited sequences of *Lactocollybia* are available until now.

Lactocollybia variicystis, originally reported from South Africa (Reid & Eicker 1998), is morphologically close to the new species *L. subvariicystis* from subtropical south China. However, the South African species differs from the Chinese one in having obviously shorter but broader basidiospores measuring 6.6–8 × 4–6 µm, and found on the stump of *Salix* sp. *Lactocollybia pilicystis* D.A. Reid & Eicker, also originally reported from South Africa, although has small-sized white basidiomata and commonly present of gloeocystidia like *L. subvariicystis*, differs from the latter in having a usually longer stipe up to 120 mm, rare or scanty of pleurocystidia, and much smaller basidiospores measuring 4.75–6.2(–6.75) × 3.2–4.5 µm (Reid & Eicker 1998).

Previously, only a single species of the genus, i.e. *L. epia* has been reported from Yunnan Province in China (Yang 2000). The Chinese collection *L. epia* also has similar color of basidiomata but differs considerably from the new species *L. subvariicystis* in having closer adnate to decurrent lamellae, longer and thicker stipe (10–60 × 2–5 mm), fusiform to occasionally clavate or subcylindrical cystidia, shorter but broader basidiospores 6.5–8.5 × 4–5.5(–6) µm (Yang 2000). Furthermore, Yang (2000) suggested that the Chinese '*L. epia*' collection is more closely related to *L. cycadicola* (Joss.) Singer, if they are not conspecific.

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