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ORIGINAL ARTICLE

Clitopilus in southern China: two new species and comments on *C. subscyphoides*

Si-Peng Jian[1,2] · Xiang-Hua Wang[2,3] · Wang-Qiu Deng[4] · Zhu L. Yang[2,3]

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Abstract
In this study, two new species, namely *Clitopilus highlandensis* and *C. subalbidus*, are proposed based on morphological and molecular analyses. *Clitopilus highlandensis* differs from *C. abprunulus* by its greyish yellow and convex pileus, and larger basidiospores. *Clitopilus subalbidus* is similar to *C. albidus* but distinguished from the latter by its inflated hyphae in the lamellar trama and bigger basidiospores. The evidence from the phylogenetic tree constructed with RNA polymerase II second largest subunit (*RPB2*) further supports the morphological taxonomy. The phylogenetic tree, comprehensive descriptions, photographs, macro- and microscopic comparisons are presented.

Keywords *Entolomataceae* · Morphology · Phylogeny · Taxonomy · New taxa

Introduction

The genus *Clitopilus* (Fr. ex Rabenh.) P. Kumm. (*Entolomataceae*, *Agaricales*) is characterized by its clitocyboid, omphaloid to pleurotoid basidiomata, white to pinkish decurrent lamellae, flesh-pink spore deposits, and evenly cyanophilic wall of basidiospores with 5–12 longitudinal ridges (Singer 1986; Kluting et al. 2014; Jian et al. 2020a). About 40 species have been incorporated into this small genus to date (Kirk et al. 2008; Kluting et al. 2014; Kumla et al. 2019; Jian et al. 2020a, 2020b; Baroni et al. 2020). Species of this small genus are widely distributed and usually considered saprotrophic, but some species (e.g., *C. hobsonii* (Berk.) P.D. Orton) have also been reported to form ectomycorrhiza with *Quercus* L. (Peng et al. 2021).

A suite of taxonomic studies on *Clitopilus* has been conducted in China, especially in recent decades. Specifically, Yang (2000) first recorded a tropical species with a detailed description in Yunnan, namely *C. crispus* Pat., relative to simple records of the predecessors (Chang and Mao 1995; Bi et al. 1997). Then, Zang (2001) published the first new species, *C. gigantosporus* M. Zang. However, the basidiospores recorded in the original document are typical of basidiospores found in the bolete genus *Boletellus* Murrill. Furthermore, the saprophytic type and smooth basidiospores observed from the holotype did not match the characteristic longitudinally ridged basidiospores of species of *Clitopilus* (Jian et al. 2020a). Yang (2007) also published a new species, *C. amygdaliformis* Zhu L. Yang, another tropical species found in Yunnan and Taiwan, China. Afterwards, Deng et al. (2013a, 2013b) proposed two new species, *C. subscyphoides* W.Q. Deng et al. and *C. ravus* W.Q. Deng & T.H. Li. Wang et al. (2017) discovered a subalpine new species named *C. fusiformis* Di Wang & Xiao L. He. Recently, Jian et al. (2020a, 2020b) systematically studied this genus

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✉ Zhu L. Yang
fungi@mail.kib.ac.cn

[1] State Key Laboratory of Grassland Agro-Ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, Engineering Research Center of Grassland Industry, Ministry of Education, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, People's Republic of China

[2] CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[3] Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming 650201, Yunnan, China

[4] Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, State Key Laboratory of Applied Microbiology Southern China, Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou 510070, China

and contributed five new species. To date, there are nearly ten species in China.

This study examines specimens collected from Yunnan and Guangdong provinces. They are all similar to formerly published species. The specimens obtained from northwestern Yunnan were close to the European species *C. abprunulus* S.P. Jian et al. The other samples gathered from Guangdong resembled to *C. albidus* K.N.A. Raj & Manim. and *C. subscyphoides*, respectively. Further morphological research and molecular phylogenetic studies demonstrated two new species of *Clitopilus*, and one represented the rediscovery of *C. subscyphoides*. Therefore, all three species are described herein.

Materials and methods

Sample collections and morphological studies

Table 1 contains detailed information about the specimens utilized in this study. Macroscopic descriptions were based on field notes and digital images. We got the colour codes (hex triplet) from ColorHexa (https://www.colorhexa.com), using the digital images of basidiomata. The code represents a colour using six characters, ranging from 0 to 9 and a to f. It consists of three parts, each with two characters, that correspond to the red, green, and blue components of the colour. The description of basidiomata, like shape and size, and the morphological classification method of *Clitopilus* followed the rules stipulated by Jian et al. (2020a). The voucher specimens were placed in the two herbaria: the Cryptogamic Herbarium of the Herbaria of Kunming Institute of Botany, the Chinese Academy of Sciences (KUNHKAS), and the Fungarium of the Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China (GDGM).

Sections cut from dried samples were rehydrated for microscopic examinations in a 5% KOH solution. Basidiospore measurements followed Jian et al. (2020a). The ornamentation of basidiospores was observed using a ZEISS Sigma 300 scanning electron microscope (SEM) (Oberkochen, Germany), and the detailed SEM procedure was followed by Jian et al. (2020a).

Molecular phylogenetic analyses

Genomic DNA was extracted from the materials dried with silica gel using the CTAB (cetyltrimethylammonium bromide) procedure (Doyle and Doyle 1987). *RPB2* (the second largest subunit of RNA polymerase II) was used to determine the phylogenetic position due to its sufficient nucleotide variation to infer evolutionary relationships. This was one of the fragments recommended by Co-David et al. (2009), Baroni and Matheny (2011), and Kluting et al. (2014) in *Entolomataceae* Kotl. & Pouzar. The primer pairs bRPB2-6F (5'-TGGGGYATGGTNTGYCCYGC-3') and bRPB2-7.1R (5'-CCCATRGCYTGYTTMCCCATDGC-3') were used for PCR (Polymerase Chain Reaction) amplification. Besides the above gene, other genes were also sequenced, uploaded and made available in GenBank in NCBI (National Center for Biotechnology Information) to support the extra reliability for these new species, such as the internal transcribed spacers 1 and 2 with the 5.8S rDNA (ITS), the large subunit of nuclear ribosomal RNA gene (LSU), the translation elongation factor 1-α gene (*TEF1*) and the ATPase subunit 6 (*ATP6*). The PCR protocol was chiefly referred to as a touchdown method in Kluting et al. (2014) and was executed as follows:

- an initial incubation of 94 °C for 5 min;
- 12 cycles of 94 °C for 1 min, 67 °C for 1 min, decreasing 1 °C each cycle and 72 °C for 1.5 min;
- 36 cycles of 94 °C for 45 s, 55 °C for 1 min, 72 °C for 1.5 min;
- a final extension period at 72 °C for 7 min.

The Gel Extraction and PCR Purification Combo Kit (Spin-column, Bioteke, Beijing) were used to purify PCR products, which were then sequenced on an ABI-3730-XL sequence analyzer (Applied Biosystems, Foster City, CA) using the same primers as in the amplification. Newly generated and previous sequences used in our research are shown in Table 1.

Sequencher 4.1.4 (Gene Code Corp., Ann Arbor, MI) was employed to concatenate sequences obtained from forward and reverse directions, and to delete some regions contained heavy peaks at the beginning or end. Sequences were aligned using MAFFT 6.8 (Katoh et al. 2005) and then manually checked in BioEdit 7.0.9 (Hall 1999). Under the Akaike Information Criterion (AIC), the best-fitted substitution model for *RPB2* was found by MrModeltest 2.3 (Nylander 2004). Phylogenetic analyses were carried out using the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses in RAxML 7.2.6 (Stamatakis 2006) and MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003), respectively. *Clitocella mundula* (Lasch) Kluting et al. and *Clitocella colorata* L. Fan & N. Mao were chosen as outgroups due to the close kinship between *Clitocella* Kluting et al. and *Clitopilus*. The GTRGAMMAI model (Stamatakis 2006) was used for ML analyses, with statistical support for internodes acquired via non-parametric bootstrapping with 1000 replications. The best-selected model was employed for BI analyses, and the Markov Chain Monte Carlo (MCMC) chain ran for two million generations. The STOPRULE command was set to STOPVAL = 0.01, and trees were sampled every 100 generations. To achieve suitably large Effective Sample Size (ESS) values (> 200), Tracer 1.5

Table 1 Collection information of voucher specimen and GenBank accession numbers for sequences used in phylogenetic analyses

Species	Collection or collector no.	Location and year	*RPB2* accession	References
Clitopilus abprunulus (Holotype)	KUN-HKAS 107040 [a]	Macedonia 2019	MT349666	Jian et al. 2020b
C. abprunulus	KUN-HKAS 107041 [a]	Macedonia 2019	MT349667	Jian et al. 2020b
C. albidus (Holotype)	CAL 1319 [b]	Kerala State, India 2001	MF946579	Raj and Manimohan 2018
C. amygdaliformis	KUN-HKAS 60406 [a]	Yunnan, China 2008	MN148120	Jian et al. 2020a
C. amygdaliformis (Epitype)	KUN-HKAS 81125 [a]	Yunnan, China 2014	MN148119	Jian et al. 2020a
C. cf. *argentinus*	MTB 4804/2 [c]	Monchengladbach, Germany 2011	KC816907	Kluting et al. 2014
C. baronii	K(M)179703 [d]	UK 2012	MN856160	Consiglio and Setti 2019
C. baronii (Holotype)	AMB 18363 [e]	Mantova, Italy 2007	MN856167	Consiglio and Setti 2019
C. brunneiceps	KUN-HKAS 80211 [a]	Hubei, China 2013	MN148121	Jian et al. 2020a
C. brunneiceps (Holotype)	KUN-HKAS 104510 [a]	Yunnan, China 2018	MN148123	Jian et al. 2020a
''*C. cinerascens*''	8024 TJB [c]	Florida, USA 1996	KC816908	Kluting et al. 2014
''*C. cinerascens*''	8133 TJB [c]	Louisiana, USA 1996	KC816909	Kluting et al. 2014
C. crispus	KUN-HKAS 90508 [a]	Yunnan, China 2015	MN148140	Jian et al. 2020a
C. crispus	KUN-HKAS 97509 [a]	Yunnan, China 2016	MN148145	Jian et al. 2020a
C. cystidiatus	ME Noordeloos 200350	Slovakia 2003	GQ289220	Co-David et al. 2009
C. fusiformis	SAAS 1038 [f]	Yunnan, China 2015	KY385632	Wang et al. 2017
C. fusiformis (Holotype)	SAAS 1892 [f]	Yunnan, China 2015	KY385633	Wang et al. 2017
C. hobsonii	K(M) 167650 [d]	UK 2010	MN856169	Consiglio and Setti 2019
C. hobsonii (Epitype)	K(M) 122842 [d]	UK 2004	MN856170	Consiglio and Setti 2019
C. hobsonii	K(M) 199928 [d]	UK 2015	MN856171	Consiglio and Setti 2019
"*C. hobsonii*"	DLL 9586	Queensland, Australia 2009	KC816912	Kluting et al. 2014
"*C. hobsonii*"	DLL 9635	Queensland, Australia 2009	KC816913	Kluting et al. 2014
"*C. hobsonii*"	DLL 9643	Queensland, Australia 2009	KC816914	Kluting et al. 2014
"*C. hobsonii*"	DLL 9746	Queensland, Australia 2010	KC816915	Kluting et al. 2014
"*C. hobsonii*"	DLL 9779	Queensland, Australia 2010	KC816916	Kluting et al. 2014
"*C. hobsonii*"	5967 TJB [c]	New York, USA 1988	KC816917	Kluting et al. 2014
C. aff. *hobsonii*	7051 TJB [c]	North Carolina, USA 1993	KC816918	Kluting et al. 2014
C. aff. *hobsonii*	UC 1860830 [g]	California, USA 2011	KC816928	Kluting et al. 2014
C. aff. *hobsonii*	K:M 195388 [d]	UK 2014	MN856172	–
C. lampangensis (Holotype)	SDBR-CMUJK 0147 [h]	Lampang, Thailand 2018	MK784129	Kumla et al. 2019
C. lampangensis	SDBR-CMUNK 0047 [h]	Lampang, Thailand 2018	MK784128	Kumla et al. 2019
C. passeckerianus	K:M 134571 [d]	UK 2005	MN856173	–
C. prunulus (Epitype)	KUN-HKAS 96158 [a]	Austria 2016	MN148129	Jian et al. 2020a
C. prunulus	HMJAU 4521 [i]	Kirov, Russia 2006	MN148117	Jian et al. 2020a
"*C. prunulus*"	TB8229 [c]	New York, USA 1996	GU384650	Baroni et al. 2011
"*C. prunulus*"	TB9663 [c]	USA –	GU384648	Baroni et al. 2011
"*C. prunulus*"	6805 TJB [c]	New York, USA 1992	KC816924	Kluting et al. 2014
C. ravus (Epitype)	KUN-HKAS 107043 [a]	Yunnan, China 2019	MT349668	Jian et al. 2020b
C. reticulosporus (Holotype)	WU 27150 [j]	Vienna, Austria 2004	HM164416	Morgado et al. 2016
C. rugosiceps	KUN-HKAS 57003 [a]	Yunnan, China 2009	MN148131	Jian et al. 2020a
C. rugosiceps (Holotype)	KUN-HKAS 73232 [a]	Yunnan, China 2011	MN148132	Jian et al. 2020a
C. cf. *scyphoides*	KUN-HKAS 104511 [a]	Gansu, China 2016	MN148157	Jian et al. 2020a
C. sinoapalus	KUN-HKAS 102807 [a]	Yunnan, China 2017	MN148147	Jian et al. 2020a
C. sinoapalus (Holotype)	KUN-HKAS 101191 [a]	Yunnan, China 2017	MN148151	Jian et al. 2020a
Clitopilus sp.	7130 TJB [c]	New York, USA 1993	KC816929	Kluting et al. 2014
Clitopilus sp.	TB8024 [c]	Florida, USA 1996	GU384647	Baroni et al. 2011
Clitopilus sp.	TB8067 [c]	Florida, USA 1996	GU384649	Baroni et al. 2011
Clitopilus sp.	KUN-HKAS 104512 [a]	Yunnan, China 2018	MN148158	Jian et al. 2020a
Clitopilus sp.	QYL10	– –	MN092372	Peng et al. 2021
C. highlandensis	KUN-HKAS 68389 [a]	Yunnan, China 2010	MN148137	Jian et al. 2020a

Table 1 (continued)

Species	Collection or collector no.	Location and year	*RPB2* accession	References
***C. highlandensis* (Holotype)**	**KUN-HKAS 117632** [a]	**Yunnan, China 2021**	**OP006563**	**This study**
***C. subalbidus* (Holotype)**	**GDGM 72219** [k]	**Guangdong, China 2018**	**ON959185**	**This study**
C. subalbidus	**GDGM 72229** [k]	**Guangdong, China 2018**	**ON959186**	**This study**
C. subscyphoides	CAL 1326 [b]	Kerala State, India 2011	MF946581	Raj and Manimohan 2018
C. subscyphoides	**GDGM 72195** [k]	**Guangdong, China 2018**	**ON959188**	**This study**
***C. subscyphoides* (Epitype)**	**GDGM 73056** [k]	**Guangdong, China 2018**	**ON959187**	**This study**
C. umbilicatus (Holotype)	KUN-HKAS 80310 [a]	Anhui, China 2013	MN148153	Jian et al. 2020a
C. umbilicatus	KUN-HKAS 104509 [a]	Yunnan, China 2017	MN148156	Jian et al. 2020a
C. velutinus (Isotype)	CORT 014618 [c]	Dominican Republic 2015	MN893321	Baroni et al. 2020
C. venososulcatus	8111 TJB [c]	Louisiana, USA 1996	KC816930	Kluting et al. 2014
C. yunnanensis	KUN-HKAS 82076 [a]	Yunnan, China 2012	MN148134	Jian et al. 2020a
C. yunnanensis (Holotype)	KUN-HKAS 104518 [a]	Yunnan, China 2018	MN148136	Jian et al. 2020a
Clitocella mundula	**KUN-HKAS 107049** [a]	**Anhui, China 2019**	**ON959189**	**This study**
Clitocella colorata	BJTC FM1891 [l]	Shanxi, China 2021	OL989914	Mao et al. 2022

[a] The Cryptogamic Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China (KUN-HKAS)

[b] Central National Herbarium, Kolkata, India (CAL)

[c] State University of New York College at Cortland Herbarium, Cortland, New York, USA (CORT)

[d] The Royal Herbarium, Royal Botanic Garden, Kew, Richmond, Surrey, England, UK (KEW)

[e] Associazione Micologica Bresadola, Trento, Italy (AMB)

[f] The Herbarium of Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, Sichuan, China (SAAS)

[g] Jepson Herbarium, University of California, Berkeley, California, USA (JEPS)

[h] The Herbarium of the Sustainable Development of Biological Resources Laboratory, Chiang Mai University, Thailand (SDBR-CMU)

[i] The Herbarium of Mycology, Jilin Agricultural University, Changchun, Jilin, China (HMJAU)

[j] Herbarium, Institute of Botany, University of Vienna, Austria (WU)

[k] The Mycological Herbarium of the Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China (GDGM)

[l] Herbarium Biology Department, Capital Normal University (BJTC)

(Newly generated information of specimens and sequences are in bold)

(http://tree.bio.ed.ac.uk/software/tracer/) was used to determine chain convergence. The trees were summarised using the sump and sumt commands with a 25% burn-in, and statistical results were produced.

Results

Phylogenetic analyses

There was no topological inconsistency between ML and BI. Consequently, only the phylogenetic tree inferred from the ML strategy is shown, with the statistical results of both ML (Bootstrap Supports, BS) and BI (Posterior Probabilities, PP) shown on the branches (Fig. 1). GTR + I + G model was the best model for ML and BI analyses, and the *RPB2* sequence dataset comprised 722 characters including gaps (see Supp. Info.). In the phylogenetic tree, the specimens collected from Yunnan were clustered in a distinct clade within the *Clitopilus* sect. *Clitopilus* (Fr. ex Rabenh.) P. Kumm.; some specimens collected from Guangdong were located in the *Clitopilus* sect. *Crispi* S.P. Jian & Zhu L. Yang; furthermore, the residual specimens gathered from Guangdong were placed in the *Clitopilus* sect. *Scyphoides* Singer gathering with "*C. subscyphoides*" (MF946581).

Morphological observations and SEM

Besides the molecular phylogenetic analyses, the morphological characters provide more valuable information. The images of fresh basidiomata and habitats of the new species and *C. subscyphoides* are provided in Fig. 2. Characteristics of the basidiospore ornamentations of three species are shown in Fig. 3. The comparison of morphological characters among these new species and their similar species are shown at Table 2.

Taxonomy

Clitopilus highlandensis S.P. Jian, X.H. Wang & Zhu L. Yang sp. nov. Figs. 2a–b, 3a–b, 4a–c

MycoBank MB844797

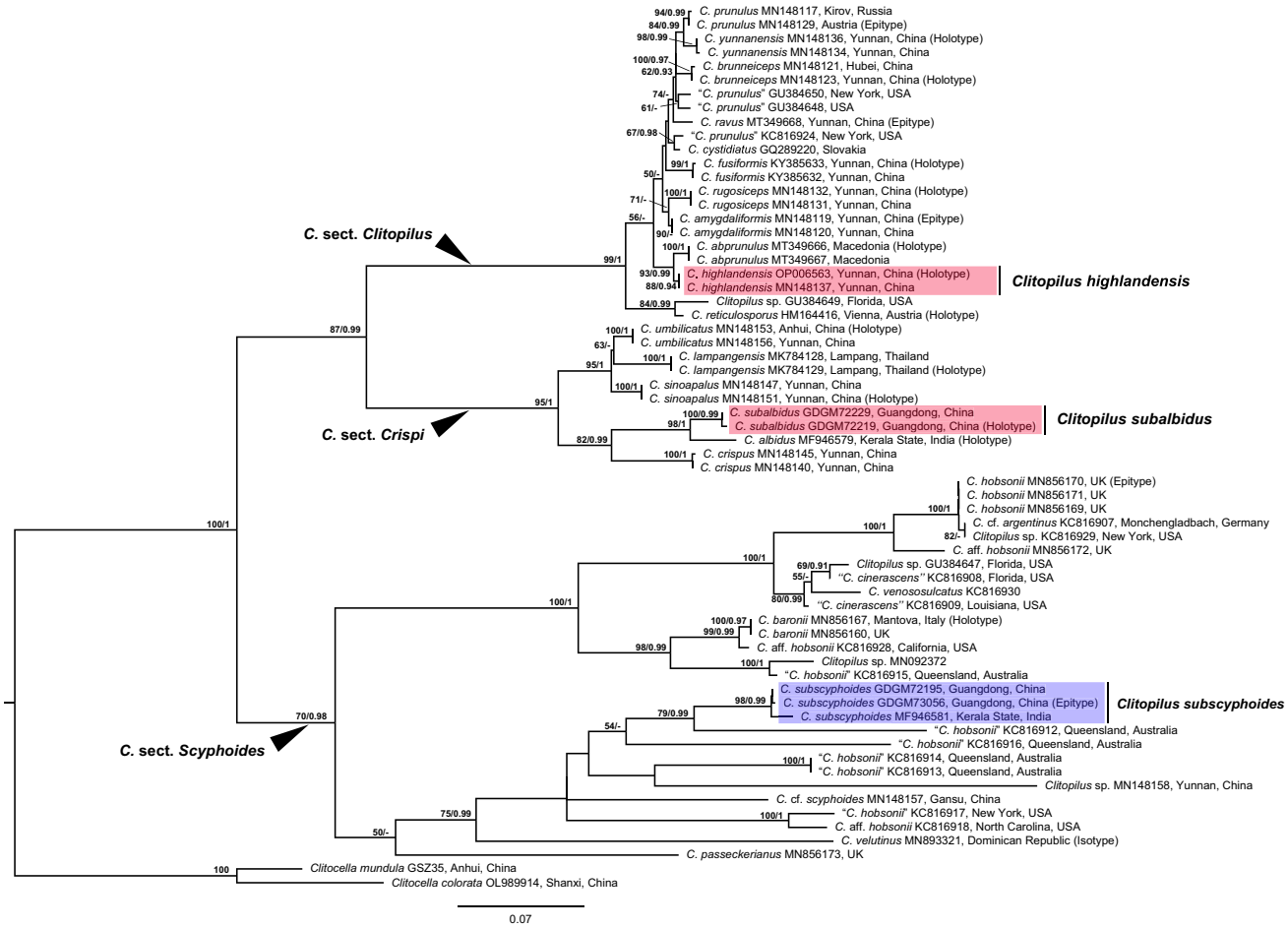

Fig. 1 Phylogenetic relationships among representative species of *Clitopilus* are inferred from *RPB2* dataset through both ML and BI methods (only show the ML tree). The supported branches show bootstrap supports (BS > 50%) and posterior probabilities (PP > 0.90). Sequences from type specimens (holotype, epitype or isotype) are marked. The red-covered portion means new taxa, and the blue-covered portion means the rediscovered species

Holotype: China, Yunnan Province, Lanping Bai and Pumi Autonomous County, Tongdian Town, Longtan Village, E 99°27′, N 26°36′, alt. 3039 m, single on soil, in mixed broadleaf-coniferous (*Quercus* and *Pinus* L.) forest, 17 August 2020, X.H. Wang 8007 (KUN-HKAS 117632). GenBank: ITS = ON999061; LSU = ON999062; *RPB2* = OP006563; *TEF1* = OP006564.

Etymology: "*highlandensis*" is proposed due to its occurrence on Yunnan Plateau.

Diagnosis: *Clitopilus highlandensis* is close to *C. abprunulus* but differs from the latter by its smaller size of basidiomata and larger basidiospores.

Description: Basidiomata clitocyboid, small to medium-size. Pileus 21–42 mm wide, convex; surface greyish (#807d84) to greyish yellow (#f1e2cb), minutely tomentose; margin slightly incurved, even; context about 3 mm thick, white (#ffffff). Lamellae decurrent, yellowish white (#e7d7b6) to pinkish (#e4c084), dense and crowded, edges entire and concolorous, lamellulae numerous. Stipe 10–25 × 2–6 mm, eccentric, subcylindrical or little twisty, whitish yellow or yellowish, smooth or minutely pruinose; the base slightly inflated, with white (#ffffff) mycelium. Odor none.

Basidiospores (8) 9–12.5 × (5) 5.5–7 (7.5) µm, $L_m \times W_m = 10.26 (\pm 0.93) \times 6.25 (\pm 0.47)$ µm, Q = 1.33–1.92 ($Q_{avg} = 1.64 \pm 0.14$) [60/3/2], pale yellow–brown, broadly fusiform, subovoid in profile and face view, strongly angled in polar view with 6–8 obvious longitudinal ridges. Basidia 30–45 × 8–12 µm, clavate, hyaline, 2- or 4-spored. Lamellar trama subregular, composed of two types of hyphae, one type cylindrical about 2–7 µm in diam., the other shorter and inflated 20–27 × 10–13 µm. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis composed of radially arranged regular hyphae, hyphae thin-walled, hyaline, smooth, cylindrical, 2–6 µm in diam.; pileal trama composed of hyaline, filamentous, and thin-walled hyphae, hyphae 2–5 (8) µm in diam. Stipitipellis a cutis composed of compactly arranged, regular, thin-walled, and

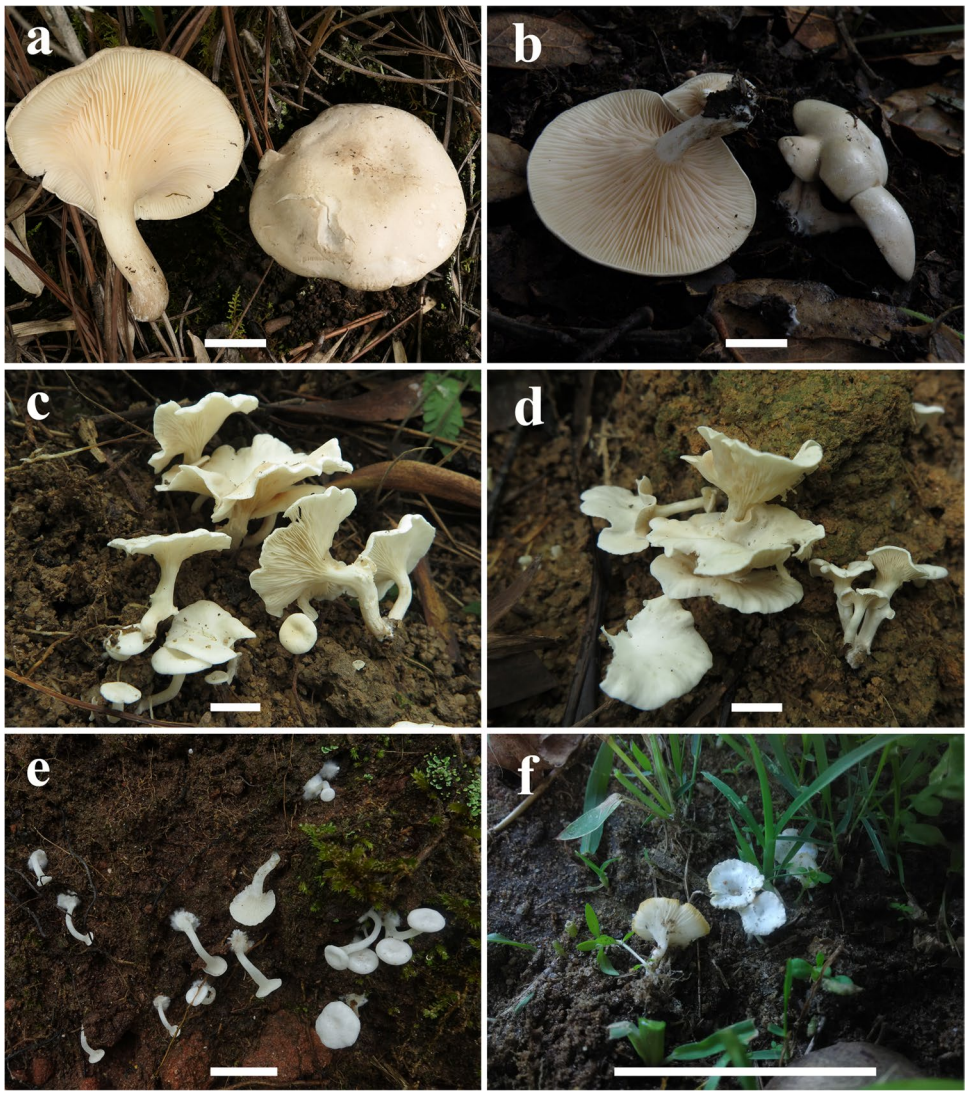

Fig. 2 Basidiomata of *Clitopilus*. **a, b**: *Clitopilus highlandensis* (a, KUN-HKAS 117632, holotype; b, KUN-HKAS 68389); **c, d**: *Clitopilus subalbidus* (c, GDGM 72219, holotype; d, GDGM 72229); **e, f**: *Clitopilus subscyphoides* (e, GDGM 73056, epitype; f, GDGM 72683). Bar = 10 mm

hyaline hyphae 3–6 µm in diam., terminal hyphae slightly cylindric or clavate, 20–36 × 4–6 µm. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Single, scattered or in groups on soil in mixed broadleaf-coniferous (*Quercus* and *Pinus*) forest, distributed in Yunnan Province, China, August.

Additional specimens examined: China, Yunnan Province, Lijiang City, Gucheng District, Jinshan Town, alt. 2600 m, scattered or in group on soil, in mixed broadleaf-coniferous (*Quercus* and *Pinus*) forest, 18 August 2010, X.T. Zhu 213 (KUN-HKAS 68389).

Notes: *Clitopilus highlandensis* belongs to *C.* sect. *Clitopilus* (Fig. 1). This new taxon is similar to *C. abprunulus*, *C. brunneiceps* S.P. Jian & Zhu L. Yang, *C. fusiformis*, *C. griseobrunneus* T.J. Baroni & Halling, *C. prunulus* (Scop.) P. Kumm. and *C. yunnanensis* S.P. Jian & Zhu L. Yang. *Clitopilus abprunulus* is found across Europe (UK, Switzerland and North Macedonia), which differs from *C. highlandensis* by its larger basidiomata (medium-sized to large), smaller basidiospores ($L_m \times W_m = 9.39 \times 6.34$ µm), and lower altitude (1000 m) (Jian et al. 2020b). *Clitopilus brunneiceps* has a wide distribution from southwest to northeast China, differing from *C. highlandensis* by its grey pileus, filamentous hyphae (4–10 µm in diam.) in lamellar trama and pigments in pileipellis (Jian et al. 2020a). *Clitopilus fusiformis* is a subalpine species, but grows at higher places (> 3400 m) with larger and fusiform basidiospores (10.5–14 × 5–7 µm) (Wang et al. 2017). The neotropical species *C. griseobrunneus* occurs in Costa Rica, characterized with medium to large-sized fruit body, brown pileus, larger and amygdaliform basidiospores (9.7–16.9 × 5.7–7.9 µm) (Baroni and Halling 2000). For *C. prunulus*, which is supposed to distribute in Europe and North America (Kummer 1871; Singer 1946), it is characterized by white and convex pileus, and fusiform basidiospores. The last species *C. yunnanensis* is also described

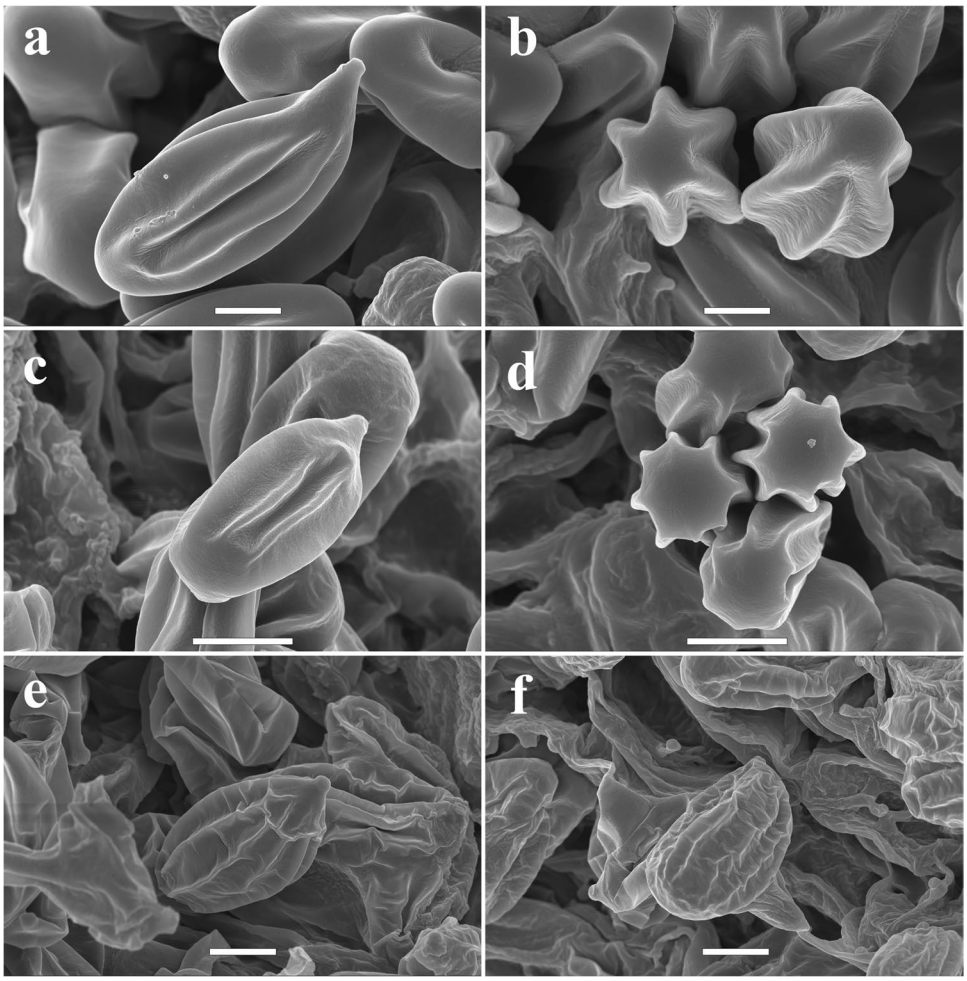

Fig. 3 Basidiospores of *Clitopilus* reveal by SEM. **a, b**: *Clitopilus highlandensis* (KUN-HKAS 68389); **c, d**: *Clitopilus subalbidus* (GDGM 72229); **e, f**: *Clitopilus subscyphoides* (e, GDGM 72683; f, GDGM 73056, epitype). Bar = 2 µm

from Yunnan province, but differs from *C. highlandensis* by its brown pileus with a central stipe, and gelatinized pileipellis (Jian et al. 2020a).

Clitopilus subalbidus S.P. Jian, W.Q. Deng & Zhu L. Yang sp. nov. Figs. 2c–d, 3c–d, 5a–c

MycoBank MB844783

Holotype: China, Guangdong Province, Guangzhou City, Tianhe District, South China Botanical Garden, Chinese Academy of Sciences, E 23°11′, N 113°21′, alt. 31 m, scattered or in group on soil, in evergreen broad-leaved forest, 15 June 2018, Ming Zhang & W.Q. Deng (GDGM 72219). GenBank: ITS = ON963951; LSU = ON963945; *RPB2* = ON959185; *TEF1* = ON959190; *ATP6* = ON959179.

Etymology: "sub-" (Latin) means "near", referring to its morphological and phylogenic similarity to *C. albidus*.

Diagnosis: *Clitopilus subalbidus* is close to *C. albidus* but differs from the latter by its inflated hyphae in lamellar trama and larger basidiospores.

Description: Basidiomata omphaloid to clitocyboid, small to medium-size. Pileus 7–42 mm wide, concave; surface white (#ffffff) to dirty white (#e0e4d2), usually minutely tomentose or smooth; margin slightly incurved when young, then straight, even or undulate; context about 1 mm thick, whitish (#fcfcfc) to white (#ffffff). Lamellae decurrent, white (#ffffff) when young, then pinkish (#ffc0cb) or carnation (#ff748c), dense and crowded, edges entire and concolorous, lamellulae numerous. Stipe 10–21 × 4–5 mm, eccentric, subcylindrical, usually concolorous with pileus, smooth or minutely pruinose; the base slightly inflated, with white (#ffffff) mycelium. Odor none. Taste a little bitter.

Basidiospores 4.6–5.5 (6) × 4–5 µm, $L_m \times W_m = 5.10 (\pm 0.24) \times 4.33 (\pm 0.32)$ µm, Q = (1.00) 1.04–1.30 (1.37) ($Q_{avg} = 1.18 \pm 0.08$) [44/2/2], hyaline, broadly ellipsoid, subglobose to globose, subovoid to ellipsoid in profile and face view, slightly angled in polar view with 8–9 inconspicuous or obscure longitudinal ridges. Basidia 18–22 × 6–8 µm, clavate, hyaline, 4-spored; sterigmata up to 3 µm. Lamellar trama subregular, composed of two types of hyphae, one is cylindrical and interwoven 3–6 µm in diam., the other is shorter and inflated hyphae, 15–20 × 10–18 µm; oleiferous hyphae rare. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis composed of more or less radially arranged regular hyphae, hyphae thin-walled,

Table 2 The comparison of morphological characters among *C. highlandensis*, *C. subalbidus*, *C. subscyphoides* and similar species

Taxa	Pileus	Stipe	Basidiospores	Ridges	Lamellae trama	Locality	References
Clitopilus sect. *Clitopilus*							
C. abprunulus (Holotype)	Greyish to grey, 35–90 mm	White, 50–70 × 5–15 mm	Broadly fusiform, 8.5–11 × 5.5–8 µm (Q_{avg} = 1.49)	6–7	Including inflated cells, 10–40 × 10–30 µm	North Macedonia	Jian et al. 2020b
C. brunneiceps (Holotype)	Grey to brownish grey, 30–45 mm	Yellowish white, 20–30 × 5–6 mm	Broadly fusiform, 8–12 × 5–7 µm (Q_{avg} = 1.63)	5–6	More or less regular	China	Jian et al. 2020a
C. fusiformis (Holotype)	Pale greyish, 25–45 mm	Pale greyish, 22–40 × 2.5–3.5 mm	Fusiform, 10.5–14 × 5–7 µm (Q_{avg} = 2)	5–6	Interwoven	China	Wang et al. 2017
C. griseobrunneus (Holotype)	Brown, 30–70 mm	Greyish to white, 15–40 × 5–10 mm	Amygdaliform, 9.7–16.9 × 5.7–7.9 µm (Q_{avg} = 1.91)	6	Regular	Costa Rica	Baroni and Halling 2000
***C. hihglandensis* (Holotype)**	**Greyish yellow, 21–42 mm**	**Yellowish, 22–40 × 2.5–3.5 mm**	**Broadly fusiform, 9–12.5 × 5.5–7 µm (Q_{avg} = 1.64)**	**6–8**	**Including inflated cells, 20–27 × 10–13 µm**	**China**	**This study**
C. prunulus (Epitype)	White, 25–70 mm	White, 10–25 × 5–10 mm	Fusiform, 10–13 × 5–7 µm (Q_{avg} = 1.94)	5–7	More or less regular	Austria	Jian et al. 2020a
C. yunnanensis (Epitype)	Yellowish brown, 30–50 mm	Greyish, 20–55 × 4–14 mm	Subovoid, 7–11 × 5–8 µm (Q_{avg} = 1.45)	5–7	Regular	China	Jian et al. 2020a
Clitopilus sect. *Crispi*							
C. albidus (Holotype)	White, 7–34 mm	White, 11–21 × 1–4 mm	Subglobose to ellipsoid, 4–5.5 × 3–4.5 µm (Q_{avg} = 1.26)	7–8	Subregular	India	Raj and Manimohan 2018
C. apalus (Holotype)	Chalk white, 10–50 mm	White, 15–45 × 3–15 mm	Ovoid to short ellipsoid, 6–8.5 × 4.5–5.5 µm (Q_{avg} = 1.4)	9–11	More or less regular	Sri Lanka	Pegler et al. 1977
C. chalybescens (Holotype)	White with bluish stain, 15–45 mm	White, 20–50 × 2–4 mm	Ellipsoid, 5.5–7.5 × 3.6–4.8 µm (Q_{avg} = 1.5)	8–10	Regular or interwoven	Thailand	Baroni et al. 2001
C. crispus (Holotype)	White with fine ridges, 30–50 mm	White, 30–50 × 4–6 mm	Ellipsoid, 6.2–7.6 × 4–5.3 µm (Q_{avg} = 1.46)	9–12	Regular	Vietnam	Baroni and Watling 1999
C. orientalis (Holotype)	White to chalk white, 12–40 mm	White, 15–30 × 3–8 mm	Ellipsoid to short-ellipsoid, 6–9 × 3.8–5.5 µm (Q_{avg} = 1.65)	8–11	Regular	Malaysia	Baroni and Watling 1999
C. sinoapalus (Holotype)	White to whitish yellow, 5–60 mm	White, 15–40 × 2–5 mm	Globose to subglobose, 4–6 × 3.5–5 µm (Q_{avg} = 1.16)	8–10	More or less regular	China	Jian et al. 2020a
***C. subalbidus* (Holotype)**	**White to dirty white, 7–42 mm**	**White, 10–21 × 4–5 mm**	**Broadly ellipsoid, 4.6–5.5 × 4–5 µm (Q_{avg} = 1.18)**	**8–9**	**Including inflated cells, 15–20 × 10–18 µm**	**China**	**This study**
Clitopilus sect. *Scyphoides*							
C. peri (Holotype)	Pure white, 8–22 mm	White, 10–20 × 1–2 mm	Ellipsoid, 6.7–8.5 × 3–4 µm (Q_{avg} = 2.14)	6–9	Regular	Sri Lanka	Pegler et al. 1977
C. scyphoides (Holotype)	White, 5–25 mm	White, 4–10×0.5–1.8 mm	Ellipsoid to oblong, 7.5–11.5 × 4–5.5 µm	8	Interwoven	Europe	Singer 1946
C. scyphoides (Neotype)	White, 5–25 mm	White, 5–20 × 0.5–2 mm	Ellipsoid, 6.5–8.5 × 3.5–5 µm	6–9	–	Sweden	Noordeloos 1984
C. subscyphoides (Holotype)	Chalk white, 7–10 mm	White, 10–14×1.4–1.6 mm	Ellipsoid, 5.2–7.4×3.9–5.0 µm (Q_{avg} = 1.47)	8–10	More or less regular	China	Deng et al. 2013b
C. subscyphoides	White, 3–6 mm	White, 4–9 × 1–1.5 mm	Ellipsoid, 5–7 × 3.5–4.5 µm (Q_{avg} = 1.48)	8–9	Subregular	India	Raj and Manimohan 2018
***C. subscyphoides* (Epitype)**	**Chalk white, 3–10 mm**	**White, 8–13 × 1–2 mm**	**Ellipsoid, 6–8 × 4–5.0 µm (Q_{avg} = 1.46)**	**8–10**	**More or less regular**	**China**	**This study**

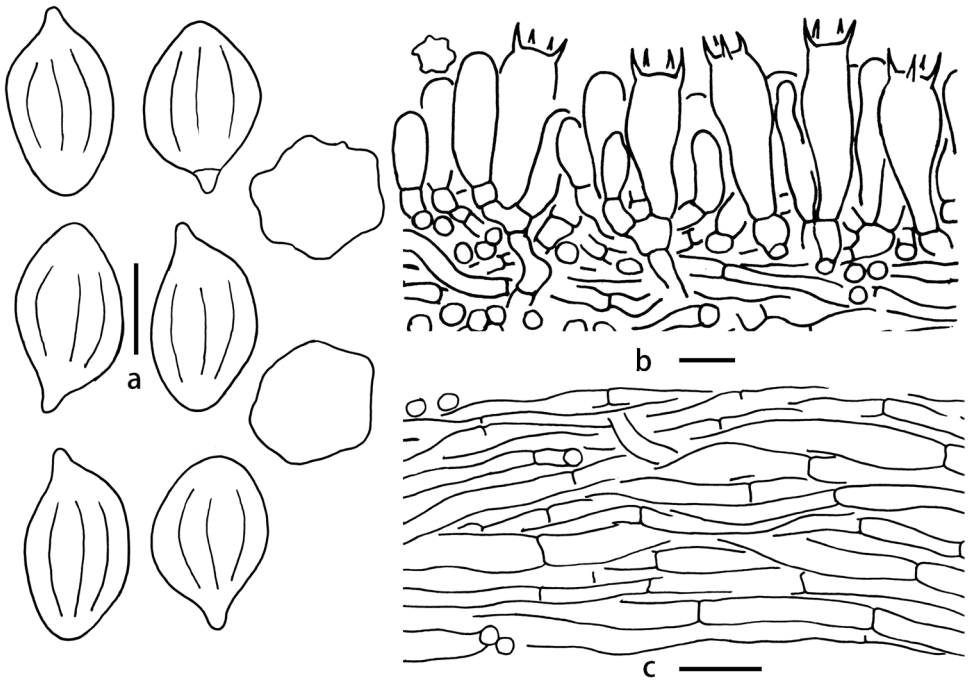

Fig. 4 Microscopic features of *Clitopilus highlandensis* (KUNHKAS 117632, holotype). **a** Basidiospores; **b** Hymenium and subhymenium; **c** Lamellar trama. Bars: **a** = 5 µm, **b** = 10 µm, **c** = 20 µm. Drew by XH Wang

hyaline, smooth, cylindrical, 4–6 µm in diam., with some oleiferous hyphae; the subpellis non-obvious separated; pileal trama composed of hyaline, oblong-ovoid or sausage-shape, inflated hyphae, hyphae 7–12 µm in diam., oleiferous hyphae present. Stipitipellis a cutis composed of compactly arranged, regular, thin-walled, and hyaline hyphae 3–6 µm in diam. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Scattered or in groups on soil in evergreen broad-leaved forest, distributed in Guangdong Province, China, June.

Additional specimens examined: China, Guangdong Province, Guangzhou City, Tianhe District, South China Botanical Garden, Chinese Academy of Sciences, E 113°21′, N 23°11′, alt. 31 m, scattered or in group on soil, in evergreen broad-leaved forest, 15 June 2018, Ming Zhang (GDGM 72229).

Notes: *Clitopilus subalbidus* belongs to *C.* sect. *Crispi* (Fig. 1). This species is similar to *C. albidus*, *C. apalus* (Berk. & Broome) Petch, *C. chalybescens* T.J. Baroni & Desjardin, *C. crispus* Pat., *C. orientalis* T.J. Baroni & Watling, and *C. sinoapalus* S.P. Jian & Zhu L. Yang. *Clitopilus albidus*, found at Kerala State (India), has slightly gelatinized hyphae in pileipellis, without inflated cells in lamellar trama, and smaller basidiospores (4–5.5 × 3–4.5 µm) (Raj and Manimohan 2018). *Clitopilus apalus*, discovered at Sri Lanka, is characterized by the solid and longer stipe (15–45 × 3–15 mm), and larger basidiospores (6–8.5 × 4.5–5.5 µm) with 9–11 longitudinal ridges (Pegler 1977). *Clitopilus chalybescens*, firstly depicted at Thailand, has infundibuliform, scissile and white with pale greyish blue staining pileus, longer and slender stipe (20–50 × 2–4 mm), and larger basidiospores (5.5–7.5 × 3.6–4.8 µm) (Baroni et al. 2001). The tropical species *C. crispus*, originally described at Vietnam, differs by the fine ridges on the pileus margin, and larger basidiospores (6.2–7.6 × 4–5.3 µm) with 9–12 longitudinal ridges (Patouillard 1913; Baroni and Watling 1999). *Clitopilus orientalis*, distributed at Malaysia and India, is distinct from *C. subalbidus* by its radiately ridged pileus, ellipsoid or short-ellipsoid and larger basidiospores (6–9 × 3.8–5.5 µm) (Baroni and Watling 1999; Raj and Manimohan 2018). *Clitopilus sinoapalus*, known from South China, differs from *C. subalbidus* by its white to yellowish pileus, narrower hyphae (4–12 µm in diam.) in lamellar trama and by its presence at higher elevations (> 1000 m) (Jian et al. 2020a).

Clitopilus subscyphoides W.Q. Deng, T.H. Li & Y.H. Shen Figs. 2e–f, 3e–f, 6a–b

MycoBank MBT10007955

Epitype: China, Guangdong Province, Shaoguan City, Renhua County, Danxia Mountain, E 113°43′, N 25°2′, alt. 281 m, scattered or in group, on soil, in laurisilvae forest, 9 June 2018, X.R. Zhong (GDGM 73056, here designated). GenBank: ITS = ON963954; LSU = ON963948; *RPB2* = ON959187; *TEF1* = ON959191; *ATP6* = ON959182.

Description: Basidiomata omphaloid, small. Pileus 3–10 mm wide, applanate to concave; surface chalk white (#ffffff), usually smooth; margin incurved when young, then straight, even or undulate, sometimes striate; context less than 1 mm thick, chalk white (#ffffff). Lamellae

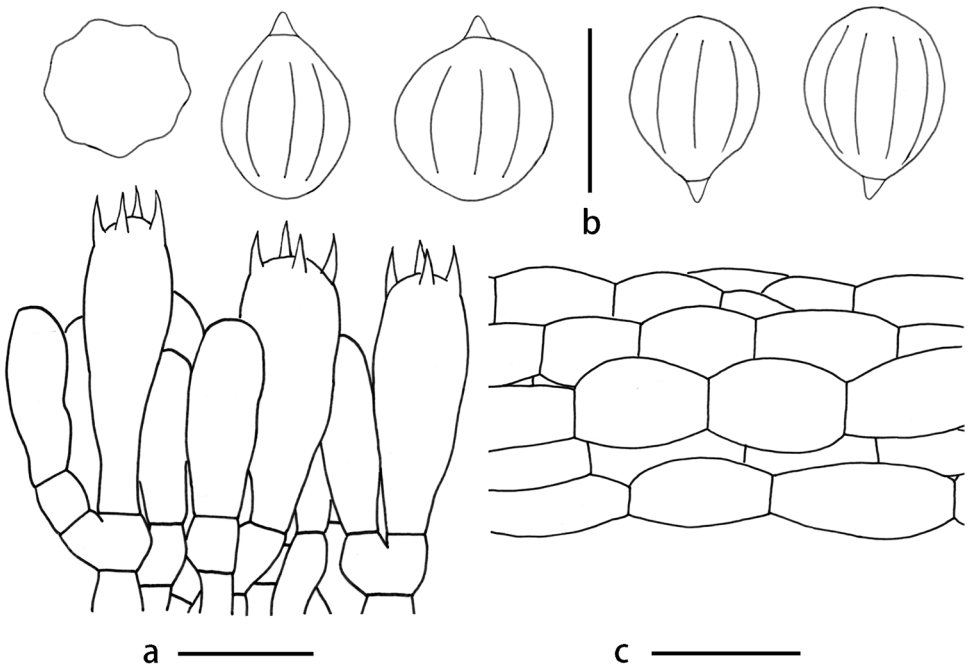

Fig. 5 Microscopic features of *Clitopilus subalbidus* (GDGM 72219, holotype). **a** Hymenium and subhymenium; **b** Basidiospores; **c** Lamellar trama. Bars: **a** = 10 µm, **b** = 5 µm, **c** = 20 µm. Drew by SP Jian

subdecurrent to decurrent, white (#ffffff) to very light gray (#ebebeb), close, edges entire, concolorous with lamellae, or occasionally very pale yellow (#ffffeb), lamellulae numerous. Stipe 8–13 × 1–2 mm, central, cylindrical, usually concolorous with pileus, finely pruinose all over; the base slightly inflated, with white (#ffffff) mycelium. Odor none.

Basidiospores 6–8 × 4–5 µm, $L_m \times W_m = 6.68 (\pm 0.48) \times 4.58 (\pm 0.33)$ µm, Q = 1.28–1.67 (1.76) ($Q_{avg} = 1.46 \pm 0.11$) [60/3/3], hyaline, broadly ellipsoid in profile and face view, slightly angled in polar view with 8–10 inconspicuous facets produced by obscure longitudinal ridges, also with minute transverse folds under SEM. Basidia 16–25 × 6–9 µm, clavate, hyaline, 4-spored; sterigmata up to 4 µm long. Lamellar trama regular, composed of 3–7 µm in diam., thin-walled, hyaline hyphae, oleiferous hyphae also present. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis composed of radially arranged subregular hyphae, hyphae thin-walled, hyaline, smooth, cylindrical, 3–6 µm in diam., with some oleiferous hyphae; pileal trama regular, composed of hyaline, thin-walled, cylindrical hyphae, hyphae 4–7 µm in diam. Stipitipellis a cutis composed of compactly arranged, regular, thin-walled, and hyaline hyphae 3–5 µm in diam., sometimes crossed with some erect cylindrical end cells; Stipe trama regular, with thin-walled, and hyaline hyphae 4–6 µm in diam. Caulocystidia absent. Clamp connections absent.

Ecology and distribution: Scattered or in groups on soil in broad-leaved forest, distributed from Southeast China to India, May to June.

Additional specimens examined: China, Guangdong Province, Yangchun City, Ehuangzhang Nature Reserve, alt. 200 m, scattered on soil in laurisilvae forest, 18

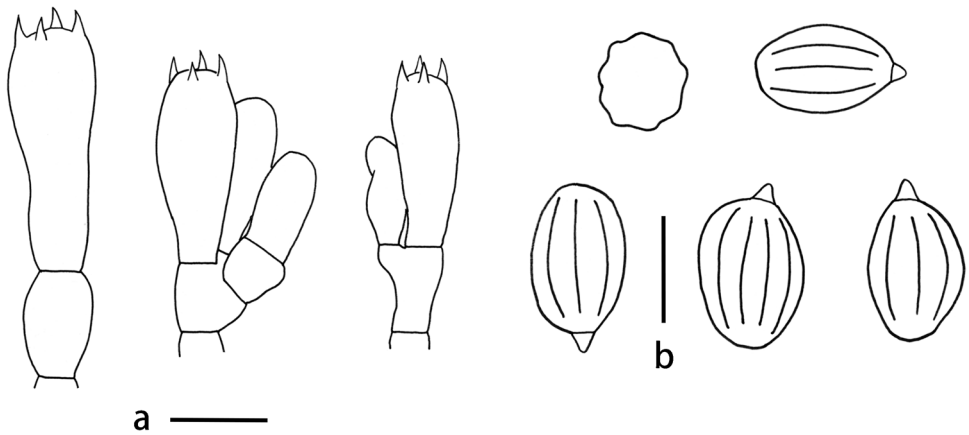

Fig. 6 Microscopic features of *Clitopilus subscyphoides* (GDGM 73056, epitype). **a** Hymenium and subhymenium; **b** Basidiospores. Bars: **a** = 10 µm, **b** = 5 µm. Drew by SP Jian

August 2004, T.H. Li & L.M. Wu (GDGM 24141, holotype); Shaoguan City, Renhua County, Danxia Mountain, E 113°43′, N 25°2′, alt. 281 m, scattered or in group on soil, in laurisilvae forest, 8 June 2018, C.Q. Wang (GDGM 72195); Zhanjiang City, Nansan Island, Aimin Park, E 110°46′, N 21°17′, alt. 21 m, scattered or in group on soil, in laurisilvae forest, 15 May 2018, Ting Li, Hao Huang & Yong He (GDGM 72683).

Notes: *Clitopilus subscyphoides* belongs to *C.* sect. *Scyphoides* due to the small omphaloid fruiting body, and transverse folds on basidiospores (Figs. 1, 3e–f). It is characterized by its small, white, omphaloid basidiomata, concave pileus with a central stipe, absence of hymenial cystidia, and broadly ellipsoid basidiospores with 8–10 obscure longitudinal ridges and transverse folds.

We have carefully studied the holotype of *C. subscyphoides* (GDGM 24141), but this specimen was in a poor condition and only the immature basidiospores connected with basidia were observed. The efforts to obtain the sequences of holotype failed. The newly collected samples have the key characters of this species given in the original description (Deng et al. 2013b). Moreover, the sequences from our samples coincided with sequences of the new record of *C. subscyphoides* from India (Raj and Manimohan 2018). In order to elaborate the concept of *C. subscyphoides* consistently, the specimen (GDGM 73056) is designated here as an epitype of *C. subscyphoides* with new DNA nucleotide sequences and a more complete morphological description presented.

This species is similar to *C. peri* (Berk. & Broome) Petch and *C. scyphoides* (Fr.) Singer. *Clitopilus peri*, originally described at Sri Lanka, differs from *C. subscyphoides* by its higer Q_{avg} (2.14) and fewer longitudinal ridges (6–9) (Pegler 1977). Besides, *C. peri* is often gregarious on rotting leaves, while *C. subscyphoides* occurs only on soil (Deng et al. 2013b). The temperate species *C. scyphoides* usually has higher Q value, fewer lamellae (Noordeloos 1984, 1988) and occurs in Europe (Singer 1986).

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Author contribution Si-Peng Jian conceived, designed, and complete the experiments under the guidance of Zhu L. Yang. Si-Peng Jian and Xiang-Hua Wang made the line drawings. Si-Peng Jian wrote the manuscript, and Wang-Qiu Deng, Xiang-Hua Wang, and Zhu-Liang Yang revised it.

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Data availability In this study, DNA sequences have been deposited in GenBank. Specimens were placed at Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS) and the Fungarium of the Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China (GDGM).

Declarations

Conflict of interest The authors declare no competing interests.

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