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# Morphological and molecular evidence for a new species of *Psilocybe* from southern China

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Abstract—*Psilocybe cinnamomea* sp. nov. is reported from China. It is characterized by its campanulate to plano-convex pileus, ellipsoid spores with a distinctly broad germ pore, clavate basidia, polymorphic cheilocystidia, and clavate to fusiform pleurocystidia with a short unbranched neck. Phylogenetic relationships among the new species and allied species in the genus were inferred from the internal transcribed spacer (ITS) region.

Key words—*Agaricales, Strophariaceae,* taxonomy, phylogeny

## Introduction

*Psilocybe* (Fr.) P. Kumm. was formerly a broadly circumscribed genus, including bluing, hallucinogenic species and non-bluing, non-hallucinogenic species (Guzmán 1983, Allen et al. 1992, Stamets 1996, Guzmán 2005, Borovička et al. 2011, Guzmán 2012). Recently, this broad polyphyletic genus was divided: *Psilocybe* sensu stricto (with *P. semilanceata* as its conserved type) was restricted to the bluing species, while the non-bluing species were assigned to *Deconica* (W.G. Sm.) P. Karst. (Redhead et al. 2007, Norvell 2010).

*Psilocybe* s. s. contains more than 150 species worldwide (Guzmán 2005, 2009). However, only 11 species have been reported from China, of which only three were originally described from the country (Bau & Sarentoya 2009, Guzmán & Zhu L. Yang 2010, Ma et al. 2014). In the present study, a new species of *Psilocybe* is described from southern China. In order to confirm the phylogenetic uniqueness of this species, sequences of its internal transcribed spacer (ITS) were generated and compared with sequences of other *Psilocybe* species.

## Material & methods

Field collections were conducted in 2010. Notes and photographs were taken on macro-morphological features, and specimens were dried using a Dörrex dehydrator at 50°C. Specimens were deposited in the Research Institute of Tropical Forestry (RITF),

Chinese Academy of Forestry. Macroscopic descriptions were based on the photos and notes made in the field. Descriptive terminology follows Vellinga (1988), and colours are designated according to Kornerup & Wanscher (1981).

For microscopic observations, sections of specimens were cut by hand and mounted in 5% aqueous KOH solution or 1% aqueous Congo red solution. Basidia, basidiospores, cheilocystidia, pleurocystidia, caulocystidia, and pileocystidia were measured using the MShot Digital Imaging System (n=25 for each character). The abbreviation [n/m/p] indicates that measurements were made on n basidiospores in m basidiomata from p collections. Basidiospore dimensions follow the form (a–)b–c(–d) with b–c representing 95% of the measured values and extreme values shown in parentheses.

DNA was extracted from herbarium materials (Zhou & Liang 2011). The internal transcribed spacer (ITS) regions were amplified by polymerase chain reaction (PCR) with the primers ITS1 and ITS4 (White et al. 1990). Both strands were sequenced with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Beijing AuGCT DNA-SYN Biotechnology Co., Ltd, Beijing). The newly generated sequences were submitted to GenBank (accession numbers KJ433483 and KJ433484). DNA sequences were edited and aligned using SeqMan (DNA STAR Package) and Clustal X (Thompson et al. 1997) and manually checked and adjusted. Ambiguous positions were excluded from the matrix. The dataset was analyzed with the RAxML BlackBox online server (Stamatakis et al. 2008) for the maximum likelihood and MrBayes 3.1 for bayesian inference (Huelsenbeck & Ronquist 2005).

## Taxonomy

***Psilocybe cinnamomea*** J.F. Liang, Yang K. Li & Ye Yuan, **sp. nov.** Fig. 1

MycoBank 807941

Differs from *Psilocybe zapotecorum* by its smaller basidiomata, its greenish white to pale green lamellae, and its smaller basidia.

Type: China, Guangdong Province, Lechang County, Yangdongshan Shierdushui Nature Reserve, 4 October 2010, Wang 189 (**Holotype**, RITF 746; GenBank KJ433484).

Etymology: The Latin word "*cinnamomea*" refers to the cinnamon-like color of young basidiomata.

Basidiomata small. Pileus 20–40 mm in diameter, campanulate when young, expanding to subconvex or plano-convex, slightly papillate at the apex, glabrous, hygrophanous, cinnamon (5B3–5C6) when young, surface pale (2A2) to cream (4A2–4), light orange (5A4–5B7) at the disk when mature, yellowish brown (5C4–8) in dry specimens, blackish blue with age or when bruising, margin with white floccose remnants from the veil. Lamellae subadnate or sinuate, crowded, unequal, greenish white to pale green (28A2–3). Stipe 20–80 × 2–5 mm, uniform or slightly thickened at the base, somewhat flexuous, hollow, whitish to pale green (5B3) at the upper, grayish orange (5B3–5) to brownish yellow (5C6–8) at the lower part, covered with milk white (1A2) fibrillose, bluing, blackish when dried. Context thin, whitish. Odor or Taste not recorded. Annulus absent.

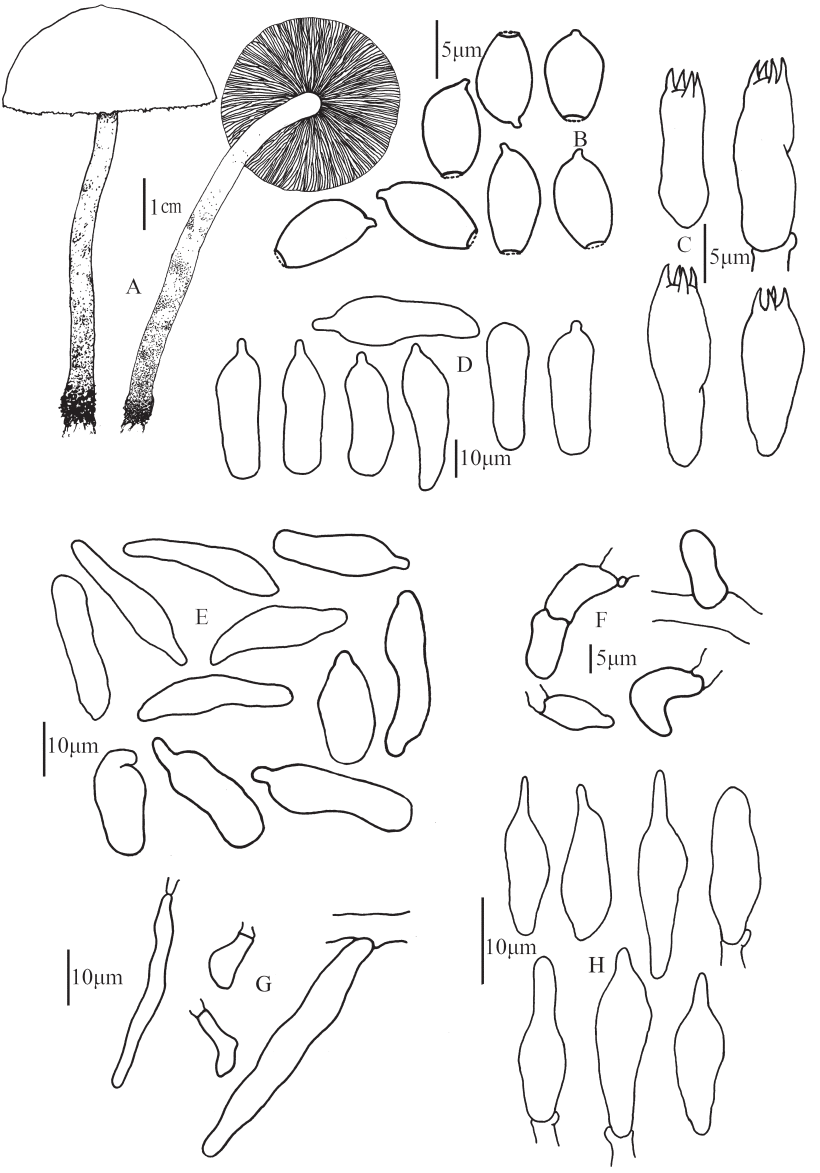


Fig. 1 *Psilocybe cinnamomea.* A. Basidiomata. B. Basidiospores. C. Basidia. D&H. Cheilocystidia. E. Pleurocystidia. F. Pileipellis. G. Caulocystidia. (A–G from holotype, RITF 746; H from RITF 726).

Basidiospores (5.5–)6.5–7.5(–8) × (3–)4–4.5(–5) μm, ellipsoid to subovoid in face view, subellipsoid in side view, sometimes subventricose, smooth, thin-walled, wall about 0.3–0.5 μm thick, with a distinctly broad germ pore and a short apical appendage, yellowish-brown pigment in KOH. Basidia 7–13.5 × 3.0–6.0 μm, 4-spored, rarely 2-spored, hyaline, clavate, sometimes with a median constriction. Cheilocystidia common, (17.5–)20–30 × 6.5–10 μm, unbranched, polymorphic (subcylindrical, clavate-mucronate, utriform, lageniform, fusiform), frequently with a median constriction and with a short or long neck, hyaline in 5% KOH. Pleurocystidia abundant, 17.5–32 × 5.5–10.0 μm, clavate, narrowly fusiform to fusiform, frequently with a short neck, the neck ≤5 μm long, unbranched, hyaline, walls smooth. Pileipellis a subcutis, not subgelatinized, 9–13 μm thick, hyaline, hyphae 3–5 μm diam., rarely with surface subcylindric or fusiform cystidioid elements 7.5–17.5 × 4.5–7.5 μm. Caulocystidia rare, 9.5–85 × 5–11 μm, variable in shape (subcylindrical or attenuate). Clamp connections common.

Additional specimen examined: **CHINA**: Guangdong Province, Lechang County, Yangdongshan Shierdushui Nature Reserve, 4 October 2010, Wang 234 (RITF 726; GenBank KJ433483).

Habitat & Distribution – Gregarious to caespitose on rotten wood or nutrient rich soils in a subtropical evergreen broad-leaved forest. Known only from the type locality.

### Phylogenetic analyses

A dataset of ITS sequences with 700 nucleotide sites was analyzed for 42 taxa (including 41 from GenBank). The dataset contained available sequences of *Psilocybe* species; *Stropharia rugosoannulata* Farl. ex Murrill and *S. hornemannii* (Fr.) S. Lundell & Nannf. were chosen as outgroup.

Bayesian and RAxML phylogenetic analyses (Fig. 2) clustered together two *P. cinnamomea* sequences on a well-supported branch (bootstrap = 100%; posterior probability = 1.00) and placed *P. cinnamomea* with *P. antioquiensis* Guzmán et al., *P. zapotecoantillarum* Guzmán et al., *P. zapotecorum* R. Heim emend., *P. thaizapoteca* Guzmán et al., and *P. argentipes* K. Yokoy. in the same subclade /zapotecorum (bootstrap = 87%; posterior probability = 1.00).

### Discussion

*Psilocybe cinnamomea* is easily recognized by its campanulate to plano-convex pileus, ellipsoid spores with a distinctly broad germ pore, clavate basidia, polymorphic cheilocystidia, and clavate to fusiform pleurocystidia with a short unbranched neck. Its ellipsoid thin-walled spores and bluing discoloration suggest placement of *P. cinnamomea* in *P.* sect. *Zapotecorum* (Guzmán 1983).

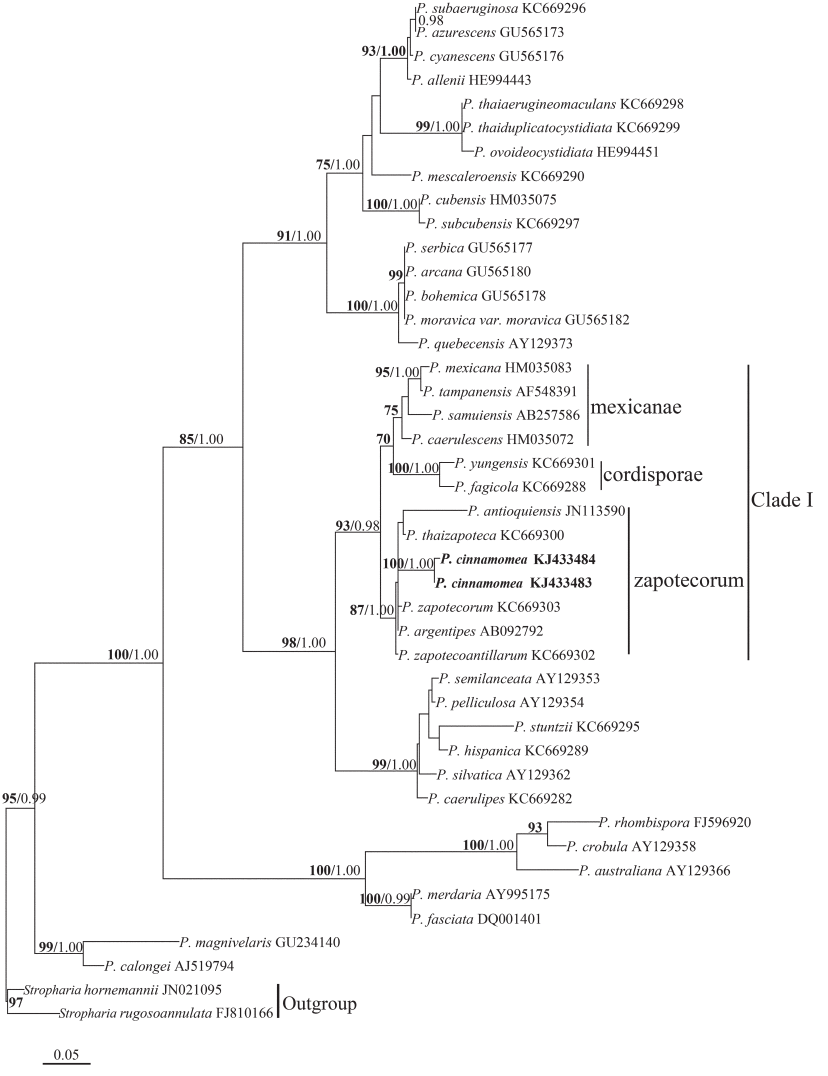


Fig. 2 One of 100 RAxML likelihood trees (–In L 5242.090489) based on the *Psilocybe* ITS dataset. Support values in bold type are RAxML likelihood bootstrap (≥70%). Values in normal type are Bayesian posterior probabilities (≥0.95).

Observations of both collections suggest that *P. cinnamomea* shows considerable variation in shape and size of cheilocystidia. In young specimens (Fig. 1H), the cheilocystidia are utriform to lageniform (17.5–22.5 × 3.5–5.5 μm) with a long neck (≤6.5 μm long), while those in mature specimens (Fig. 1D) are subcylindrical to clavate-mucronate with a short neck. Although there are differences between young and mature specimens, they are confirmed as representing the same species based on their same habitat and an ITS sequence similarity between young and mature specimens of 99%.

Bayesian and RAxML phylogenetic analyses (Fig. 2) supported *P. cinnamomea* in Clade I, representing hallucinogenic and mainly tropical and subtropical taxa (Guzmán et al. 2012) that were divided into three subclades (/cordisporae, /mexicanae, /zapotecorum) that grouped together with 93% bootstrap support and 0.98 posterior probability. Within /zapotecorum, *P. antioquiensis*, which has slightly thick-walled and angular spores, has been placed in *P.* sect. *Mexicanae* (Guzmán et al. 1994), while the other species, which share ellipsoid, thin-walled, non-angular spores, have been assigned to *P.* sect. *Zapotecorum* (Guzmán et al. 2003, 2012).

As *P. cinnamomea* bruises or dries blackish blue, it might also be hallucinogenic.

Closely related to *P. cinnamomea* are *P. subcaerulipes* Hongo (from Japan), which differs by its distinctly subumbonate brown to orangish brown pileus, gray-brown to dark violaceous brown lamellae, longer basidia (15–25 × 4.5–6 μm), and irregularly branched pleurocystidia (Guzmán et al. 2013); *P. zapotecorum* (from Mexico), which has larger basidiomata, whitish-brown to dark violaceous lamellae, and larger basidia (Guzmán 2012); and *P. zapotecoantillarum* (from Puerto Rico), which is easily distinguished by light brown to dark reddish-brown adnexed lamellae, longer basidia, and smaller ventricose pleurocystidia (Guzmán et al. 2003).

*Psilocybe antioquiensis,* described from Colombia, is close to *P. cinnamomea* in phylogenetic analyses but is distinguished by its reddish brown to fulvous pileus, gray brownish or violaceous brown lamellae, bigger angular spores (6–10.6 × 4.5–7 μm), and longer basidia (20–30 × 6–8 μm; Guzmán et al. 1994).

Morphologically, the new species is similar to the Caribbean species *Psilocybe guilartensis* Guzmán et al. and the Mexican species *P. moseri* Guzmán. However, *P. guilartensis* (placed in *P.* sect. *Brunneocystidiatae*) has thick-walled rhomboid spores and longer basidia (20–32 × 4–7 μm; Guzmán et al. 2003), while *P. moseri* has a dark buff to brownish pileus, brownish lamellae, and smaller spores (4–6.5 × 3–3.5 μm) and pleurocystidia (12–17 × 4–5.5 μm; Guzmán 1995).

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