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Hygrocybe griseobrunnea, a new brown species from China

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Abstract — *Hygrocybe griseobrunnea*, a new species in *Hygrocybe* subsect. *Squamulosae,* is described and illustrated based on the morphological characters and molecular data. The fungus is characterized by numerous greyish brown to brown or dark brown squamules on the pileus surface, adnate to shortly decurrent lamellae, and a trichodermal pileipellis.

Key words — *Basidiomycetes*, *Hygrophoraceae*, taxonomy

Introduction

The genus *Hygrocybe* (Fr.) P. Kumm. (*Hygrophoraceae*, *Agaricales*, *Basidiomycota*) is distributed worldwide, with ~150 accepted species (Kirk et al. 2008) and ~670 proposed names (http://www.indexfungorum.org). *Hygrocybe* sect. *Squamulosae* (Bataille) Singer is characterized by the dry fruitbody, squamulose or tomentose pileus, smooth stipe, and trichodermal pileipellis (at least in the pileus centre) (Boertmann 2010). More than 15 species in subsect. *Squamulosae* have been reported from different parts of the world (Singer 1986, Arnolds 1995, Borgen & Senn-Irlet 1995, Desjardin & Hemmes 1997, Young & Wood 1997, Borgen & Arnolds 2004, Cantrell & Lodge 2004, Leelavathy et al. 2006, Boertmann 2010, Ronikier & Borgen 2010, Vrinda et al. 2013). Only three species of the subsection — *Hygrocybe cantharellus* (Schwein.) Murrill, *H. coccineocrenata* (P.D. Orton) M.M. Moser, and *H. turunda* (Fr.) P. Karst. — have been recorded from China (Zeng & Yang 1991, Bi et al. 1993, Shao & Xiang 1997, Chen & Li 2013).

Recently, a representative of subsect. *Squamulosae* was collected and studied during an investigation of the agaric flora in Chebaling National Nature Reserve, Guangdong province, China. Morphological examinations and molecular

analyses based on the internal transcribed spacer (ITS) sequences indicate that the fungus is distinct from any other known species of *Hygrocybe;* it is therefore described here as a new species.

Materials & methods

Specimens were photographed and annotated in the field and then dried in an electric oven. Macroscopic depictions were gained from the original field notes and photographs. The holotype is preserved in the Fungal Herbarium of the Guangdong Institute of Microbiology (GDGM). Colour descriptions are according to Kornerup & Wanscher (1978). Tissue sections were immersed in 5% potassium hydroxide (KOH) or 1% Congo Red for microscopical examination. From a mature specimen, 20 basidiospores and 10 basidia were randomly selected and measured in KOH. The notation (a–)b–c(–d) is used to describe basidiospore dimensions where the range b–c represents 90% or more of the measured values, and a and d are the extreme values. The length/width ratio of spores and basidia is presented as Q, and the mean ratio is presented as Q_m. Elements of immersed pileipellis, stipitipellis, and hymenophoral trama are also described.

Genomic DNA was extracted from the herbarium specimen using the Sangon Fungus Genomic DNA Extraction kit according to the manufacturer's instructions (Sangon Biotech Co., Ltd., Shanghai, China). The ITS region was amplified by PCR, using universal primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993). Amplified products were electrophoresed on 1% agarose gels with a known standard DNA marker and directly sequenced by Beijing Genomic Institute (BGI), and the holotype sequence was submitted to GenBank. These and GenBank reference sequences were used in phylogenetic analysis after being edited and aligned using Clustal 1.81 (Thompson et al. 1997) and MEGA5.1 (Tamura et al. 2011). The dataset was analyzed with maximum parsimony by PAUP* 4.0b10 (Swofford 2003) following He & Li (2013).

Taxonomy

Hygrocybe griseobrunnea T.H. Li & C.Q. Wang, **sp. nov.** Figs 1–2

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Differs from *Hygrocybe caespitosa* by its applanate pileus with a partly uplifted margin, longer basidia, and shorter spores and from *H. melleofusca* by its shorter spores and rarity of clamp connections in its hymenophoral trama.

Type: China, Guangdong Province, Chebaling National Nature Reserve, 27 August 2010, B. Song, C.S. Qiu & H. Huang (**Holotype**, GDGM 28755; Genbank, KC914883).

Etymology: from Latin, griseus = grey, brunneus = brown.

Pileus 16–32 mm broad, slightly convex when young, expanding to applanate with a partly uplifted margin, often cracked or even perforated in the centre with age, weakly striate from the margin to half way to the centre when mature, with greyish brown to brown or dark brown (6E3–5, 6F4–8) squamules on orange-grey to brownish grey (6B2, 6C2, 6D2) background, often with some very slight purple to violet (14A2, 15A2, 16A2) tint here and there, becoming paler grey (6B1, 6C1, 6D1) surface where the exfoliated scales have fallen off

Fig. 1. *Hygrocybe griseobrunnea* (holotype). Basidiomata in situ.

with age, usually fissile, and with violet-grey to greyish violet (18B2–3, 18C2–3) tint at margin. Lamellae adnate to short-decurrent, waxy, transparent, thick, fragile, up to 3 mm broad, concolorous at edge, white or near so, sometimes inconspicuously yellowish white (4A2 to 5A2), partially violet-white (18A2) especially near the pileus margin, with about 4 complete lamellae per cm, and usually with 1–3 lamellulae of different lengths between two complete lamellae. Stipe 12–28 × 3–6.5 mm (the length often shorter than the pileus diameter), central, hollow, terete or more often compressed terete, usually with a narrower base, in large fruitbodies irregularly furrowed, smooth, occasionally curved at base, white to pale violet or violet-grey to greyish violet (18A1–3, 18B2–3), becoming brownish orange to light brown (6C3, 6D4) at lower part when mature. Context thin, concolourous with the lamellae, fragile. Smell insignificant.

Basidiospores 6.0–8.5(–9.0) × 4.0–6.5(–7.0) µm, Q = 1.1–1.6, Q_m = 1.41, ellipsoid to oblong, smooth, thin walled, hyaline, inamyloid. Basidia narrowly clavate, 45–63 × 8–12 µm, Q = 4.9–5.6, Q_m = 5.2, thin-walled, with 2 or 4 sterigmata up to 8.0 µm long, with a basal clamp connection. Hymenophoral trama subregular, composed of hyaline, thin-walled, cylindrical elements (30–110 × 9–20 µm), sometimes inflated, and rarely with clamp connections. Pileipellis a trichoderm, made up of septate, thin-walled hyphae about 27–75 × 6–20 µm, with brown intracellular pigment, clamp connections usually absent; underlying hyphae repent, cylindrical, devoid of any pigments. Stipitipellis a cutis, with cells 2–7 µm broad.

Ecology & distribution — On soil, in broad-leaved forest, and so far known only from the type locality in China.

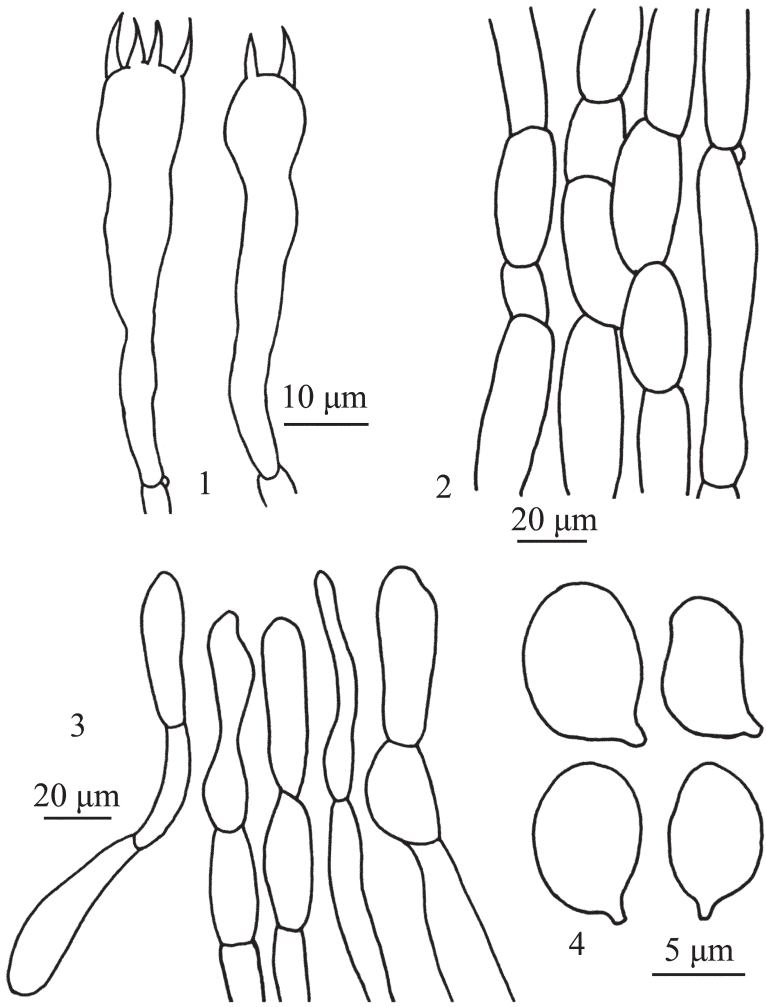

Fig. 2. *Hygrocybe griseobrunnea*.
1. Basidia. 2. Hymenophoral trama. 3. Elements of pileipellis. 4. Basidiospores.

Comments — *Hygrocybe griseobrunnea* is well characterized by its squamulose greyish brown to brown or dark brown pileus, subregular hymenophoral trama, and upturned pileipellis tufts. The trichodermal pileus squamules and subregular hymenophoral trama comprising hyphae <200 μm long place this fungus in *Hygrocybe* subsect. *Squamulosae* (Borgen & Arnolds 2004, Boertmann 2010).

Hygrocybe brunneosquamulosa Leelav. et al., described from India, is similar to *H. griseobrunnea* in sharing a grey-brown pileus with darker brown scales and sometimes having a central perforation in the mature pileus, but the two species are quite different because *H. brunneosquamulosa* has bright yellow, greyish brown or olive-brown lamellae, light yellow, amber-yellow or egg-yellow stipe, longer spores (7–10.5(–11) × 4.5–6.5(–7.5) μm), clamp-connections present in all parts of the basidioma, and numerous dark yellow-brown intracellular pigment in all tissues (Leelavathy et al. 2006; P. Manimohan, unpublished photo). *Hygrocybe caespitosa* Murrill, described from North America, also with a brownish squamulose pileus, is distinguished by its convex to obconic pileus with an inrolled margin, sinuate lamellae, yellow context, longer spores

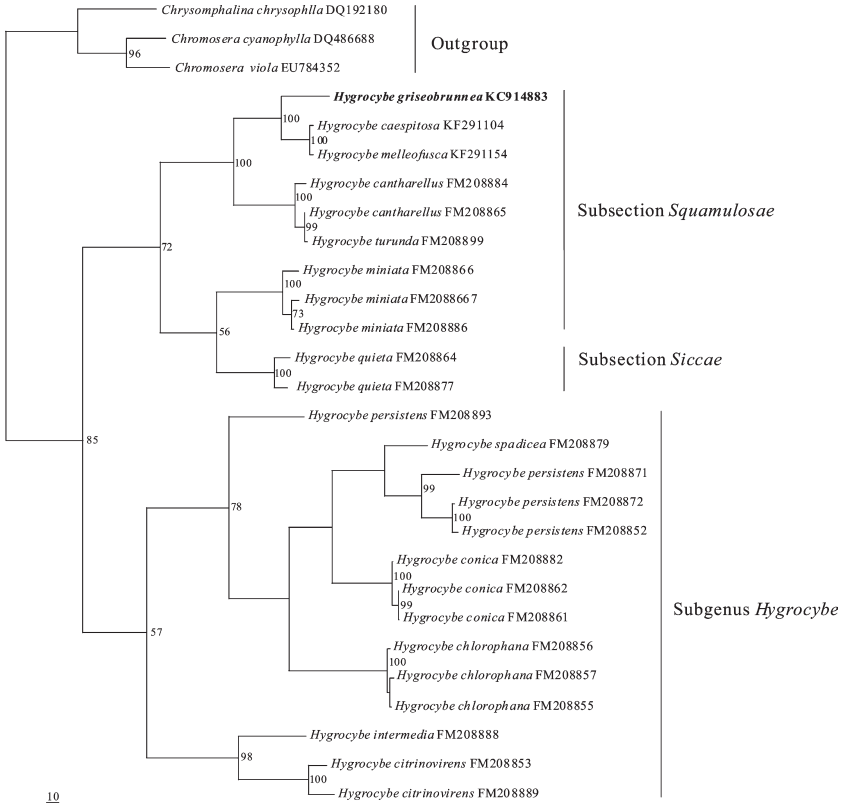

Fig. 3. The phylogenetic tree obtained from Maximum Parsimony analysis of ITS sequences of *Hygrocybe* species. *Chromosera cyanophylla*, *C. viola*, and *Chrysomphalina chrysophylla* were selected as outgroup. Parsimony bootstrap values (>50%) are shown. The unpublished ITS sequences of *H. caespitosa* and *H. melleofusca* were provided by Dr. D. Jean Lodge.

(7.5–10 × 5–6.5 μm), and smaller basidia (34.5–43 × 6–7.5 μm) (Murrill 1914, Cantrell & Lodge 2004). *Hygrocybe melleofusca* Lodge & Pegler, described from Puerto Rico, which resembles the new species with its dull coloured and often perforated pileus, has longer spores (7.8–10(–12) × 5–7(–8) μm) and prominent clamp-connections in the hymenophoral trama (Lodge & Pegler 1990).

The phylogenetic analysis (Fig. 3) clusters *H. griseobrunnea, H. caespitosa,* and *H. melleofusca* in the same clade with a 100% bootstrap value. This well-supported clade is sister to the clade with *H. turunda* (type species of subsect. *Squamulosae*). Bootstrap support for subsection *Squamulosae* (excluding *H. miniata*) is 100%, indicating that *H. griseobrunnea* belongs in the same subsection as *H. caespitosa* and *H. melleofusca*. Unfortunately, we were unable to obtain sequences from *H. brunneosquamulosa*. Furthermore, the ITS sequences of the *Hygrocybe* species formed two separated sister genetic groups that correspond to the two subgenera (*Hygrocybe* and *Pseudohygrocybe*) distinguished by morphological features (85% bootstrap support).

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