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Inocybe hainanensis, a new lilac-stiped species from tropical China

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Abstract: *Inocybe hainanensis* is described as a new species based on morphological and molecular data. It is characterized by its small basidioma, lilac stipe, slightly thick-walled pleurocystidia and the lack of caluocystidia. The similarities and differences of the new species and its close relatives from tropical Asia and adjacent region were discussed. The nLSU sequence of the holotype was generated and analyzed with available sequences of closely related taxa, which indicated it has closer relationship with south temperate (Australian) species *I. violaceocaulis* than with other north temperate taxa.

Key words: Inocybaceae, taxonomy, new species, nLSU phylogeny

海南丝盖伞，一个产自中国热带的淡紫色柄新种

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摘 要：结合形态和分子数据对产自我国热带地区的新种海南丝盖伞 *Inocybe hainanensis* 进行了描述，其主要特征是子实体小、菌柄淡紫色，侧生囊状体壁稍厚，无柄生囊状体。讨论了海南丝盖伞和热带亚洲及邻近地区相似种在形态上的区别和联系。利用自测 nLSU 序列和下载序列对该新种及其他具厚壁侧囊体、紫色菌柄的丝盖伞之间进行了系统学分析，发现海南丝盖伞与产自南温带（澳大利亚）的 *I. violaceucaulis* 亲缘关系最近。

关键词：丝盖伞科，分类，新种，nLSU 序列系统发育

INTRODUCTION

Inocybe is a cosmopolitan genus with a large number of described species (Kirk *et al.* 2008; Matheny *et al.* 2009). In recent years, new taxa have been continuously discovered from

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different regions of the world, including Asia (Kobayashi & Onishi 2010; Fan & Bau 2013, 2014), Europe (Kokkonen & Vauras 2011; Vauras & Larsson 2012), North America (Kropp *et al.* 2010, 2013; Matheny *et al.* 2013), South America (Matheny *et al.* 2012) and Australia (Bougher *et al.* 2012; Braaten *et al.* 2014). The increasing knowledge of species diversity, ecology and distribution will provide more evidences for its evolutionary history.

Species diversity and ecology of the genus to date is poorly documented in China (Fan & Bau 2010, 2013, 2014; Fan *et al.* 2013), especially in tropical areas. The accurate identification and taxonomy of *Inocybe* is a difficult task for most mycologists, even for the experienced ones. Nevertheless, species with lilac to violet color make an exception because only a few species share this feature. The polyphyly of smooth-spored species with lilac coloration is revealed by multilocus phylogeny (Matheny 2005; Matheny & Bougher 2005). During our taxonomic study on Inocybaceae in China, we encountered a new *Inocybe* species with lilac stipe from Hainan Province. In this paper, we aim to elucidate the phylogenetic relationships between the new species and other lilac species and to describe it.

1 MATERIALS AND METHODS

1.1 Morphological study

Fresh materials were collected in a highland tropical forest in Jianfengling Nature Reserve in Hainan Province, China (18°44′42″N, 108°50′09″E). Basidiomata were photographed before collecting, a field note was taken soon after collecting. Voucher specimens were deposited in the Mycological Herbarium of Jilin Agricultural University (HMJAU). Macro-characters were described based on the field note and photographs. Color notations were according to Kornerup & Wanscher (1981). Micro-characters were examined in a 5% KOH mounted slide preparation. Measurement methods follow Kobayashi (2002, 2009).

1.2 DNA extraction, PCR and sequencing

Genomic DNA was extracted from dried material of the holotype using a modified CTAB method (Doyle & Doyle 1987). The internal transcribed space gene (ITS) fragment and nuclear large submit gene fragment (nLSU) were generated. Primer pairs ITS1/ITS4 (White *et al.* 1990) and LR0R/LR7 (Vilgalys & Hester 1990) were used for the amplification. PCR reaction was performed using a Bio-Rad MyCycler thermal cycler (Bio-Rad, California, USA). Sequencing work was finished by Sangon Biotech (Shanghai) Co., Ltd.

1.3 Phylogenetic analysis

The ITS sequences of selected taxa were too variable to be included in the analysis. Therefore, the ITS sequence of the new species was used for a BLASTn similarity search. The available nLSU sequences of lilac *Inocybe* taxa and closely related taxa were selected and downloaded from the GenBank for phylogenetic analysis. All these downloaded sequences and newly generated sequence were aligned using

ClustalX 1.8 (Thompson *et al.* 1997). The aligned dataset was visually adjusted by BioEdit 7.0.9 (Hall 1999). A Maximum Liklihood (ML) tree was generated by using MEGA version 5 (Tamura *et al.* 2011). The Kimura 2-parameter was selected as the nucleotide substitution model. The Bootstrap method was used to test the phylogeny with 1 000 replicateds.

2 RESULTS

2.1 Molecular phylogeny

BLASTn results of the ITS sequence showed a large number of unidentified sequences (84%–85% identity) together with a few identified ones. An unidentified environment sample associated *Populus×berolinensis* from Estonia reach a highest maximum score (592) and 84% identity. Among the identified matches, *Inocybe fuscidula* determined by E. Larrson (AM882888 and AM882887) reach a highest maximum score (547) and 85% identity, *I. griseovelata* (JF908237, JF908182 and FN550931) reach 82% identity. None of the lilac taxa occurred in the ITS BLASTn results.

In the unrooted ML tree inferred from nLSU sequences, two well supported clades were found. *Inocybe hainanensis* clusters with *I. violaceocaulis* from Australia in a well supported clade (95%). Another well supported clade (99%) includes several north temprate taxa *I. pusio*, *I. cincinnata* and *I. cincinnata* var. *major*. The north temperate taxa *I. lilacina* and *I. griseolilacina* nested in a less support clade (67%) (Fig. 1). The phylogenetic relationships of *I. flocculosa*, *I. griseovelata* and *I. fuscidula* could not be inferred due to low bootstrap values.

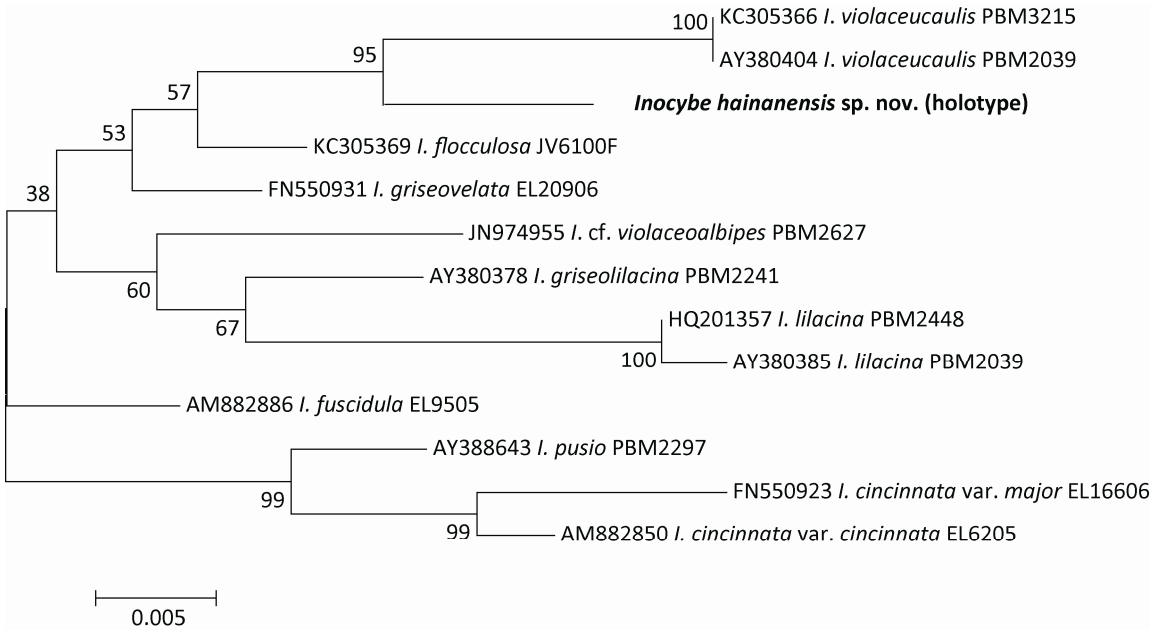

Fig. 1 An unrooted maximum likelihood tree inferred from nLSU sequences of *Inocybe hainanensis* and closely related taxa.

2.2 Taxonomy

Inocybe hainanensis T. Bau & Y.G. Fan sp. nov. Figs. 2, 3

MycoBank MB 808763

Etymology: refers to the type locality of the fungus.

Holotype: China, Hainan Province, Ledong Country, Jianfengling Nature Reserve, alt. 1 200m, in Fagaceae forest, 6 Jul. 2012, T. Bau & Y.G. Fan 2012009 (HMJAU 26067, ITS: GenBank, KJ816350; nLSU: GenBank, KJ816351).

Diagnosis: Basidiomata small. Pileus straw-yellow to pale brown. Stipe lilac with ochraceus tinge towards base. Lamellae pallid to yellowish brown with paler edges. Basidospores smooth. Pleurocystidia thick-walled. Cheilocystidia rare. True caulocystidia absent. Differs from *Inocybe subgeophylla* in having pallid to lilac stipe, wider basidiospores, thinner-walled pleurocystidia and in having thick-walled cheilocystidia.

Basidiomata small. Pileus 18–22mm in diam., conical when young, then subcampanulate to umbonate or subglobose, occasionally without umbo; dry, smooth or nearly so at disc, outwards woolly-fibrillose to sub-squamose, margin appressed fibrillose near margin, usually split in age, pallid, straw-yellow (1B7-4B6) to pale brown (6D8), paler towards margin (1B1). Cortina present in young specimens. Lamellae adnexed, moderately crowded, 1.5–2mm in width, thin, pallid (1A1-1B1) when young, yellowish brown (4B6) to brown (6C7-8) in age, paler at gill edge. Stipe 20–25×2–3mm, equal with indistinct swollen base, pruinose at stipe apex, fibrillose downwards; pale lilac (15A2-3) with staw-yellow (2A5) tinge towards base. Context cream white (1A1) to pale yellow (1A2) in pileus, fleshy, up to 2mm in thickness; white in stipe, base with pale yellow tinge (1A3), fibrillose. Odor indistinct.

Fig. 2 Basidiomata of *Inocybe hainanensis* (HMJAU 26067, holotype). Scale bar=10mm.

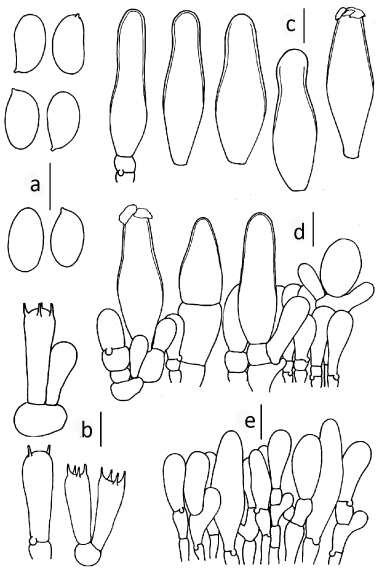

Fig. 3 Microscopic structures of *Inocybe hainanensis* (drawn from HMJAU 26067, holotype). a: Basidiospores; b: Basidia with probasidium; c: Pleurocystidia; d: Cheilocystidia and cheiloparacystidia; e: Cauloparacystidia. Scale bars: a–e=10μm.

Basidiospores 8.0–10.0(–10.5)×5.0–6.0μm, Q=(1.50–)1.55–1.98, smooth, ellipsoid, usually with obtuse apex and distinct apiculus. Basidia 20–27×7–9μm, clavate to slenderly clavate, colorless, with 4(2) sterigmata. Pleurocystidia 35–59×12–2μm, fusiform to ventricose, apices well-rounded to obtuse and crystalliferous, at times with sub-capitate apices, base obtuse to truncate; slightly thick-walled, walls less than 1(1.5)μm, pale yellow to yellow; hyline or with intracellular contents. Cheilocystidia 43–58× 13–17μm, similar to pleurocystidia, scattered among cheiloparacystidia, not abundant. Cheiloparacystidia 14–23×7–11μm, abundant, thin-walled, clavate to broadly clavate or ovoid, at times septate, hyline. Lamellae trama sub-regular, hyaline to pale yellowish, composed of inflated hyphae up to 25μm wide. Caulocystidia absent. Cauloparacystidia abundant at stipe apex, septate, translucent; terminal cells 20–41×9–12μm, clavate, occasionally fusiform. Pileipellis a cutis, irregular, pale brown, composed of thin-walled, cylindric to incrusted hyphae 5–10μm in diam. Clamp connects present, common, but not observed at the base of pleurocytidia.

Habitat and ecology: scattered under Fagaceae in highland tropical forest, fruits in July in type locality, not common.

Additional speciemen examined: China, Hainan Province, Ledong Country, Jianfengling Nature Reserve, alt. 1 200m, in Fagaceae forest, 6 Jul. 2012, T. Bau & Y.G. Fan 2012015 (paratype: HMJAU 26073).

3 DISCUSSION

The new species could be easily recognized by its lilac stipe in the field. It is characterized by small habit, slightly thick-walled hymenial cystidia and the lack of caulocystidia. A combination of smooth basidiospores and metuloid pleurocystidia indicates its membership in subgenus *Inocybe* sensu Kuyper (Kuyper 1986). In subg. *Inocybe* sensu Kuyper, only a few species with lilac stipe documented in tropical Asia and adjacent geographic areas (Horak 1980; Matheny & Bougher 2005). *Inocybe subgeophylla* Hennings, described from Indonesia, is similar in appearance and ecology, however, differs by its brown to red-brown pileus, cinnamon or pale red-brown stipe, narrower basidiospores [Horak (1980) gives 7.5–9×4–5μm], thicker walls of pleurocystidia and by the lack of cheilocystidia. *Inocybe violeipes* E. Horak and *I. violaceovelata* E. Horak, described from Papua New Guinea, both share lilac stipe. However, the former has a distinct acute papilla in pileus, belted stipes and amygdaliform basidiospores; the latter has lilac belts towards stipe base and thicker walls (up to 3μm thick) of pleurocystidia. *Inocybe ionides* Corner & E. Horak, described from Sabah, differs by its lilac to violet pileus and lamellae in young specimens and the lack of cortina and pleurocystidia. *Inocybe violaceucaulis* Matheny & Bougher, a recent described species from Western Australia, has robuster habit, lilac tinge in pileus, pedicel hymenial cystidia and different associated host plants. Among this set of species (except for the non-metuloid species *I. ionides*), an unambiguous tendency of infrequent or the lack of true cheilocystidia and the lack of true

caulocystidia could be inferred.

Among the north temperate taxa, *Inocybe flocculosa* Sacc., *I. griseovelata* Kühner and *I. fuscidula* Velen. share no lilac tinge in basidiomata. Several north temperate taxa do exhibit a lilac to violet coloration. *Inocybe griseolilacina* J. Lange shares infrequent cheilocystidia and the lack of caulocystidia, but differs in having sub-amygdalifrom basidiospores and pedicel pleurocystidia (Kuyper 1986; Stangl 1989). *Inocybe lilacina* (Peck) Kauffman (=*Inocybe geophylla* var. *lilacina*) has silky smooth and lilac pileus and caulocystidia (Kuyper 1986). *Inocybe pusio* P. Karst. has caulocystidia (Kuyper 1986; Stangl 1989). *Inocybe cincinnata* (Fr.) Quél. (including var. *cincinnata* and var. *major*) and *I. violaceoalbipes* G.F. Atk. have slender and longer hymenial cystidia (Atkinson 1918; Kuyper 1986).

Phylogenetically, *I. hainanensis* clusters with its south temperate relatives *I. violaceucaulis* in a well supported clade, the latter was suggested to be derived from its north temperate ancestry by long-distance dispersal (Matheny *et al.* 2009). In this case, *I. hainanensis* and its allies from tropical Asia and adjacent regions may play an important role during such dispersal course. The discovery of the new species in tropical China provides more chance for a better known of long-distances dispersal from north-to south-temperate in this species group.

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