

## Six new combinations of lepiotaceous fungi from China

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**Abstract:** During the compilation of lepiotaceous fungal flora of China, we have found that several taxonomic treatments should be done. Based on morphological data and partial molecular phylogenetic evidence inferred from ITS and nLSU, six new combinations, namely *Cystolepiota squamulosa*, *Lepiota ompnera*, *Leucoagaricus atrosquamulosus*, *Leucoagaricus lacrymans*, *Leucoagaricus nivalis* and *Leucoagaricus purpureoruber*, were proposed.

**Key words:** Basidiomycetes, morphology, new combinations, systematics, taxonomy

## 中国环柄菇类真菌新组合

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**摘要:** 在中国真菌志编研中, 作者发现有必要对其中几种环柄菇类真菌进行必要的分类处理。根据形态特征和部分 ITS 和 nLSU 序列分析结果, 本文提出 6 个新组合, 即红鳞囊小伞 *Cystolepiota squamulosa*、暗色锥鳞环柄菇 *Lepiota ompnera*、黑鳞白环蘑 *Leucoagaricus atrosquamulosus*、滴泪白环蘑 *Leucoagaricus lacrymans*、雪白小白环蘑 *Leucoagaricus nivalis* 和紫红白环蘑 *Leucoagaricus purpureoruber*。

**关键词:** 担子菌, 形态学, 新组合, 系统学, 分类学

## INTRODUCTION

Lepiotaceous fungi are a group of mushrooms in the family Agaricaceae (Agaricales). This group is very rich in species, and systematic treatments of many species of the group are often controversial

(Singer 1986; Bi *et al.* 1987; Candusso & Lanzoni 1990; Vellinga 2001, 2003, 2004; Bau & Li 2004; Hausknecht & Pidlich-Aigner 2004; Kumar & Manimohan 2004, 2009; Liang *et al.* 2010; Ge *et al.* 2015). Considering the present achievements in the phylogeny of lepiotaceous fungi, many molecular

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phylogenetic analyses and other studies should be carried out before a consensus treatment of the species can be reached.

During the compilation of lepiotaceous fungal flora of China, we have found that several taxonomic treatments should be done before they can be included in the fungal flora. In this account, six species were studied morphologically, and partially molecular-phylogenetically. Based on morphological data and molecular phylogenetic evidence, six new combinations were proposed.

## 1 MATERIALS AND METHODS

Seventeen collections of lepiotaceous fungi deposited in the Herbarium of Guangdong Institute of Microbiology (GDGM), the Cryptogamic Herbarium of Kunming Institute of Botany of the Chinese Academy of Sciences (HKAS), the Herbarium of Institute of Microbiology of the Chinese Academy of Sciences (HMAS), and Herbarium of Mycology of Jilin Agricultural University (HMJAU) were examined. Macro-morphological characters were described based on fresh material, colored photos, and/or field-notes. For micro-morphological studies, sections were mounted on slides in 5% KOH, and then observed, measured and illustrated under a compound microscope (Zeiss Axioskop 40, Germany). Melzer's reagent was used to test the amyloidity of

spores. Spore wall reaction to Cresyl blue was also checked. Dimensions for basidiospores are given using notation of the form (a) b–c (d). The range b–c contains a minimum of 90% of the measured values. Extreme values (a and d) are given in parentheses. Q indicates length/width ratio of a spore in side view; **Q** means average Q of all basidiospores  $\pm$  sample standard deviation.

Protocols for DNA extraction, PCR, and sequencing followed those in Yang & Feng (2013) and references therein. Newly generated sequences were aligned to the dataset used in Ge *et al.* (2015), and sequence alignments and phylogenetic analyses strategies used were the same as detailed therein. Generic names of *Cystolepiota*, *Lepiota*, *Leucoagaricus* and *Leucocoprinus* are abbreviated as *C.*, *L.*, *La.* and *Lc.* respectively.

## 2 RESULTS AND TAXONOMIC TREATMENTS

Data of morphological observations were provided in the following species descriptions. In total, 10 sequences were newly generated (Table 1). Initial analyses suggested that LSU provided very limited phylogenetic information, thus only ITS tree was demonstrated. Phylogenetic analysis (Fig. 1) indicated that *L. nivalis* W.F. Chiu and *Lc. lacrymans* T.K.A. Kumar & Manim. should be treated in the

Table 1 Taxa used in molecular phylogenetic analyses and their GenBank accession numbers for DNA sequences

Taxon	Voucher	GenBank accession	
		ITS	nrLSU
<i>Heinemanomyces splendidissimus</i>	HKAS 60216 /Ge2540	KY039570	KY039575
<i>Heinemanomyces splendidissimus</i>	HKAS 90465/Zhao2591	KY039571	KY039576
<i>Leucoagaricus japonicus</i>	HKAS 91249/Li 221	KY039572	KY039577
<i>Leucoagaricus nivalis</i>	HKAS 82691/Yang5792	KY039573	KY039578
<i>Leucocoprinus lacrymans</i>	HKAS 52738/Zhang 599	KY039574	KY039579

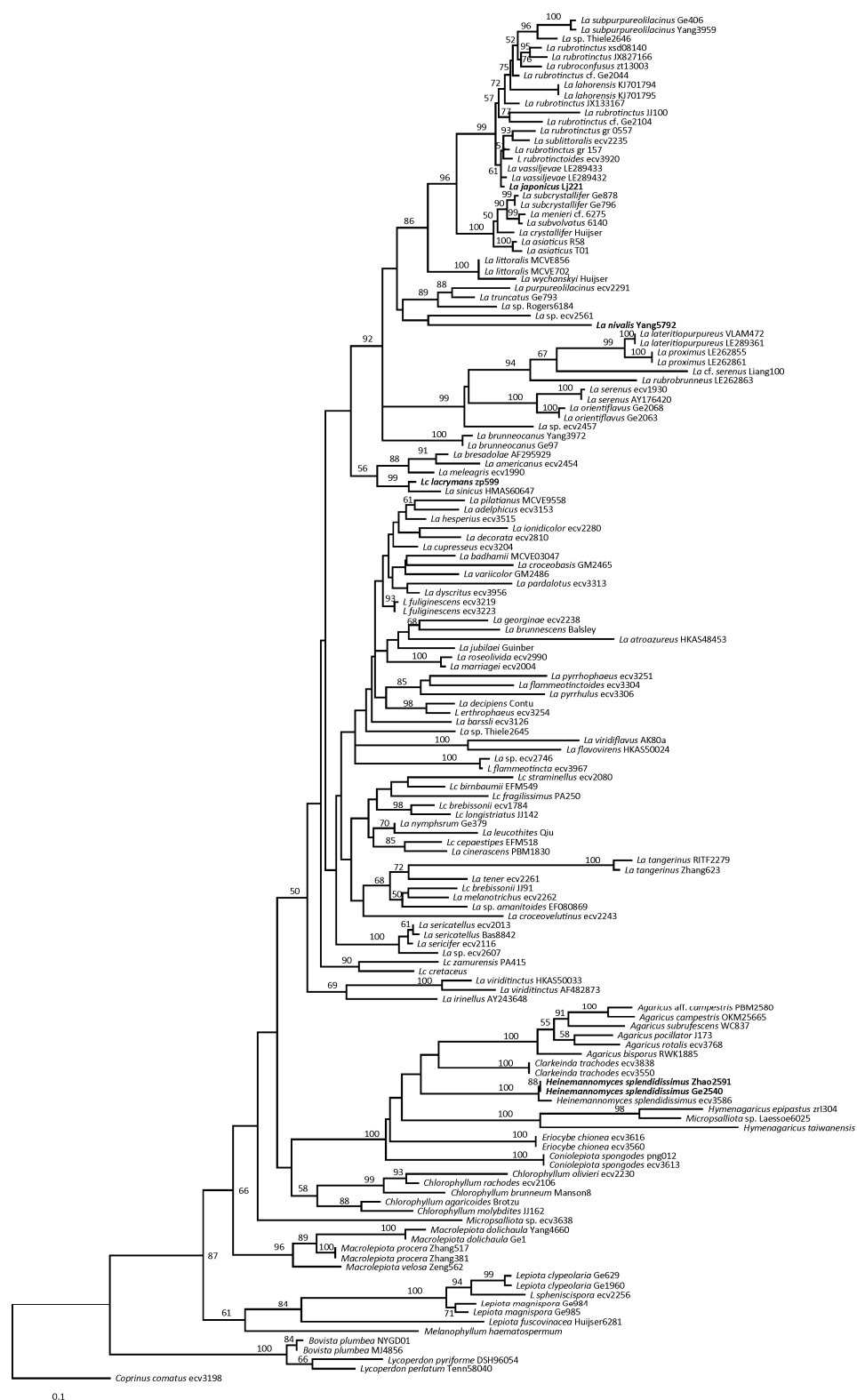


Fig. 1 Phylogenetic analysis of lepiotaceous fungi inferred from Maximum Likelihood (ML) analysis of ITS sequences. Bootstrap values (ML, > 50%) are shown above or beneath individual branches. Newly generated sequences are shown in bold.

genus *Leucoagaricus*. Based on morphological characters, *L. atosquamulosa* Hongo and *L. purpureorubra* Z.S. Bi should also be arranged in *Leucoagaricus*, while *C. ompnera* (Berk. & Broome) Pegler should be put in the genus *Lepiota*, and *L. squamulosa* T. Bau & Yu Li should be treated in the genus *Cystolepiota*. Based on morphological data and molecular phylogenetic evidence, the following treatments are made.

***Cystolepiota squamulosa*** (T. Bau & Yu Li) Zhu L. Yang, **comb. nov.** Fig. 2

MycoBank MB 818787

Basionym: *Lepiota squamulosa* T. Bau & Yu Li, Journ. Fung. Res. 2(3): 49, fig. 1/1–3, 2004.

Pileus 0.5–2cm in diam., convex to applanate, umbonate; surface pinkish, densely covered with pink, granular to warted squamules; context white. Lamellae white to cream, up to 2.5mm in height. Stipe 2–3.5cm in length, 1–2mm in diam., white to whitish, lower half with pink squamules; annulus present, white, fugacious.

Basidia 15–20×5–6µm, 4-spored, basal septa often with clamps. Basidiospores (3.5) 4–5(6)×2.5–3(3.5)µm [ $Q=(1.25)1.33–1.67(1.72)$ ,  $Q=1.51\pm 0.14$ ], ellipsoid, non-dextrinoid, non-amyloid, metachromatic in Cresyl blue, without germ pore, smooth or very finely warted. Pleurocystidia absent. Cheilocystidia not well differentiated, lamellar edge occasionally with colorless and hyaline clavate to subfusiform cells (15–25×5–7µm). Squamules on pileus composed of loosely arranged filamentous hyphae and inflated cells: inflated cells abundant, suglobose to ovoid (10–40×8–30µm), sometimes ellipsoid to pyriform (20–30×10–15µm), often 3–5 in chains; filamentous hyphae 3–7µm, fairly abundant. Clamps present in all parts of the

basidiome.

Habitat and distribution: Growing in pine forests; known from Beijing and Inner Mongolia.

Specimen examined: Beijing, Huairou, Mt. Yunmeng, 16. VIII. 2000, T. Bau (HMJAU 962, paratype).

Notes: The granular to warted squamules on the pileus and the loosely arranged filamentous hyphae and inflated cells in the squamules suggest the placement of the species in *Cystolepiota*. *Cystolepiota squamulosa* is very similar to *C. moelleri* Knudsen, originally described from Europe, but the latter species possesses slightly larger basidiomes and abundant cheilocystidia often with excrescence at apex (Candusso & Lanzoni 1990; Vellina 2001). *Lepiota chichuensis* W.F. Chiu (Chiu 1948) and *Cystolepiota rosea* Singer are also very similar to *C. squamulosa*. The type of *Lepiota chichuensis* is lost, and its concept is thus unclear. *Cystolepiota rosea*, known from South America (Argentina and Chile), has a smoother pileal surface and no cheilocystidia (Singer 1969).

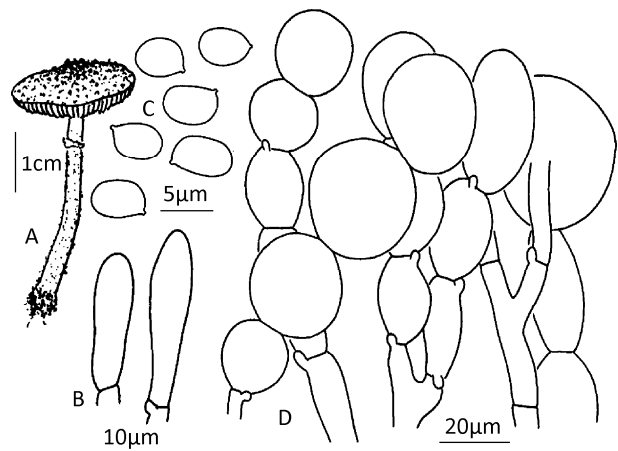


Fig. 2 *Cystolepiota squamulosa* (HMJAU 962, paratype). A: Basidioma; B: Cheilocystidia; C: Basidiospores; D: Microscopic structure of a squamule on pileus.

***Lepiota ompnera*** (Berk. & Broome) Zhu L. Yang, **comb. nov.** Fig. 3

MycoBank MB 818788

Basionym: *Agaricus ompnerus* Berk. & Broome, Journ. Linn. Soc., Bot. 11: 514, 1871.

Synonyms: *Armillaria ompnera* (Berk. & Broome) Sacc., Syll. Fung. 5: 86, 1887; *Cystolepiota ompnera* (Berk. & Broome) Pegler, Kew Bull. add. Ser. 12: 284, 1986.

Pileus 4cm in diam., convex, umbonate; surface dirty brown to yellowish brown, becoming paler towards the margin, covered with brown to grey-brown echinulate to conical squamules. Lamellae cream-colored. Stipe 4.5cm in length, 0.5cm in diam., glabrous and brownish to dirty white above annulus, densely covered with brown to grey-brown echinulate to conical squamules below annulus. Annulus membranous.

Basidia 10–13×4–5µm, 4-spored. Basidiospores 3.5–4.5×1.8–2.8µm [ $Q=1.60–2.00$ ,  $Q=1.75\pm0.17$ ], elongate, without suprahilar depression, dextrinoid, without germ pore, not metachromatic in Cresyl blue, smooth. Pleurocystidia absent. Cheilocystidia unknown. Squamules on pileus compactly composed of more or less vertically arranged filamentous hyphae and inflated cells: inflated cells very abundant, suglobose, ovoid, ellipsoid to subfusiform (10–30×8–25µm), with brownish to dark brown intracellular pigments, often in chains; filamentous hyphae 3–8µm, fairly abundant. Clamps present in all parts of the basidiome.

Habitat and distribution: Growing in tropical forests; known from Sri Lanka and tropical China.

Specimens examined: Taiwan: Nantou County, Qingjing, 7 June, 2000, JM Chen 2848 (HKAS 42308).

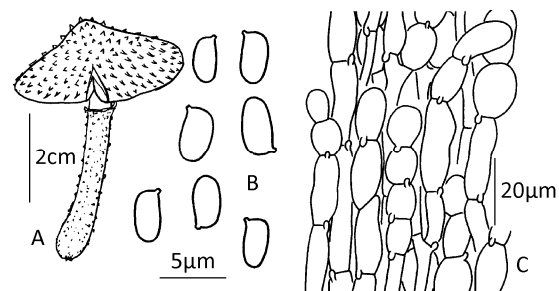


Fig. 3 *Lepiota ompnera* (HKAS 42308). A: Basidioma; B: Basidiospores; C: Microscopic structure of a squamule on pileus.

Notes: *Lepiota ompnera* was put in *Cystolepiota* by Pegler (1986). However, the squamules on the pileal surface are echinulate to conical, neither granular nor warted, and the inflated cells and the filamentous hyphae in the squamules are compactly and vertically arranged, which is similar to those of *L. echinacea* J.E. Lange and *L. jacobi* Vellinga & Knudsen. *Lepiota echinacea* is characterized by its slightly wider basidiospores, larger inflated cells in the pileal squamules and temperate distribution, while *L. jacobi* can be distinguished by its woolly squamules below annular zone, larger inflated cells in the pileal squamules and temperate distribution (Vellinga 2001).

***Leucoagaricus atosquamulosus*** (Hongo) Z.W. Ge & Zhu L. Yang, **comb. nov.** Fig. 4

MycoBank MB 818789

Basionym: *Lepiota atosquamulosa* Hongo, Journ. Jap. Bot. 34: 239, fig. 1 (d–f), 1959.

Pileus 1.5–2.5cm in diam., convex to applanate, umbonate; surface dirty white, covered with blackish brown to fuliginous fibrillose, warted or felted squamules, margin often radially striate when mature; context white, unchanging. Lamellae white to cream-colored. Stipe 4–5cm in length, 0.2–0.3cm in diam., subcylindrical, dirty white, nearly glabrous; annulus membranous, white, fugacious.

Basidia 15–22×6.5–7.5µm, 4-spored. Basidiospores 5–7(8)×3.5–4 [Q=(1.35)1.45–1.75(2.0),  $Q=1.63\pm0.13$ ], ovoid to ellipsoid, dextrinoid, non-amyloid, metachromatic in Cresyl blue, smooth, with a slightly attenuate apex, without germ pore. Pleurocystidia absent. Cheilocystidia narrowly clavate, clavate to broadly clavate (20–55×10–15µm), colorless. Squamules of the pileal surface a loosely arranged cutis of frequently septate cylindrical hyphae 5–10µm in diam., with terminal elements barely differentiated, subcylindrical (20–50×5–10µm), thin-walled, with brown to yellowish brown intracellular pigments. Clamps absent in all parts of the basidiome.

Habitat and distribution: Growing in broad-leaved forests or mixed forests; known from China, Japan, and Papua New Guinea.

Specimens examined: Beijing: Zhongguancun, 13 August 1973, L.W. Xu 92 (HMAS 35904). Yunnan: Kunming, Heilongtan, 30 June 1991, Z.L. Yang 1073 (HKAS 24841); Kunming: Kunming Institute of Botany, 27 August 1993, Z.L. Yang 1932 (HKAS 26151); same location, 31 July 2007, Z.L. Yang 4784 (HKAS 52299); same location, 1 August 2007, Z.L. Yang 4789 (HKAS 52304); Zhanyi, Mt. Maxiong, 29 June 2008, X.H. Wang 2164 (HKAS 54136).

Notes: *Leucoagaricus atrosquamulosus* is characterized by its very small basidiome, blackish brown to fuliginous pileal surface with radially arranged filamentous elements and metachromatic basidiospores without germ pore. Hongo (1986) put it in *L. sect. Ovisporae*. However, the radially and repeatedly arranged elements in the squamules on the pileus and the metachromatic basidiospores with slightly attenuate apex and without germ pore indicate that this taxon should be transferred to *Leucoagaricus*.

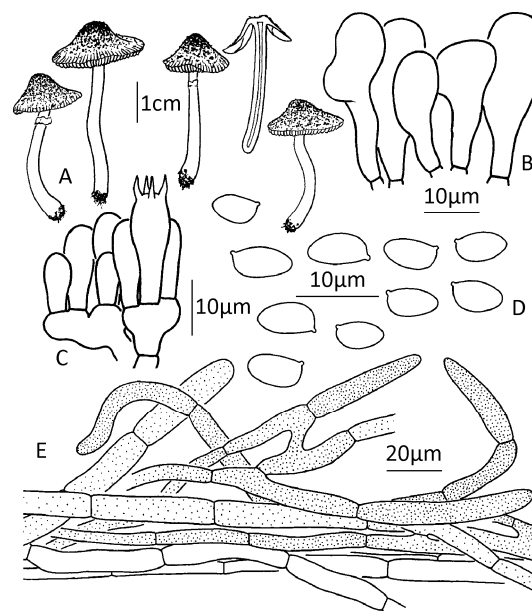


Fig. 4 *Leucoagaricus atrosquamulosus*. A: Basidiomata (HKAS 26151); B: Cheilocystidia (HKAS 52304); C: Basidia (HKAS 52304); D: Basidiospores (HKAS 52304); E: Microscopic structure of a squamule on pileus (HKAS 52304).

***Leucoagaricus lacrymans*** (T.K.A. Kumar & Manim.)  
Z.W. Ge & Zhu L. Yang, **comb. nov.** Fig. 5

MycoBank MB 818790

Basionym: *Leucocoprinus lacrymans* T.K.A. Kumar & Manim., Mycotaxon 90: 393, 2004.

Pileus 2–6cm in diam., obtusely conical when young, applanate when mature; surface white to cream-colored, covered with minute brown to purplish brown squamules; margin finely radially striate; context up to 0.3cm in thickness, white to whitish, becoming pale orange when cut. Lamellae white to cream-colored, becoming grey to greenish grey when dried. Stipe 5–10cm in length, 0.3–0.5cm in diam.; surface dirty white to brownish, often with brown drops; annulus membranous, brown, persistent.

Basidia 22–30×9–12µm, 4-spored. Basidiospores (8) 8.5–10.5 (11)×(5.5) 6–7.5 (8)µm [Q=(1.29) 1.33–1.50 (1.58)],  $Q=1.43\pm0.06$ , broadly ellipsoid to ellipsoid



in side view, ellipsoid to subovoid in frontal view, with a rounded apex, with apical germ pore but without cap on germ pore, smooth, slightly thick-walled, strongly dextrinoid, metachromatic in Crysel blue. Pleurocystidia absent. Cheilocystidia  $70\text{--}120 \times 4\text{--}8\mu\text{m}$ , contorted subcylindrical, with a round apex, colorless or sometimes with ochreous yellow intracellular pigments, often in groups. Squamules on pileus a trichoderm composed of vertically arranged lanceolate elements ( $80\text{--}120 \times 6\text{--}12\mu\text{m}$ ) with dark brown to deep ochreous yellow intracellular pigments. Clamps absent in all parts of the basidiome.

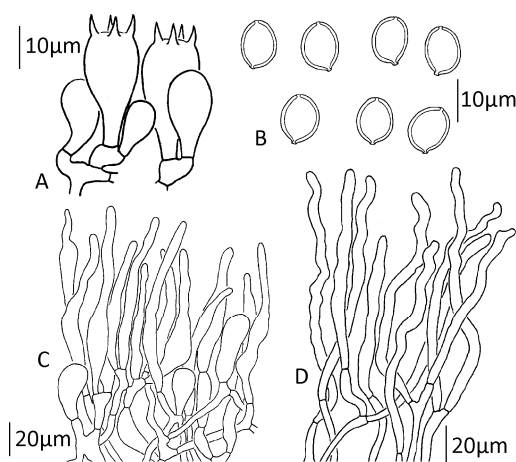


Fig. 5 *Leucoagaricus lacrymans* (HKAS 52739). A: Basidia; B: Basidiospores; C: Elements in squamules on pileus; D: Cheilocystidia.

**Habitat and distribution:** Growing in tropical forests; known from India and tropical China.

**Specimens examined:** Guangdong: Guangzhou, campus of Guangdong University of Foreign Studies, 30 March, 2007, P. Zhang 599 (HKAS 52738); South China Botanical Garden, 30 March, 2007, P. Zhang 601 (HKAS 52739). Taiwan: Taizhong, campus of Zhongxing University, 25 April, 2001, JM Chen 2744 (HKAS 42306); same location, 5 June 2002, JM Chen 3182 (HKAS 42312).

**Notes:** *Leucoagaricus lacrymans* is characterized by the presence of drops on the surface of the stipe, whitish context becoming paler orange when cut and lanceolate elements in the squamules on the pileus (Kumar & Manimoha 2004). It is close to *La. holospilotus* and *La. sinicus* (Pegler 1986; Reid 1990; Yang 2007). However, *La. holospilotus* is characterized by its unchanging context, non-cylindrical cheilocystidia and broadly lanceolate elements in the squamules on the pileus (Reid 1990), while *La. sinicus* possesses a flesh-colored pileal context and subcylindrical elements in the squamules on the pileus (Yang 2007). Molecular data indicated that *La. sinicus* and its allied should be treated in *Leucoagaricus* (Fig. 1).

***Leucoagaricus nivalis*** (W.F. Chiu) Z.W. Ge & Zhu L. Yang, **comb. nov.** Fig. 6

**MycoBank MB 818792**

**Basionym:** *Lepiota nivalis* W.F. Chiu, Sci. Rep. Natl. Tsing Hua Univ. Ser. B 3: 177, 1948.

Pileus 1.2–4.5cm in diam., convex to applanate, umbonate. Surface white, glabrous to radially fibrillous; margin finely striate when mature. Context white, unchanging. Lamellae white, up to 3mm in height. Stipe 2–7cm in length, 0.2–0.5cm in diam.; surface white, glabrous above annulus, finely fibrillose to near glabrous below annulus; annulus membranous, persistent.

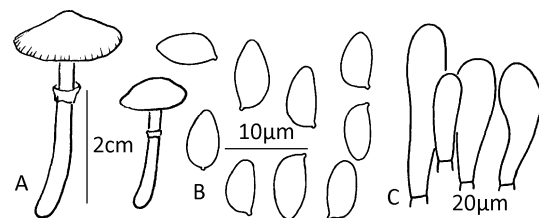


Fig. 6 *Leucoagaricus nivalis* (HMAS 4237, type). A: Basidiomata; B: Basidiospores; C: Cheilocystidia.

Basidia  $17\text{--}20 \times 6\text{--}7\mu\text{m}$ , 4-spored. Basidiospores  $6\text{--}7.5(9) \times (3) 3.5\text{--}4.5(5)\mu\text{m}$  [ $Q=1.50\text{--}2.00(2.03)$ ,  $Q=1.71 \pm 0.13$ ], subamygdaliform in side view, subovoid in frontal view, often with a narrow apex, without germ pore, smooth, slightly thick-walled, dextrinoid, metachromatic in Crysyl blue. Pleurocystidia absent. Cheilocystidia  $15\text{--}35 \times 6\text{--}11\mu\text{m}$ , clavate to narrowly clavate, colorless and hyaline. Squamules of the pileal surface a loosely arranged cutis of frequently septate cylindrical hyphae  $3\text{--}5\mu\text{m}$  in diam., with terminal elements barely differentiated, subcylindrical ( $4\text{--}6\mu\text{m}$  in diam.) or sometimes nearly clavate ( $10\text{--}15\mu\text{m}$  in diam.), thin-walled, colorless. Clamps absent in all parts of the basidiome.

Habitat and distribution: Growing in subtropical broad-leaved forests or mixed forests; known from China.

Specimens examined: Yunnan: Kunming, Dapuji, 20 July 1942, W.F. Chiu s.n. (HMAS 4237, type); Kunming Botanical Garden, 1 August 2007, Z.L. Yang 4791 (HKAS 52306); Kunming Botanical Garden, 10 July 2014, Z.L. Yang 5792 (HKAS 82961); Tengchong, National Forest Park, 22 July 2003, Z.L. Yang 3826 (HKAS 42665).

Notes: *Leucoagaricus nivalis* is very close to *La. serenus* (Fr.) Bon & Boiffard, originally described from Europe. However, the latter differs from the former by its wider basidiospores and wider cheilocystidia often with a long pedicel (Vellinga 2001). Phylogenetic analysis indicated that *La. nivalis* should be treated in *Leucoagaricus* (Fig. 1).

***Leucoagaricus purpureoruber* (Z.S. Bi) Z.W. Ge & Zhu L. Yang, comb. nov.** Fig. 7

MycoBank MB 818794

Basionym: *Lepiota purpureorubra* Z.S. Bi, Acta Mycologica Sinica Supplement I: 291, 1987 (1986).

Pileus  $4\text{--}12\text{cm}$  in diam., convex; surface purplish red, covered with grey-brown squamules; margin striate. Context white, unchanging. Lamellae  $0.3\text{--}0.7\text{cm}$  in height, cream, becoming purple or brown when dried. Stipe  $4\text{--}13\text{cm}$  in length,  $0.3\text{--}1.7\text{cm}$  in diam., upper portion cream, middle portion pinkish purple; annulus greyish brown, membranous.

Basidia  $22\text{--}40 \times 9.5\text{--}11\mu\text{m}$ , 4-spored. Basidiospores  $(7.5)8.5\text{--}11.5(12.5) \times 6\text{--}7.5(8)\mu\text{m}$  [ $Q=(1.25)1.35\text{--}1.54(1.67)$ ,  $Q=1.45 \pm 0.07$ ], ellipsoid or occasionally subamygdaliform in side view, ellipsoid or occasionally ovoid in frontal view, with apical germ pore but without cap on germ pore, smooth, slightly thick-walled, dextrinoid, metachromatic in Crysyl blue. Pleurocystidia absent. Cheilocystidia  $45\text{--}80 \times 12\text{--}18\mu\text{m}$ , clavate to broadly clavate to subfusiform, with brownish to yellow-brown intracellular pigments, often with a terminal appendage  $3\text{--}15 \times 3\text{--}5\mu\text{m}$ . Squamules on pileus a trichoderm composed of more or less vertically arranged fusiform or sometimes clavate elements  $45\text{--}180(250) \times 12\text{--}23(30)\mu\text{m}$ , often with a short appendage, with yellow-brown intracellular pigments. Clamps absent in all parts of the basidiome.

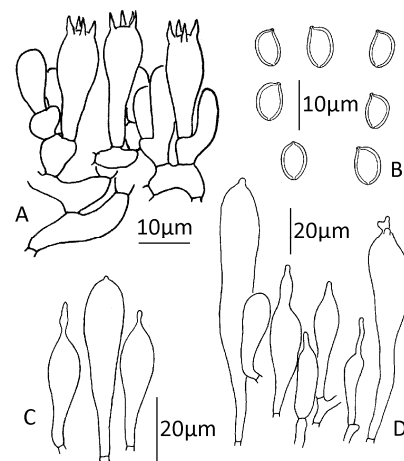


Fig. 7 *Leucoagaricus purpureoruber* (GDGM 8660, type). A: Basidia; B: Basidiospores; C: Cheilocystidia; D: Elements in squamules on pileus.



Habitat and distribution: Growing on sawdusts; known from southern China.

Specimen examined: Guangdong: Qujiang, Xiaokeng, 27 July 1985, T.H. Li s.n. (GDGM 8660, type).

Notes: Although the type of *Leucoagaricus purpureoruber* was heavily damaged by insects, a few important characters, like the shape and the size of cheilocystidia, basidiospores and elements in the squamules on the pileus, could be observed. Microscopic characters indicate that this taxon should be transferred to *Leucoagaricus*. In fact, it is very similar to *La. americanus* (Peck) Vellinga and *La. meleagris* (Sowerby) Singer, both of which have, however, a discoloring context when cut (Vellinga 2001).

### 3 DISCUSSION

Molecular techniques have revolutionized knowledge of fungal evolution and can provide sound evidence for fungal taxonomy (Yang 2011, 2013). Systematic treatments of lepiotaceous fungi should be based on both morphological and molecular phylogenetic data (Vellinga 2003, 2004; Liang *et al.* 2010; Ge *et al.* 2010; Vellinga *et al.* 2011; Sysouphanhong *et al.* 2012; Ge *et al.* 2015). Considering the present achievements in the phylogenetic studies of lepiotaceous fungi, many molecular phylogenetic studies should be carried out before a consensus treatment of the species can be reached.

In this study DNA sequences were unfortunately only generated from two of the six taxa (Fig. 1, Table 1). In the near future, well-dried materials of lepiotaceous fungi should be made available for molecular phylogenetic researches, which will shed new lights on the evolution and systematic arrangements of them.

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