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Lepiota nigrosquamosa, a new species from China

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With 2 figures

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Abstract: A new species, *Lepiota nigrosquamosa*, is described from southwestern China. It is characterized by black brownish to black squamules on its pileus and stipe, fusiform or broadly fusiform spores, and a pileus covering of a trichoderm consisting of elongate, subcylindrical terminal elements in between with short clavate elements.

Kew words: Agaricales, Agaricaceae, lepiotaceous fungi, taxonomy.

Introduction

The genus *Lepiota* (Pers.) Gray is characterized by the following morphological characteristics: pileus and stipe context not confluent, universal and partial veils present, lamellae free, spore print white to cream, microscopically with (rarely without) clamp connections, dextrinoid (rarely non-dextrinoid), non-metachromatic (rarely metachromatic), bi-nucleate (rarely uni-nucleate) generally non-ornamented spores, without a germ pore, with pileus covering made up of either long elements (trichodermally arranged or in chains and radially adnate or ascending), or narrowly clavate elements (hymeniderm-like), regular lamella trama (Vellinga 2003). The genus *Lepiota* contains more than 400 described species (Kirk et al 2008).

Taking the rich mycota of China into account, it is reasonable to expect a large number of species of this cosmopolitan genus in China. However, rather few taxa of the genus were originally described from China (Chiu 1948, Bi et al 1986, Yang 1994, Tolgor

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& Li 2004, Wang & Yang 2005a, Wang & Yang 2005b). The diversity of the genus in this country is understudied. In our monographic work of *Lepiota* in China, besides several new species which have been published recently (Liang et al 2009, Liang et al 2010, Liang et al 2011, Liang & Yang 2011), interesting and undescribed taxa are still waiting for documentation yet. The present paper belongs to a series of studies devoted to *Lepiota* diversity of China.

Materials and methods

Material was borrowed from the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) and the Herbarium Mycologicum Academiae Sinicae (HMAS). Terminology for descriptive terms follows Vellinga (2001). Color designations are from Kornerup and Wanscher (1981). Herbarium abbreviations follow Thiers (2011, continuously updated).

Morphology: For microscopic observations, sections of specimens were cut by hand and mounted in 5% KOH, Congo red, and Melzer's reagent. Basidiospores were mounted in cresyl blue to test for a metachromatic reaction (Singer 1986). Size ranges were determined for basidia, basidiospores, cheilocystidia, and elements of the pileus covering, based on ocular micrometer measurements of at least 20 elements of each character. The abbreviation [n/m/p] indicates that measurements were made on n basidiospores measured from m basidiocarps of p collections. Dimensions of basidiospores are given using a notation of the form (a) b–c (d). The range b–c contains 90% of the measured values. Extreme values are given in parentheses. The following abbreviations are used: Q refers to the length/breadth ratio of basidiospores; **Q** refers to the average Q of all basidiospores ± sample standard deviation.

Molecular identification: DNA was extracted from herbarium material and the internal transcribed spacer I (ITS I) of the nuclear ribosomal repeat was amplified by the polymerase chain reaction (PCR) with the fungal specific primers ITS1 and ITS2 (White et al 1990). Sequencing of both strands was performed with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services CO., Ltd, Shanghai). The sequence of the holotype has been submitted to GenBank (accession number JN203140). DNA sequences were edited and aligned using SeqMan (DNASTAR Package) and ClustalX (Thompson et al 1997) and manually checked and modified. Ambiguous positions were excluded from matrix. The dataset was analyzed with maximum likelihood with the RAxML BlackBox online server (Stamatakis et al 2008) and Bayesian inference using the parallel version of MrBayes 3.1.2 with default priors (Ronquist and Huelsenbeck 2003, Altekar et al 2004).

Descriptive Part

Lepiota nigrosquamosa J.F.Liang & Zhu L.Yang, **sp. nov.** Fig. 1

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Pileus initio subcampanulatus, deinde late conicus, albidus, squamulis nigro-brunneis. Lamellae liberae, albae. Stipes subcylindricus, albus, annulatus, squamulis nigro-brunneis infra annulum. Annulus lanatus vel submembranaceus, sursum albidus, deorsum squamulosus. Basidiosporae 13–16 × 5–7 µm, late fusoideae. Basidia clavata, 4-sporigera, raro 2-sporigera. Cheilocystidia subfusoidea. Pleurocystidia nulla. Squamulae pilei ex trichodermiis subcylindricis terminalibus compositae. Fibulae praesentes.

Holotypus: China, Sichuan Prov., Hongyuan Co., Shuajing Temple, alt. 3500 m, 22 Aug 1998, M.S.Yuan 3468 (HKAS 33874).

Etymology: Named because of its black squamules on the pileus.

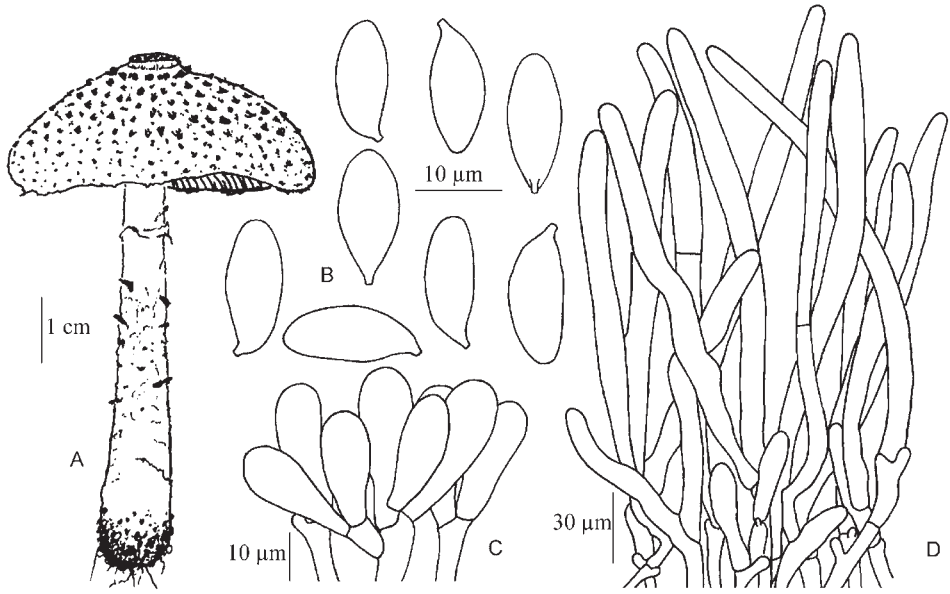

Fig. 1. *Lepiota nigrosquamosa* A. Basidioma B. Basidiospores C. Cheilocystidia D. Pileus covering. All from Holotype.

Basidiomata (Fig . 1A) medium-sized to big. Pileus 4–9 cm in diameter, convex, with broad low umbo, at centre glabrous, uplifted and black; around centre with concentrical zones of more or less uplifted, discrete, small, black brownish to black squamules on a whitish background. Lamellae free, whitish, moderately to crowded, with lamellulae of two lengths; subventricose to ventricose. Stipe 6–9.5 × 0.5–1 cm, subcylindrical to attenuate, slightly inflated at base, hollow, cream and glabrous at the apical part; surface cream, ochraceous, woolly, with some girdles of squamules in lower part, concolorous with those on pileus. Annulus whitish, membranous, covered with black brownish squamules at lower surface; evanescent. Odor none. Taste not recorded.

Basidiospores (Fig. 1B) [64/3/3] (12)13–16 × 5–7 µm [Q = 2.00–2.80, Q = 2.45 ± 0.21], fusiform or broadly fusiform, with a suprahilar depression in side view, without germ pore, fusiform in frontal view, rarely narrowed at the apex; colorless, hyaline, smooth, slightly thick-walled, strongly dextrinoid, congophilous, not metachromatic in Cresyl Blue, with guttulate contents. Basidia 25–40 × 9.5–16 µm, clavate, 4-spored, rarely intermixed 2-spored. Lamella edge sterile. Cheilocystidia (Fig. 1C) 17–29 × 6–13 µm, clavate to fusiform, often capitate, occasionally ellipsoid; walls smooth, thin, colorless, hyaline in KOH, congophilous. Pleurocystidia absent. Pileus covering (Fig. 1D) a trichodermium consisting of elongate, subcylindrical terminal elements 97–385 × 9.5–18 µm, sometimes septate, in between with some short elements; pigment pale yellow brown, parietal and intracellular in upper part, sometimes basal part also encrusted with a black pigment. Clamp connections present in all tissues.

ADDITIONAL SPECIMEN EXAMINED: CHINA, Sichuan Prov., Yajiang Co., Jianziwan Mount., alt. 4000 m, 22 July 1984, H.A.Wen & J.J.Shu 1274 (HMAS 59667); Tibet Autonomous Region, Bomi Co., Palong, alt. 2400 m, 4 Oct 1982, X.L.Mao 749 (HMAS 53368).

HABITAT: solitary or gregarious in small group, saprotrophic and terrestrial on nutrient-rich soils, in coniferous and broad leaf forests at high altitude localities.

DISTRIBUTION: Only known from southwestern China.

DISCUSSION: *Lepiota nigrosquamosa* is well characterized by its dirty white pileus and stipe densely covered black brownish to black squamules consisting of elongate, subcylindrical terminal elements in between with short clavate elements, fusiform to broadly fusiform basidiospores, and clavate to fusiform cheilocystidia. Due to the trichodermium type of squamules on the pileus and the fusiform basidiospores, *L. nigrosquamosa* may be placed in *L.* sect. *Lepiota* (Singer 1986, Vellinga 2001).

The type was reported as *L. acutesquamosa* (Weinm.) Gill. [*L. aspera* (Pers.) Quél.] (Yuan & Sun 2007), but the latter was distinguished from the new species by its pyramidal warts on the pileus made up of chains of globose to ellipsoid elements and significantly smaller cylindrical spores. HMAS 59667, and HMAS 53368 were regarded as *L. clypeolaria* (Bull.) P.Kumm, and *L. magnispora* Murrill (*L. ventriosospora* D.A.Reid) by Mao (1995) seperately. However, *L. clypeolaria* has no black squamules and real annulus, while *L. magnispora* posseses penguin-shaped basidiospores.

In the genus *Lepiota*, black squamules at the pileus can also be found from *Lepiota atrosquamulosa* Hongo, a species originally described from Japan. However, the latter has smaller basidiocarps, ellipsoid spores and a pileus covering composed of chains of cylindric to subcylindric, often branched, elements (Hongo 1959).

Several other species with fusiform-amygdaliform spores and a pileus covering made up of long cylindrical elements are also worth noting here. These species include *L. kuehneriana* Locq. (Candusso & Lanzoni 1990, Bon 1996), *L. erminea* (Fr.) Kumm., *L. oreadiformis* Velen. and *L. subgracilis* Kühner (Vellinga 2001). However, black squamules coupled with the fusiform cheilocystidia of the new species can be used to distinguish it from the above species. In addition, the penguin-shaped spores of *L. cortinarius* J.E.Lange (Vellinga 2001), *L. metulispora* (Berk. & Broome) Sacc. and *L. attenuata* J.F.Liang & Zhu L.Yang (Liang et al 2011) can serve as characters to separate them from the new species.

Bayesian and RAxML phylogenetic analyses (Fig. 2) showed that *L. nigrosquamosa* fell into clade I which included most species of *L.* sect. *Lepiota* shared with a trichodermial pileus covering, and fusiform-amygdaliform basidiospores (e.g. *L. clypeolaria* and *L. erminea*) or penguin-shaped basidiospores (e.g. *L. magnispora* and *L. cortinarius*). Although the sect. *Lepiota* is not monophyletic (Vellinga 2003, Liang et al 2011), the species included in the clade I always cluster together and belong to the section, indicating that the new species should be placed in the section.

Phylogenetic results showed that the new species was different from the previously described species for which ITS sequences were available. The obvious divergence between the new species and the previously described species indicates that it is significantly distinguished from these known species.

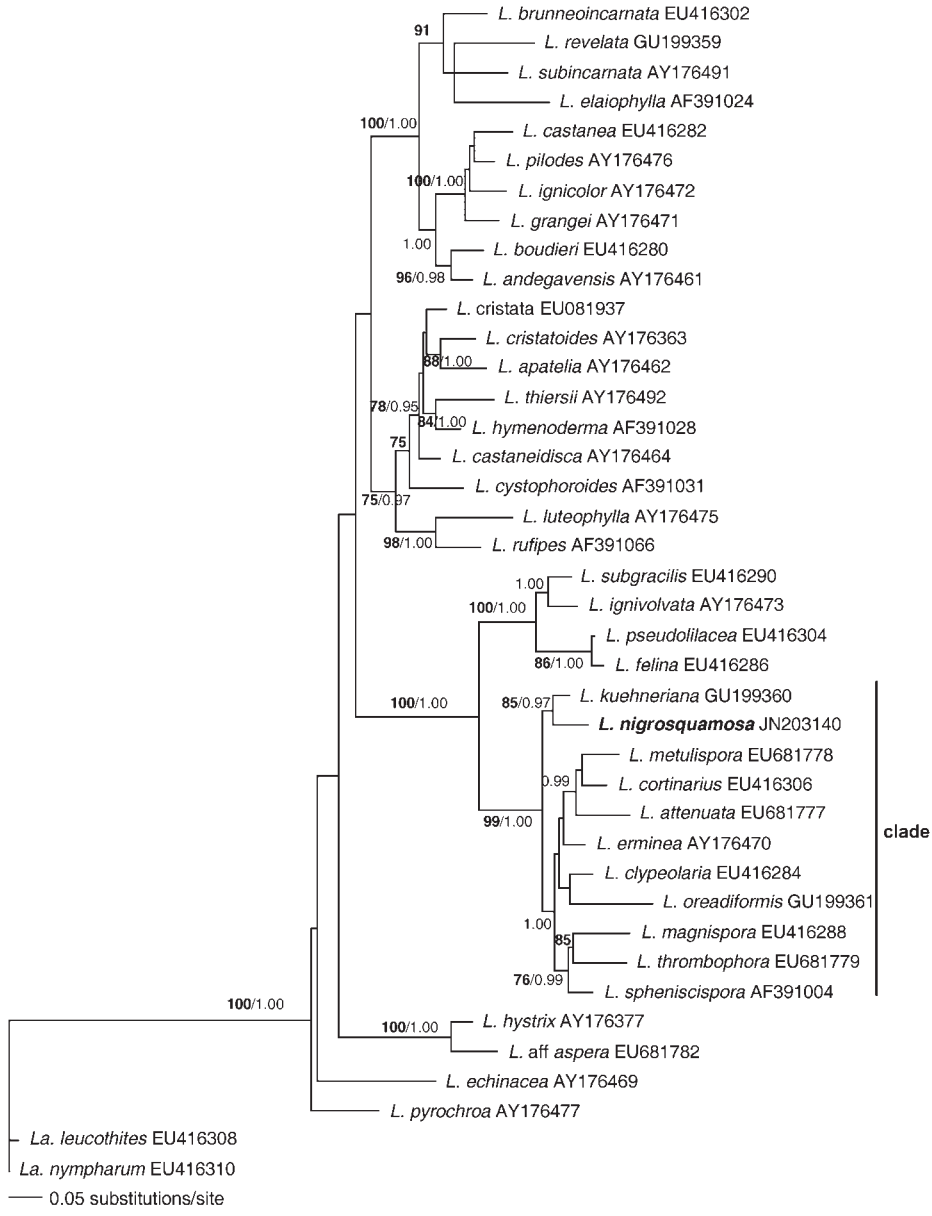

Fig. 2. One of 100 RAxML likelihood trees (-In L 9078.622636) based on the ITS dataset. Support values in bold type are RAxML likelihood bootstrap (≥ 70%). Values in normal type are Bayesian posterior probabilities (≥ 0.95). L = *Lepiota*; La = *Leucoagaricus*

Key to the species in *Lepiota* sect. *Lepiota* of China

1. Spores fusiform-amygdaliform, with convex abaxial and adaxial sides 2
1. Spores 'penguin-shaped' with straight abaxial side, convex adaxial side and suprahilar depression..... 7

2. Pileus without discrete squamules, white to pale brown at centre 3
2. Pileus with brown to pale brown squamules contrasting with background..... 4

3. Pileus white, pale ochraceous to yellowish brown at centre..... ***L. erminea***
3. Pileus pale brown to brown at centre..... ***L. oreadiformis***

4. Stipe with annulus **5**
4. Stipe without real annulus, but with annular zone, and lanate covering below... ***L. clypeolaria***

5. Pileus without black brownish to black squamules 6
5. Pileus and stipe with black brownish to black squamules..... ***L. nigrosquamosa***

6. Pileus rarely covered with pyramidal squamules at centre; stipe with densely squamules in lower part; spores 11–16 × 5–7 µm ..... ***L. kuehneriana***
6. Pileus densely covered with small fibrillose or pyramidal squamules at centre; stipe with bands or squamules in lower part; spores 9–12.5 × 4.5–6 µm..... ***L. subgracilis***

7. Basidiomata slender to robust; pileus without sulcate striations..... 8
7. Basidiomata slender; pileus with sulcate striations 9

8. Spores 14–18.5 × 4–6 µm..... ***L. magnispora***
8. Spores (8.)8.5–11(13) × 3–4.5 µm..... ***L. cortinarius***

9. Spores never narrowed at the apex; pileus covering without inflated submoniliform or catenulate elements 10
9. Spores distinctly narrowed at the apex; pileus covering with inflated submoniliform or catenulate elements ..... ***L. attenuata***

10. Pileus with light ochraceous buff, concentric squamules, made up of a trichodermium of elongate, apically attenuate terminal elements, in between often with some short elements.. ***L. metulispora***
10. Pileus with densely crowded dark brown squamules, made up of a trichodermium of elongate, apically attenuate terminal elements, rarely with short elements..... ***L. thrombophora***

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