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Pseudoarmillariella bacillaris, a new species with bacilliform basidiospores in Asia

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Abstract: A new species, *Pseudoarmillariella bacillaris*, is described from southwestern China based on both morphological and molecular phylogenetic evidence. This species differs phenotypically from the remaining two known species in the genus by its bacilliform basidiospores. The discovery of the new taxon in Asia indicates the genus *Pseudoarmillariella* has a much wider geographical distribution range than had previously been known.

Key words: biogeography, Hygrophoraceae, new species, taxonomy

长孢假小蜜环菌，一种孢子杆状的亚洲产新种

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摘 要：根据形态和分子系统发育证据，描述采自我国西南的新种长孢假小蜜环菌 *Pseudoarmillariella bacillaris*。与假小蜜环菌属 *Pseudoarmillariella* 已知分布于北美-中美洲和大洋洲的2个物种相比，该新种的突出特征是担孢子杆状。在亚洲发现假小蜜环菌属真菌，表明该属的地理分布范围与过去已知的相比要广泛得多。

关键词：生物地理，蜡伞科，新种，分类学

Pseudoarmillariella Singer (1956) is a small genus consisting of only 2 known species distributed in North and Central America, and New Zealand (Horak 1971; Singer 1986; Kirk *et al*. 2008). The systematic position of the genus was unclear and long debated until recently. Based on morphological,

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anatomical and molecular phylogenetic evidence, Norvell *et al*. (1994) and Redhead *et al*. (2002) defined the generic boundaries. Later, molecular phylogenetic studies indicated that *Pseudoarmillariella* is a member of the family Hygrophoraceae (Matheny *et al*. 2007; Lawrey *et al*. 2009).

During our study of the species diversity of agarics in southwestern China, we encountered a new species of *Pseudoarmillariella*, which is very unique in morphology. The objective of the present study is to elucidate its phylogenetic position and to describe it based on morphological and molecular phylogenetic evidence.

1 MATERIALS AND METHODS

1.1 Specimens and morphological descriptions

Macro-morphological descriptions are based on the field notes and images of basidiomata. Color codes of the form "10D7", indicating the plate, row, and color block, are from Kornerup & Wanscher (1981). Specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany of the Chinese Academy of Sciences (HKAS). Micro-morphological data were obtained from the dried specimens after sectioning and mounting in 5% KOH solution. In the descriptions of basidiospores, the abbreviation [*n*/*m*/*p*] shall mean *n* basidiospores measured from *m* basidiomata of *p* collections; **Q** is used to mean "length/width ratio" of a spore in side view; **Q** means average **Q** of all basidiospores ± sample standard deviation.

1.2 DNA extraction, PCR and sequencing

Genomic DNA was extracted from materials dried with silica gel using CTAB method (Doyle & Doyle 1987). Two gene markers, the internal transcribed spacer (ITS) and the large nuclear ribosomal RNA subunit (nrLSU), were selected for phylogenetic analyses. Universal primers ITS1/ITS4 (White *et al.* 1990) were used for the amplification of the ITS region, and primers LR0R/LR7 (Vilgalys & Hester 1990) were applied for the amplification of the nrLSU region. Amplification reactions were performed in an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR program was as follows: pre-denaturation at 95℃ for 3min; then followed by 35 cycles of denaturation at 94℃ for 30s, annealing at 50℃ (ITS and nrLSU) for 50s, elongation at 72℃ for 90s; afterwards, a final elongation at 72℃ for 8min was included. PCR products were depurated with the Gel Extraction & PCR Purification Combo Kit (Spin-column, Bioteke, Beijing, China), and then sequenced on an ABI-3730-XL sequence analyzer (Applied Biosystems, Foster City, CA, USA) using the same primers as those used in amplifications.

1.3 Sequence alignments and phylogenetic analyses

Sequences of representatives of Hygrophoraceae (Matheny *et al*. 2007; Seitzman *et al*. 2011) were retrieved from GenBank and combined with our newly generated sequences (KC222315 for ITS and KC222316 for nrLSU) to make up two datasets, 5.8S and nrLSU datasets. Each dataset was aligned using Muscle v3.8.31 (Edgar 2004) and manually optimized on BioEdit v7.0.9 (Hall 1999). The two datasets were then concatenated to conduct combined analyses. Due to high divergences in the ITS1 and ITS2 regions among the selected genera, the sequences of the ITS1 and ITS2 were not used in the analyses.

Maximum likelihood (ML) and Bayesian inference (BI) were applied for molecular phylogenetic analyses using RaxML (Stamatakis *et al*. 2008) and MrBayes (Ronquist & Huelsenbeck 2003), respectively. SYM+G and GTR+I+G were chosen as best fit models for 5.8S and nrLSU partitions respectively by using Mrmodeltest2.3 (Nylander 2004). Partitioned analyses were

conducted for both ML and BI. For ML analysis, all parameters were kept at their default values except for the model choice as GTRGAMMAI, and the statistical supports were calculated using nonparametric bootstrapping with 1,000 replicates. BI analysis was carried out with MrBayes by setting the number of generations to 5 million and using the stoprul command with the value of stopval set to 0.001, while other parameters were kept at their default values. Subsequently, trees were summarized and statistical supports were obtained by using the sumt command implemented in MrBayes by discarding the first 10% of generations as burn-ins.

2 RESULTS

2.1 Molecular phylogeny

In the phylogeny of nrLSU/5.8S sequences, both ML and BI analyses strongly supported the monophyly of *Pseudoarmillariella* with two lineages: *P. ectypoides* lineage and *P. bacillaris* lineage, and the genus *Pseudoarmillariella* was clustered within the Hygrophoraceae with high statistical supports (Fig. 1).

2.2 Taxonomy

Pseudoarmillariella bacillaris Zhu L. Yang, B. Feng & Y.J. Hao, **sp. nov**. Figs. 2, 3

MycoBank MB 802410

Etymology: referring to the bacilliform basidiospores.

Holotypus: China, Sichuan Province, Muli County, on the way from Muli to Daocheng, alt. 3,480m, on rotten wood, 2 August 2012, Y.J. Hao 719 (HKAS 76377; ITS sequence generated from the holotype: KC222315; nrLSU sequence generated from the holotype: KC222316).

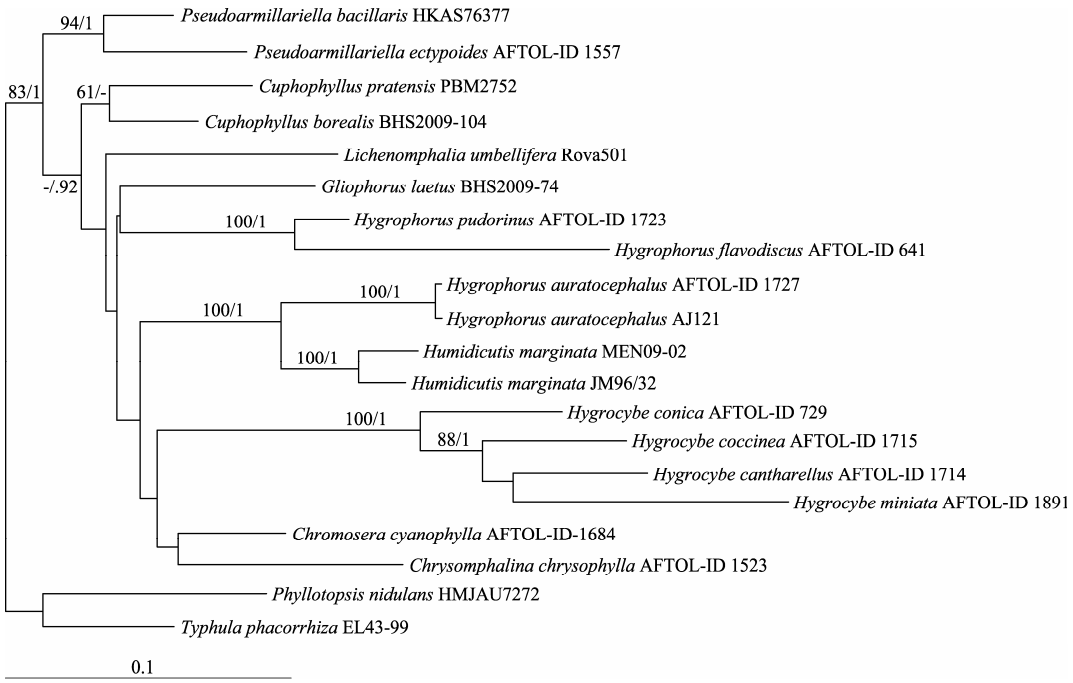

Fig. 1 Phylogenetic position of *P. bacillaris* inferred from Maximum Likelihood (ML) analysis of nrLSU/5.8S sequences. Bootstrap values (ML, > 50%)/posterior probabilities (BI, > 0.90) are shown above or beneath individual branches.

Fig. 2 Basidiomata of *Pseudoarmillariella bacillaris* (HKAS 76377, holotype).

Pileus 2.5–6.5cm latus, umbilicatus vel infundibuliformis, rufo-brunneus vel brunneolus, squamulibus rufo-brunneis ornatus. Lamellae decurrentes, angustae, flavae vel flavidae. Stipes 3–7×0.3–0.6(1)cm, subcylindricus vel sursum atenuatus, flavus vel pallide flavus. Caro pallide flava. Basidia 28–38×5.5–7µm, clavata, 4-sporigera. Basidiosporae 8.5–12×2.5–3.5µm, bacilliformis, amyloideae. Cheilocystidia et pleurocystidia absentes. Fibulae praesentes.

Basidiomata small to medium-sized, subcaespiotose. Pileus 2.5–6.5cm in diam., broadly umbilicate to funnel-shaped; surface reddish brown (10D7) to brownish (8E7) or ochraceous (7D7), covered with reddish brown (10D7) to brownish (8E7) to dark brown (7E6), recurved, pointed squamules; margin waved and incurved. Lamellae decurrent, narrow, crowded to subdistant, pale yellow (3A6) to yellow (4A6) but becoming reddish brown (10D7) when injured, sometimes forked or veined; edge even. Stipe centrally attached to excentric, 3–7×0.3–0.6(1)cm, subcylindric or slightly attenuate upwards, yellow (4A6) to yellowish (3A6), cartilagenous, solid; surface covered with yellow (2A3) to yellowish (2A2), minute floccose squamules; base slightly enlarged, with whitish mycelium. Context thin, yellowish. Odour and taste mild.

Hymenium and subhymenium thickened, composed of 3–5µm wide frequently branching hyphal segments. *Basidia* 28–38×5.5–7µm, clavate, hyaline, thin-walled, 4-spored; sterigmata 3–4 µm long; basal septum with clamp. Basidiospores [80/4/2] (8)8.5–12(17)×2.5–3.5(4)µm, **Q**=(2.43)2.57–4.44(6.80) (**Q**=3.09±0.31), bacilliform, narrowly fusiform to nearly boletoid, hyaline, thin-walled, smooth, amyloid, non-metachromatic, no germ pore; apiculus small. *Lamellar trama* regular to sub-regular, composed of branching filamentous hyphae 3–7µm wide. Cheilocystidia and pleurocystidia absent. Pileipellis a cutis composed of radially arranged, yellowish to brownish vacuolar-pigmented, occasionally also finely pigment-encrusted, thin-walled, filamentous hyphae 3–6(10)µm wide. Squamules on the surface of the pileipellis composed of compactly arranged, yellowish to brownish vacuolar-pigmented, occasionally also finely pigment-encrusted, filamentous hyphae 2.5–7µm wide; terminal cells not differentiated, 30–60×3–7µm. Stipitipellis composed of vertically arranged, branching filamentous hyphae 3–7(12)µm in width, outer surface of the stipitipellis often with more or less irregularly and somewhat loosely arranged yellowish to brownish vacuolar-pigmented, occasionally also finely pigment-encrusted, filamentous hyphae (2.5–7µm wide) forming squamules on the surface of the stipe. Clamp connections abundant in every part of basidioma.

Habitat and known distribution: Subcaespitose, lignicolous, in forest dominated by *Abies*, *Betula*, *Salix*, etc.; fruiting in summer in subalpine areas in southwestern China.

Additional specimen examined: China, Sichuan Province: Luding County, Moxi, Hailuogou, alt. 3000m, on rotten wood, 12 August 1997, P.Q. Sun 2929 (HKAS 31335, as "*Cantharellus melanoxeros* Desm." in Yuan & Sun 2007).

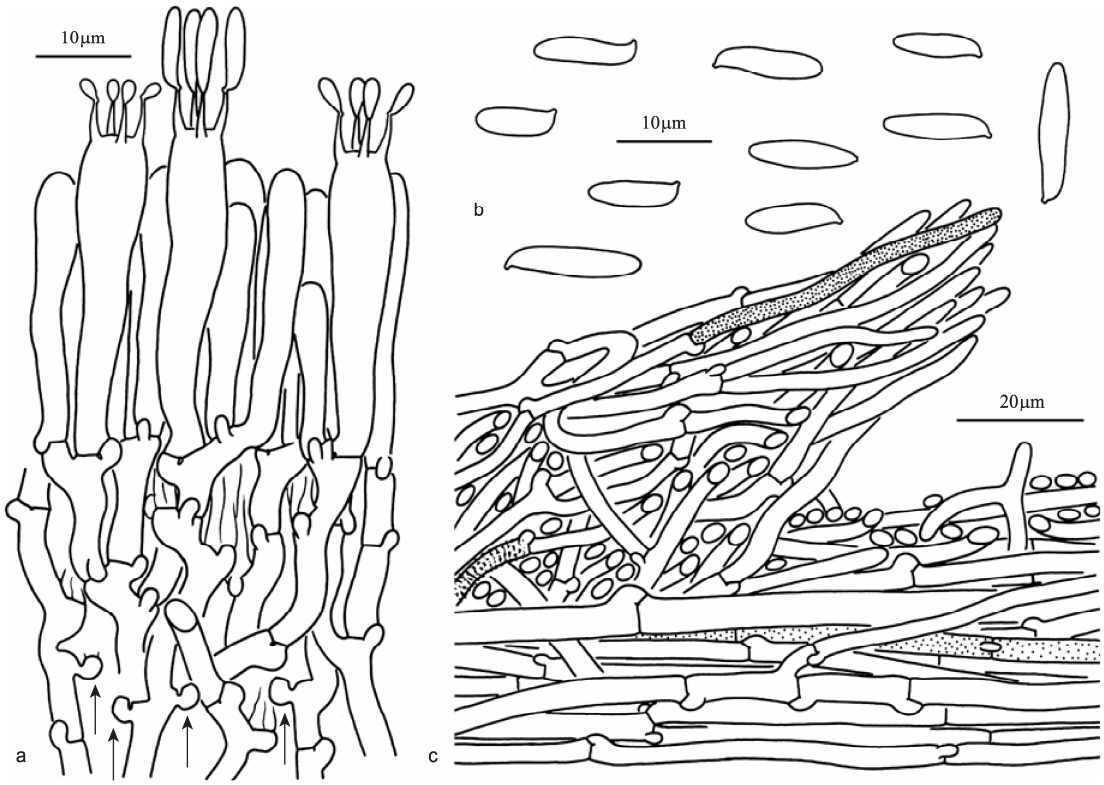

Fig. 3 Microscopic characters of *Pseudoarmillariella bacillaris* (HKAS 76377, holotype). a: Hymenium and subhymenium. In the lower part of the subhymenium, some basidia have disappeared completely and only the basal clamps remain (arrow); b: Basidiospores; c: Pileipellis and a part of a squamule on the pileus in radial-vertical section.

3 DISCUSSION

There were two known species, *P. ectypoides* (Peck) Singer and *P. fistulosa* (G. Stev.) E. Horak in the genus (Horak 1971; Singer 1986; Kirk *et al*. 2008). *Pseudoarmillariella ectypoides*, originally described from the United States of America, differs from *P. bacillaris* by its paler pileal surface, paler-colored lamellae and stipe, and the ellipsoid basidiospores measuring 6.5–9×3.5–5μm (Peck 1872; Bigelow 1982; Phillips 1991; Bessette *et al*. 1997), while *P. fistulosa*, originally described from New Zealand, differs from *P. bacillaris* by its hollow stipe, ovoid basidiospores measuring 5–6×3.5–4μm and the common presence of clavate cheilocyscydia 20–55×10–18μm (Stevenson 1964; Horak 1971). The available DNA sequence data showed that *P. ectypoides* and *P. bacillaris* form a monophyletic group with strong statistical supports (Fig. 1). DNA sequences of *P. fistulosa* are still unavailable.

The hymenium and subhymenium of *P. bacillaris* had thickened during the development of the basidioma, and many mature basidia in the lower part of the subhymenium had disappeared and only the clamps remained (Fig. 3-a, arrows). Such features were also observed in *P. ectypoides* (Singer 1956; Norvell *et al*. 1994; Redhead *et al*. 2002).

To our knowledge, *Pseudoarmillariella* has not been reported from Asia previously, although a collection regarded as "*Clitocybe ectypoides* (Peck) Sacc." was listed in Yuan & Sun (2007). Reexamination

of the collection, viz. M.S. Yuan 4721 (HKAS 37230) (as "HKAS 37030" in Yuan & Sun 2007), showed that the basidiospores are 3.5–4.5×2.5–3.5μm, colorless and non-amyloid. This collection may be a representative of *C. acromelalga* Ichimura. The discovery of a *Pseudoarmillariella* species in Asia indicates the genus has a much wider geographical distribution range than previously supposed.

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