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Agaricus taeniatus sp. nov., a new member of *Agaricus* sect. *Bivelares* from northwest China

Sai-Fei Li[1,2], Ya-Li Xi[3], Cai-Xia Qi[3], Qian-Qian Liang[3], Sheng-Long Wei[3], Guo-Jie Li[1, 2], Dong Zhao[1], Shao-Jie Li[1A*], & Hua-An Wen[1B*]

[1] *State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No 31st Beichen West Road, Chaoyang District, Beijing 100101, China*

[2] *University of Chinese Academy of Sciences, Beijing 100049, China*

[3] *Gansu Engineering Laboratory of Applied Mycology, Hexi University, 846th Huancheng North Road, Ganzhou District, Zhangye 734000, Gansu, China*

* Correspondence to: [A]*lisj@im.ac.cn* [B]*wenha@im.ac.cn*

Abstract—*Agaricus taeniatus* from Qilian Mountains, northwest China, is described as a new species. It is distinguished by its larger basidia and basidiospores, band-like velar remnants on the stipe surface, and persistent single rings with eroded edges. ITS1-5.8S-ITS2 rDNA sequence analyses fully support the establishment of the new species within *A.* sect. *Bivelares*.

Key words —*Agaricales*, *Agaricaceae*, phylogeny, taxonomy

Introduction

Agaricus L. is a genus with nearly 400 species comprising both edible and poisonous species (Bas 1991). It has a long history and has been subdivided into different taxonomic groups (Møller 1950, Heinemann 1978, Cappelli 1984, Singer 1986, Parra 2008). Kauffman (1919) first described *Agaricus* sect. *Bivelares* (Kauffman) L.A. Parra as a section of *Psalliota* typified by *P. rodmanii* (Peck) Kauffman [= *A. bitorquis* (Quél.) Sacc.]. Later studies reorganized this section (Heinemann 1978, Wasser 1980, Challen et al. 2003, Didukh et al. 2004) and characterized its species by negative Schäffer and KOH reactions, red discoloration of the context, and a mild or indistinct odor (Parra 2008). The species most widely consumed, *A. bisporus* (J.E. Lange) Imbach, is included in this section (Kerrigan et al. 2008).

The taxonomy of *Agaricus* has been studied intensively in Europe, America, and southeast Asia (Hotson & Stuntz 1938; Freeman 1979a, 1979b; Heinemann 1956; Kerrigan 1985, 1989, 2005; Chen et al. 2012; Zhao et al. 2012a, 2012b). Despite China's long history of *Agaricus* consumption, its taxonomy remains little studied, and only a few *Agaricus* species have thus far been described from China (Chiu 1973, Li 1990). In our effort to further *Agaricus* taxonomy in the country, we collected specimens from the Qilian Mountains, an area of high macrofungal diversity (Xi et al. 2011). As a result of our research, we present here a new species of *A.* sect. *Bivelares.*

Materials & methods

Photos were taken and main morphological characters were recorded in situ. Other macrocharacters and chemical tests were carried out immediately after the specimens were brought back to the laboratory. Color names and codes follow Ridgway (1912). Specimens were air-dried overnight in a dehydrator prior to microscopic examination. Tissues were hand-sectioned and rehydrated in 5% KOH. Particular attention was paid to the basidia, basidiospores, and cystidia and the anatomy of the pileipellis, annulus, and band-like remnants on the stipe surface. Line drawings were prepared from tissues stained with Congo red. For basidiospores, (*n/m/p*) refers to *n* spores from *m* basidiocarps of *p* specimens, with dimensions given as (a–)b–c(–d), with b–c including 90% of the measured values between the extreme values shown in parentheses. Sterigma lengths are not included. Q = spore length/width quotient in side view; avQ = average Q ± standard deviation. Specimens cited were deposited in Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

DNA extractions were made with a DNA extraction kit (Plant Genomic DNA Rapid Extraction kit, BioTeke, China). Internal transcribed spacers (ITS) were amplified by PCR reactions with primers ITS1/ITS4 (White et al. 1990, Gardes & Bruns 1993), and sequenced by SinoGenoMax Co. Ltd. Sequences obtained were deposited in GenBank.

Sequences of representative and closely related *Agaricus* taxa were retrieved from GenBank. Sequences were aligned using BioEdit (Hall 1999) and MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/ Katoh & Toh 2010). The alignment has been submitted to TreeBase (submission ID 15563).

Maximum-likelihood (ML) analysis was performed in PhyML3.0 (Guindon et al. 2010), via the ATGC bioinformatics platform (http://www.atgc-montpellier.fr/phyml/). The GTR substitution model was selected with 6 substitution rate categories to account for rate heterogeneity across sites. Likelihood was increased by using the SPR tree improvement. The analysis included 100 bootstrap replicates. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) under the estimated evolutionary model. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The first 2,000 resulting trees were discarded as burn-in and the remaining 8,000 trees were used for calculating the posterior probabilities in the majority rule consensus tree. Trees were visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/).

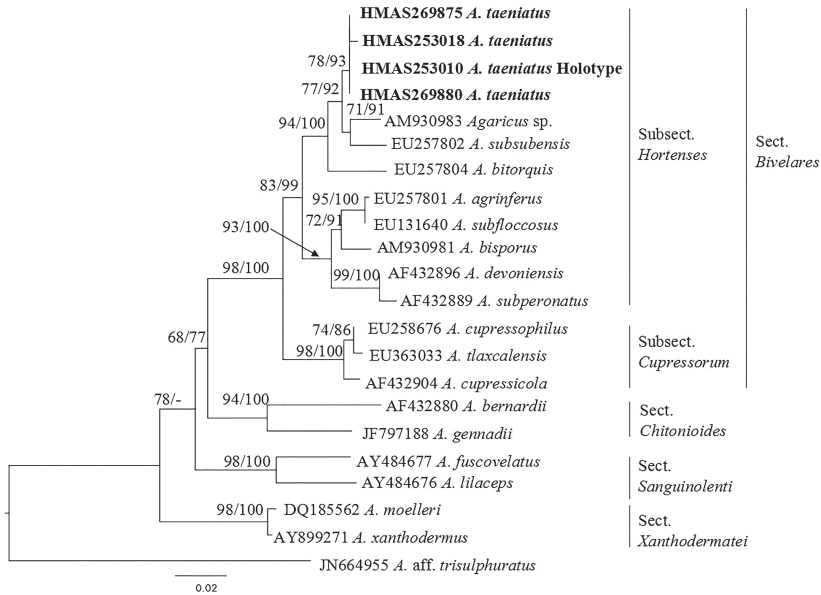


Fig. 1. Phylogenetic tree of *Agaricus* spp. generated from a maximum-likelihood analysis based on the ITS nrDNA sequence. Bootstrap support values (>50%) followed by Bayesian posterior probability values (>50%) are given at the internodes. The tree is rooted by *A.* aff. *trisulphuratus*. Sequences generated in this study shown in bold.

Results

The ITS alignment comprised 22 sequences representing 19 *Agaricus* taxa. *Agaricus* aff. *trisulphuratus* Berk., representing *A.* subg. *Lanagaricus* Heinem., was chosen as outgroup (Zhao et al. 2012b). The ML tree, which showed a topology identical to Bayesian tree, was selected to show the phylogeny of the new species (Fig. 1). The new taxon is well supported with good bootstrap and posterior probability values and is sister to the subclade of *A. subsubensis* and *Agaricus* sp. (AM930983).

Taxonomy

Agaricus taeniatus Sai F. Li, Shao J. Li & H.A. Wen, **sp. nov.** Figs 2–4

Fungal Names FN 570120

Differs from other species of *Agaricus* sect. *Bivelares* by its larger basidia and basidiospores, band-like velar remnants on the stipe surface, and persistent single rings with eroded edges.

Fig. 2. *Agaricus taeniatus* habit.
A. HMAS253016; B. HMAS253010 (holotype); C. HMAS269876; D. HMAS269875.

Type: China, Gansu Province, Minle County, Haichaoba, 38°23′50.03″N 100°40′02.90″E, alt. 2593 m, in forest dominated by *Picea crassifolia*, 10 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS60 (**Holotype**, HMAS253010; GenBank KJ623317); QLS15 (**Isotype**, HMAS253017; GenBank KJ623319).

Etymology: refers to the band-like velar remnants on the stipe surface.

Pileus 6–17 cm diam., irregularly hemispheric with a flat top when young, then expanding to plano-convex, and finally applanate and marge wavy; surface covered with fibril or fibrillose squamules, adpressed, Cinnamon (XXIX15″) to Mikado Brown (XXIX13″i) upon a barely discernible background, squamules patch-like on the disc, decreasing in size towards the margin; edge appendiculate; no discoloration on bruising. Context 7–20 mm thick from the centre of pileus to margin, firm, white, becoming Orange-Pink (II11f) at the disc, Jasper Red (XIII3′) at the lamellar/context interface. Lamellae free, 6–8 mm broad, 13–24/cm at the edge, white when young, maturing from light pink, light brown, brown and to finally dark brown. Annulus 6–17 mm broad, persistent, single, thick near the stipe and thinner toward an eroded edge, upper surface striate, lower surface fibrillose-woolly, having obvious cortinate fibrils

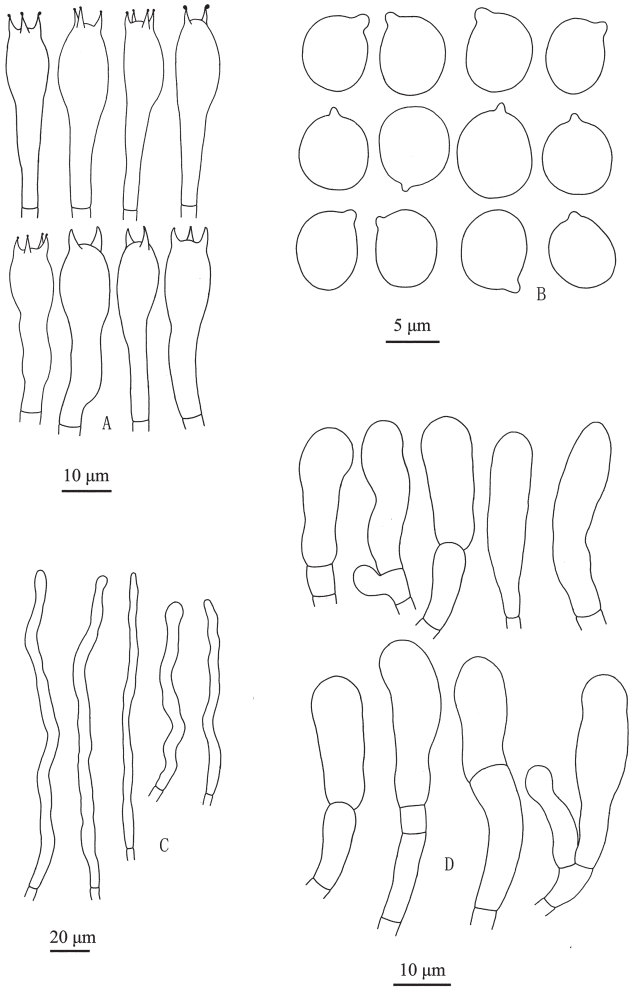
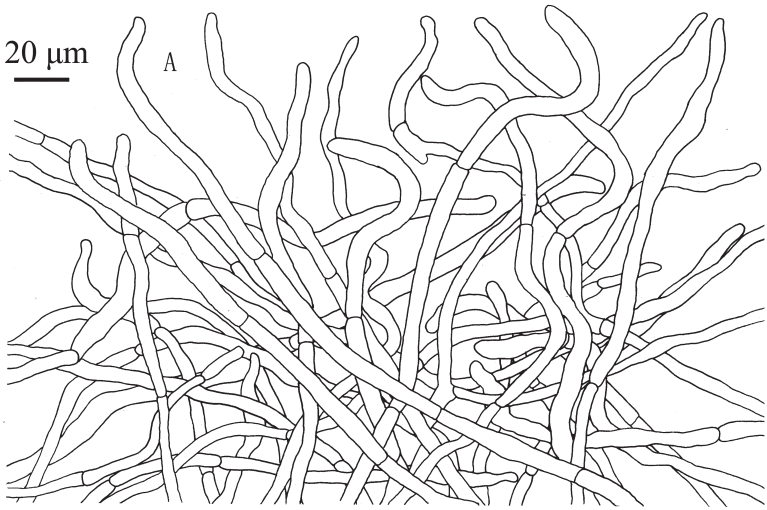


Fig. 3. *Agaricus taeniatus* (HMAS253010, holotype).
A. Basidia; B. Basidiospores; C. Hyphae from band-like remnants; D. Cheilocystidia.

at the stipe, generally superior, white. Stipe 2–6 × 7–22 cm, cylindrical, ± equal or (sometimes) distinctly bulbous with pointed base, solid, surface covered with small, appressed squamules above annulus, heavily floccose-fibrillose below annulus, white overall, Eosine Pink (I1d) around some squamules, bruising Ferruginous (XIV9′i); veils decorating stipe in a series of complete or partial transverse bands concolorous with the surface squamules, discernible when young; context white, becoming Vinaceous-Rufous (XIV7′i) when cut at



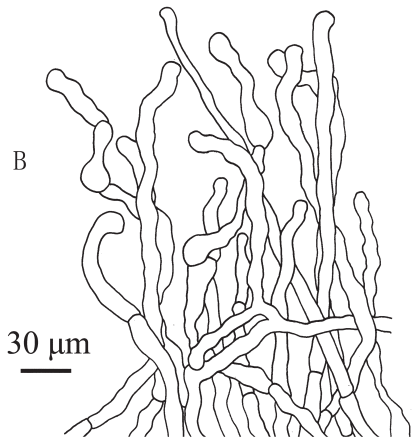


Fig. 4. *Agaricus taeniatus* (HMAS253010, holotype).
A. Hyphae from pileipellis; B. Hyphae from annulus.

the stipe base of stipe, Pale Flesh (XIV7′f) above base. Odor indistinct. Taste pleasant.

Macrochemical reactions: KOH and Schäffer reaction negative.

Basidiospores [120/8/8] (5.1–)5.7–7.1(–8.4) × (6.1–)6.8–8.6(–10.7) µm, average 6.3 × 7.6 µm, Q = (1.04–)1.11–1.30(–1.38), avQ = 1.20 ± 0.07, subglobose to broadly ellipsoid, without germ pore, thick-walled, smooth, dark brown when mature. Basidia 8–11(–12) × (32–)33–41(–42) µm, clavate, 4-spored; sterigmata 2.5–4.6 µm long. Cheilocystidia clavate to cylindrical, sometimes flexuose, usually septate with 1–3 basal septa, hyaline, smooth; terminal elements measuring (5–)6–9(–10) × (16–)18–38(–45) µm. Pleurocystidia absent. Pileipellis a cutis, composed of hyphae 3–14 µm diam., cylindrical, hyaline, smooth, not or slightly constricted at the septa; terminal cells 3–9 × 33–154 µm, slightly attenuated toward apex. Annulus hyphae 4–14 µm diam., cylindrical or irregularly cylindrical, inflated or constricted at some places of the hyphae, hyaline, smooth, not constricted, slightly constricted, or inflated at the septa; terminal cells 4–16 × 39–270 µm, slightly or sometimes obviously inflated toward apex. Band–like remnants composed of hyphae 2.3–6.6 µm diam., cylindrical, hyaline, smooth; terminal cells 4–9 × 83–163 µm, slightly attenuated toward apex. Clamp connection not observed.

Habit: Scattered or solitary in fluffy soil covered with lush moss in *Picea crassifolia* Kom. forest.

Additional specimens examined: **CHINA, Gansu Province,** Minle County, Haichaoba, 38°23′50.03″N 100°40′02.90″E, alt. 2593 m, 6 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS04 (HMAS253016; GenBank KJ623318); Sunan County, Kangle, 38°48′22.02″N 99°55′58.57″E, alt. 2766 m, 8 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS20 (HMAS269876; GenBank KJ623321); QLS21 (HMAS269875; GenBank KJ623322); QLS28 (HMAS253018; GenBank KJ623323); Haichaoba, 10 August 2013, S.F. Li, Y.L. Xi, C.X. Qi & Q.Q. Liang QLS64 (HMAS269880; GenBank KJ623320); QLS65 (HMAS253019; GenBank KJ623324).

Discussion

The brownish pileus squamules, superior annulus with veil remnants, reddish discoloration when cut, indistinct odor, and negative KOH and Schäffer reactions place *A. taeniatus* in *A.* subsect. *Hortenses* Heinem. (Kerrigan et al. 2008), which the ITS-based phylogeny supports.

Among the species of this section, the squamulose pileus and single annulus of *A. taeniatus* easily separate it from *A. bitorquis* and *A. devoniensi*s P.D. Orton, both of which have a smooth pileus surface and double rings. *Agaricus subperonatus* (J.E. Lange) Singer, which shares similar pileus characters and a stipe with complete or incomplete transverse bands, differs from *A. taeniatus* by forming a double-edged annulus that is <5 mm broad and slightly fibrillose on the underside (Bohus 1975, Parra 2008).

A stipe decorated with heavily floccose-fibrillose squamules and a series of complete or incomplete transverse bands are characters that easily separate *A. taeniatus* from other species of *A.* subsect. *Hortenses* characterized by a

relatively simple stipe surface (e.g., *A. maleolens* F.H. Møller, *A. subfloccosus* (J.E. Lange) Hlaváček, *A. agrinferus* Kerrigan & Callac, and *A. tlaxcalensis* Callac & G. Mata). Additional differences are: *A. maleolens* has stems with few ochraceous spots or small ochre squamules towards the base (Cappelli 1984, Lacheva & Stoichev 2004); *A. subfloccosus* occasionally has sparse woolly remnants on glabrous stipes (Parra 2008); *A. agrinferus* has glabrous to minutely floccose stipes sometimes with fibrillose zones below (Kerrigan et al. 2008); and *A. tlaxcalensis* has a longitudinally fibrillose and silky stipe surface (Kerrigan et al. 2008).

Agaricus cupressicola Bon & Grilli has been placed into different sections: either *A.* sect. *Bivelares* based on phylogenetic analysis (Kerrigan 2008) or *A.* sect. *Sanguinolenti* based on morphology (Parra 2008). This species differs from *A. taeniatus* by its much smaller basidiocarps, relatively strongly rufescent context, and thin annulus with an almost smooth lower surface.

Microscopically, *A. taeniatus* is easily separated from *A. bisporus* by its 4-spored basidia and from *A. cupressophilus* Kerrigan (3.7–3.9 × 4.6 µm) and *A. devoniensis* subsp. *bridghamii* Kerrigan (5.0–5.5 × 5.9–6.6 µm) by its much larger spores and longer cheilocystidia (19–23 µm in the two latter taxa). *A. cupressophilus* and *A. devoniensis* subsp. *bridghamii* are further distinguished by smaller pilei (3–7.5 cm diam.) and shorter stipes (1–2 × 4–8 cm) (Kerrigan et al. 2008). *Agaricus subedulis* Heinem. differs from *A. taeniatus* in forming shorter (<33 µm) basidia, wider (>9 µm) cheilocystidia, and irregularly shaped cheilocystidia with constricted ends (Heinemann 1956).

The phylogeny (Fig. 1) shows *A. taeniatus* most closely related to *A. subsubensis* Kerrigan and *Agaricus* sp. (AM930983). *Agaricus taeniatus* resembles *A. subsubensis* in the brownish pileus, reddish discoloration when sectioned and a series of thin broken ring-like remnants on the stipe. However, *A. subsubensis* has shorter (24–30 µm) basidia, a much less robust (0.5–1.5 × 0.5–4.5 cm) stipe, and a much more narrow, indistinct, band-like annulus (Kerrigan et al. 2008). *Agaricus* sp. (AM30983), collected from Lake Baikal district in southern Siberia, Russia, has been tentatively identified as *A. balchaschensis* Samgina & G.A. Nam but lacks sufficient taxonomic data for a positive identification (Hildén et al. 2013); *A. balchaschensis* differs from *A. taeniatus* in forming a stouter stipe (7–8 × 10–14 cm), movable annulus, a context that more strongly discolors (orange to cherry red) upon exposure, and shorter (23–34 µm) basidia (Samgina & Nam 1989).

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