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The edible wide mushrooms of *Agaricus* section *Bivelares* from Western China

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Abstract

Agaricus is a genus of macrofungi containing species with highly edible and medicinal values. A mushroom survey was recently carried out in Qilian Mountain National Natural Reserve, in Gansu Province of China, and yielded 21 *Agaricus* specimens. The morphological examination and phylogenetic analysis based on four-gene sequences from those specimens were conducted. The result shows they belong to four species in *A.* section *Bivelares*: *A. sinotetrasporus* sp. nov. and *A. qilianensis* sp. nov. are new species for science; *A. devoniensis* is a new record from China; and the famous button mushroom, *A. bisporus* is found in the wild. All of them are described and illustrated in details. A brief comparison with similar taxa or previous records are addressed too.

Key words – Agaricaceae – Multi-gene – Phylogeny – Taxonomy

Introduction

Agaricus L. is the type genus of the family Agaricaceae in the phylum Basidiomycota, it presently includes more than 500 edible or poisonous species (Zhao et al. 2016). The genus is distributed worldwide, and several species are served as food, including the commercially cultivated species such as *A. bisporus* (J.E. Lange) Imbach, the famous button mushroom, and *A. subrufescens* Peck, the almond mushroom (syn. *A. blazei* Murrill sensu Heinemann) and many non-cultivated species, such as *A. campestris* L. and *A. augustus* Fr. In recent years, several species, which have been described as new for science are consumed by local people for a long time or have potential edible and medicinal values, such as *A. flocculosipes* R.L. Zhao, Desjardin, Guinb. and K.D. Hyde (Zhao et al. 2012), *A. sinodeliciosus* Z.R. Wang and R.L. Zhao (Wang et al. 2015), and *A. taeniatus* Sai F. Li, Shao J. Li and H.A. Wen (Li et al. 2014).

Agaricus species have considerable ecological, nutritional and medicinal interests, yet the extent of its diversity remains poorly know in some areas, particularly in subtropical and tropical areas. The classification of *Agaricus* species was previously mainly based on species from the temperate regions of the north hemisphere and comprised eight widely accepted sections (Cappelli 1984, Parra 2008, 2013). After the inclusion of tropical samples, eleven potentially new sections were revealed by phylogenetic analysis based on ITS sequence data (Zhao et al. 2011). The recently

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reconstructed taxonomic system of *Agaricus* based on multi-gene sequences and molecular dating analysis, recognizes this genus as five subgenera and twenty sections (Zhao et al. 2016). Then Chen et al. (2017) added a sixth subgenus in this classification.

Even the taxonomic system of *Agaricus* has been updated largely in recent years, *A.* section *Bivelares* is keeping stable with its well morphological and molecular definitions. Section *Bivelares* was first described by Kauffman under the name of "*Psalliota*", and typified by *P. rodmanii* (Peck) Kauffman [Syn. of *A. bitorquis* (Quél.) Sacc.]. In 1897, Karsten formally transfer this genus under the older name of *Agaricus* L. Fr. ss. Karsten (= *Psalliota* Fr.) (Cappelli 1984), so section *Bivelares* moved into the genus *Agaricus*. The later studies well reorganized this section using morphological characters (Heinemann 1978, Wasser 1980, Cappelli 1984, Kerrigan 1986). Recent years, with the development of molecular biology, molecular phylogenetic analyses results support this section well (Challen et al. 2003, Didukh et al. 2004, Kerrigan 2008) and it was slightly modified as *A.* sect. *Bivelares* (Kauffman) L.A. Parra and characterized by negative Schäffer and KOH reactions, red discoloration of the context, a mild or indistinct odor and edibility (Parra 2008). In the new system of classification recently proposed, *A.* sect. *Bivelares* is one of the 12 sections of the *A.* subg. *Pseudochitonina* (Zhao et al. 2016).

Qilian Mountains National Natural Reserve locates at western China with the east longitude 97°25'~103°38', North latitude 36°45'~39°40', at an altitude of 2000m–4500m, with an alpine arid and semi-arid climate (Xi 2011). The sampling area was in the central area of the eastern slope of the Qilian Mountains, belonging to the continental cold and semi-arid and semi-humid forest grassland climate. We made a mushrooms survey on this area in 2016, and the sampling sites are about 2800–3000 meters above sea level. At this altitude, *Picea crassifolia* is the preponderant tree in those primary forests and scatter some shrub at the edge areas of the forests.

Materials & Methods

Morphological characters examination

Specimens were collected and photographed *in situ* and then kept separately in a box or packed by aluminum foil. Macro-morphological characters and Macro-chemical reactions were recorded on fresh specimens. Specimens were dried overnight in a food drier and sealed in plastic bags, then brought back laboratory. The micro-morphological observation was carried out under microscopy. Attention was paid to the basidiospores, basidia and cystidia and the anatomy of the pileipellis. Measurements of basidiospores, basidia and cheilocystidia are presented based on at least 20 measurements, and include the range of spore length by width x , the mean of all spores \pm SD (Standard Deviation); Q , the range of the quotient length/width of all basidiospores and Q_m , the mean Q of all spores \pm SD. Specimens cited were deposited in Herbarium Mycologicum Academiae Sinicae (HMAS), Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Molecular phylogenetic analysis

DNA extractions were made with a DNA extraction kit (Broad spectrum plant genomic DNA rapid extraction kit, Biomed, China). ITS1–5.8S–ITS2 region (ITS) of rDNA region were amplified by PCR reactions with primers ITS1/ITS4 or ITS5/ITS4 (White et al. 1990, Gardes & Bruns 1993), Large Subunit of rDNA region (LSU) amplified with primers LROR/LR5, elongation factor-1 α (EF1) gene sequence amplified with primers 983F/1953R, mitochondrial intermediate peptidase (MIP) gene sequence amplified with primers 91BF/91BR based on the previous studies (Thongklang et al. 2014, Zhao et al. 2016). All PCR products were sent to commercial biotechnological company (Biomed Co. Ltd, Beijing) for sequencing. The alignment is deposited to TreeBase (submission ID: 21021). (Reviewer access URL: <http://purl.org/phylo/treebase/phylovs/study/TB2:S21021?x-access-code=9f75b34777b3ef3c637feed580be30d7&format=html>)

The datasets were constructed by sequences from authority specimens retrieved from GenBank, along with those sequences produced from this study (indicated in Table 1). Sequences of each gene were aligned using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) separately, cut off the head and tail fragments to make it consistent in length. Then those four genes sequences were combined by SequenceMatrix1.7.8 (Gaurav 2010). Maximum likelihood (ML) phylogenetic tree and bootstrap values (BS) obtained from 1000 replicates were performed using RAXmlGUI 1.3 (Silvestro & Michalak 2012). Bayesian analyses were performed using MrBayes v3.2.6 (Ronquist & Huelsenbeck 2003) with the substitution model selected by MrModeltest 2.3 (Johan A. A. Nylander 2004). Four simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100th generation. The first 5,000 resulting trees were discarded as burn-in and the remaining trees were used to calculate Bayesian posterior probabilities (PP) in the majority rule consensus tree. Trees were viewed in FigTree1.4.3 (Andrew Rambaut 2006).

Table 1 Materials used in the phylogenetic analysis.

Collection NO.	Species	LSU GenBank	ITS GenBank	EF1 GenBank	MIP GenBank	Location
RWK1397_T	<i>A. agrinferus</i>	–	EU257801	–	–	California, USA
Bs423_T	<i>A. bisporus</i>	–	KF848699	–	–	Olonne-sur-mer, France
Bs261	<i>A. bisporus</i>	–	AF465404	–	–	Dinard, France
JB3-83	<i>A. bisporus</i>	–	AF465401	–	–	California, USA
MYA-4627	<i>A. bisporus</i>	–	GU327643	–	–	California, USA
ZRL20160021	<i>A. bisporus</i>	KY885133	KY885112	KY905103	KY905124	Gansu, China
ZRL20161813	<i>A. bisporus</i>	KY885138	KY885117	KY905108	KY905129	Gansu, China
ZRL20161854	<i>A. bisporus</i>	KY885139	KY885118	KY905109	KY905130	Gansu, China
ZRL20162049	<i>A. bisporus</i>	KY885143	KY885122	KY905113	KY905134	Gansu, China
LAPAG446	<i>A. bisporus</i>	KR006611	KM657920	KR006640	–	Burgos, Spain
LAPAG486	<i>A. bisporus</i>	-	KM657921	-	–	–
WZR2012827	<i>A. bitorquis</i>	KY885130	KY885109	KY905100	KY905121	Gansu, China
CA427	<i>A. bitorquis</i>	KT951491	KT951320	KT951646	–	–
RWK1462	<i>A. bitorquis</i>	–	AF432898	–	–	USA
LAPAG889	<i>A. cupressicola</i>	KT951465	KT951334	KT951649	–	Roma, Italy
G84.112_T	<i>A. cupressicola</i>	–	EU363031	–	–	Italy
FS-22_T	<i>A. cupressophilus</i>	–	EU258676	–	–	California, USA
ZRL20162196	<i>A. devoniensis</i>	KY885147	KY885126	KY905117	KY905138	Gansu, China
CA445	<i>A. devoniensis</i>	–	EU363036	–	–	Sardegna, Italy
ZRL20161021_T	<i>A. qilianensis</i>	KY885135	KY885114	KY905105	KY905126	Gansu, China
ZRL20161024	<i>A. qilianensis</i>	KY885136	KY885115	KY905106	KY905127	Gansu, China
ZRL20161797	<i>A. qilianensis</i>	KY885137	KY885116	KY905107	KY905128	Gansu, China
ZRL20161019	<i>A. qilianensis</i>	–	–	–	–	Gansu, China
ZRL20161810	<i>A. qilianensis</i>	–	–	–	–	Gansu, China
ZRL20162094	<i>A. qilianensis</i>	–	–	–	–	Gansu, China
WZR2012821_T	<i>A. sinodeliciosus</i>	KY885129	KY885108	KY905099	KY905120	Gansu, China
ZRL20152598	<i>A. sinodeliciosus</i>	KY885131	KY885110	KY905101	KY905122	Gansu, China
ZRL20160001	<i>A. sinodeliciosus</i>	KY885132	KY885111	KY905102	KY905123	Gansu, China
ZRL20161020_T	<i>A. sinotetrasporus</i>	KY885134	KY885113	KY905104	KY905125	Gansu, China
ZRL20161862	<i>A. sinotetrasporus</i>	KY885140	KY885119	KY905110	KY905131	Gansu, China
ZRL20162148	<i>A. sinotetrasporus</i>	KY885144	KY885123	KY905114	KY905135	Gansu, China

Table 1 Continued.

Collection NO.	Species	LSU GenBank	ITS GenBank	EF1 GenBank	MIP GenBank	Location
ZRL20162192	<i>A. sinotetrasporus</i>	KY885146	KY885125	KY905116	KY905137	Gansu, China
ZRL20162019	<i>A. sp</i>	KY885141	KY885120	KY905111	KY905132	Gansu, China
ZRL20162178	<i>A. sp</i>	KY885145	KY885124	KY905115	KY905136	Gansu, China
CA103	<i>A. sp</i>	–	EU363037	–	–	Oléron Island, France
RWK1994	<i>A. subfloccosus</i>	–	EU131640	–	–	New Mexico, USA
RWK1701	<i>A. subperonatus</i>	–	AF432902	–	–	Toronto, Canada
RWK1789	<i>A. subsubensis</i>	–	EU257802	–	–	California, USA
QLS60_T	<i>A. taeniatus</i>	KY885127	KJ623317	KY905097	KY905118	Gansu, China
QLS15	<i>A. taeniatus</i>	KY885128	KJ623319	KY905098	KY905119	Gansu, China
CA221_T	<i>A. tlaxcalensis</i>	–	EU363033	–	–	Tlaxcala, Mexico
CA339_OUT	<i>A. gennadii</i>	–	KT951318	KT951575	–	(=LAPAG 8) Burgos, Spain
LAPAG257_OUT	<i>A. nevoi</i>	KR006606	KM657922	KR006635	–	Burgos, Spain

T refers to holotype; OUT refers outgroup; **Bold** refers to the sequences produced from this study.

Results

The dataset consists of 4 genes sequences from 43 specimens representing 15 species of *A.* section *Bivelares* and two species of the close related section *A.* sect. *Chitonioides*, *A. gennadii* and *A. nevoi*, which were chosen as outgroups for rooting purposes (details see Table 1). The dataset consists of 2346 characters, of which 2055 are constant, 76 variable characters are parsimony–uninformative and 215 are parsimony–informative. The ML and Bayesian topologies were almost identical and the Bayesian tree was showed in Fig. 1. All taxa of *A.* section *Bivelares* form a well-supported monophyletic lineage (100% BS, 100% PP), and sister to the outgroup taxa *A. gennadii* and *A. nevoi*.

Specimen ZRL20162196 nests with CA445 (*A. devoniensis*) under a fully supports (100% BS, 1 PP). Specimens ZRL20161020, ZRL20161862, ZRL20162148 and ZRL20162192 form a monophyletic clade under the statistic supports of 97% BS, 1 PP, which represents as a new species *A. sinotetrasporus* in this study. Specimens ZRL20161019, ZRL20161021, ZRL20161024, ZRL20161797 and ZRL20161810, ZRL20162094 form another monophyletic clade under the statistic supports of 80% BS, 0.99 PP, which represents as another new species *A. qilianensis*. Specimens ZRL20160021, ZRL20161813, ZRL20161854 and ZRL20162049 cluster with *A. bisporus* from Europe and North America under 99% BS, 1 PP supports.

Taxonomy

Agaricus sinotetrasporus Y.L. Xi, M.Z. Zhang & R.L. Zhao, sp. nov.

Fig. 2

Fungal Names: FN570462

Etymology – the epithet of “sino” refers to the location of this species from China and “tetrasporus” refers to the 4–spored basidia.

Typus – China, Gansu Province, Zhangye City, Qilian Mountain National Natural Reserve, collected by R.L. Zhao, 31 August 2016, ZRL20161020 (HMAS 255155, holotype).

Pileus 20–40 mm in diam. parabolic, pulvinate, top flat when young; then 85–95 mm in diam. when mature, convex, plano–convex, centre subumbonate or slightly depressed, circular margin inrolled to decurved in age, exceeding, crenate in some cases; surface dry, covered by scales completely, appressed, often reddish brown or dark brown, sometimes brown.

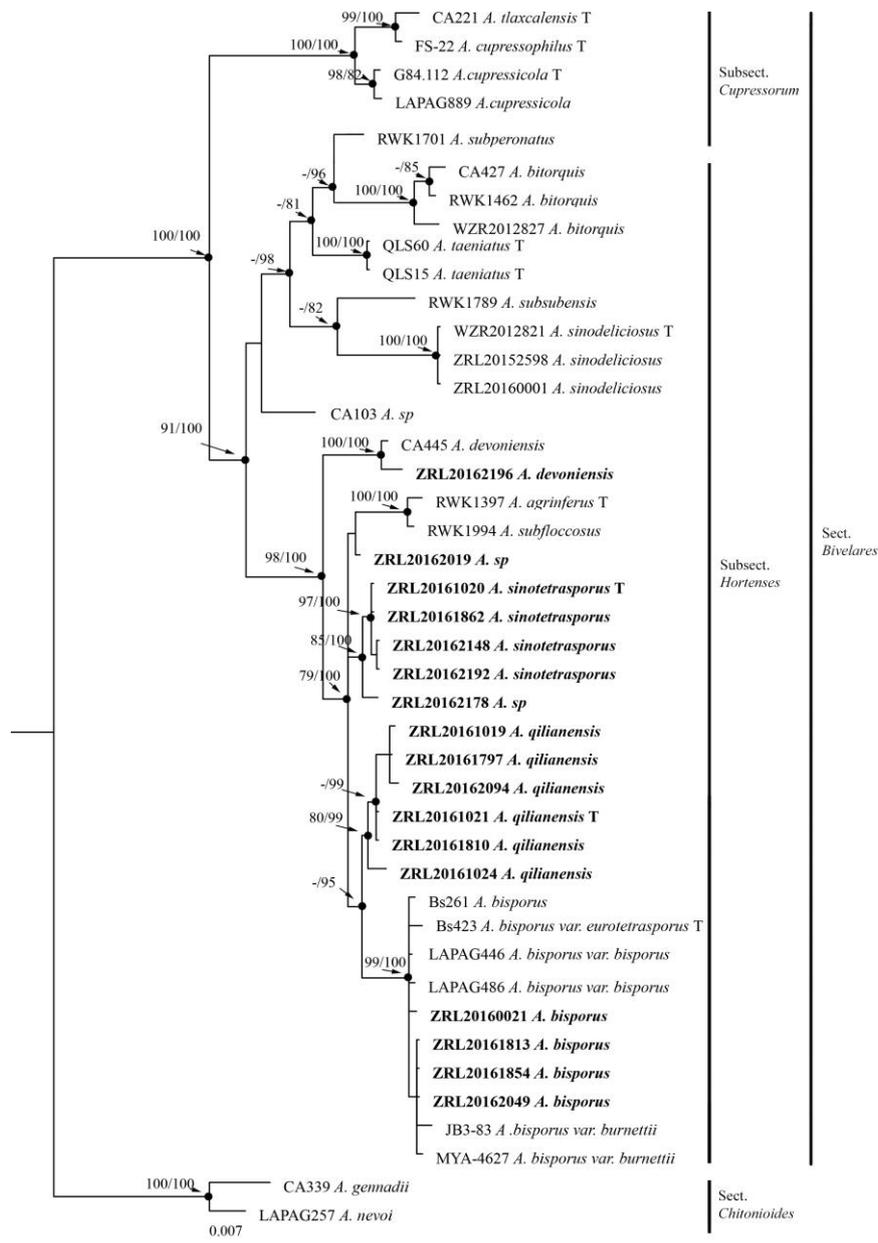


Figure 1 – Phylogeny of *Agaricus* section *Bivelares* generated from Bayesian analysis of four genes sequences, *A. gennadii* (CA339) and *A. nevoi* (LAPAG257) are outgroups. Parsimony bootstrap (BS) and Bayesian posterior probability (PP) values >79% are given at the internodes (BS/PP). Bolt refers to the sequences produced from this study; T refers to the type specimen.

Lamellae free, crowded, unequal, pink, brown to dark brown. Stipe 25–40 × 15–20 mm when young 55–65 × 8–11 (apex) – 25–30 (base) mm, cylindrical, clavate, solid to narrow hollow; surface fibrillose and heavily fibrillose scales towards base, erected, somehow concentric, white. Annulus membranous, superous, cottonous, thick, double, upper side often striate and lower side cogwheel towards margin, up to 15 mm broad, pendant, upper side smooth or slightly striate, white, lower side fibrillose and form discrete squames, white or light brown at the edge; Context 6–8 mm thick at the disc, white. Slightly reddish brown on scratching, and distinct rubescent discoloration on cutting. Odour pleasant, mushroomy.

Macrochemical reactions – KOH and Schäffer's reactions negative.

Basidiospores (5.8–) 6.3–7.6 × 4.5–5.7 μm [\bar{x} = 6.9 ± 0.4 × 5.0 ± 0.3, Q = 1.2–1.6, Q_m = 1.4 ± 0.1, n = 20], ellipsoid, brown, smooth and thick-walled, without germ pore. Basidia 24–29 (–33) × 7.6–12 μm, clavate, mostly 4-spored and rarely 2-spored, hyaline. Cheilocystidia 27–35 × 13–

16µm, mostly clavate, pyriform in some cases with long stipe, hyaline. Pleurocystidia absent. Pileipellis a cutis composed of hyphae 6.7–11 µm in diam., long cylindrical, curved, unbranched, containing brown pigments.

Habitat – scattered in *Picea crassifolia* forest.

Other materials examined – China, Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Kangle, collected by R.L. Zhao, 28 August 2016, ZRL20161862 (HMAS 281180); Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Dayekou reserve station, Alt. 2810~2980m, E 100°14'31.13", N 38°32'52.71", collected by R.L. Zhao, 31 August 2016, ZRL20162148 (HMAS 255153), ZRL20162192 (HMAS 281181).

Notes – This new species is similar to *A. bisporus* in morphology because both present a variable colour on pileus, such as from reddish brown, dark brown to near white; similar basidiospores and cheilocystidia in shape and size (Parra 2013). Furthermore, the tetrasporic basidia and basidiospores with an average of longer than 6 µm make *A. sinotetrasporus* similar to *A. bisporus* var. *eurotetrasporus* mostly (Callac et al. 2003). The distinct morphological characters to separate this new species from the known variety *A. bisporus* var. *eurotetrasporus* is the character of annulus, which is double and superous in new species vs single and intermediate or inferous for *A. bisporus* (Parra 2008). On the other hand *A. sinotetrasporus* only known from western China, while *A. bisporus* var. *eurotetrasporus* only known from Europe (Callac et al. 2003; Parra 2008). In the molecular phylogeny, the topology clearly shows *A. sinotetrasporus* is not related to *A. bisporus* var. *eurotetrasporus* which is represented by its type specimen Bs423 (Fig. 1). Then we propose those Chinese specimens as a novel species and characterized by 4-spored basidia and its Asia distribution. A preliminary cloning experiment for its *MIP* gene PCR amplification products shows the presence of SNP (Single nucleotide polymorphism). Then we could exclude that *A. sinotetrasporus* has a homothallic life cycle.

The unnamed specimen ZRL20162178 has a sister position with *A. sinotetrasporus* clade (Fig. 1) and phylogenetically it was possibly the same species to this new species. However we did not name it, as we lack its morphological information.

Agaricus qilianensis S.L. Wei, M.Z. Zhang & R.L. Zhao, sp. nov.

Fig. 3

Fungal Names: FN570460

Etymology – epithet “qilian” refers to Qilian Mountain where the type specimen is from.

Typus: China, Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Kangle, collected by R.L. Zhao, 28 August 2016, ZRL20161021 (HMAS 255156, holotype).

Pileus 30–45 mm when young, 97–102 mm in diam. parabolic to cushion when young, then convex, plano-concave, centre slightly depressed in old, margin straight and entire; surface dry, covered by fibrils completely, broken into fine squamoses towards the margin, and often woolly at the margin, brown or light brown against white background. Lamellae free, crowded, unequal, pink, reddish brown to dark brown in age. Stipe 15–25 mm when young; 65–75 × 9–11 mm, equal with a round base, solid, surface, fibrillose squamose, and floccose when young below the ring. Annulus pendant, membranous, thick, median, entire, pendant or subperonate, white, 5–7 mm in diam; Context 7–10 mm thick at the disc, white. Reddish brown on touching; rubescent on cutting. Odour pleasant, mushroomy.

Macrochemical reactions – KOH and Schäffer's reactions negative.

Basidiospores 6.0–7.3 × 4.6–6.5 µm [$x = 6.6 \pm 0.4 \times 5.5 \pm 0.4$, $Q = 1.1–1.4$, $Q_m = 1.2 \pm 0.1$, $n = 20$], ellipsoid, brown, smooth and thick-walled, without germ pore. Basidia 22–33 × 7.5–12 µm, clavate, 2-spored, hyaline. Cheilocystidia 17–41 × 7.9–14 µm, mostly clavate, pyriform in some cases with narrow stipe, hyaline. Pleurocystidia absent. Pileipellis a cutis composed of hyphae 4.5–15.9 µm in diam., long cylindrical, curved, branched, containing brown pigments.

Habitat – scattered in *Picea crassifolia* forest.

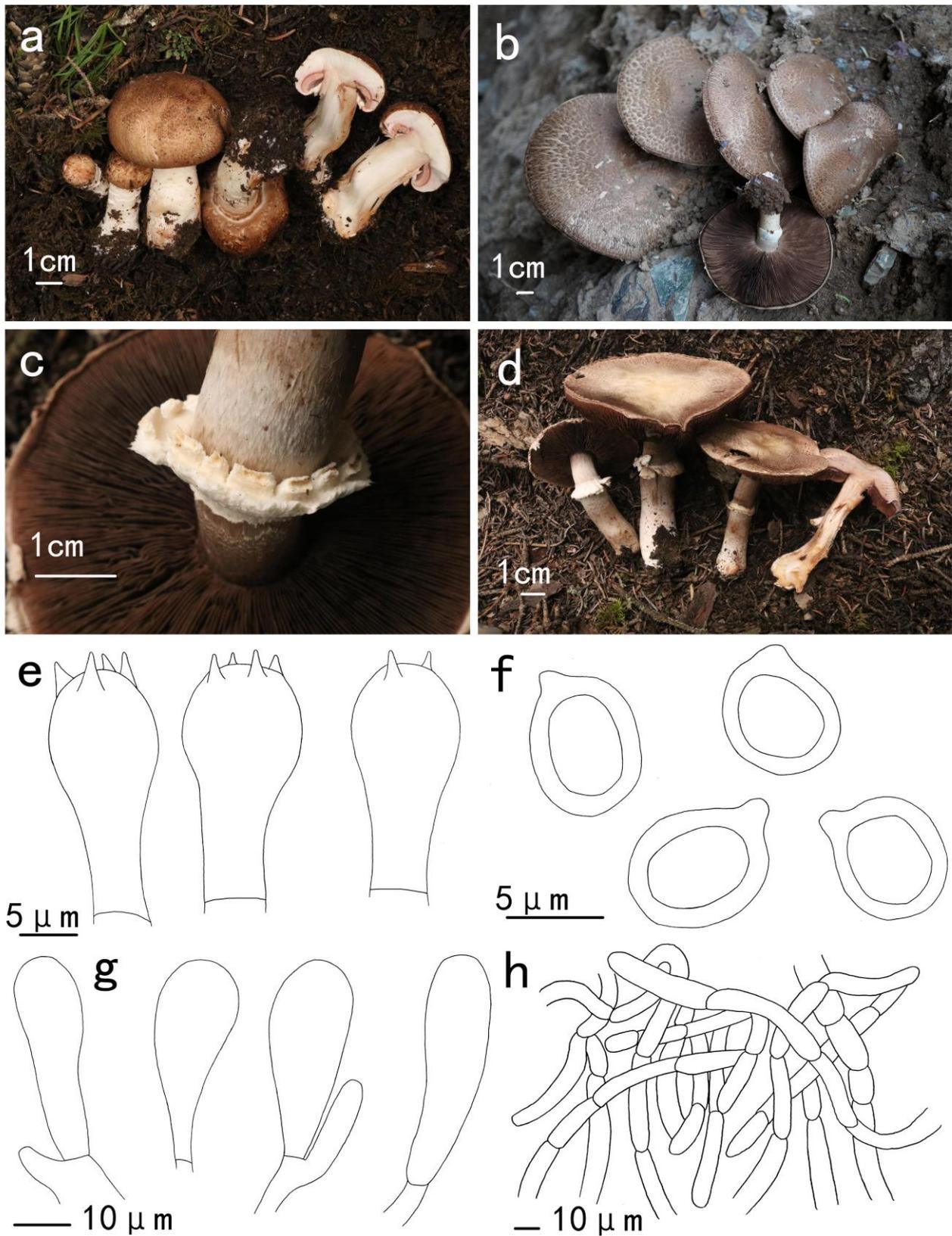


Figure 2 – Morphological characters of *Agaricus sinotetrasporus*. (b, e–h Holotype ZRL20161020; a specimen ZRL20162192; c, d ZRL20161862). a–d Morphology in field. e Basidia. f Basidiospores. g Cheilocystidia. h Pileipellis hyphae.

Other materials examined – China, Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Kangle grassland, Alt. collected by R.L. Zhao, 28 August 2016,

ZRL20161019 (HMAS 255213), ZRL20161024 (HMAS 255158); ZRL20161797 (HMAS 255157); ZRL20161810 (HMAS 255212). Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Yanzhi Forest Park, Alt. 2785 m, E101°14'20", N38°25'36", collected by R.L. Zhao, 31 August 2016, ZRL20162094 (HMAS 255214).

Notes – This new species is similar to *A. bisporus* var. *bisporus* in morphology, such as characters of cheilocystidia, basidiospores and pileus, especially both have the 2-spored basidia. However, the proposed new species *A. qilianensis* presents floccose to fibrillose squamose on the stipe surface especially when young, and related long stipe compared with the width of pileus; while those of *A. bisporus* is smooth or silky and related shorter stipe (Parra 2013). Furthermore, the phylogenetic analysis indicates they are different species (Fig. 1). A preliminary cloning experiment for its *MIP* gene PCR amplification products shows the presence of SNP (Single nucleotide polymorphism). Then we could exclude *A. qilianensis* has a homothallic life cycle.

Agaricus bisporus (J.E. Lange) Imbach, Mitt. naturf. Ges. Luzern 15: 15 (1946) Fig. 4

Pileus 30–80 mm in diam., 8–15 mm thick at the disc, circular, convex to planate, margin decurved, edge exceeding and entire; surface dry, covered by fibrils completely and broken into fibrillose scales, thick at disc and fading toward the margins, brown, ochreous brown. Lamellae free, narrow and crowded, unequal, intercalated with lamellulae, pink, pinkish brown, brown to dark brown. Stipe 40–70 × 8–13 mm, cylindrical, hollow, surface smooth or slightly fibrillose. Annulus membranous when young, then thick, intermediate, 5–8 mm in diam, entire, in median or apical position of stipe, slightly striate at the upper side; Context fresh, firm, white. Discoloration reddish brown on touching and cutting, Odour pleasant, mushroomy.

Macrochemical reactions – KOH and Schäffer's reactions negative.

Basidiospores 6.6–8.3 × 4.9–6.3 μm [$x = 7.3 \pm 0.6 \times 5.4 \pm 0.4$, $Q = 1.2–1.5$, $Q_m = 1.4 \pm 0.1$, $n = 20$], ellipsoid, brown, smooth and thick-walled, without germ pore. Basidia 16–22 × 5.8–8.0 μm, clavate, 2-spored. Cheilocystidia 18–36 × 9.2–13 μm, mostly clavate, a few pyriform, hyaline. Pleurocystidia absent. Pileipellis a cutis composed of hyphae 7.8–15 μm in diam., long cylindrical, curved, unbranched, containing brown pigments.

Habitat – scattered in *Picea crassifolia* forest.

Materials examined – China, Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Dayekou reserve station, Alt. 2810~2980m, collected by R.L. Zhao, 1 September 2016, ZRL20162049 (HMAS 255152); Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Kangle grassland, Alt. collected by R.L. Zhao, 28 August 2016, ZRL20161813 (HMAS 281179), ZRL20161854 (HMAS 279134).

The morphology of those specimens is matching *A. bisporus* well, and especially match the variety of *A. bisporus* var. *bisporus*, due to their 2-spored basidia and basidiospores with a length more than 6 μm (Callac et al. 2003; Parra 2008). However, in the molecular analysis they cluster with *A. bisporus* var. *burnettii*, which is a variety only known from western of the USA and characterized by 4-spored basidia and basidiospores with a length shorter than 6 μm (Callac et al. 2003; Parra 2008). In our present study, all those three varieties of *A. bisporus* are clustered together with full support (Fig. 1). However, the separation of varieties is still not clear in phylogeny. Then in this study we identified those Chinese specimens as *A. bisporus*, and the definition of variety needs a further study on their lifecycles even if a homothallic life cycle could be excluding, based on preliminary cloning analysis on *MIP* gene sequences.

Agaricus devoniensis P.D. Orton, Trans. Br. Mycol. Soc. 43(2): 173 (1960) Fig. 5

Pileus 25–30 mm in diam., 5–7 mm thick at the disc, circular, globose, convex, subumbonate, margin inflexed and entire surface dry, silky fibrillose to smooth, white to grey–white in age. Lamellae free, close, unequal, pink, pinkish brown. Stipe 30–35 × 8–10 mm, cylindrical, hollow, surface smooth or slightly fibrillose, white. Annulus membranous, 1–2 mm in diam, simple, fringed, inferous, superiors, peronate or subperonate, white; Context fresh, firm, white. Discoloration unknown on touching and cutting. Odour pleasant, mushroomy.

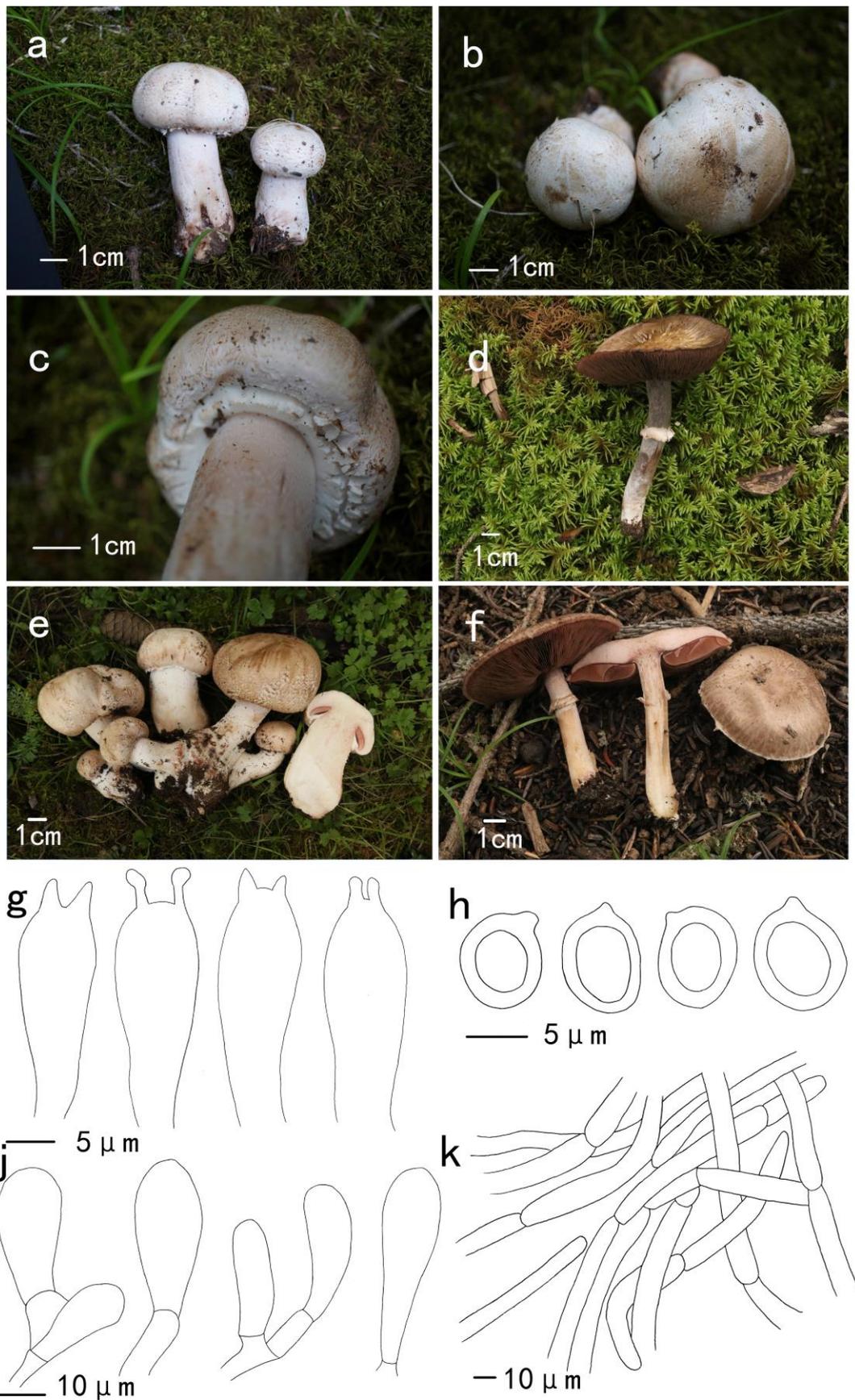


Figure 3 – Morphological characters of *A. qilianensis* sp. nov. (a–c, g–k Holotype ZRL20161021, d specimen ZRL20161797, e specimen ZRL20161810, f specimen ZRL20162094) a–f Morphology in field. g Basidia. h Basidiospores. j Cheilocystidia. k Pileipellis hyphae.

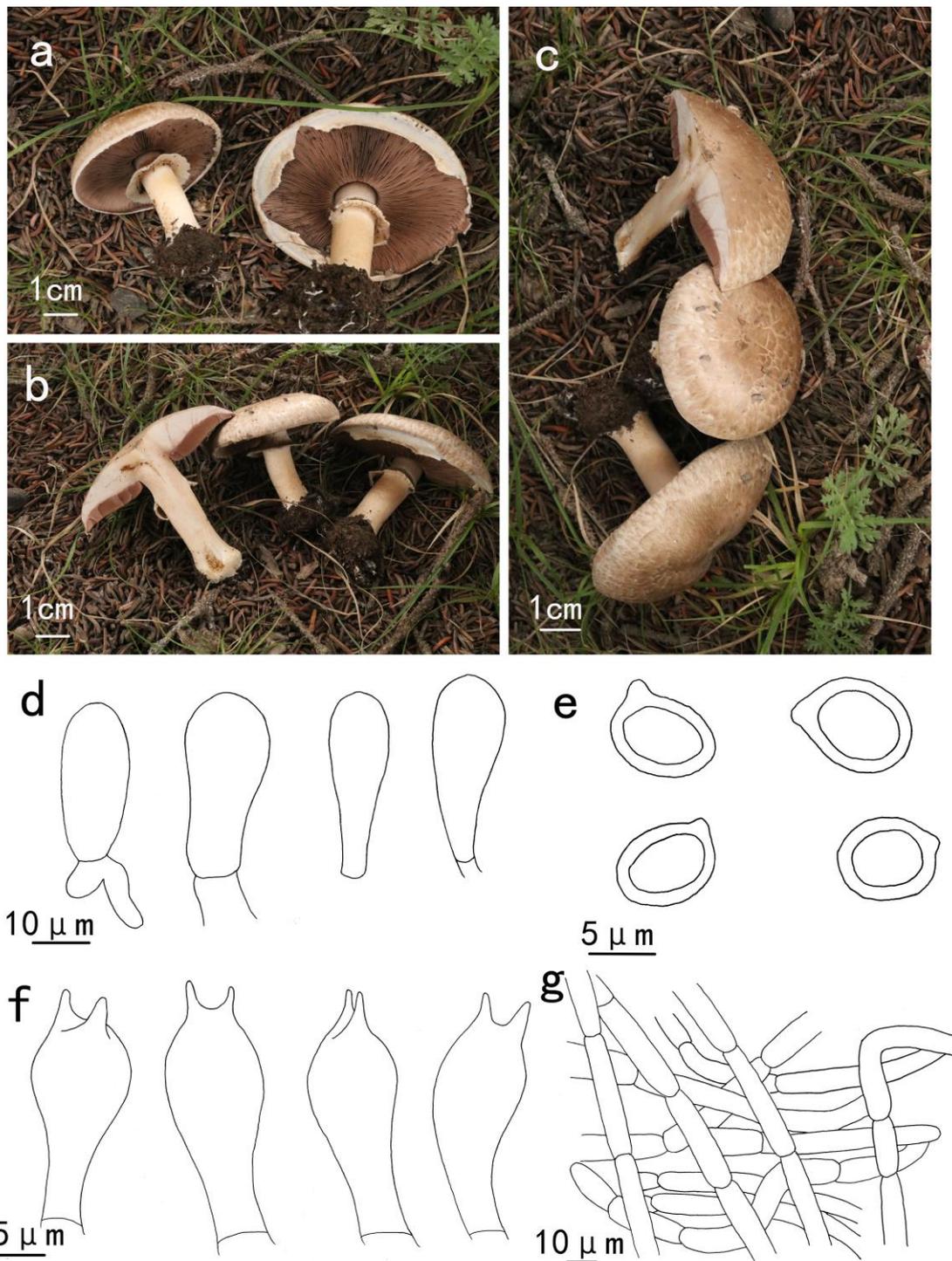


Figure 4 – Morphological characters of *A. bisporus* (specimen ZRL20162049) a–g Morphology in field. d Cheilocystidia. e Basidiospores. f Basidia. g Pileipellis hyphae.

Macrochemical reactions – KOH and Schäffer’s reactions negative.

Basidiospores $3.9\text{--}5.9 \times 3.4\text{--}5.2 \mu\text{m}$ [$x = 5.0 \pm 0.4 \times 4.2 \pm 0.4$, $Q = 1.1\text{--}1.3$, $Q_m = 1.2 \pm 0.1$, $n = 20$], ellipsoid, brown, smooth and thick-walled without germ pore. Basidia $15\text{--}20 \times 5.6\text{--}7.5 \mu\text{m}$, clavate, mostly 4-spored and rare 2-spored. Cheilocystidia $22\text{--}30 \times 7.9\text{--}12 \mu\text{m}$, mostly clavate, pyriform in some case with long stipe, hyaline. Pleurocystidia absent. Pileipellis a cutis composed of hyphae $8.5\text{--}20.5 \mu\text{m}$ in diam., long cylindrical, curved, unbranched, containing brown pigments.

Habitat – scattered in *Picea crassifolia* forest.

Materials examined – China, Gansu Province, Zhangye City, Sunan county, Qilian Mountain National Natural Reserve, Dayekou reserve station, Alt. 2810~2980m, E100°14'31.13", N 38°32'52.71", collected by R.L. Zhao, 31 August 2016, ZRL20162196 (HMAS 279135).

Note – The morphology of Chinese specimen matches the description of *A. devoniensis* well (Parra 2008) and the molecular analysis also confirms it is *A. devoniensis* because Chinese specimen cluster with *A. devoniensis* from Europe under fully support (Fig. 1). This species originally described in Europe is firstly recorded from China.

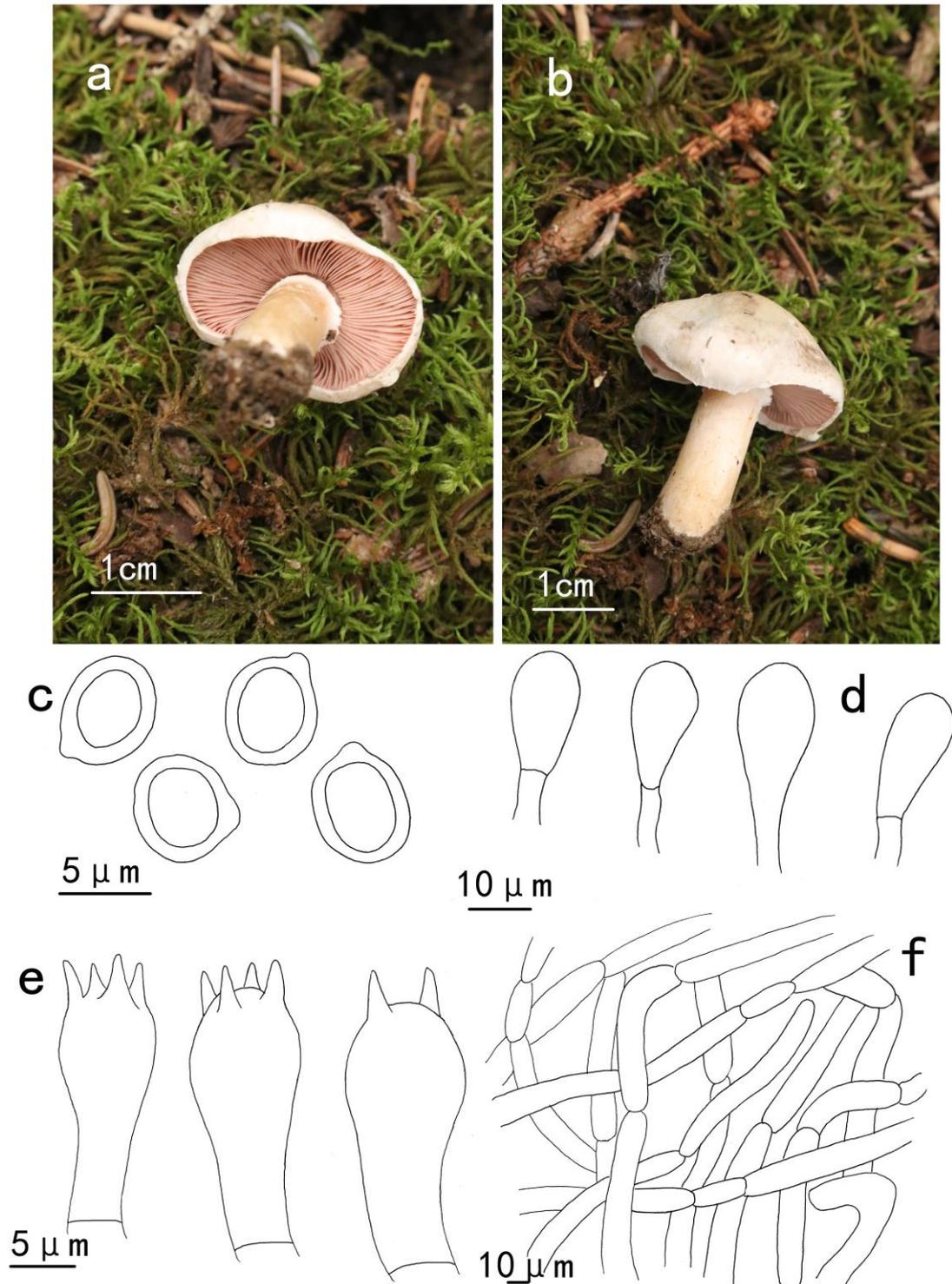


Figure 5 – Morphological characters of *A. devoniensis* (specimen ZRL20162196) a–f. Morphology in field, c. Basidiospores d. Cheilocystidia, e. Basidia, f. Pileipellis hyphae.

Discussion

As mentioned in the above the members of *A.* section *Bivelares* are edible and some of them are widely cultivated. Species from this section mostly distribute in temperate areas. Similarly, all species reported from this paper are from primary forests of Gansu Province where is in the western China and northern edge of Tibet Plateau, which belong to temperate climate too. In those areas, the vegetational types are simple and almost only composed of *Picea* species, there are highly species diversity in *A.* section *Bivelares*. Up to now three new species from this section have been reported from this area, and they are *A. sinotetrasporus* and *A. qilianensis* from this study, and *A. taeniatus* in a previous study (Li et al. 2014). Some known species which origin from Europe and Northern America also have been found in Qilian Mountain, such as *A. devoniensis*, *A. bisporus*. Then their biogeographic information would be interesting to the further study on *Agaricus* species origin and dispersal.

The previous studies showed the important cultivated species *A. bisporus* has several varieties and their lifecycles represent a critical character to separate this species into variety level (Callac et al. 2003, Challen et al. 2003). For example, *A. bisporus* var. *bisporus* is bisporic amphithallic and mostly pseudohomothallic; while *A. bisporus* var. *eurotetrasporus* and *A. bisporus* var. *burnettii* are both tetrasporic but the former is homothallic and the latter is amphithallic and mostly heterothallic with smaller spores (Callac et al. 2003). In this study, we could exclude Chinese *A. bisporus* has a homothallic life cycle. In the future work, their lifecycle should be studied in details, and that would be helpful not only in the identification at the varietal level, but also to develop them as newly cultivated food source.

Supplementary note – In the paper "Characterization of four species including one new species of *Agaricus* subgenus *Spissicaules* from Eastern China", published in the volume 7(4) of *Mycosphere*, the authors failed to indicate the registration number of the species *Agaricus catenariocystidiosus* which they newly described, so here is indicated a registration number for this name.

Agaricus catenariocystidiosus R.C. Dai & R.L. Zhao, *Mycosphere* 7(4): 407
Names of Fungi: NF570332

Fig. 2

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