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Two new species of *Acervus* (Pezizales) with a key to species of the genus

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Abstract: Studies on the genus *Acervus* from China are reviewed briefly. Six of the seven known species of the genus are currently recorded in this country. *Acervus beijingense* and *A. changchunense* are described as new based on morphological features and molecular data, and *A. flavidus* with minor emendation is added to the Chinese fungus flora. Phylogenetic relationships among four of the seven species of the genus are investigated based on the 28S rDNA sequence analysis. Species of the genus formed a single clade with high bootstrap support. The sequences of *A. epispartius* forma *epispartius* and that of *A. flavidus* obtained from materials in different geographical regions together formed a group with 100% bootstrap support. Ascospore size and shape are important criteria in the taxonomy of the genus and are phylogenetically informative. A dichotomous key to all described species of the genus is provided.

Key words: China, morphology, sequence analysis, taxonomy

INTRODUCTION

The fungal genus *Acervus* Kanouse is characterized by medium- to small-sized apothecia, various tints of yellow or orange of the hymenium, presence of a subiculum, mycelial pad or root-like structure at the apothecial base, thin-walled asci, which are not common in other members of Pyronemataceae, and small ascospores. It is probably saprobic and occurs on soil, duff and plant debris. A previous taxonomic and nomenclatural revision of the genus included only two species, *Acervus epispartius* (Berk. & Broome) Pfister and *A. flavidus* (Berk. & M.A. Cutris) Pfister, and it was determined that genus *Phaedropezia* Le Gal was a synonym (Pfister 1975). Since then a few taxa have been added to the genus (Moravec 1983, Zhuang and Korf 1989, Zhuang and Wang 1998) and a total of four species are currently accepted (Kirk et al. 2008). Species have been found in several tropical and subtropical regions around the world (Le Gal 1953, Korf 1963, Pfister 1975, Moravec 1983, Korf and Zhuang 1985). The phylogenetic position of the genus in the Pyronemataceae recently was confirmed by the 18S and 28S rDNA sequence analyses. The genus represents an independent lineage and seems not closely related to any other genera of the family (Liu and Zhuang 2006, Perry et al. 2007).

The first collection of *Acervus* in China was made in 1981 from the Qingcheng Mountains of Sichuan province in southwestern China by Prof R.P. Korf and identified as *A. epispartius* (Berk. & Broome) Pfister (Korf and Zhuang 1985). This species later was collected in different areas of China. Other taxa were added to the Chinese fungus flora based on collections from Xishuangbanna, Yunnan province, as *A. epispartius* forma *albus* Korf & W.Y. Zhuang (Zhuang and Korf 1989) and *A. xishuangbannicus* W.Y. Zhuang & Zheng Wang (Zhuang and Wang 1998) (Fig. 1). Liu et al. (2008) and Zhong et al. (2009) reported for the first time that *A. epispartius* forma *epispartius* is a disease-causing agent in the large-scale edible mushroom cultivation (''*Agaricus brasiliensis*'') of Fujian province in southeastern China (Fig. 2a). In the present study collections from Changchun of Jilin province in northeastern China and from Beijing are described as new species based on the combined data of morphology and DNA sequence analysis. Diagnos-

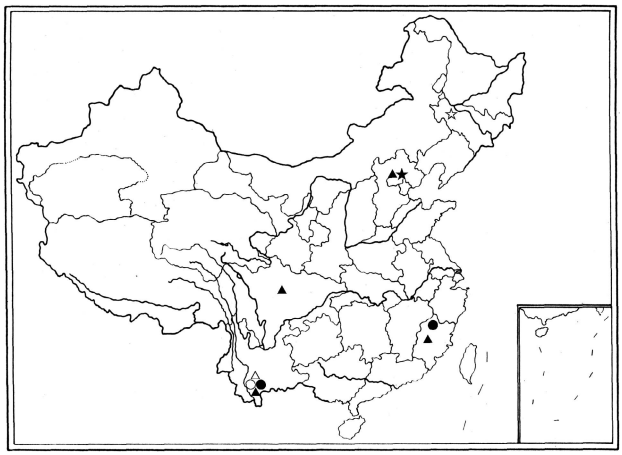

Fig. 1. China showing the localities of *Acervus* collections. ★*A. beijingense,* ☆*A. changchunense,* ▲*A. epispartius* f. *epispartius,* △*A. epispartius* f. *albus,* ●*A. flavidus,* ○*A. xishuangbannicus.*

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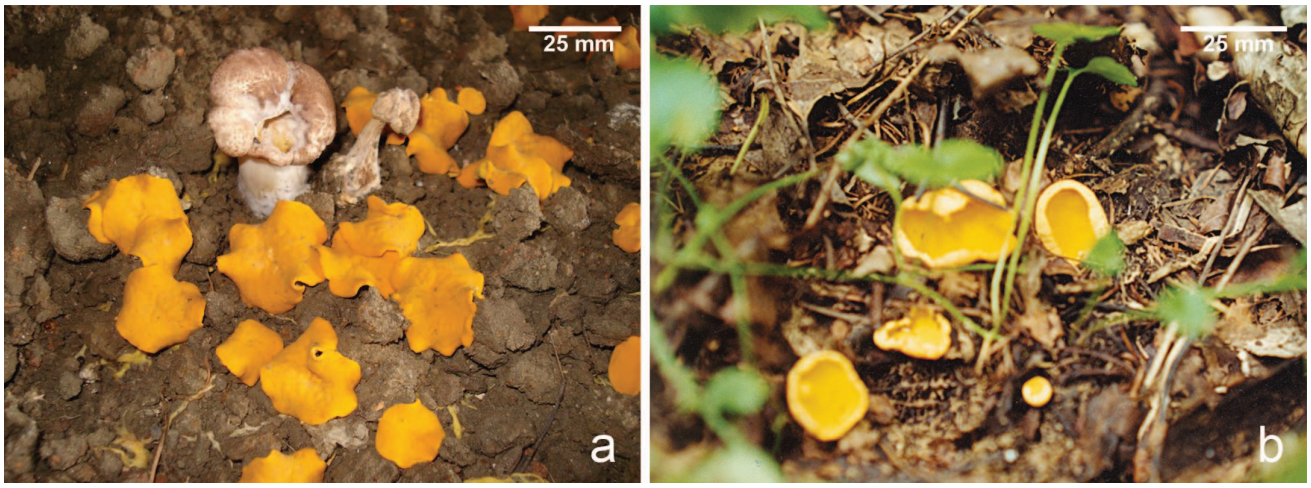

FIG. 2. Apothecia of *Acervus* spp. a. *A. epispartius* forma *epispartius* in mushroom cultivation farm in Fujian, provided by R.K. Lin (HMAS 173242). b. *A. changchunense* on duff (HMAS 78146) from Jilin.

tic features of *A. flavidus*, a new record for China, are provided based on observations of the Chinese materials. So far all species of the genus, except *A. lusakianus* J. Moravec known only from type locality in Zambia, have been recorded in different areas of China. It appears that it is in China that the genus attains its highest species diversity.

All species of *Acervus* share similar anatomic structures of excipulum and have thin-walled asci. Shape and size of ascospores are considered to be the most important characters in taxonomy of species in this genus along with apothecial gross morphology. A dichotomous key to the seven currently known taxa of the genus is provided.

MATERIALS AND METHODS

Morphological study.—Materials from China examined were collected 1981–2008. Apothecia were rehydrated and sectioned on a freezing microtome (YD-1508A, Yidi Medical Instrument Co., Jinhua, China) at ca. 15 μm thick. Measurements were taken from sections mounted in lactophenol cotton blue solution and from squash mounts in the same medium. Photographs were taken with Canon G5 digital camera connected to a Zeiss Axioskop 2 plus microscope.

DNA extraction, amplification, sequencing and phylogenetic analysis.—Chelex-100 chelating resin (Sigma, USA) was applied to extract genomic DNA from the dried apothecia according to the method of Zhang et al. (2006) with modifications. The apothecia or fragments of an apothecium were transferred to a 1.5 mL Eppendorf tube, mixed with equal volume of quartz sand and thoroughly ground with a glass pestle 10 min. Then 200 μL 10% w/v Chelex-100 was added and vortexed 10 s. The tube was incubated at 56 C for 2 h, mixed 10 s and incubated at 99 C for 10 min. After centrifuging at 12 000 rpm 10 min the supernatant was transferred into another 1.5 mL tube filled with four-fifths volume of 100% isopropanol. The mixture was placed at −20 C overnight and centrifuged at 12 000 rpm 15 min. After rinsing with 200 μL 75% ethanol the precipitant was dried at room temperature and dissolved in 30 μL TE or ddH_2O as PCR template.

28S rDNA were amplified with the primer pairs, LROR–LR5 (Vilgalys and Hester 1990). The PCR reaction mixture (50 μL) contained 5.0 μL 10× PCR buffer, 3.0 μL $MgCl_2$ (25 mM), 2.5 μL sense primer (10 μM), 2.5 μL antisense primer (10 μM), 1.0 μL dNTP (10 mM each), 2.5 μL DNA template, 0.5 μL Taq polymerase (5.0 U/μL) and 33 μL ddH_2O. Reactions were performed on the 2720 Thermal Cycler (Applied Biosystems, USA) with cycling conditions of denaturation at 95 C for 5 min, followed by 35 cycles of denaturation at 94 C for 30 s, annealing at 55 C for 30 s and elongation at 72 C for 60 s, with a final extension step at 72 C for 5 min to complete the reactions. Amplicons were purified with the PCR Product Purification Kit (Biocolor BioScience & Technology Co., Shanghai) and sequenced with the BigDye Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730XL DNA Sequencer. The amplifying primers served as sequencing primers. Final sequences were checked and edited manually with BioEdit 7.0.5 (Hall 1999). DNA sequences were mostly from our research group plus two retrieved from GenBank (TABLE I).

All sequences were aligned with Clustal X 1.8 (Thompson et al. 1997). A neighbor joining tree was generated with MEGA 4.10 (Tamura et al. 2007) based on 28S rDNA with *Aleuria aurantia* (Pers.) Fuckel and *Otidea onotica* (Pers.) Fuckel as outgroup taxa. Kimura 2 parameter was selected as the nucleotide substitution model, and gaps or missing data were pairwise deleted. Bootstrap method was performed with 1000 replicates to test phylogeny branch support.

TAXONOMY

Acervus beijingense W.Y. Zhuang, sp. nov. FIG. 3a–c
MycoBank MB518352

TABLE I. 28S rDNA sequences of *Acervus* analyzed in this study

Species	Geographic origin	28S nrDNA	
		Collection number	GenBank number
Acervus beijingense	China	HMAS 78150	**HM197754**[a]
Acervus changchunense	China	HMAS 78146	**HM197752**
Acervus epispartius f. *epispartius*	China	HMAS 78149	**HM197753**
	China	HMAS 173242	**HM197755**
	USA	A. Bessete 1984	DQ220305
Acervus flavidus	Puerto Rico	DHP PR98.2	DQ220306
	China	HMAS 188443	**HM197756**
Aleuria aurantia	USA	AFTOL-ID 65	AY544654
Otidea onotica	USA	s.n. 2.13.98	DQ220387

[a] Numbers in boldface indicate the newly submitted sequences.

Ab *Acervus epispartius* f. *epispartius* apotheciis cupulatis vel disciformibus, 10–30 mm diam; hymeniis flavis; ascis subcylindicis, 86–95 × 6–8 μm; ascosporis late ellipsoideis, guttulatis, 6.5–7.8 × 4.4–5.8(–6.2) μm differt.

Apothecia arising from a mycelia pad consisting of subhyaline to pale yellow hyphae, cupulate to discoid, sessile, up to 30 mm diam; hymenium surface lemon yellow (Ridgway 1912) when fresh, cadmium yellow (Ridgway) when dry; receptacle surface lighter than hymenium surface, nearly smooth. Ectal excipulum of textura angularis to textura epidermoidea, 70–100 μm thick, cells angular to nearly isodiametric, thin-walled, hyaline, 20–32 × 8–20 μm, 10–32 μm diam if isodiametric. Medullary excipulum of textura intricata, 100–1130 μm thick and thicker near the apothecial base, hyphae thin-walled, hyaline, 5–13 μm wide. Subhymenium not clearly distinguished from medullary excipulum, ca. 0–25 μm thick. Hymenium 100–110 μm thick. Asci subcylindrical, thin-walled, apical apparatus not clearly seen under light microscope, eight-spored, J− in Melzer's reagent, 86–95 × 6–8 μm. Ascospores broadly ellipsoid, with blunt ends, smooth-walled, hyaline, unicellular, usually with 1 or 2 guttules, uniseriate, 6.5–7.8 × 4.4–5.8(–6.2) μm. Paraphyses filiform, 3.5–5.5 μm wide.

Etymology. The specific epithet refers to the type locality.

Holotype. CHINA. BEIJING: outside the Beijing Botanical Garden, on soil and duff among grass, 29 Aug 2000, *W.Y. Zhuang, Z.H. Yu, Y.H. Zhang* and *X.M. Zhang 3622*, HMAS 78150.

Notes. Among the known species of *Acervus A. beijingense* is somewhat similar to *A. epispartius* forma *epispartius* in apothecial shape and size, but its disk is yellow and paler than that of the latter fungus. They are also similar in ascospore length, but the ascospores of *A. beijingense* appear to be wider (6.5–7.8 × 4.4–5.8[–6.2] μm vs. 5.8–7.5[–9] × 3.2–4 μm [from Pfister 1975]), and its spore is thus broadly ellipsoid instead of ellipsoid. The separation of *A. beijingense* from *A. epispartius* forma *epispartius* also was supported by our 28S rDNA sequence analysis; this will be discussed below.

Acervus changchunense W.Y. Zhuang, sp. nov.

FIGS. 2b, 3d–i

MycoBank MB518353

Ab *Acervus beijingense* apotheciis cupulatis, 11–25 mm diam; ascis subcylindicis, 97–114 × 7–9 μm; ascosporis ellipsoideis, guttulatis, 6.7–8.9 × 4.4–5.6 μm differt.

Apothecia arising from a thick mycelia pad consisting of subhyaline to pale yellow hyphae, cupulate, sessile, 11–25 mm diam; hymenium pale lemon yellow to lemon yellow (Ridgway) when fresh, orange-buff (Ridgway) when dry; receptacle surface lighter than hymenium surface, nearly smooth. Ectal excipulum of textura angularis to textura epidermoidea, 150–250 μm thick, cells angular to isodiametric, thin-walled, hyaline, 15–50 × 11–40 μm. Medullary excipulum of textura intricata mixed with textura angularis, 250–1200 μm thick at flanks, thicker near base, hyphae thin-walled, subhyaline, 4–15 μm wide. Subhymenium 0–30(–38) μm thick. Hymenium 95–110 μm thick. Asci subcylindrical, thin-walled, apical apparatus not clearly seen under light microscope, eight-spored, J− in Melzer's reagent, mostly 97–114 × 7–9 μm. Ascospores ellipsoid, with blunt ends, smooth-walled, hyaline, unicellular, uni- to biguttulate or eguttulate, uniseriate, 6.7–8.9 × 4.4–5.6 μm. Paraphyses filiform, 3.5–4.5 μm wide.

Etymology. The specific epithet refers to the type locality.

Holotype. CHINA. JILIN: Changchun, Jingyuetan, on duff, 18 Aug 2000, *W.Y. Zhuang* and *Z.H. Yu 3580*, HMAS 78146.

Notes. Among the known species of the genus *Acervus changchunense* is most similar to *A. beijingense* in apothecial shape, size and disk color, but the ascospores of *A. changchunense* are longer and

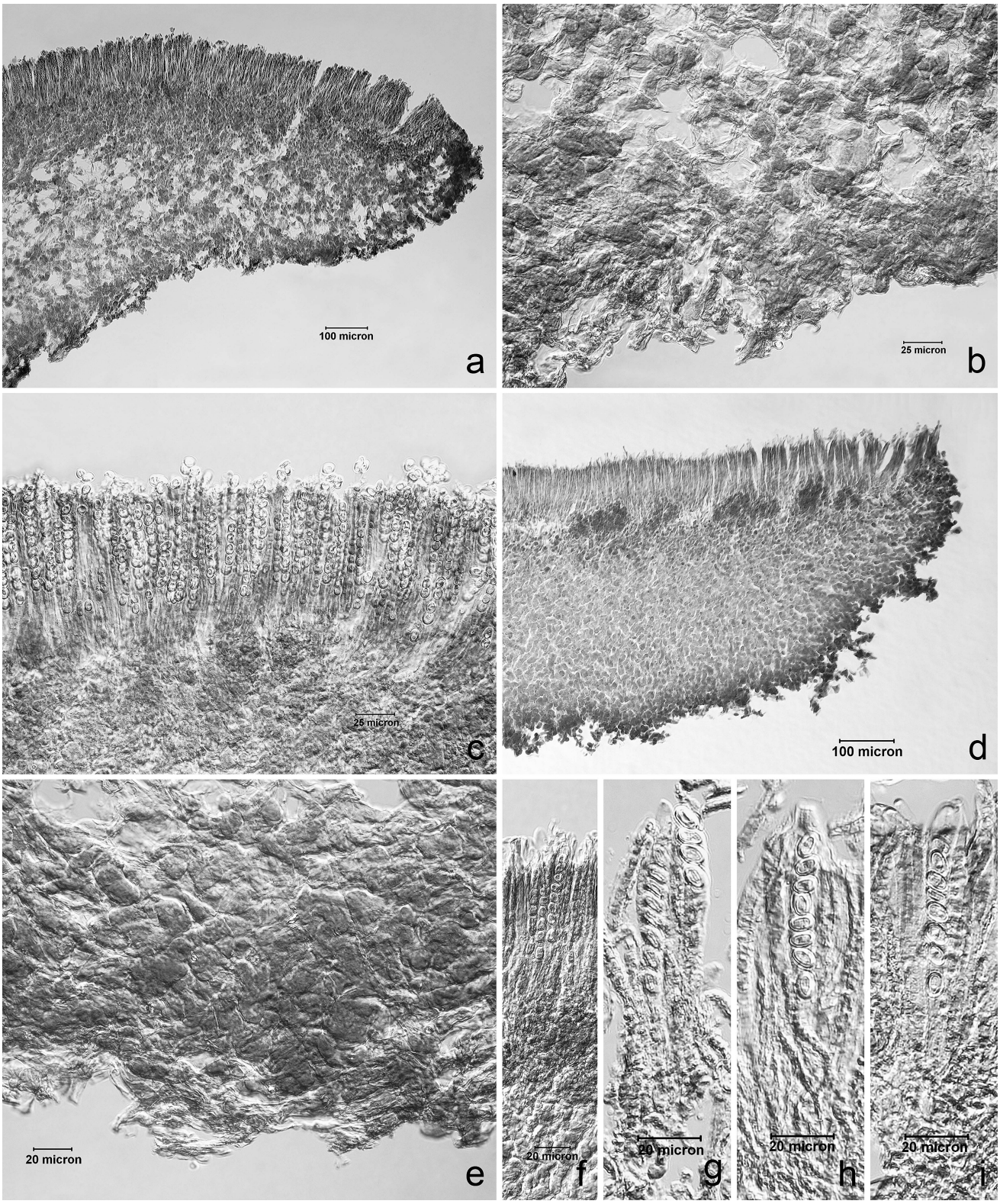

FIG. 3. *Acervus* spp. a–c. *A. beijingense* (HMAS 78150). a. Structure of apothecium at and near margin. b. Ectal excipulum at flank. c. Hymenium and a portion of medullary excipulum. d–i. *Acervus changchunense* (HAMS 78146). d. Structure of apothecium at and near margin. e. Ectal excipulum at flank. f. Hymenium and a portion of medullary excipulum. g–i. Asci and ascospores.

ellipsoid instead of broadly ellipsoid as shown in *A. beijingense* (6.7–8.9 μm vs. 6.5–7.8 μm long). Differences also are found in the size of asci, which are longer in *A. changchunense*. The morphological distinctions between the above two species are again supported by the sequence analysis of 28S rDNA, in which *A. changchunense* is distantly related to *A. beijingense*; this will be discussed below in the phylogenetic study.

Acervus flavidus (Berk. & M.A. Curtis in Berk.) Pfister, Occ. Pap. Farlow Herb. Crypt. Bot. 8 : 5, 1975. (with minor emendation)

≡ *Psilopezia flavida* Berk. & M.A. Curtis, in Berkeley, Grevillea 4 : 1, 1875.

Apothecia discoid, nearly sessile, up to 8 mm diam; hymenium lemon yellow (Ridgway) when young, becoming discolored, slightly pinkish or light orange; receptacle surface nearly white, villose. Ectal excipulum of textura angularis to textura globulosa, 50–75 μm thick, cells nearly isodiametric, thin-walled, hyaline, mostly 10–18 μm diam. Medullary excipulum of textura intricate, 100–160 μm thick or thicker, hyphae thin-walled, subhyaline, 4–10 μm wide. Subhymenium not well developed, 0–25–40 μm thick. Hymenium 140–170 μm thick. Asci subcylindrical, thin-walled, eight-spored, J− in Melzer's reagent, 124–152 × 7.5–10.5 μm. Ascospores regularly ellipsoid, with blunt ends, smooth, thin-walled, hyaline, unicellular, biguttulate, uniseriate, 10.5–12.5 × 6–7.5 μm. Paraphyses filiform, ca. 3 μm wide.

Specimens examined. CHINA. YUNNAN: ''53 km'' mark on the road from Mengyang to Xishuangbanna Botanical Garden, on rotten roots, 24 Oct 1988, *R.P. Korf, M. Zang, K.K. Chen* and *W.Y. Zhuang 303*, HMAS 72133; FUJIAN: Wuyi Mountains, on soil, 21 Sep 2006, *W.Y. Zhuang, J. Luo, W.Y. Li* and *L. Wang 6921*, HMAS 188443.

Notes. The Chinese collections are similar in apothecial shape and ascospore size to *Acervus flavidus* described by Pfister (1975) based on materials from Congo, Madagascar, Peru, Puerto Rica and Venezuela. The Yunnan collection was recorded previously as ''*Acervus* cf. *flavidus*'' due to the variations in ascospore features (Zhuang and Wang 1998). A later collection from Fujian province is identical in morphology to the Yunnan collection. The ascospores of the Chinese specimens are regularly ellipsoid, thin-walled and 10.5–12.5 × 6–7.5 μm, whereas ascospores of specimens from other countries are regularly to irregularly ellipsoid, thick-walled, and (7.5–)9–13 × 5 × 5–7 μm (Pfister 1975). However when the 28S rDNA sequence of HMAS 188443 from China was obtained it turned out to be exactly the same as that of *A. flavidus* (DQ220306) from Puerto Rico (Perry et al. 2007). These collections should be conspecific. The distinction in spore outline is thus treated as infraspecific variations. *Acervus flavidus* is added to the Chinese fungus flora.

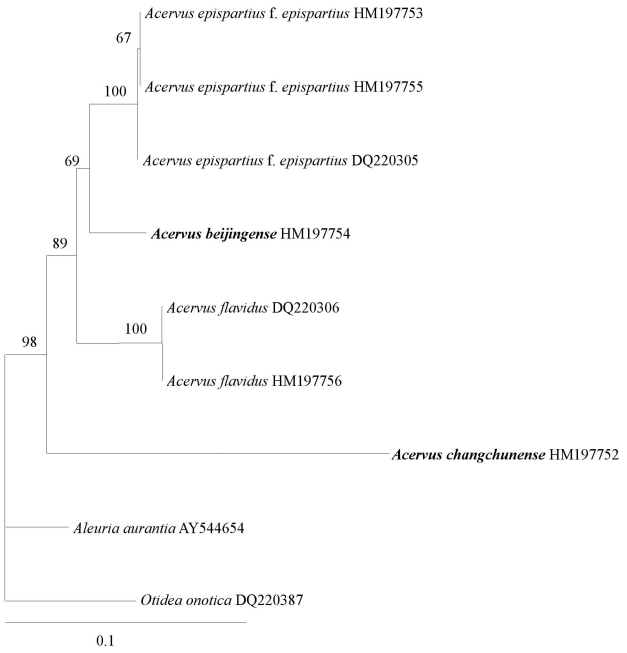

FIG. 4. Neighbor joining tree based on 28S rDNA, showing the relationships among four *Acervus* species. Bootstrap value ≥ 50% are noted above internode. TreeBase No. 10713.

DISCUSSION

Only two 28S rDNA sequences representing two species of *Acervus* were available previously at GenBank (Perry et al. 2007). During this study five more sequences belonging to four species were added to the analysis (TABLE I). The neighbor joining tree with *Aleuria aurantia* and *Otidea onotica* as outgroup taxa showed that all *Acervus* species formed a clade with 98% bootstrap support (FIG. 4). Three sequences of *A. epispartius* forma *epispartius* from different geographical regions grouped together with 100% bootstrap support. This indicates that this species is widespread and shows stable morphology among collections. *Acervus beijingense*, *A. changchunense* and *A. flavidus* are distinguishable, which gives additional support to taxonomy based on morphological characteristics, especially the size and shape of ascospores.

Among the Chinese *Acervus* specimens examined two are from Beijing. *Acervus epispartius* forma *epispartius* (HMAS 78149) was collected in woods inside the Beijing Botanical Garden, and *A. beijingense* (HMAS 78150) was located outside the gate of the same garden. They are distinct morphologically in spore shape and size, as well as in apothecial color.

This agrees with our molecular data. Sequences of all three collections of *A. epispartius* forma *epispartius* grouped together with a bootstrap value of 100%, but *A. beijingense* was related to them with only 69% bootstrap value (FIG. 4). *Acervus changchunense*, even though similar to *A. beijingense* in shape and color of apothecia, has different spore size and shape, is distantly related to the latter species and forms a basal lineage in the neighbor joining tree (FIG. 4). In both cases the ascospore features appear to carry the phylogenetic information.

In spite of the minor variations in spore character, collections of *Acervus flavidus* from China and Puerto Rico shared the same 28S rDNA sequence, were together with 100% bootstrap support and composed an independent lineage in the neighbor joining tree (FIG. 4). Having a similar fruit body color but larger ascospores (10.5–12.5 × 6–7.5 μm) and smaller apothecia, *A. flavidus* is related to and distinct from *A. beijingense* (6.5–7.8 × 4.4–5.8[–6.2] μm) and *A. changchunense* (6.7–8.9 × 4.4–5.6 μm), which reveals again that the size and shape of ascospores are phylogenetically informative.

It seems that apothecial color is also taxonomically distinguishable. We unfortunately failed to obtain DNA from the scanty type materials of *A. epispartius* forma *albus* having pure white apothecia and *A. xishuangbanicus* possessing egg yolk-yellow hymenium. Whether apothecial color is of phylogenetic importance awaits more extensive investigations of *Acervus* in nature and intensive studies on species diversity of the genus worldwide.

KEY TO THE KNOWN TAXA OF *ACERVUS*

1. Ascospores globose to subglobose, 3.7–5.2(–5.4) μm diam or up to 5.4 × 4 μm . . . . . ***A. lusakianus***
1. Ascospores ellipsoid to broadly ellipsoid 2
2. Apothecia more than 10 mm diam 3
2. Apothecia less than 10 mm diam 6
3. Hymenium light yellow to pale yellow; ascospores 4.4–5.8 μm wide 4
3. Hymenium yellow to orange yellow; ascospores 6–7.5(–8) × (3.3–)3.7–4.5 μm . . . . . ***A. epispartius*** 5
4. Ascospores ellipsoid, 6.7–8.9 μm long . . . . . ***A. changchunense***
4. Ascospores broadly ellipsoid, 6.5–7.8 μm long . . . . . ***A. beijingense***
5. Apothecia yellow to orange yellow; ascospores 6–7.5(–8) × (3.3–)3.7–4.5 μm . . . . . ***A. epispartius*** f. ***epispartius***
5. Apothecia white; ascospores 6–6.5 × 3.5–4 μm . . . . . ***A. epispartius*** f. ***albus***
6. Ascospores ellipsoid to irregular-ellipsoid, 10.5–12.5 × 6–7.5 μm or (7.5–)9–13 × 5 × 5–7 μm . . . . . ***A. flavidus***
6. Ascospores broadly ellipsoid, 12.5–13.5 × 9–10 μm . . . . . ***A. xishuangbannicus***

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