

## A new species of *Acervus* (Pezizales) from China

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**Abstract:** A new species of *Acervus* from Heilongjiang Province of China is described and illustrated. It is characterized by apothecia cupulate, sessile, 5–10mm diam., hymenium yellow when fresh, ectal excipulum of textura angularis to textura epidermoidea and medullary excipulum of textura intricata mixed with textura angularis; asci operculate, 8-spored, J– in Melzer’s reagent and 98–119×6.5–8.5µm, ascospores ellipsoid, unicellular and 9–10.5×3.9–5.5µm. Both morphological features and 28S rDNA sequence data support the establishment of *A. heilongjiangensis* as a new taxon.

**Key words:** morphology, sequence analysis, taxonomy

## 中国小孢盘菌属一新种

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**摘要:**报道了源于我国黑龙江的小孢盘菌属 *Acervus* 一新种,即黑龙江小孢盘菌 *A. heilongjiangensis*。该种的显著特征为子囊盘杯状,无柄,直径 5–10mm;子实层新鲜时黄色;外囊盘被为角胞组织至表层组织;子囊具囊盖,具 8 个子囊孢子,孔口在 Melzer 试剂中不变色,98–119×6.5–8.5µm;子囊孢子椭圆形,无分隔,9–10.5×3.9–5.5µm。对该种的形态学特征进行了描述和图示,并对其分类地位提供了 DNA 序列分析的佐证。

**关键词:** 形态学, 序列分析, 分类

## INTRODUCTION

*Acervus* Kanouse is a genus of Pyronemataceae (Pezizales) and typified by *A. aurantiacus* Kanouse [=

*A. epispartius* (Berk. & Broome) Pfister]. Two species, *A. epispartius* and *A. flavidus* (Berk. & M.A. Curtis) Pfister, were accepted, and *Phaedropezia* Le Gal was treated as a synonym of *Acervus* by Pfister (1975).

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More taxa were subsequently added to the genus (Moravec 1983; Zhuang & Korf 1989; Zhuang & Wang 1998). Recently, Zhuang *et al.* (2011) described additional two species from China, and provided a dichotomous key to the known *Acervus* species. The genus is characterized by medium- to small-sized apothecia seated on a mycelial pad, subiculum-like structure or root-like structure, yellow to orange hymenium, thin-walled asci which are not in common with other members of Pyrenomataceae. It is probably saprobic and occurs on soil, duff and plant debris. Currently, 6 species are recognized (Kirk *et al.* 2008; Zhuang *et al.* 2011), and 5 of them were previously recorded from China (Korf & Zhuang 1985; Zhuang & Korf 1989; Zhuang & Wang 1998; Zhuang *et al.* 2011). In connection with our present work on the Chinese fungus flora, a new species is further discovered based on the combined data of morphology and DNA sequence analysis. It turns out that China has high species diversity of the genus.

## 1 MATERIALS AND METHODS

### 1.1 Morphological study

The apothecia were rehydrated and sectioned

at a thickness of 10–20 μm with a freezing microtome (YD-1508A, Jinhua, China). Measurements were taken from longitudinal sections and from squash mounts in lactophenol cotton blue solution using an Opton microscope (Germany). Iodine reaction of ascus apparatus was observed in Melzer's reagent. Photographs were taken using a Canon G5 digital camera (Tokyo, Japan) connected to a Zeiss Axioshop 2 Plus microscope (Göttingen, Germany).

### 1.2 Sequence analysis

Genomic DNA was extracted from dried apothecia using the CTAB procedure (White *et al.* 1990) with some modification. Sequences of 28S rDNA were amplified and sequenced with the primers LROR and LR5 (White *et al.* 1990). DNA sequencing was performed on an ABI 3730 XL DNA Sequencer (Applied Biosciences, Foster City, USA) at the Shanghai Majorbiopharm Technology Ltd., Beijing Branch, China.

DNA sequences of the reference taxa along with their collection numbers and GenBank accession numbers are listed in Table 1.

Sequences were assembled, aligned and manually edited using the program BioEdit 7.0.5.3 (Hall 1999) and converted to nexus files in ClustalX

**Table 1** Materials used in this study

Species	Geographic origin	Collection No.	28S nrDNA GenBank No.
<i>Acervus beijingensis</i> W.Y. Zhuang	China	HMAS 78150	HM197754
<i>A. changchunensis</i> W.Y. Zhuang	China	HMAS 78146	HM197752
<i>A. epispertius</i> (Berk. & Broome) Pfister f. <i>epispertius</i>	China	HMAS 78149	HM197753
<i>A. epispertius</i> f. <i>epispertius</i>	China	HMAS 173242	HM197755
<i>A. epispertius</i> f. <i>epispertius</i>	USA	A. Bessete 1984	DQ220305
<i>A. flavius</i> (Berk. & M.A. Cutris) Pfister	Puerto Rico	DHP PR98.2	DQ220306
<i>A. flavius</i>	China	HMAS 188443	HM197756
<i>A. heilongjiangensis</i> F. Ren & W.Y. Zhuang	China	HMAS 271281	<b>KP243162</b>
<i>Aleuria aurantia</i> (Pers.) Fuckel	USA	AFTOL-ID 65	AY544654
<i>Otidea onotica</i> (Pers.) Fuckel	USA	s. n. 2. 13 .98	DQ220387

Note: Number in bold indicating the newly submitted sequence.

1.83 (Thompson *et al.* 1997). Neighbor-joining tree was generated using 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 1 000 replicates to test phylogeny branch support.

## 2 TAXONOMY

***Acervus heilongjiangensis*** F. Ren & W.Y. Zhuang, sp. nov. Fig. 1

**Fungal Name FN 570148**

Holotype: China, Heilongjiang, Jiayin, Qingshan, on soil and duff among grass, 25 August 2014, H.D. Zheng, Z.Q. Zeng & W.T. Qin 9163, HMAS 271281.

Etymology: The specific epithet refers to the type locality Heilongjiang Province of the fungus.

Apothecia arising from a mycelia pad consisting of subhyaline to pale yellow hyphae, cupulate, sessile, 5–10mm diam.; hymenium yellow when fresh, orange to orange-red when dry; receptacle surface lighter than hymenium, nearly smooth. Ectal excipulum of textura angularis to textura epidermoidea, 55–120 $\mu$ m thick, cells angular to isodiametric, thin-walled, hyaline, 6–21 $\times$ 5–15 $\mu$ m; medullary excipulum of textura intricata mixed with textura angularis, 55–240 $\mu$ m thick, hyphae, thin-walled, subhyaline, 4–15mm wide; subhymenium 0–20 $\mu$ m thick; hymenium 105–135 $\mu$ m thick. Asci operculate, subcylindrical, thin-walled, apical apparatus not clearly seen under light microscope, 8-spored, J– in Melzer's reagent, 98–119 $\times$ 6.5–8.5 $\mu$ m. Ascospores ellipsoid, smooth-walled, hyaline, unicellular, eguttulate or 2- to 3-guttulate, uniseriate, 9–10.5 $\times$ 3.9–5.5 $\mu$ m. Paraphyses filiform, 2–3.5 $\mu$ m wide.

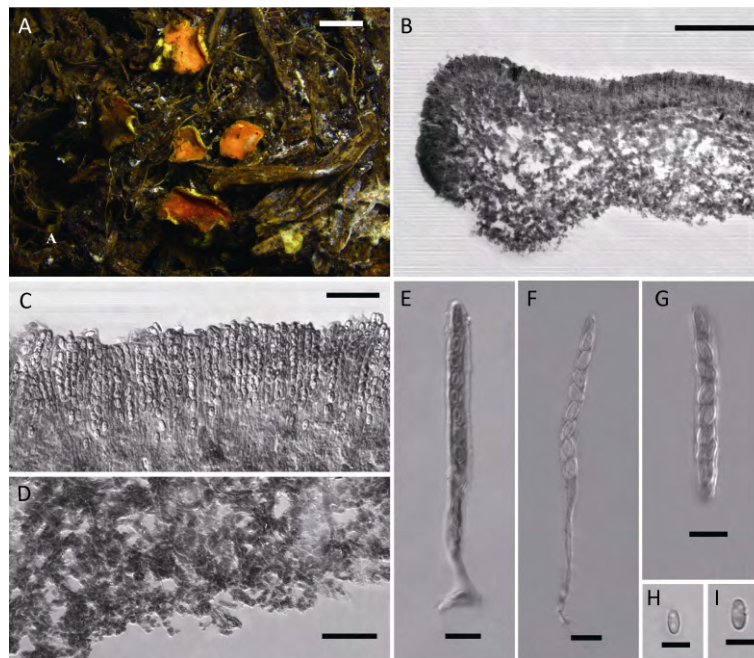


Fig. 1 *Acervus heilongjiangensis* (HMAS 271281). A: Apothecia on soil and duff among grass; B: Median vertical section of an apothecium; C: Hymenium; D: Structure of ectal excipulum; E, F: Asci; G, H, I: Ascospores. Bars: A=5mm; B=200 $\mu$ m; C=50 $\mu$ m; D=20 $\mu$ m; E–I=10 $\mu$ m.

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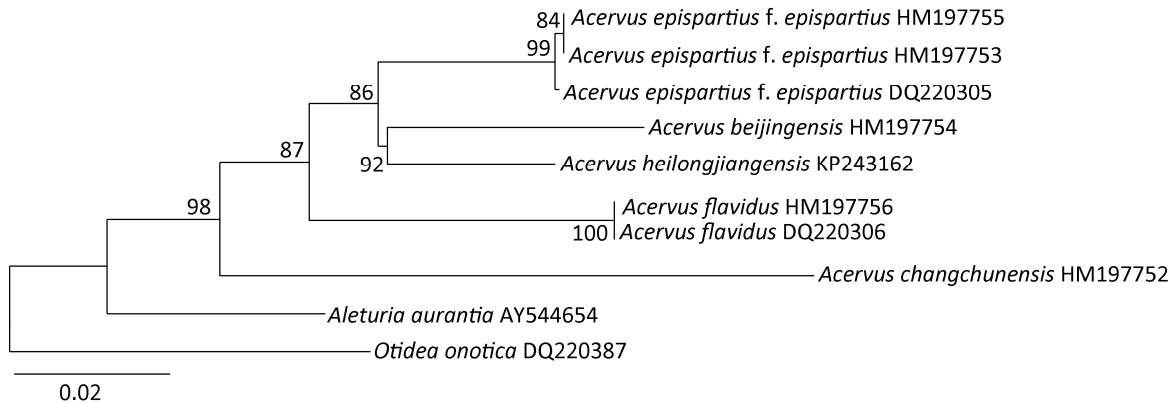


Fig. 2 Neighbor-joining tree inferred from sequences of 28S rDNA, showing the relationships among *Acervus* species. Bootstrap values  $\geq 50\%$  from 1 000 replicates are shown at internodes.

Notes: Among the known species of the genus, *A. heilongjiangensis* is most similar to *A. beijingensis* in apothecial shape, structure of ectal excipulum and ascus shape, but differs in longer ascospores which are ellipsoid instead of broadly ellipsoid in *A. beijingensis* (9–10.5 $\mu\text{m}$  vs. 6.5–7.8 $\mu\text{m}$  long). Moreover, *A. beijingensis* has larger apothecia (10–30mm vs. 5–10mm diam.) and shorter asci (86–95 $\mu\text{m}$  vs. 98–119 $\mu\text{m}$ ). The morphological distinctions between the above two species are also supported by the sequence analysis of 28S rDNA, in which *A. heilongjiangensis* is related to *A. beijingensis* but belongs to a different species (Fig. 2).

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