

## The Genus *Acervus* from Southwestern China and Northern Thailand

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### ABSTRACT

*Acervus* (Pyronemataceae, Pezizales) is a saprobic genus in Pezizomycetes, characterized by colored apothecia, subcylindrical to cylindrical asci and guttulate ascospores. We collected four *Acervus* samples from China and Thailand. Descriptions and illustrations are introduced for all fresh samples. One new record of *A. globulosus* from Thailand, one new species, *A. rufus*, two known species, *A. epispartius* and *A. stipitatus* from China are reported. Phylogenetic analysis based on five genes, the large subunit rRNA (LSU), the translation elongation factor-1 alpha (*tef1- $\alpha$* ), the second largest subunit of RNA polymerase II (*rpb2*), the largest subunit of RNA polymerase II (*rpb1*), and the small subunit rRNA (SSU), revealed the distinct position of the new species. The new species is set apart by its red apothecia. A key to *Acervus* species is also given.

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One new species; new record; Pezizales; taxonomy; phylogeny

## 1. Introduction

*Acervus* Kanouse includes nine species and is characterized by sessile or stipitate, discoid to cup-shaped or pulvinate, vivid colored apothecia, inamyloid asci, and guttulate ascospores [1–3]. The type species of the genus is *Acervus aurantiacus* Kanouse, which was collected from soil under an elm tree in the USA [4]. When Kanouse [4] examined this species, he did not observe operculate asci and placed the genus within Dermateaceae. Seaver [5] moved *Acervus* to the tribe Ascotremelleae in Helotiaceae owing to its rubbery sclerotiform base and inoperculate asci. Korf [6] reexamined type material and placed *Acervus* in Sarcosomataceae. Korf [7] placed *Acervus* into Pyronemataceae on account of its thin-walled asci, while Denison [8] treated *Acervus* in Sarcosyphaceae. Pfister [9] included *Phaedropezia* Le Gal in the genus and proposed two combinations: *Acervus epispartius* (Berk. & Broome) Pfister (= *Phaedropezia epispartia* (Berk. & Broome) Le Gal) and *Acervus flavidus* (Berk. & M.A. Curtis) Pfister (= *Phaedropezia flavida* (Berk. & M.A. Curtis) Le Gal). Upon examination of holotype of *A. aurantiacus* and *P. epispartia*, Pfister [9] concluded that the two species have no notable differences. Due to *P. epispartia* taking precedence over *A. aurantiacus*, the names of the two species were combined as *A. epispartius*, which is the current name for the type

species in *Acervus* [9]. Moravec [10] provided descriptions of his collection of *A. epispartius* and of a new species, *Acervus lusakianus* J. Moravec. Moravec [10] observed that the *A. epispartius* he collected was identical to *P. epispartia*, but congeneric with *Acervus*. Based on previous discussions on *Acervus* and *Phaedropezia*, he adopted Pfister's recommendations regarding the emendation of *Acervus* [10].

For a long time, the establishment of new species depended only on morphology. For example, Zhuang and Wang collected *Acervus xishuangbannicus* W.Y. Zhuang and Zheng Wang in rotten wood from tropical China and provided morphological description [11]. Nonetheless, the classification of *Acervus* was varied because the views of various researchers differed [4–8,12]. This was illustrated when an examination of the same material resulted in different classification schemes due to alternative points of view [6,9,12]. Initial phylogenetic analysis placed *Acervus* within Pyronemataceae [13] and *Acervus* species formed a highly-supported monophyletic group. Zhuang et al. [1] introduced *A. beijingensis* W.Y. Zhuang and *A. changchunensis* W.Y. Zhuang from China, using evidence from morphology and phylogeny. *Acervus heilongjiangensis* F. Ren & W.Y. Zhuang was collected from soil from a temperate area in China [2]. Shortly afterwards,

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*Acervus globulosus* Ekanayaka, Q. Zhao & K.D. Hyde and *A. stipitatus* Ekanayaka, Q. Zhao & K.D. Hyde were described and a detailed comparative table of morphological features of *Acervus* species was provided [3]. Subsequently, extensive phylogenetic analysis placed *Acervus* in Otideaceae within the Pezizomycetes, along with eight additional genera [3,14,15]. In the updated outline of fungi, Otideaceae is synonymized to Pyronemataceae [16].

Herein, we introduce four new collections from China and Thailand. To obtain an insight into the phylogenetic position of these morphologically similar species, sequences of the large subunit rRNA (LSU), translation elongation factor 1-alpha (*tef1-α*), RNA polymerase II second largest subunit (*rpb2*), RNA polymerase II largest subunit (*rpb1*) and small subunit rRNA (SSU) were analyzed jointly with sequences of closely related taxa within *Acervus*. We introduce a new species of *Acervus*, provide a detailed account of morphological characteristics for all collections, as well as a key to species of the genus.

## 2. Materials and methods

### 2.1. Sample collection, morphological studies, and deposition

Four fresh samples were collected from soil and humus. Three samples were collected from Yunnan Province, southwest China, and the rest one was collected from Chiang Mai Province, Thailand. These samples were brought to the laboratory in plastic bags containing allochroic silica gel. In addition, we loaned an Herbarium specimen (MFLU 16-0607) from the Herbarium of Mae Fah Luang University (MFLU). Samples and herbarium were observed using a Motic SMZ-168 stereoscope and photographed by Nikon Eclipse 80i compound microscope with a Cannon EOS 600 D camera. Cotton Blue, Melzer's, Gongo Red, and 5% KOH, was used to stain and observe morphological characteristics. Measurements were made using the Tarosoft® Image Frame Work program v. 0.9.7. New herbarium specimens were deposited at the Herbarium of Mae Fah Luang University (MFLU) and the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS). Facesoffungi and Index Fungorum numbers were obtained as in Jayasiri et al. [17] and Index Fungorum (2020).

### 2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA of fresh samples was extracted directly from ascomata using a *Trelief*<sup>TM</sup> Plant

**Table 1.** Primers used for amplification.

Locus	Primer	Primer sequence (5'-3')
LSU [18]	LROR	5'-ACC CGC TGA ACT TAA GC-3'
	LR5	5'-TCC TGA GGG AAA CTT CG-3'
<i>tef1-α</i> [19]	983F	5'-GCY CCY GGH CAY CGT GAY TTY AT-3'
	2218R	5'-AT GAC ACC RAC RGC RAC RGT YTG-3'
<i>rpb2</i> [20]	frPB2-5f	5'-GAY GAY MGW GAT CAY TTY GG-3'
	frPB2-7cR	5'-CCC ATR GCT TGY TTR CCC AT-3'
<i>rpb1</i> [21]	gRPB1-A	5'-GAK TGT CCK GGW CAT TTT GG -3'
	frPB1-Crev	5'-CNG CDA TNT CRT TRT CCA TRT A -3'
SSU [22]	NS1	5'-GTA GTC ATA TGC TTG TCT C-3'
	NS4	5'-CTT CCG TCA ATT CCT TTA AG-3'

Genomic DNA Kit. The large subunit rRNA (LSU), translation elongation factor 1-alpha (*tef1-α*), RNA polymerase II second largest subunit (*rpb2*), RNA polymerase II largest subunit (*rpb1*), and small subunit rRNA (SSU) regions were amplified by polymerase chain reaction (PCR) using the primers listed in Table 1. Total reaction volume was 25 μl containing 9.5 μl sterile deionized water, 12.5 μl of 2 × Power Taq PCR MasterMix, 1 μl of each primer (10 μM stock) and 1 μl of DNA template. Amplifications were carried out using an Applied Biosystems 2720 thermocycler (Foster City, CA, USA). Conditions of PCR for each gene are shown in Table 2. PCR products were visualized using 1% agarose gel electrophoresis and distinct bands viewed in Gel documentation system. The PCR products were purified using a *Trelief*<sup>TM</sup> DNA Gel Extraction Kit and sequenced by Tsingke Company, Beijing, P.R. China.

### 2.3. Phylogenetic analysis

Relevant sequences from GenBank are listed in Table 3 [1–3,13]. Sequences were assembled using BioEdit v.7.0.5.3 [23] and aligned using MAFFT v.7.110 online program (<http://mafft.cbrc.jp/alignment/server/>) [24]. Dataset was trimmed by TrimAl v.1.3 using the gappout option (<http://phylemon.bioinfo.cipf.es/utilities.html>) [25].

A maximum likelihood (ML) phylogenetic analysis was carried out by RAXMLGUI v.1.3 [26] under the GTR + GAMMA nucleotide substitution model with 1000 rapid bootstrap replicates.

Maximum-parsimony analysis was performed using PAUP v.4.0b10 [26]. Gaps were treated as missing data with the heuristic search option with 1000 random sequence additions and tree bisection reconnection (TBR) branch-swapping. Maxtrees were unlimited, branches of zero length were collapsed and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa.

Bayesian inference (BI) analysis was performed using MrBayes v.3.2.6 [27]. Model of evolution was estimated by using MrModeltest v.2.3 [28] as

**Table 2.** PCR conditions used for amplification.

Procedure	Genes				
	LSU	<i>tef1-<math>\alpha</math></i>	<i>rpb2</i>	<i>rpb1</i>	SSU
Pre-denaturation			94 °C for 5 min		
Denaturation			94 °C for 50 s		
Annealing	55 °C for 50 s	56 °C for 50 s	55 °C for 2 min	55 °C for 2 min	55 °C for 50 s
Extension			72 °C for 1 min		
Final extension			72 °C for 8 min		
Cycles	Number of cycles for LSU was 35 and for the rest 40				

**Table 3.** Sequences used in this study.

Species name	Distribution	Voucher	GenBank No.				
			LSU	<i>tef1</i>	<i>rpb2</i>	<i>rpb1</i>	SSU
<i>Acervus beijingsensis</i>	China, Beijing	HMAS 78150	HM197754	KP993492	KP993484	KP993500	DQ787815
<i>Acervus changchunensis</i>	China, Jilin	HMAS 78146	KP993477	KP993490	KP993482	KP993498	DQ787813
<i>Acervus epispartius</i>	China	HMAS 78149	HM197753	KP993491	KP993483	KP993499	DQ787814
<i>Acervus epispartius</i>	China	HMAS 173242	HM197755	–	–	–	–
<i>Acervus epispartius</i>	USA, New York	s.n. (FH)	DQ220305	–	–	–	–
<i>Acervus epispartius</i>	China, Yunnan	MFLU 20-0257	MT165625	MT336154	MT210807	–	MT165629
<i>Acervus flavidus</i>	Puerto Rico	DHP PR98.2 (FH)	DQ220306	–	–	–	–
<i>Acervus flavidus</i>	China	HKAS 90046	KX765259	KX765252	KX765260	–	–
<i>Acervus flavidus</i>	China, Fujian	HMAS 188443	HM197756	KP993487	KP993479	KP993495	–
<i>Acervus globulosus</i>	China, Yunnan	HKAS 88987	KX765253	KX765255	KX765254	–	–
<i>Acervus globulosus</i>	Thailand, Chiang Mai	MFLU 20-0258	MT165626	MT336155	MT210808	–	MT165630
<i>Acervus heilongjiangensis</i>	China, Heilongjiang	HMAS 271281	KP243162	KP993493	KP993485	KP993501	–
<i>Acervus rufus</i>	China, Yunnan	MFLU 20-0259	MT192219	MT227372	MT210811	MT210810	MT192216
<i>Acervus stipitatus</i>	China, Yunnan	MFLU 16-0607	KX765256	KX765258	KX765257	–	–
<i>Acervus stipitatus</i>	China, Yunnan	MFLU 20-0260	MT165627	MT336156	MT210809	MT210806	MT165631
<i>Monascella botryosa</i>	Spain	CBS 233.85	MH873558	KC109256	JX943831	JX943733	–
<i>Warcupia terrestris</i>	Canada	CBS 891.69	DQ220467	KC109308	–	–	–

Names in red indicate new species, while those in blue indicate new collections in this study.

CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DHP: Donald H. Pfister; HKAS: Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences. HMAS: Chinese Academy of Sciences, Beijing, China; MFLU: Mae Fah Luang University Herbarium, Chiang Rai, Thailand.

performed by MrMTgui [29] based on the Akaike information criterion [30]. Posterior probabilities (PP) [31] were calculated by Markov Chain Monte Carlo Sampling (MCMC) [32]. Six simultaneous Markov Chains were run for 50,000,000 generations and tree sampling occurred every 100th generations [33]. 25% of these trees were discarded as burn-in and analysis was stopped when the standard deviation of split frequency reached 0.01.

Alignment and trees were submitted to TreeBASE (submission ID: 25938). New sequences have been submitted to GenBank and accession numbers have been obtained (Table 3).

### 3. Results

#### 3.1. Phylogenetic analyses

The alignment contained 17 taxa spanning the current molecular diversity of *Acervus* along with *Monascella botryosa* Guarro & Arx (CBS 233.85) and *Warcupia terrestris* Paden & J.V. Cameron (CBS 891.69) as outgroup taxa. The combined matrix of LSU, *tef1- $\alpha$* , *rpb2*, *rpb1*, and SSU included 5257 sites (LSU: 1 – 906 bp, *tef1- $\alpha$* : 907 – 1872 bp, *rpb2*: 1873 – 2763 bp, *rpb1*: 2764 – 3550 bp, SSU: 3551 – 5257 bp). Of these, 4212 characters were constant and 705 were parsimony informative. The phylogenetic tree can be seen in Figure 1.

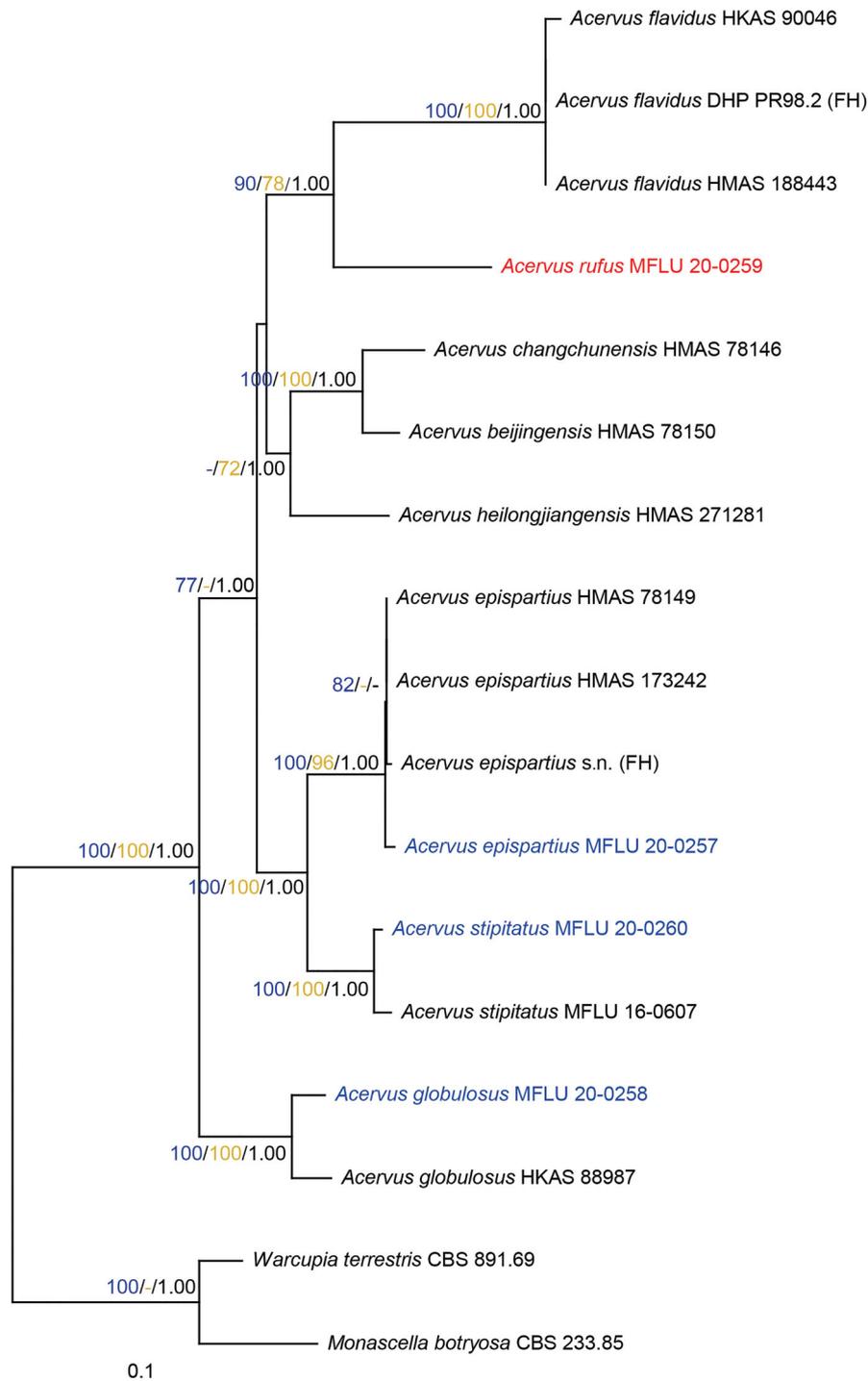
Likelihood value of the best scoring RaxML tree was –16128.204161. The most parsimonious trees (MPTs) showed tree length of 1908 steps [CI = 0.680, RI = 0.632, RC = 0.430, HI = 0.320]. *Acervus* is monophyletic and this relationship has maximum support with all three methods of analysis (100ML/100MP/1.00BI). *Acervus epispartius* (MFLU 20-0257) grouped with the rest of the isolates of this species with strong support (100ML/96MP/1.00BI). *Acervus stipitatus* (MFLU 20-0260) and *A. globulosus* (MFLU 20-0258) grouped with isolates of the same species also with maximum support (100ML/100MP/1.00BI). *Acervus rufus* (MFLU 20-0258) grouped as sister to *A. flavidus* strains. The clade support was 90ML/78MP/1.00BI. *Acervus rufus* (MFLU 20-0258) and *A. flavidus* (HKAS 188443), differ by 61 base pairs including 9 gaps in the LSU region (888 bp), 47 base pairs including 3 gaps in the *tef1- $\alpha$*  region (432 bp), 150 base pairs without gaps in the *rpb2* region (897 bp) and 68 base pairs including 4 gaps in the *rpb1* region (594 bp).

#### 3.2. Taxonomy

*Acervus epispartius* (Berk. & Broome) Pfister, Occ. Pap. Farlow Herb. Crypt. Bot. 8: 3 (1975) (Figure 2)

Index Fungorum No: IF 308081

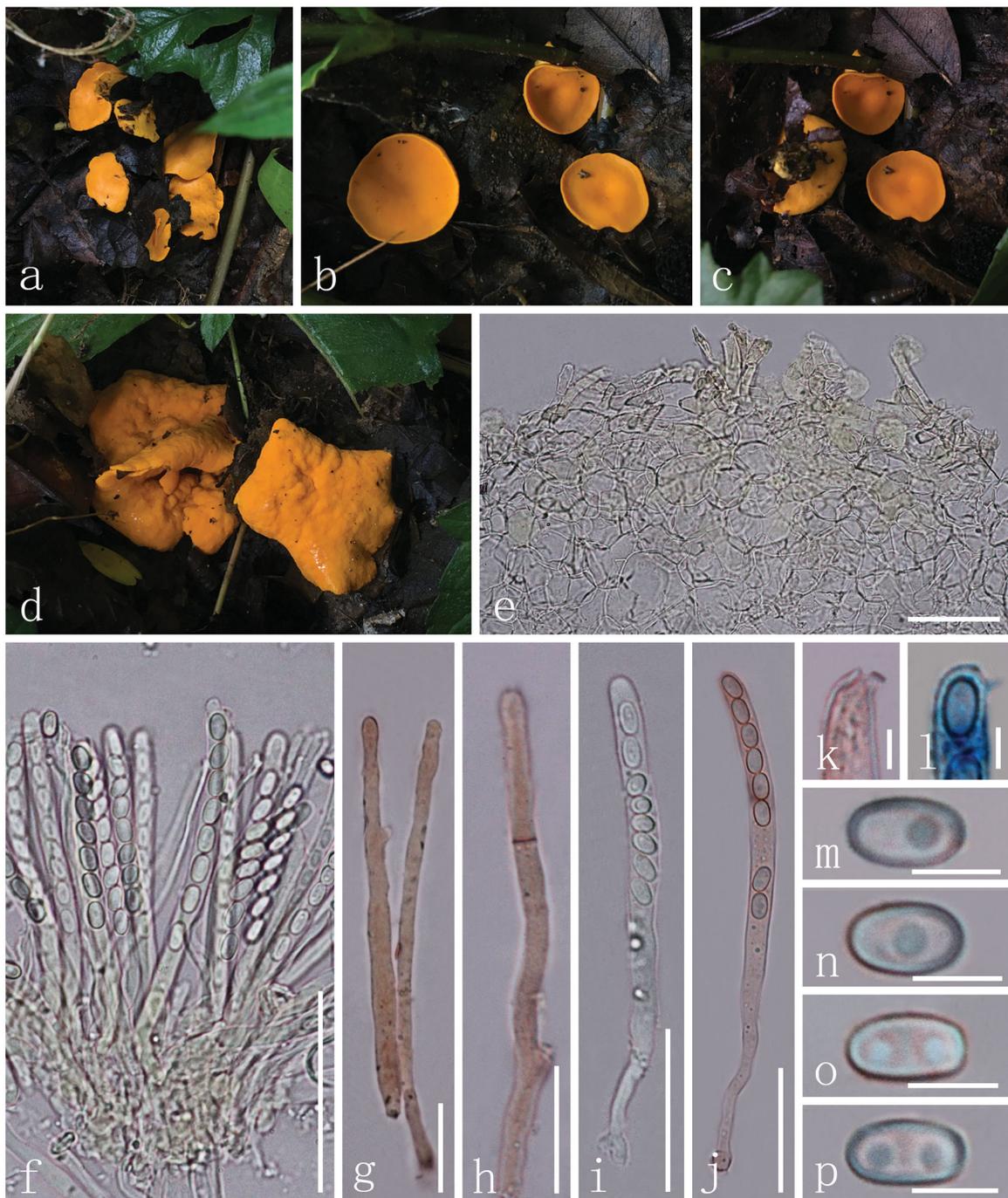
**Description:** *Saprobic* on soil and humus. **Sexual morph:** *Apothecia* 20–50 mm broad, scattered to



**Figure 1.** Phylogenetic tree of combined LSU, *tef1- $\alpha$* , *rpb2*, *rpb1*, and SSU sequence data inferred from 17 taxa and 5257 sites under the GTR (general time reversible) + GAMMA model of nucleotide substitution. Numerical values at the nodes indicate maximum likelihood bootstrap support (blue), maximum parsimony bootstrap support (yellow), and posterior probabilities (black). Only values greater than 70% (maximum likelihood and maximum parsimony) and 0.95 (Bayesian analysis) are indicated. Name in red indicates new species. Names in blue indicate new collections. Tree is artificially rooted to *Monascella botryosa* (CBS 233.85) and *Warcupia terrestris* (CBS 891.69) taxa.

gregarious, arising from a yellowish mycelia pad, forming a short stalked, up to 3 mm long, cupulate to pulvinate, hymenium yellow, receptacle surface yellow. *Medullary excipulum* 440–650  $\mu$ m broad, of *textura intricata*, yellowish, composed of 7–11  $\mu$ m broad hyphae. *Ectal excipulum* 90–195  $\mu$ m broad, of *textura angularis* to *textura globulosa*, mix with *textura epidermoidea*, yellowish, composed of 20–28  $\times$

16–24  $\mu$ m broad cells, slightly pubescent. *Paraphyses* 4–5  $\mu$ m diam, yellowish, branched or unbranched, septate, filiform. *Asci* 79–98  $\times$  5–6  $\mu$ m, 8-spored, operculate, subcylindrical, J. *Ascospores* [20/1/1, in 5% KOH] (5.4–)5.6–6.6(–7.7)  $\times$  (3.0–)3.2–4.1(–4.9)  $\mu$ m ( $Q$  = 1.42–1.85  $\mu$ m,  $Q$  = 1.68  $\pm$  0.13  $\mu$ m), ellipsoid with blunt ends, uniseriate, hyaline, smooth-walled, 1–2 guttules. **Asexual morph:** Unknown.



**Figure 2.** *Acervus epispartius* (HKAS 107301). (a–d) Typical mature specimens. (e) Receptacle surface of pileus in 5% KOH. (f) Asci and paraphyses in 5% KOH. (g, h) Paraphyses in Congo red. (i, j) Asci. (j) Asci in Congo red. (k, l) Apexes of asci. (k) Apex in Congo red. l) Apex in Cotton blue. m–p) Ascospores. Scale bars: e–f = 50  $\mu\text{m}$ . g–h = 20  $\mu\text{m}$ . i–j = 30  $\mu\text{m}$ . k–p = 5  $\mu\text{m}$ .

**Type:** Southern Zambia, the Victoria Falls near Livingstone, on the ground mixed with putrid leaves and debris, 23 January 1981, Jiri Moravec (TRH:F:11406, isotype)

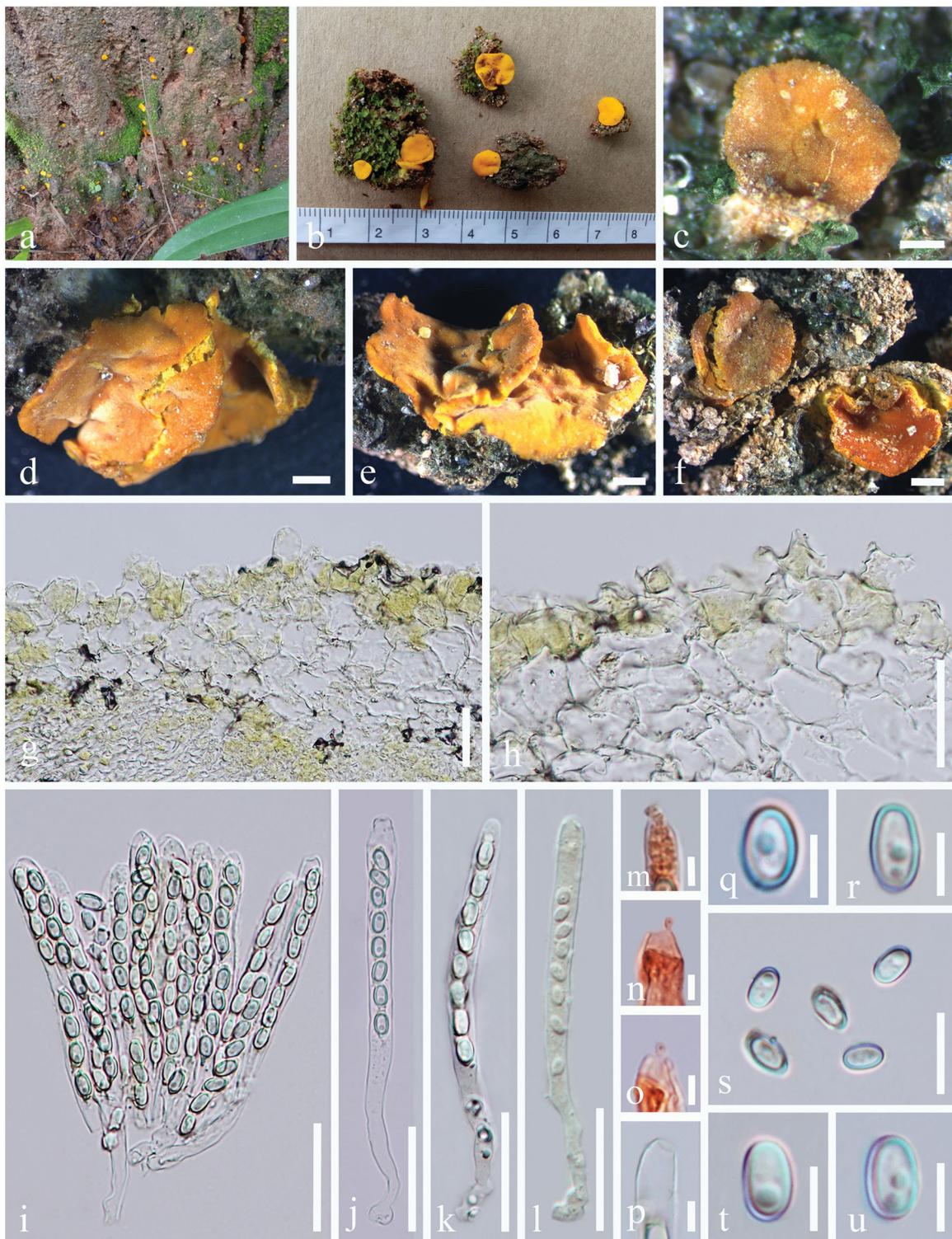
**Material examined:** China, Yunnan, Xishuangbanna, 101°25' E, 21°41' N, on soil, 27 August 2019, M. Zeng, ZM355 (HKAS 107301; MFLU 20-0257).

**Notes:** Originally, this was considered as a *Phaedropezia* species and named *P. epispartius* [34,35]. *Acervus epispartius* is the current name for the type species of *Acervus*. This species has cupulate to pulvinate apothecia with substipitate, small

ascospores with guttules. In Jiri Moravec's collection (TRH:F:11406), the size of ascospores are 6.5–7.6  $\times$  3.8–4.9  $\mu\text{m}$ , but we observed smaller ascospores in our collection (HMAS 107301). In addition, multiguttulate ascospores were found in collection TRH:F:11406, but we only found uniguttulate and biguttulate ascospores in our collection [10].

*Acervus globulosus* Ekanayaka, Q. Zhao & K.D. Hyde, in Ekanayaka, Zhao, Jones, Pu & Hyde, *Phytotaxa* 283(1): 78 (2016) (Figure 3)

Index Fungorum No: IF 552369; Facesoffungi No: FoF 02524



**Figure 3.** *Acervus globulosus* (MFLU 20-0258). (a–f) Typical mature specimens. (g, h) Receptacle surface of pileus. (i) Asci and paraphyses. (j–l) Asci (l Asci in Melzer's reagent). (m–p) Apexes of asci (m–o Apexes in Congo red.). (q–u) Ascospores. Scale bars: c = 1500  $\mu\text{m}$ . d–f = 1000  $\mu\text{m}$ . g–l = 30  $\mu\text{m}$ . m–u = 5  $\mu\text{m}$ .

**Description:** Saprobic on soil. **Sexual morph:** *Apothecia* 5–10 mm broad, 1–2 mm high, scattered to gregarious, arising from a yellowish mycelia pad, sessile, discoid to concave, hymenium yellow, receptacle surface orange to yellow. *Medullary excipulum* 138–270  $\mu\text{m}$  broad, of *textura angularis* to *textura globulosa*, hyaline to yellowish, composed of 15–24  $\times$  12–20  $\mu\text{m}$  broad cells. *Ectal excipulum* 51–106  $\mu\text{m}$  broad, of *textura angularis*, mix with

*textura epidermoidea*, yellowish, composed of 18–23  $\times$  13–18  $\mu\text{m}$  broad, J-. *Paraphyses* 4–5  $\mu\text{m}$ , yellow, unbranched, septate, filiform. *Asci* 82–100  $\times$  6–8  $\mu\text{m}$ , 8-spored, suboperculate, subcylindrical to clavate, J-. *Ascospores* [20/1/1, in H<sub>2</sub>O] (5.2–)5.4–6.1(–6.3)  $\times$  (3.0–)3.3–4.0(–4.3)  $\mu\text{m}$  (Q = 1.41–1.78  $\mu\text{m}$ , Q = 1.59  $\pm$  0.18  $\mu\text{m}$ ), ellipsoid, uniseriate, hyaline, smooth-walled, 1–2 guttules. **Asexual morph:** Unknown.

**Table 4.** Morphological differences for two collections of *Acervus globulosus*.

	Ekanayaka <i>et al.</i> 's descriptions	Our descriptions
Medullary excipulum	hyaline of <i>textura intricata</i> cells	hyaline to yellowish of <i>textura angularis</i> to <i>textura globulosa</i>
Paraphyses	hyaline	yellow
Apex of asci	inoperculate	suboperculate
Size of asci	72–96 × 5–6 μm	82 – 100 × 6 – 8 μm

**Type:** China, Yunnan, Xishuangbanna Botanical Garden, Menglun, Mengla County, 600 m alt., 101°16'06.87" E, 21°55'11.69" N, on soil near the vegetations of *Millettia leptobrya*, *Garcinia cowa*, *Castanopsis indica*, 5 July 2014, W. Gang (HKAS 88987, holotype).

**Material examined:** Thailand, Chiang Mai, Samoeng, 98° 36' E, 18° 56' N, on soil, 13 July 2017, Y.P. Xiao, MM10 (MFLU 20-0258, HKAS 97493).

**Notes:** Ekanayaka *et al.* [3] introduced this species which is distinguished by discoid to concave apothecia, *textura angularis* to *textura globulosa* cells in the excipulum, and ellipsoid ascospores. We observed some differences in our collection (MFLU 20-0258) of *A. globulosus* (Table 4). This species is the first recorded in Thailand.

***Acervus rufus*** M. Zeng, Q. Zhao & K.D. Hyde, sp. nov. (Figure 4)

Index Fungorum No: IF 557490; Facesoffungi No: FoF 07739

**Etymology:** The epithet refers to its reddish pileus and receptacle.

**Description:** *Saprobic* on soil with debris of decayed leaves. **Sexual morph:** *Apothecia* 7–10 mm broad, 8–10 mm high, scattered, arising from a white mycelia pad, forming a short stalked, 5–7 mm long, up to 5 mm broad, white to reddish. *Receptacle* discoid, red, hymenium red, receptacle surface reddish to orange. *Medullary excipulum* 87–163 μm broad, of *textura angularis* to *textura globulosa* mix with rarely swollen elongated hyphae, hyaline to reddish, composed of 19–30 × 12–18 μm broad cells. *Ectal excipulum* 127–201 μm broad, of *textura angularis* to *textura epidermoidea*, hyaline to reddish, 27–41 × 20–27 μm broad. *Paraphyses* 4–6 μm diam, hyaline, branched or unbranched, septate, filiform. *Asci* 121–140 × 8–9 μm, 8-spored, suboperculate to operculate, subcylindrical to clavate, J-. *Ascospores* [20/1/1, in H<sub>2</sub>O] (8.5–)9.1–10.6(–11.3) × (5.9–)6.5–7.6(–8.0) μm (Q = 1.20–1.59 μm, Q = 1.41 ± 0.12 μm), broadly ellipsoid, uniseriate, hyaline, smooth-walled, with 1–2 guttules. **Asexual morph:** Unknown.

**Material examined:** China, Xishuangbanna, Yunnan, 100°47' E, 21°59' N, on soil, 9 June 2018, M. Zeng, Zeng 012 (HKAS 107302, holotype); *ibid.* (MFLU 20-0259, isotype).

**Notes:** This species is characterized by red apothecia with short stalks, *textura angularis* cells in the excipulum, operculate asci and ellipsoid ascospores with 1–2 guttules. Based on phylogenetic tree, *A. rufus* groups with the *A. flavidus*, but *A. flavidus* differs in lemon yellow apothecia, sessile, medullary excipulum cells of *textura intricata* and 1–3-guttulate ascospores [1,3].

***Acervus stipitatus*** Ekanayaka, Q. Zhao & K.D. Hyde, in Ekanayaka, Zhao, Jones, Pu & Hyde, *Phytotaxa* 283(1): 78 (2016) (Figure 5)

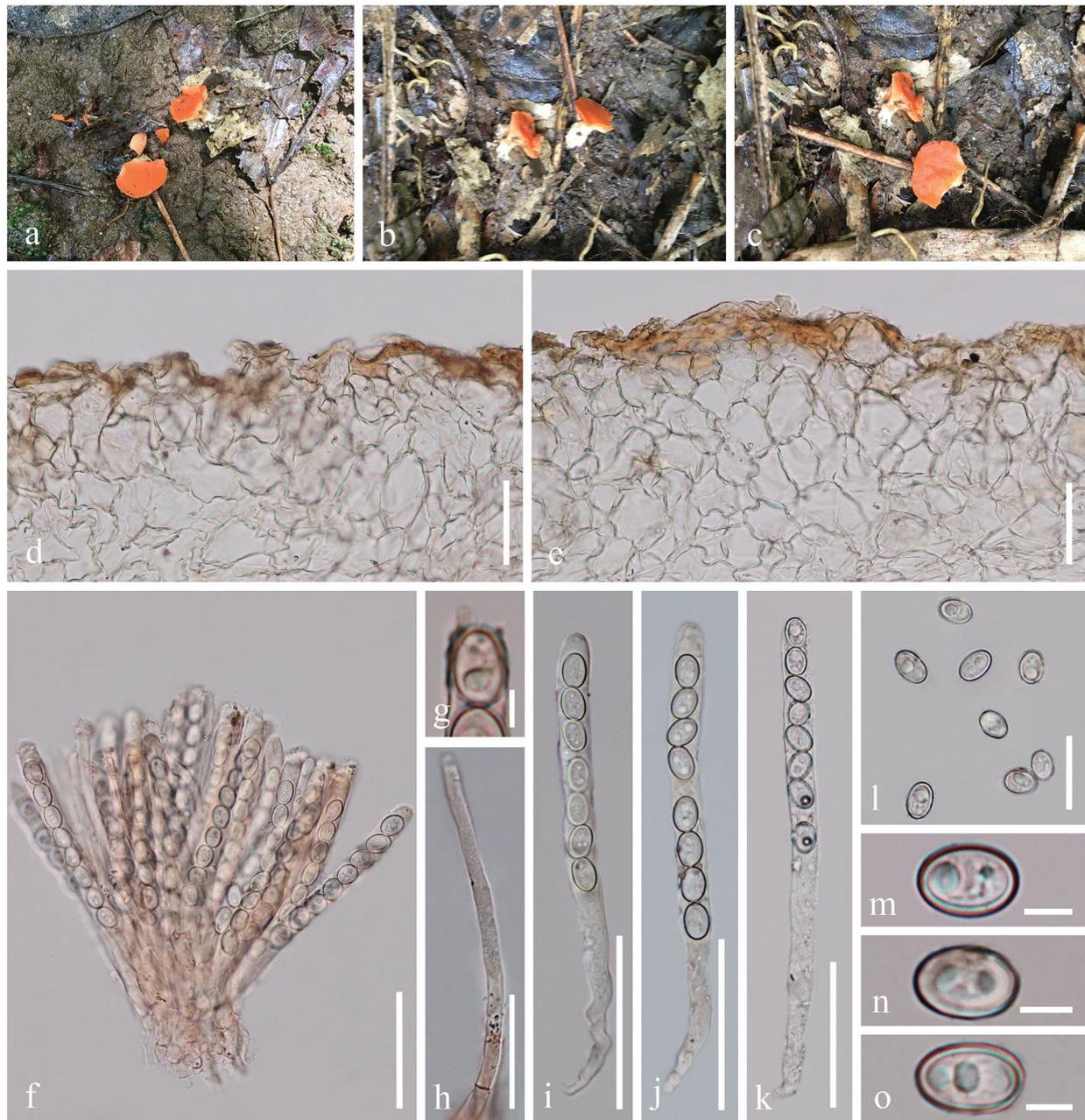
Index Fungorum No: IF 552370; Facesoffungi No: FoF 02525

**Description:** *Saprobic* on soil. **Sexual morph:** *Apothecia* 0.7–1.0 cm broad, 1–2 mm high superficial, scattered, arising from a subhyaline to pale yellow mycelia pad, substipitate, discoid to concave, orange to yellow, hymenium yellowish, receptacle yellow. *Medullary excipulum* 107–168 μm broad, of *textura angularis* to *textura globulosa*, hyaline to yellowish, composed of 19–25 × 13–17 μm broad cells. *Ectal excipulum* 106–217 μm broad, of *textura angularis* to *textura epidermoidea*, hyaline to yellowish, 21–28 × 15–19 μm broad, J-. *Paraphyses* 3–5 μm, hyaline to yellowish, branched, septate, filiform. *Asci* 89–100 × 5–7 μm, 8-spored, suboperculate to operculate, subcylindrical to clavate, J-. *Ascospores* [20/1/1, in H<sub>2</sub>O] (8.3–)8.9–11.0(–11.6) × (6.1–)6.4–7.8(–8.2) μm (Q = 1.08–1.62 μm, Q = 1.41 ± 0.13 μm), broadly ellipsoid, uniseriate, hyaline, smooth-walled with 1 to 2 or more small oil drops. **Asexual morph:** Unknown.

**Type:** China, Yunnan Province, Xishuangbanna, buffer zone of the Nabanhe National Nature Reserve (NNNR), 100°32'–100°44' E, 22°04'–22°17' N, 30 August 2015, S.C. Karunarathna (MFLU 16-0607, holotype).

**Material examined:** China, Yunnan, Xishuangbanna, 100°47' E, 21°59' N, on soil in a cave, 9 June 2018; M. Zeng, Zeng 013 (HKAS 107302, MFLU 20-0260).

**Notes:** *Acervus stipitatus* is characterized by discoid to concave apothecia with substipitate, uniguttulate to multiguttulate ascospores. *A. stipitatus* was established by Ekanayaka *et al.* [3]. In her descriptions, it is saprobic on dead stem with aseptate paraphyses, inoperculate asci, and uniguttulate ascospores. We reexamined holotype of *A. stipitatus*



**Figure 4.** *Acervus rufus* (HKAS 107302, holotype). (a–c) Typical mature specimens. (d, e) Receptacle surface of pileus. f Hymenium in Congo red. (g) Apex of asci in Congo red. (h) Paraphyses in Congo red. (i–k) Asci (j Asci in Congo red. k Asci in Melzer's reagent.). (l–o) Ascospores. Scale bars: d–f, i–k = 50  $\mu$ m. h = 30  $\mu$ m l = 20  $\mu$ m. g, m–o = 5  $\mu$ m.

(MFLU 16-0607) and illustration is provided herein (Figure 6). The *A. stipitatus* (MFLU 16-0607) has apothecia in which aseptate, unbranched, and hyaline paraphyses, operculate asci, and 1 to 3 guttules. The apothecial photo of the loaned herbarium differs from the previously published one [3].

#### 4. Discussion

The diversity of fungi in northern Thailand and southern China is proving to be extremely high [36,37]. Herein, we introduce one new species of

*Acervus* and new collections from Yunnan Province and Thailand. Most *Acervus* species have been found on soil, though some species have also been reported from rotten wood, including *A. flavidus*, *A. stipitatus*, and *A. xishuangbannicus*, suggesting a saprobic lifestyle [1,3,11]. Among all known *Acervus* species, nine have been reported in China including seven type species [1–3,11]. This indicates a high diversity of *Acervus* in China [1,3].

*Acervus* is a small group in the family Pyronemataceae [16]. Phylogenetic methods have resolved some classification problems in this genus

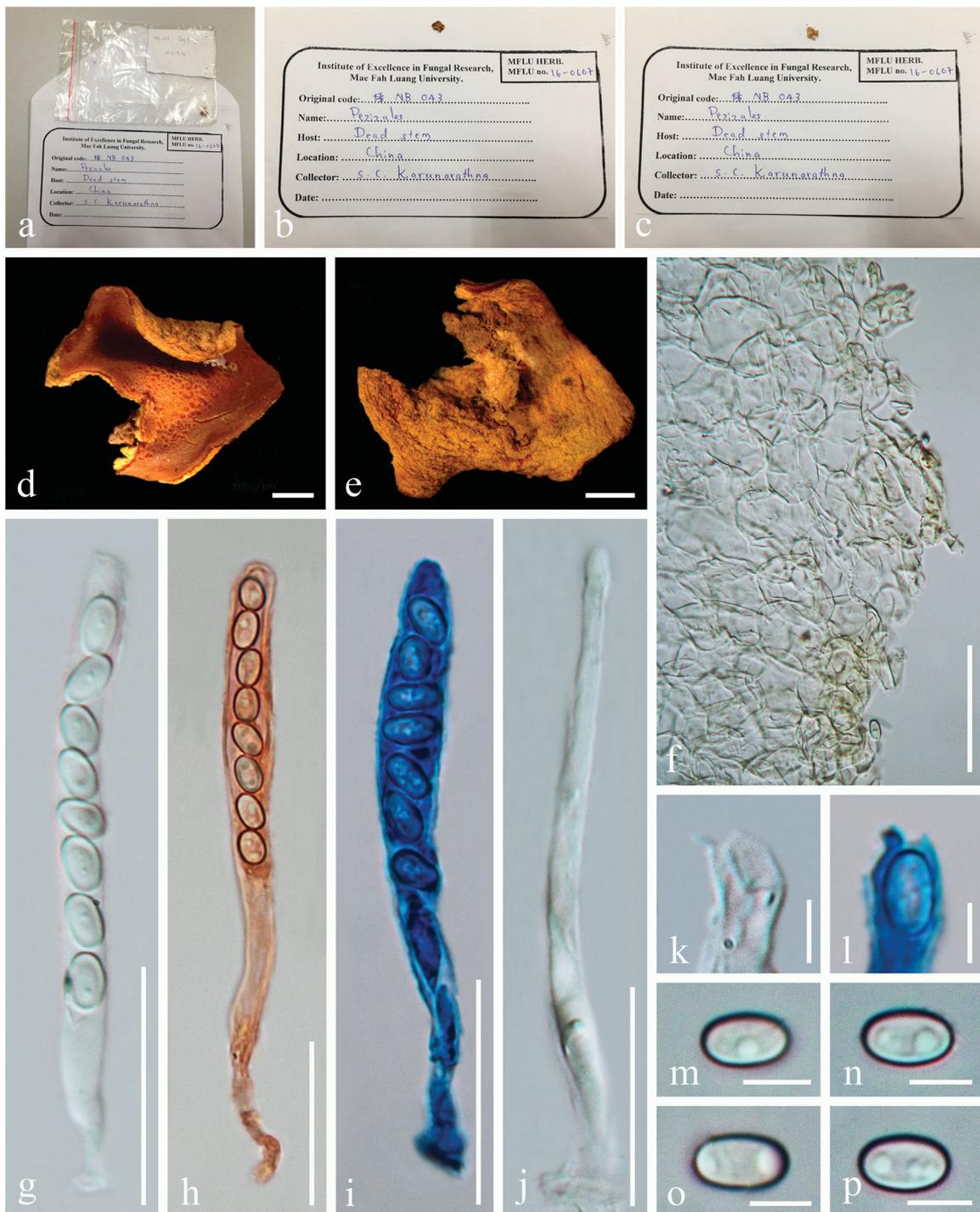


**Figure 5.** *Acervus stipitatus* (HKAS 107302). (a) Habitat. (b, c) Typical mature specimens. (d) Receptacle surface of pileus. (e–g) Paraphyses (g Paraphyses in Congo red.). (h–j) Asci (i–j Asci in Congo red.). (k–m) Apexes of asci. (n–q) Ascospores. Scale bars: d, h–j = 30  $\mu$ m. e–g = 20  $\mu$ m. k–m, o–q = 5  $\mu$ m. n = 15  $\mu$ m.

[1–3,13,14]. Nonetheless, some features of *Acervus* remain problematic. For instance, ascus dehiscence, and whether asci are inoperculate, suboperculate or operculate are topics of debate [4,6,9,12]. In their description of *A. beijingensis* and *A. changchunensis*, Zhuang et al. [1] stated that the “apical apparatus was not clearly seen”. Ren and Zhuang [2] considered *A. heilongjiangensis* as an operculate species, but authors still stated, “apical apparatus not clearly seen under light microscope”. Thus, it is necessary to make additional collections of this species. Although Pezizomycetes are operculate

discomycetes, the apical apparatus of some taxa in the class is in doubt [12]. Thus, it is important to understand the apical apparatus for each group in this class. Regarding *Acervus*, Ekanayaka et al. [3] considered the genus as an inoperculate discomycetes group. Nonetheless, an examination of our collections indicated distinct suboperculate to operculate structures for species in this genus.

Pfister [9] provided a detailed explanation of *A. epispartius* as an older epithet and current name for the type in this genus. These observations were later confirmed by others [1,2,10,11]. Ekanayaka et al. [3]



**Figure 6.** *Acervus stipitatus* (MFLU 16-0607, holotype). (a–e) Herbarium materials. (f) Receptacle surface of pileus. (g–i) Asci (h Asci in Congo red. i Asci in Cotton blue). (k–l) Apexes of asci. (l) Apex of asci in Cotton blue). (m–p) Ascospores. Scale bars: d–i = 30  $\mu$ m. j–l, n–p = 5  $\mu$ m. m = 15  $\mu$ m.

treated *A. epispartius* and *A. aurantiacus* as separate without examining the holotype. We also did not observe the holotype of *A. epispartius* and *A. aurantiacus*. Given the disagreement, reexamination of holotype is recommended.

Herein a key to *Acervus* species is provided [1–3,10,11]. Shape and size of ascospores are important characters to identify species [1,2]. In descriptions of Ren and Zhuang [2], the size of

ascospores is the primary morphological feature for distinguishing species. Up to now, the yellow to orange color of apothecia was considered an important trait to identify this genus. However, the new species *A. rufus* shows distinct red apothecia, thus expanding the range of apothecia colors in the genus. All species in the key have available sequence data, with the exception of *A. xishuangbannicus* and *A. lusakianus*. Therefore, future collections should

focus on obtaining these species along with others to uncover the diversity of the genus.

#### Key to species of *Acervus*

1. Apothecia substipitate to stipitate .....2
1. Apothecia sessile.....3
2. Apothecia red.....*A. rufus*
2. Apothecia yellow .....4
3. Apothecia ellipsoid .....5
3. Apothecia broadly ellipsoid.....6
4. Ascospores smooth or verrucose .....*A. lusakianus*
4. Ascospores smooth .....7
5. Ascospores eguttulate or with 1–2 guttules.....*A. changchunensis*
5. Ascospores with 1–2 guttules .....8
6. Ascospores 13.1–15.6 × 7.5–7.8 μm ..... *A. flavidus*
6. Ascospores 9–10.5 × 3.9–5.5 μm .....*A. heilongjiangensis*
7. Apothecia discoid to cupulate.....*A. xishuangbannicus*
7. Apothecia discoid to concave or pulvinate .....9
8. Ascospores 6.5–7.8 × 4.4–5.8 .....*A. beijingensis*
8. Ascospores 5.4–6.1 × 3.3–4.0 .....*A. globulosus*
9. Ascospores 5.6–6.6 × 3.2–4.1 .....*A. epispartius*
9. Ascospores 8.9–11.0 × 6.4–7.8 ..... *A. stipitatus*

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