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Acrocordiella yunnanensis sp. nov. (Requienellaceae, Xylariales) from Yunnan, China

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Abstract

Acrocordiella yunnanensis sp. nov. is introduced here from dead twigs of an unidentified dicotyledonous host from Xishuangbanna Prefecture, Yunnan Province, China. Phylogenetic analyses based on LSU and ITS sequence data revealed that this new species with a distinct sexual morph belongs to Requienellaceae (Sordariomycetes, Ascomycota). *Acrocordiella yunnanensis* is closely related to *Acrocordiella omanensis* in Requienellaceae. This novel taxon is illustrated, described, and a morphology-based key is provided to facilitate the identification of species in *Acrocordiella*.

Keywords: 1 new species, microfungi, phylogeny, saprobic, Sordariomycetes, taxonomy

Introduction

Requienellaceae was introduced by Boise (1986) in the class Dothideomycetes, with *Requienella* as the type genus. The taxonomic placement of Requienellaceae was uncertain, and Barr (1990) and Aptroot (1991) considered it as a member of Melanommatales based on its trabeculate-like paraphyses (Liew *et al*. 2000). However, Jaklitsch *et al*. (2016) transferred Requienellaceae to Xylariales as a distinct family based on phylogenetic analysis and morphological comparison. Subsequently, Maharachchikumbura *et al*. (2018), and Wijayawardene *et al*. (2020) accepted four genera in Requienellaceae *viz*. *Acrocordiella*, *Lacrymospora*, *Parapyrenis* and *Requienella*. However, Jaklitsch *et al*. (2016) and Voglmayr *et al*. (2019) accepted only *Acrocordiella* and *Requienella* within Requienellaceae. The sexual morph of the family has solitary or aggregated, perithecioid ascomata with a prominent papillate, a peridium with thick-walled angular cells, numerous, apically free, scarcely branched paraphyses, unitunicate, asci with thick-walled apex and wide ocular chamber and olivaceous or brown, ellipsoid to oblong ascospore with round or acute ends, with one or several transverse distosepta and large lumina (Jaklitsch *et al*. 2016, Hyde *et al*. 2020). The morphological features of asexual morphs of Requienellaceae remain undetermined. Members of this family mainly occur as saprobes on dead wood or lichenicolous and borderline lichens (Aptroot 1991, Hawksworth & Halici 2007, Jaklitsch *et al*. 2016, Maharachchikumbura *et al*. 2018, Voglmayr *et al*. 2019).

Acrocordiella was introduced by Eriksson (1982), with *A. occulta* as the type species. This genus was synonymized under *Requienella* based on shared morphological characteristics (Boise 1986). However, based on phylogenetic analyses, Jaklitsch *et al*. (2016) confirmed that *Acrocordiella* and *Requienella* are distinct genera. Wijayawardene *et al*. (2018) treated *Acrocordiella* in Pyrenulaceae. However, based on morphology and sequence data, Maharachchikumbura

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et al. (2018) and Hyde *et al.* (2020) placed *Acrocordiella* in Requienellaceae. They mentioned that *Acrocordiella* could be separated based on the solitary or aggregate immersed ascomata with white or black margins, a peridium comprising brown to hyaline, thick-walled cells of *textura angularis* and 3-distoseptate ascospores with rhomboid lumina (Maharachchikumbura *et al.* 2018, Hyde *et al.* 2020). There are currently only two species in *Acrocordiella viz. A. occulta* and *A. omanensis* (Maharachchikumbura *et al.* 2018, Wijayawardene *et al.* 2020).

In the present study, we surveyed wood inhabiting ascomycetes in Menghai County, Xishuangbanna, Yunnan Province, China. *Acrocordiella yunnanensis sp. nov.* is introduced in Requienellaceae, and its novelty is confirmed by morphology and phylogenetic analyses based on combined LSU and ITS sequence data.

Materials and methods

Morphological observations

The specimens were collected from dead twigs of an undetermined plants from Menghai County, Xishuangbanna, Yunnan Province, during December 2018 which is the dry season. Samples were observed using a stereomicroscope (Olympus SZ61). Collections, observation of specimens and isolations are as detailed in Senanayake *et al.* (2020). Measurements were made with the Tarosoft (R) Image Frame Work program (IFW 0.97 version), and images were processed with Adobe Photoshop CS6 software (Adobe Systems, USA). The type material is deposited in the Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica (HKAS), Chinese Academy of Science, Kunming, China. Faces of Fungi and Index Fungorum numbers were obtained as outlined in Jayasiri *et al.* (2015) and Index Fungorum (2021) (http://www.indexfungorum.org), respectively.

DNA extraction, PCR amplification and sequencing

DNA extraction, PCR amplifications, sequencing and phylogenetic analyses were followed by Dissanayake *et al.* (2020) with the following modifications. DNA was extracted from 15–20 fruiting bodies of the fungus outlined by Wanasinghe *et al.* (2018). According to the manufacturer's instructions, DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China). DNA extracts were stored at –4 °C for use in regular work and duplicated at –20 °C for long-term storage. DNA was amplified by polymerase chain reaction (PCR) for LSU and ITS gene regions using primer pairs LR0R/LR5 (Vilgalys and Hester 1990) and ITS5/ITS4 (White *et al.* 1990), respectively. The thermal cycling program followed Wanasinghe *et al.* (2020). Purification and sequencing of PCR products were done at Beijing Biomed Gene Technology Co., Ltd., Shijingshan District, TsingKe Biological Technology Co., Beijing, China. Both directions of the PCR products were sequenced using the same primer pairs used in PCR amplification to ensure the sequences' integrity.

Phylogenetic analyses

Lasergene SeqMan Pro v.7 was used to obtain consensus sequences from sequences generated from forward and reverse primers. Consensus sequences were checked with BLAST searches to recognize high similarity indices and the related literature (Table 1). Single and multiple (LSU and ITS) alignments of all reference sequences were automatically generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh & Standley 2013, Katoh *et al.* 2019) and improved manually using BioEdit v. 7.0.5.2 (Hall 1999). Phylogenetic analyses of the combined dataset were based on maximum-likelihood (ML) and Bayesian (BI) phylogenetic analysis. Both RAxML and Bayesian analyses were run on the CIPRES Science Gateway portal (Miller *et al.* 2012). Evolutionary models for Bayesian analysis and maximum-likelihood were selected independently for each locus using MrModeltest v. 2.3 (Nylander 2004) under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b10. The evaluated best-fit model was GTR + I + G for each locus in both Bayesian analysis and maximum-likelihood. ML analyses for the data-sets were performed with RAxML-HPC2 on XSEDE v. 8.2.10 (Stamatakis 2014) using a GTR + I + G substitution model with 1000 bootstrap iterations. BI analysis was computed with MrBayes v. 3.2.6 (Ronquist *et al.* 2012). Six simultaneous Markov chains were run for 2,000,000 generations, and trees were sampled at every 100th generation, ending the run automatically when the standard deviation of split frequencies dropped below 0.01. ML bootstrap values equal or greater than 60 % and Bayesian posterior probabilities (BYPP) greater than 0.95 are given above each node (FIGURE 1). Phylograms were visualized with FigTree v1.4.0 program (Rambaut 2012) and reorganized in Microsoft PowerPoint (2016). The finalized alignment and tree were deposited in TreeBASE (submission ID 27512, http://purl.org/phylo/treebase/phylows/study/TB2:S27512?x-access-code=7dd4b3d71b11cbce5e4aa365671cc1fb&format=html).

TABLE 1. Names, strain numbers, and GenBank accession numbers of the taxa used in the phylogenetic analyses.

Species	Strain no.	GenBank Accession no.		Reference
		ITS	LSU	
Acrocordiella occulta	RS10	KT949894	KT949894	Jaklitsch *et al*. 2016
Acrocordiella occulta	RS9	KT949893	KT949893	Jaklitsch *et al*. 2016
A. omanensis ^{T}	SQUCC 15091	MG584568	MG584570	Maharachchikumbura *et al*. 2018
A. omanensis	SQUCC 13852	MG584569	MG584571	Maharachchikumbura *et al*. 2018
A. yunnanensis ^{T}	**HKAS 111922**	**MW424507**	**MW424505**	**This study**
A. yunnanensis	**HKAS 111923**	**MW424497**	**MW424506**	**This study**
Amphibambusa bambusicola ^{T}	MFLUCC 11-0617	KP744433	KP744474	Liu *et al*. 2015
Amphisphaeria. umbrina	AFTOL-ID 1229	NA	FJ176863	Schoch *et al*. 2009
A. fuckelii ^{T}	CBS 140409	KT949902	KT949902	Jaklitsch *et al*. 2016, Liu *et al*. 2019
Anthostoma decipiens	CD	KC774565	KC774565	Jaklitsch *et al*. 2014
Apiospora setosa	ICMP 4207	NA	DQ368631	Tang *et al*. 2007
Arthrinium phragmites	CPC 18900	KF144909	KF144956	Crous *et al*. 2013a
Barrmaelia moravica ^{T}	Cr1	MF488987	MF488987	Voglmayr *et al*. 2018
Beltraniella acaciae ^{T}	CPC 29498	NR_147685	KY173483	Crous *et al*. 2013b
B. endiandrae ^{T}	CBS 137976	NR_148073	KJ869185	Crous *et al*. 2014
Biscogniauxia nummularia ^{T}	MUCL 51395	JX658444	KT281894	Senanayake *et al*. 2015
Broomella vitalbae	CBS 140412	KT949895	KT949895	Jaklitsch *et al*. 2016
Cainia desmazieri	CAI	KT949896	KT949896	Jaklitsch *et al*. 2016
Chaetosphaeria innumera	SMH2748	AY906956	AY017375	Huhndorf *et al*. 2001
Clypeosphaeria mamillana ^{T}	CLM1 T	KT949898	KT949898	Jaklitsch *et al*. 2016
Coniocessia maxima ^{T}	Co117	NR_137751	GU553344	Asgari *et al*. 2011
C. nodulisporioides ^{T}	CO106	GU553343	GU553351	Asgari *et al*. 2011
Creosphaeria sassafras	CBS 127876	KT949900	KT949900	Jaklitsch *et al*. 2016
Daldinia vernicosa ^{T}	CBS 119316	KY610395	KY610442	Wendt *et al*. 2018
Entosordaria quercina	RQ T	MF488994	MF488994	Voglmayr *et al*. 2018
Graphostroma platystomum	AFTOL-ID 1249	NA	DQ836906	Zhang *et al*. 2006
Idriella lunata	CBS 736.74	KP859046	KP858983	Hernandez *et al*. 2016
Iodosphaeria tongrenensis ^{T}	MFLU 15-0393	KR095282	KR095283	Li *et al*. 2015
Leiosphaerella praeclara	LP	JF440976	JF440976	Jaklitsch *et al*. 2012
Lopadostoma dryophilum	LG24	KC774572	KC774572	Jaklitsch *et al*. 2014
Melogramma campylosporum	MBU	JF440978	JF440978	Jaklitsch *et al*. 2012
Phlogicylindrium eucalyptorum ^{T}	CBS 111680	KF251204	KF251707	Quaedvlieg *et al* 2013
P. uniforme ^{T}	CBS 131312	JQ044426	JQ044445	Crous *et al*. 2011
Pseudapiospora corni	PCO1	KT949908	KT949908	Jaklitsch *et al*. 2016
Requienella fraxini ^{T}	RS3	KT949910	KT949910	Jaklitsch *et al*. 2016
R. fraxini	RS7	KT949911	KT949911	Jaklitsch *et al*. 2016
R. seminuda ^{T}	RS12	KT949912	KT949912	Jaklitsch *et al*. 2016
R. seminude	RS13	KT949913	KT949913	Jaklitsch *et al*. 2016
Seiridium marginatum ^{T}	SEI1	KT949916	KT949916	Jaklitsch *et al*. 2016
Vialaea mangfia ^{T}	MFLUCC 12-0808	KF724974	KF724975	Senanayake *et al*. 2014
V. minutella	BRIP56960	KC181927	KC181925	McTaggart *et al*. 2013
Xylaria hypoxylon ^{T}	CBS 122620	KY610407	KY610495	Wendt *et al*. 2018

^{T} EX-types strains. Newly generated sequences are indicated in black bold. “NA” sequences are unavailable.

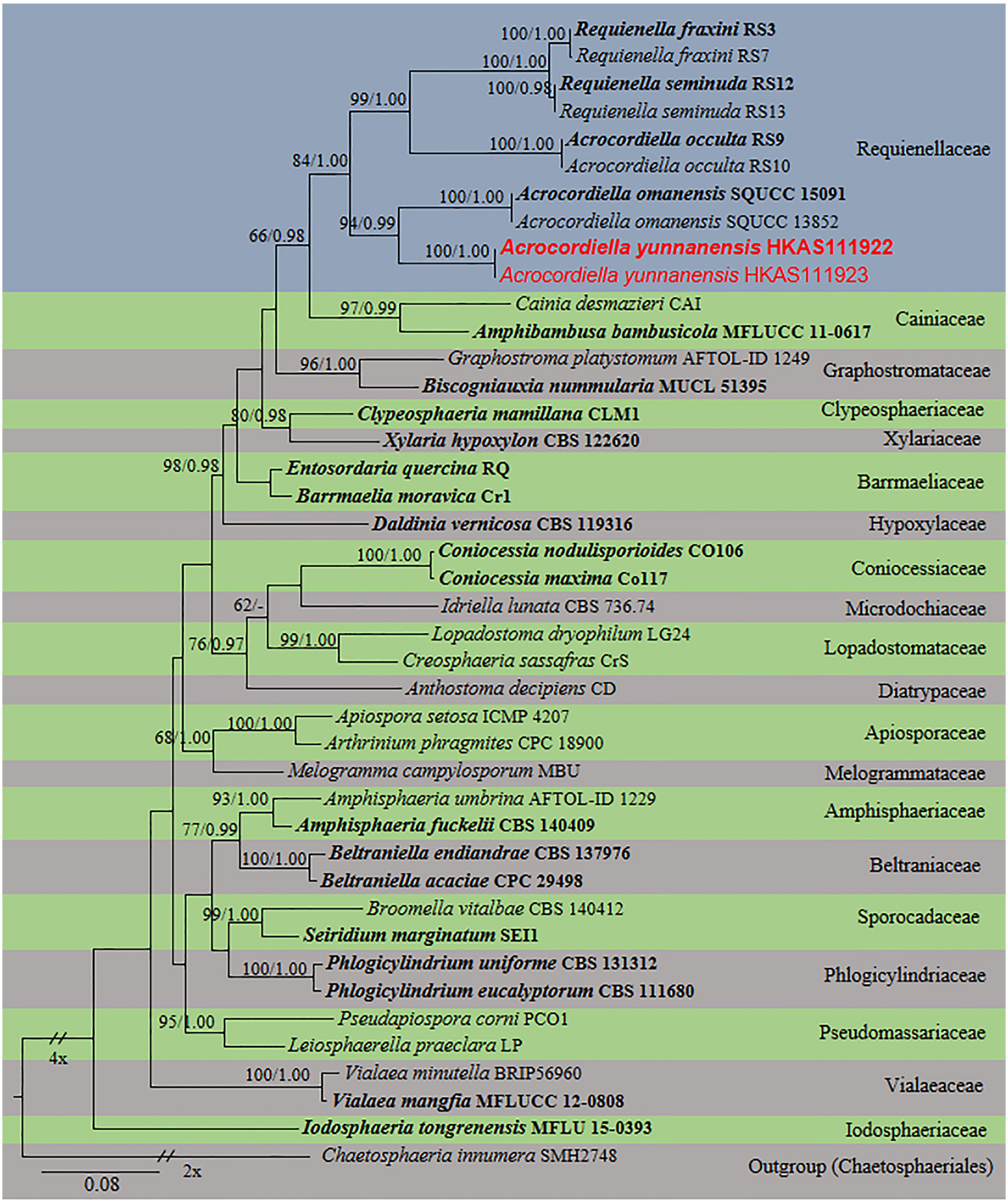

FIGURE 1. RAxML tree based on a combined dataset of partial LSU and ITS sequence analyses. Bootstrap support values for ML equal to or greater than 60 %, Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are shown as ML/ BYPP above the nodes. Type strains are in black bold and the new isolates are in red bold. The tree is rooted to *Chaetosphaeria innumera* (SMH2748). The scale bar represents the expected number of nucleotide substitutions per site.

Results

Phylogenetic analyses

The concatenated alignment contained 1343characters, including gaps (LSU: 1-875 and ITS: 876-1343), 42 strains of ingroups and one outgroup taxon (*Chaetosphaeria innumera* SMH2748). The best scoring ML tree was chosen to represent the phylogenetic relationships of the new taxon with other taxa in *Requienellaceae* (Figure 1), with a final optimization likelihood value of -11344.351548. All model parameters were estimated by the GTR + I + G best-fit model of rate heterogeneity, with 618 distinct alignment patterns and 12.72 % undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.258565, C = 0.218702, G = 0.278726 and T = 0.244007, with substitution rates AC = 1.073485, AG = 2.356041, AT = 1.737634, CG = 0.771289, CT = 5.188866 and GT = 1.000000. The proportion of invariable sites was I = 0.431814, the gamma distribution shape parameter alpha = 0.481263 and the Tree-Length = 3.071027. Phylogenetic trees obtained from ML and BYPP analyses yielded trees with similar overall topologies as found in previous work (Jaklitsch *et al*. 2016, Maharachchikumbura *et al*. 2018).

Requienellaceae clade (Figure 1) comprised species from two genera *viz*. *Acrocordiella* and *Requienella*. *Acrocordiella occulata* is closer to *Requienella* species than other species of *Acrocordiella*. *Acrocordiella omanensis* forms a strongly supported clade (94 % ML and 1.00 BYPP, Figure 1) with the newly introduced *A. yunnanensis*.

Taxonomy

Acrocordiella yunnanensis L.S. Dissan, J.C. Kang & K.D. Hyde, ***sp. nov***. (FIGURE 2)
Index Fungorum Number: IF556940, *Facesoffungi number*: FoF 09458

Etymology:—referring to the location Yunnan Province where the fungus was collected.

Holotype:—HKAS111922

Saprobic on woody substrates of undetermined plants. **Sexual morph**: Appearing as black spots on the substrate with black margins, sometimes with circular sunken perforation through the bark. *Ascomata* 290–325 × 325–355 µm ($\bar{x}$ = 307 × 336 µm, n = 10), immersed, solitary or in small groups within the bark, perithecial, globose to subglobose, black, ostiolate, papillate, periphysate. *Papilla* surrounded by a narrow darkened clypeus. *Peridium* 10–15 µm ($\bar{x}$ = 12.5 µm) thick, comprising brown to hyaline, multi-layered, thick-walled cells of *textura angularis*. *Paraphyses* 3–5 µm ($\bar{x}$ = 3.8 µm) wide, longer than asci, numerous, hardly septate, curved, hardly branched, trabeculae. *Asci* 100–160 × 30–40 µm ($\bar{x}$ = 125 × 36 µm, n = 10), arising from basal and lateral cavity-surface of the ascoma, 8-spored, in the middle often biseriately arranged ascospores, bitunicate, fissitunicate, narrowly clavate to ovoid, or fusiform, with short, simple to knob-like pedicel, with thick-walled apex, inversely funnel-shaped dome (8–10 × 10–15 µm), staining pale yellow in Melzer's solution. *Ascospores* 25–40 × 10–15 µm ($\bar{x}$ = 34 × 12.5 µm), ellipsoid, narrowly rounded to nearly acute at the ends, multi-guttulate, 3–7 transversely septate, with 1 longitudinal septum, muriform, at first hyaline, greyish olive when young, becoming olivaceous to medium brown when mature, smooth and thick-walled with a mucilaginous sheath. **Asexual morph**: Undetermined.

Material examined:—CHINA, Yunnan Province, Xishuangbanna, Menghai County, Bulangshanxiang, 21.575908 N, 100.325724 E, 1063 m absl, on dead twigs of an unidentified dicotyledonous plant, 19 December 2018, D.N. Wanasinghe (HKAS111922, **holotype**); *ibid*. (HKAS111923, **isotype**).

Known distribution:—Yunnan Province, China

Notes:—Phenotypic characterization reveals that our new species resembles *Acrocordiella* in its immersed ascomata with white or black margins and a peridium comprising brown to hyaline, thick-walled cells of *textura angularis* (Hyde *et al*. 2020). In phylogenetic analyses (Figure. 1), *A. yunnanensis* clusters to *A. omanensis* with relatively high bootstrap support (94% ML; 0.99% BYPP), but *A. yunnanensis* can easily be distinguished from *A. omanensis*. *Acrocordiella omanensis* has unitunicate, cylindrical asci with uniserriate 3-distoseptate ascospores without longitudinal septa, whereas *A. yunnanensis* has narrowly clavate to ovoid asci with biseriate, muriform ascospores with 3–7 distosepta with mucilaginous sheath. Furthermore, the ascomata dimensions of *A. omanensis* are larger than those of *A. yunnanensis* ($\bar{x}$ = 780 × 720 μm vs 307 × 336 μm).

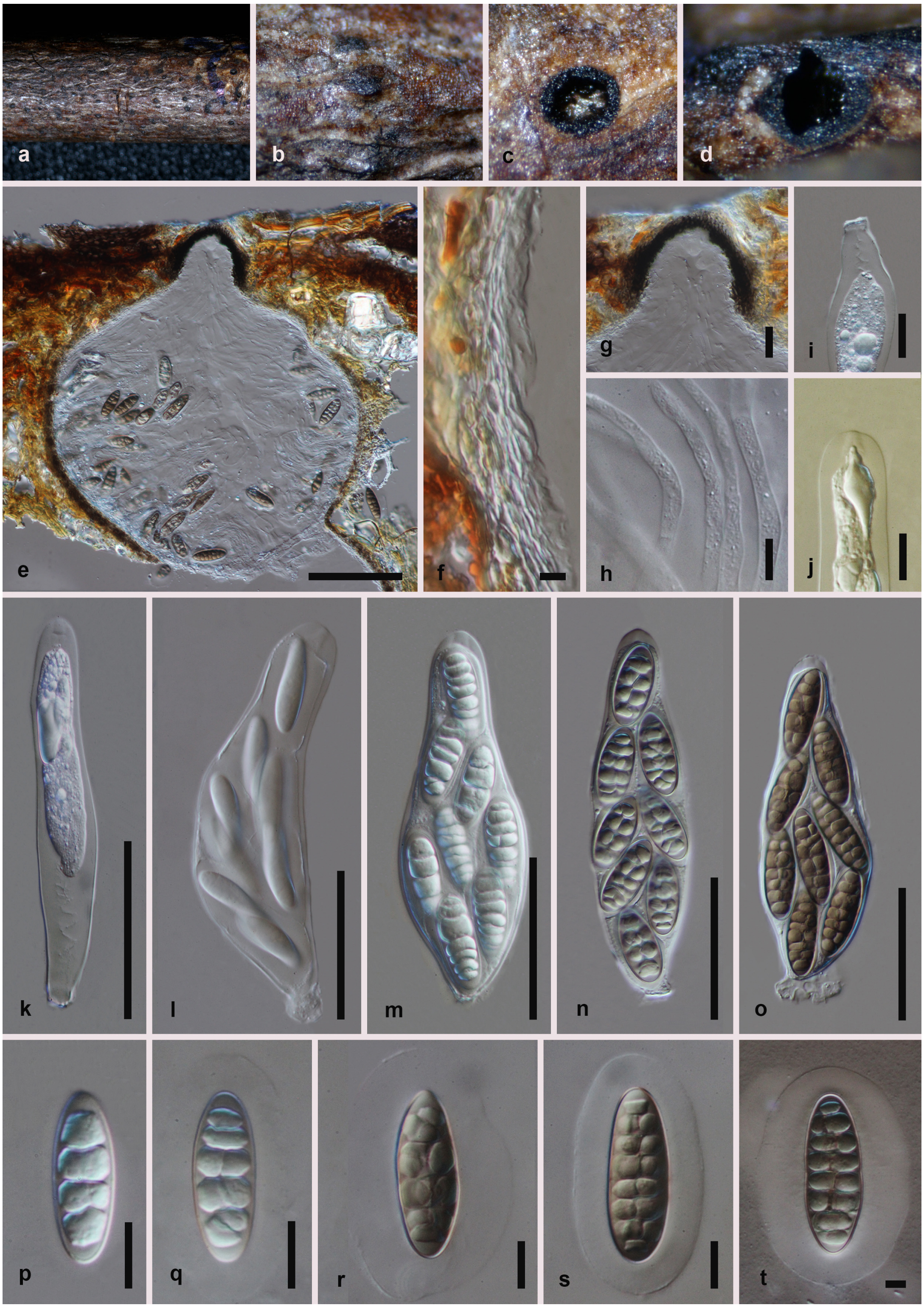

FIGURE 2. *Acrocordiella yunnanensis* (HKAS111922, holotype). a, b. Ascomata on host substrate c. Transverse section of an ascoma. d, e. Vertical sections of ascomata. f. Peridium g. Ostiolar neck. h. Paraphyses. i–o. Asci (j. in Melzer's solution). p–t. Ascospores (t. in Indian ink). Scale bars: e = 100 μm, g, i, j. = 20 μm, k–o. = 50 μm, h, p–s. = 10 μm, t–f. = 5 μm.

Key to species in *Acrocordiella*

1	Ascospores only transversely septate	2
1	Ascospores muriform	***A. yunnanensis***
2	Clypeus present around the ostiole	***A. omanensis***
2	Clypeus absent around the ostiole	***A. occulta***

Discussion

The placement of Requienellaceae has been subjected to several changes in recent years. *Acrocordiella occulta*, *Requienella fraxini* and *R. seminude* were re-described by Jaklitsch *et al*. (2016) based on taxonomy, ecology and sequence data. Jaklitsch *et al*. (2016) showed that Requienellaceae belongs to *Xylariales* in Sordariomycetes, despite its fissitunicate asci, *Requienella* is distinct from *Acrocordiella*. The resulting ML single gene trees based on ITS and LSU loci (results are not shown) and combined ML tree were not congruent in this study. In the combined analysis, the type of *A. occulta* is related to *Requienella* (Figure 1). Therefore, one could argue that *Acrocordiella* does not group in the same sense as monophyletic groups. However, our current sampling and analyses were not powerful enough to synonymize *Acrocordiella* under *Requienella.* Further collections and additional gene regions are essential to resolve the placement of *Acrocordiella* in Requienellaceae. Two other genera *viz Lacrymospora* and *Parapyrenis* were previously recognized in Requienellaceae by Wijayawardene *et al*. (2017) and Maharachchikumbura *et al*. (2018). However, Jaklitsch *et al*. (2016) and Voglmayr *et al*. (2019) only accepted *Requienella* and *Acrocordiella* for their phylogenetic analyses. *Lacrymospora* is differentiated from *Parapyrenis* by blackening of the thallus around the ascomata (Aptroot 1991) and asymmetrical ascospores with several rows of granules, indicating septation (Calatayud *et al*. 2001). *Parapyrenis* have been studied through cultures and subjected to DNA (LSU and ITS) sequence analyses. According to LSU and ITS sequence data (Vu *et al*. 2019), *Parapyrenis conica* is related to Herpotrichiellaceae (Chaetothyriales) and *P. maritima* to Hypocreales. Therefore, species in this genus should be transferred to different orders. However, until the type species of *Parapyrenis* is sequenced, placement of the genus remains doubtful. Fresh collections are needed to understand the taxonomic status and to provide support as a separate genus.

Requienellaceae appears not to be host-specific, as the family can be found on a wide range of hosts in various habitats. They usually occur on plants or lichens, e.g., *Acrocordiella occulta* was reported from the bark of *Ribes* sp., in Central Europe, *A. omanensis* on stems of *Juniper* sp. in Oman, *Parapyrenis lichenicola* on thalli of *Pertusaria erythrella*, *Requienella fraxini* on the bark of old living *Fraxinus excelsior* trunks in Northern/Western Europe and North America, and *R. seminuda* on the bark of dead *Olea europaea* trees in Western and Southern Europe (Aptroot 1997, Hawksworth & Halici 2007, Jaklitsch *et al*. 2016, Maharachchikumbura *et al*. 2018). In this study, *Acrocordiella yunnanensis* is introduced as the first *Acrocordiella* species reported on woody substrates in China.

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