Current Research in Environmental & Applied Mycology (Journal of Fungal Biology)
13(1): 1–15 (2023) ISSN 2229-2225
www.creamjournal.org **Article**
Doi 10.5943/cream/13/1/1

Bambusicolous fungi in Guangdong, China: establishing *Apiospora magnispora* sp. nov. (Apiosporaceae, Amphisphaeriales) based on morphological and molecular evidence

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Zhao HJ, Dong W, Shu YX, Mapook A, Manawasinghe IS, Doilom M, Luo M 2023 – Bambusicolous fungi in Guangdong, China: establishing *Apiospora magnispora* sp. nov. (Apiosporaceae, Amphisphaeriales) based on morphological and molecular evidence. Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 13(1), 1–15, Doi 10.5943/cream/13/1/1

Abstract

Apiospora species are cosmopolitan and often associated with various bamboo hosts. During an investigation of the diversity of bambusicolous fungi in Guangdong Province, China, an interesting coelomycete was encountered from a decaying bamboo culm of *Bambusa textilis*. Morphological comparison and multi-locus phylogeny of combined internal transcribed spacer (ITS), the partial large subunit nuclear rDNA (LSU), the translation elongation factor 1-alpha gene (*tef*1-α) and beta-tubulins (*tub2*) sequence data support it as a novel species in *Apiospora*, namely *Apiospora magnispora*. The new species is featured as having solitary, irregular-shaped, oval, subglobose, reddish brown and large conidia, which often become dry and show a large hollow on the surface. A detailed description is provided and the taxon is illustrated.

Keywords – 1 new species – bamboo – multi-locus phylogeny – saprobe – taxonomy

Introduction

Chinese bamboo is known for its economic and cultural value due to its extensive use in Asian furniture and construction of roof thatch, the edibility of bamboo shoots, as well as the positive effect of being a Chinese traditional medicine for treating infections and healing (Janssen 1991, Dransfield & Widjaja 1995, Lin 2004, Liu et al. 2010, Arbain et al. 2020, Lopes et al. 2021, Silva et al. 2021). Bamboo is often colonized by a large number of fungal species including saprobes, endophytes and pathogens, which lead to the serious loss of beauty and practical value (Hyde et al. 2002a, b, 2013, 2020, Ariyawansa et al. 2015, Dai et al. 2017, Zhang et al. 2017, Dong et al. 2020, Wijayawardene et al. 2020, Boonmee et al. 2021, Yin et al. 2021). Thus, it is of economic significance to investigate the fungal diversity, taxonomy and phylogeny of bambusicolous fungi.

Apiospora (Apiosporaceae, Amphisphaeriales) is one of the most important and commonly occurring fungal genera on bamboo (Jiang et al. 2019, Pintos & Alvarado 2021, Tian et al. 2021).

Submitted 14 October 2022, Accepted 9 November 2022, Published 9 January 2023
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Apiospora was introduced by Saccardo (1875) with *A. montagnei* as the type species. The sexual morph is characterized by multi-locular, perithecial stromata with clavate to fusoid, and hyaline ascospores surrounded by a gelatinous sheath (Saccardo 1875, Pintos & Alvarado 2021). The asexual morph has two forms, hyphomycetous and coelomycetous. The hyphomycetes is characterized by subcylindrical setae, subcylindrical, flexuous, hyaline conidiophores, basauxic conidiogenous cells and globose to subglobose, obovoid, pale brown to brown conidia. The coelomycete has irregular-shaped and dark brown conidia with a thin, longitudinal, transparent slit (Pintos & Alvarado 2021). It has been proved that many ascomycetous *Apiospora* species are fertile and can reproduce the hyphomycetous asexual status on artificial media, such as *A. balearica* on MEA, *A. phragmitis* on OA and *A. septatum* on PDA (Crous & Groenewald 2013, Dai et al. 2017, Feng et al. 2021). Therefore, the asexual-sexual morph was linked by cultural and molecular evidence.

Besides the saprophytes living on the decaying bamboo culms, *Apiospora* species are also plant pathogens and endophytes. *Apiospora kogelbergensis* can cause blight disease of *Schizostachyum* spp. (Crous & Groenewald 2013), *A. arundinis* can cause leaf necrosis and dieback of *Olea europaea* and *A. arundinis* can cause leaf edge spot of *Prunus persica* (Bagherabadi et al. 2014, Chen et al. 2014, Jiang et al. 2020). In addition, *A. montagnei*, as an endophyte, was isolated from the inner tissue of the North Sea alga *Polysiphonia violacea* (Klemke et al. 2004).

The objectives of this study were to investigate the diversity of bambusicolous fungi in the less-studied region, Guangdong Province, China, and report on novel species. A new collection possessing the characteristics of *Apiospora* was subjected to taxonomic and phylogenetic study. In order to ascertain the classification, a multi-locus analysis of a concatenated internal transcribed spacer (ITS), nuc 28S rDNA (LSU), translation elongation factor 1-alpha (*tef1*-α) and beta-tubulin (*tub2*) dataset was used.

Materials & Methods

Collecting, isolation and morphology

Decaying bamboo culms were collected from Danxia Mountain, Renhua County, Shaoguan City, Guangdong Province, China (E 113°44′22″, N 25°2′39″, altitude of 130 m) on 17 November 2020. The samples were taken to the laboratory in a zip-lock bag and were examined using a stereomicroscope (Carl Zeiss Microscopy GmbH 37081 Gottingen, Germany) to locate the fruiting bodies after one week of incubation at room temperature (25–28°C). The fungal structures were photographed using a compound microscope (Nikon Eclipse Ni-U, Japan) fitted with a digital camera (Canon 750D, Japan). All fungal structures were measured with the TaroSoft (R) Image Frame Work program (v. 0.9.0.7).

Single spore isolations were made following the method described by Chomnunti et al. (2014) and Senanayake et al. (2020). Germinated spores were aseptically transferred into fresh potato dextrose agar (PDA) plates, and then incubated at 25°C to obtain pure cultures. Colony characteristics were photographed. All pure cultures obtained in this study are deposited in Zhongkai University of Agriculture and Engineering Culture Collection, China (ZHKUCC). Herbarium materials are deposited in the herbaria of Zhongkai University of Agriculture and Engineering, China (ZHKU).

DNA extraction, PCR amplification and sequencing

The total genomic DNA was extracted from 7-day-old mycelia grown on PDA using a fungus genomic DNA extraction kit (Solarbio, China). DNA amplification was performed by polymerase chain reaction (PCR). The ITS, LSU, *tef*1-α and *tub2* sequences were amplified using primer pairs ITS1/ITS4, LR0R/LR5, EF1-728F/EF-2 and BT2a/BT2b, respectively (Vilgalys & Hester 1990, White et al. 1990, Glass & Donaldson 1995, Carbone & Kohn 1999). The amplifications were carried out in a 25 µl reaction volume containing 1 µl of DNA template, 1 µl of each primer, 12.5 µl of 2× PCR Master Mix, and 9.5 µl of double-distilled sterilized water (ddH_2O). Adjusted thermal

cycles for the amplification of each locus are given in Table 1. The PCR products were checked on 1% agarose electrophoresis gel stained with ethidium bromide (EB). The sequencing reactions were carried out by Tianyi Huiyuan Co., Guangzhou, China.

Table 1 Adjusted thermal cycler conditions used in PCR amplification (all denaturation, annealing and elongation procedures were carried out with 35 cycles).

Locus	Primer	PCR thermal cycle protocol				
		Initial denaturation	Denaturation	Annealing	Elongation	Final extension
ITS	ITS1/ITS4	95°C for 3 min	95°C for 30 s	58°C for 30 s	72°C for 1 min	72°C for 10 min
LSU	LR0R/LR5	94°C for 5 min	94°C for 1 min	56°C for 50 s	72°C for 1 min	72°C for 10 min
*tef*1-α	EF1-728F/EF-2	95°C for 3 min	95°C for 30 s	52°C for 30 s	72°C for 1 min	72°C for 10 min
tub2	BT2a/BT2b	95°C for 3 min	95°C for 30 s	58°C for 30 s	72°C for 1 min	72°C for 10 min

Sequence alignment and phylogenetic analysis

The chromatograms of sequence data obtained from the company were checked with SeqMan (v. 7.0.0) (Swindell & Plasterer 1997). BLASTn (BLAST: Basic Local Alignment Search Tool (nih.gov)) searches were made using the newly generated sequences to assist in taxon sampling for phylogenetic analyses. To confirm the phylogenetic position of our isolates, reference sequences were downloaded from NCBI GenBank (Table 2) following relevant publications (Tian et al. 2021, Samarakoon et al. 2022). Each sequence dataset of the ITS, LSU, *tef*1-α, and *tub2* was aligned using the online version of MAFFT v. 7.0362 (MAFFT alignment and NJ / UPGMA phylogeny (cbrc.jp)) (Katoh et al. 2013) with default settings and manually adjusted using BioEdit v. 7.0 (Hall 1999) when necessary. Phylogenetic analyses were implemented using maximum likelihood (ML) (Stamatakis 2014), maximum parsimony analysis (MP) (Swofford 2003) and Bayesian inference (BI) (Huelsenbeck & Ronqvist 2001).

ML analysis was performed by RAxML (Stamatakis 2014) implemented in RAxML – HPC2 on XSEDE (CIPRES) using the ML + rapid bootstrap setting and the GTRGAMMA model of nucleotide substitution with 1000 replicates.

MP analysis was performed using PAUP v. 4.0b10 (Swofford 2003). All characters were unordered and had equal weight. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. MaxTrees were set up to 1,000 branches collapsed if the minimum branch length is zero, and all multiple parsimonious trees were saved. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), homoplasy index (HI), and log likelihood (-ln L) were calculated for trees generated under different optimality criteria. The robustness of the most parsimonious trees was evaluated by 1,000 bootstrap replications resulting from maximum parsimony analysis, each with ten replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino–Hasegawa tests (Kishino & Hasegawa 1989) were carried out to determine if the inferred trees differ significantly under different optimization criteria.

MrBayes v. 3.0b4 (Huelsenbeck & Ronqvist 2001) was used for the BI analysis. Partitioning of data was initially done by locus and then the parameters of the nucleotide substitution models for every partition were selected independently using the CIPRES J model under the Akaike information criterion (AIC) executed. The models TIM3+G for ITS, TIM1+I+G for LSU, TPM2uf+I+G for *tef*1-α and HKY+G for *tub2* were set for their respective genes in the analysis. Markov chain Monte Carlo (MCMC) was run for 2,000,000 generations, and the trees were sampled every 100th generation. The first 25% of the trees that represented the burn-in phase were discarded, and the remaining 75% of the trees were used for calculating the posterior probabilities

(PP) for the majority rule consensus tree. The resulting trees were viewed in FigTree v. 1.4.0 (Institute of Evolutionary Biology, University of Edinburgh, UK) (Rambaut 2012) and annotated in Adobe Illustrator CS6.

Table 2 Details of the isolates used in the phylogenetic analyses.

Species	Strain	Substrate	Origin	GenBank accession number			
				ITS	LSU	*tef1-α*	*tub2*
Apiospora acutiapica	KUMCC 20-0210	*Bambusa bambos*	China	MT946343	MT946339	MT947360	MT947366
Apiospora aquatica	S-642	Submerged wood	China	MK828608	MK835806	-	-
Apiospora arundinis	CBS 133509	*Aspergillus flavus* sclerotium buried in sandy field	USA	KF144886	KF144930	KF145018	KF144976
Apiospora arundinis	CBS 449.92	Bamboo	Canada	KF144887	KF144931	KF145019	KF144977
Apiospora aurea	**CBS 244.83**	**Air**	**Spain**	**AB220251**	**KF144935**	**KF145023**	**KF144981**
Apiospora balearica	**CBS 145129**	**Undetermined *Poaceae***	**Spain**	**MK014869**	**MK014836**	**MK017946**	**MK017975**
Apiospora bambusae	**ICPM6889**	**Bamboo**	**New Zealand**	**MK014874**	**MK014841**	**MK017951**	**MK017980**
Apiospora bambusicola	**MFLUCC 20-0144**	**Culms of *Schizostachyum brachycladum***	**Thailand**	**MW173030**	**MW173087**	**MW183262**	**-**
Apiospora biserialis	**CGMCC 3.20135**	**Bamboo**	**China**	**MW481708**	**MW478885**	**MW522938**	**MW522955**
Apiospora camelliaesinensis	**LC 5007**	***Camellia sinensis***	**China**	**KY494704**	**KY494780**	**KY705103**	**KY705173**
Apiospora chromolaenae	**MFLUCC 17-1505**	***Chromolaena odorata***	**Thailand**	**MT214342**	**MT214436**	**MT235802**	**-**
Apiospora chiangraiense	**MFLUCC21-0053**	**Dead culms of bamboo**	**Thailand**	**MZ542520**	**MZ542524**	**-**	**MZ546409**
Apiospora cordylinae	GUCC 10026	*Cordyline fruticosa*	China	MT040105	-	MT040126	MT040147
Apiospora cyclobalanopsidis	**CGMCC 3.20136**	***Cyclobalanopsidis glauca***	**China**	**MW481713**	**MW478892**	**MW522945**	**MW522962**
Apiospora descalsii	**CBS 145130**	**Dead culms of *Ampelodesmos mauritanicus***	**Spain**	**MK014870**	**MK014837**	**MK017947**	**MK017976**
Apiospora dichotomanthi	**LC4950**	***Dichotomanthes tristaniicarpa***	**China**	**KY494697**	**KY494773**	**KY705096**	**KY705167**
Apiospora esporlensis	**CBS 145136**	**Dead culms of *Phyllostachys aurea***	**Spain**	**MK014878**	**MK014845**	**MK017954**	**MK017983**
Apiospora euphorbiae	IMI 285638b	*Bambusa* sp.	Bangladesh	AB220241	AB220335	-	AB220288

Table 2 Continued.

Species	Strain	Substrate	Origin	GenBank accession number			
				ITS	LSU	*tef1-α*	*tub2*
Apiospora gaoyouensis	**CFCC 52301**	**Living leaves and culms of *Phragmites australis***	**China**	**MH197124**	**-**	**MH236793**	**MH236789**
Apiospora garethjonesii	**KUMCC 16-0202**	**Dead culms of bamboo**	**China**	**KY356086**	**KY356091**	**-**	**-**
Apiospora gelatinosa	**KHAS 11962**	**Bamboo**	**China**	**MW481706**	**MW478888**	**MW522941**	**MW522958**
Apiospora guiyangensis	**HKAS 102403**	**grass**	**China**	**NR_175678**	**MW240577**	**MW759535**	**MW775604**
Apiospora guizhouensis	**LC5322**	**Air in karst cave**	**China**	**KY494709**	**KY494785**	**KY705108**	**KY705178**
Apiospora hispanica	**IMI 326877**	**Beach sand**	**Spain**	**AB220242**	**AB220336**	**-**	**AB220289**
Apiospora hydei	**CBS 114990**	**Culms of *Bambusa tuldoides***	**China**	**KF144890**	**KF144936**	**KF145024**	**KF144982**
Apiospora hyphopodii	**MFLUCC 15-0003**	**Culms of *Bambusa tuldoides***	**China**	**KR069110**	**-**	**-**	**-**
Apiospora iberica	**CBS 145137**	**Dead culms of *Arundo donax***	**Portugal**	**MK014879**	**MK014846**	**MK017955**	**MK017984**
Apiospora intestini	**CBS 135835**	**Gut of a grasshopper**	**India**	**KR011352**	**MH877577**	**KR011351**	**KR011350**
Apiospora italica	**CBS 145138**	***Arundo donax***	**Italy**	**MK014880**	**MK014847**	**MK017956**	**MK017985**
Apiospora jatrophae	**AMH-9557**	***Jatropha podagrica***	**India**	**JQ246355**	**-**	**-**	**-**
Apiospora jiangxiensis	**LC4577**	***Maesa* sp.**	**China**	**KY494693**	**KY494769**	**KY705092**	**KY705163**
Apiospora kogelbergensis	**CBS 113333**	**Dead culms of *Restionaceae***	**South Africa**	**KF144892**	**KF144938**	**KF145026**	**KF144984**
Apiospora locuta-pollinis	**LC11683**	***Brassica campestris***	**China**	**MF939595**	**-**	**MF939616**	**MF939622**
Apiospora longistroma	**MFLUCC 11-0481**	**Dead culms of bamboo**	**Thailand**	**KU940141**	**KU863129**	**-**	**-**
Apiospora magnispora	**ZHKUCC 22-0001**	***Bamboo***	**China**	**OM728647**	**OM486971**	**OM543543**	**OM0543544**
Apiospora marii	**CBS 497.90**	**Beach sands**	**Spain**	**AB220252**	**KF144947**	**KF145035**	**KF144993**
Apiospora mediterranea	**IMI 326875**	**Air**	**Spain**	**AB220243**	**AB220337**	**-**	**AB220290**
Apiospora minutispora	17E-042	Mountain soil	Korea	LC517882	-	LC518889	LC518888
Apiospora mytilomorpha	**DAOM 214595**	Dead blades of *Andropogon* sp.	India	KY494685	-	-	-
Apiospora neobambusae	**LC 7106**	Leaves of bamboo	China	KY494718	KY494794	KY806204	KY705186
Apiospora neochinensis	**CFCC 53036**	*Fargesia qinlingensis*	China	MK819291	-	MK818545	MK818547
Apiospora neogarethjonesii	**DQD 2019a**	Bamboo	China	MK070897	MK070898	-	-
Apiospora neosubglobosa	**KUMCC 16-0203**	Bamboo	China	KY356090	KY356095	-	-
Apiospora obovata	**LC4940**	*Lithocarpus* sp.	China	KY494696	KY494772	KY705095	KY705166

Table 2 Continued.

Species	Strain	Substrate	Origin	GenBank accession number			
				ITS	LSU	*tef1-α*	*tub2*
Apiospora ovata	**CBS 115042**	*Arundinaria hindsii*	China	KF144903	KF144950	KF145037	KF144995
Apiospora paraphaeosperma	**MFLUCC 13-0644**	Dead culms of bamboo	Thailand	KX822128	KX822124	-	-
Apiospora phragmitis	**CPC 18900**	**Culms of *Phragmites australis***	**Italy**	**KF144909**	**KF144956**	**KF145043**	**KF145001**
Apiospora phyllostachydis	**MFLUCC 18-1101**	**Dead culms of *Phyllostachys heteroclada***	**China**	**MK351842**	**MH368077**	**MK340918**	**MK291949**
Apiospora piptatheri	**CBS 145149**	**Dead culms of *Piptatherum miliaceum***	**Spain**	**MK014893**	**MK014860**	**MK017969**	**-**
Apiospora pseudomarii	**GUCC 10228**	***Aristolochia debilis***	**China**	**MT040124**	**-**	**MT040145**	**MT040166**
Apiospora pseudoparenchymatica	**LC7234**	**Leaves of bamboo**	**China**	**KY494743**	**KY494819**	**KY705139**	**KY705211**
Apiospora pseudorasikravindrae	**KUMCC 20-0208**	***Bambusa dolichoclada***	**China**	**MT946344**	**-**	**MT947361**	**MT947367**
Apiospora pseudosinensis	**CPC 21546**	**Leaves of bamboo**	**Netherlands**	**KF144910**	**KF144957**	**KF145044**	**-**
Apiospora pseudospegazzinii	**CBS 102052**	***Macaranga hullettii* stem colonised by ants**	**Malaysia**	**KF144911**	**KF144958**	**KF145045**	**KF145002**
Apiospora pterosperma	**CPC 20193**	**Leaves of *Lepidosperma gladiatum***	**Australia**	**KF144913**	**KF144960**	**KF145046**	**KF145004**
Apiospora qinlingensis	**CFCC 52303**	**Dead culms of *Fargesia qinlingensis***	**China**	**MH197120**	**-**	**MH236795**	**MH236791**
Apiospora rasikravindrae	**NFCCI 2144**	**Soil**	**Norway**	**JF326454**	**-**	**-**	**-**
Apiospora sacchari	CBS 372.67	Air	Unknown	KF144918	KF144964	KF145049	KF145007
Apiospora sacchari	CBS 664.74	Soil under *Calluna vulgaris*	Netherlands	KF144919	KF144965	KF145052	KF145008
Apiospora saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145051	KF145009
Apiospora saccharicola	CBS 831.71	-	Unknown	KF144922	KF144969	KF145054	KF145012
Apiospora septata	**CGMCC 3.20134**	**Bamboo**	**China**	**MW481711**	**MW478890**	**MW522943**	**MW522960**
Apiospora serenensis	**IMI 326869**	**Food, pharmaceutical excipients,**	**Spain**	**AB220250**	**AB220344**	**-**	**AB220297**

Table 2 Continued.

Species	Strain	Substrate	Origin	GenBank accession number			
				ITS	LSU	*tef1-α*	*tub2*
		atmosphere and home dust					
Apiospora setariae	Beilin 024	*Setaria viridis*	China	MT492005	-	MW118457	MT497467
Apiospora setostroma	**KUMCC19-0217**	**Dead branches of bamboo**	**China**	**MN528012**	**MN528011**	**MN527357**	**-**
Apiospora sichuanensis	**HKAS 107008**	***Poaceae***	**China**	**MW240648**	**MW240578**	**MW759536**	**MW775605**
Apiospora sorghi	**URM 93000**	***Sorghum bicolor***	**Brazil**	**MK371706**	**-**	**-**	**MK348526**
Apiospora subglobosa	**MFLUCC 11-0397**	**Dead culms of bamboo**	**Thailand**	**KR069112**	**KR069113**	**-**	**-**
Apiospora subrosea	**LC7292**	**Leaves of bamboo**	**China**	**KY494752**	**KY494828**	**KY705148**	**KY705220**
Apiospora thailandica	**MFLUCC 15-0202**	**Dead culms of bamboo**	**Thailand**	**KU940145**	**KU863133**	**-**	**-**
Apiospora vietnamensis	**IMI 99670**	***Citrus sinensis***	**Vietnam**	**KX986096**	**KX986111**	**-**	**KY019466**
Apiospora xenocordella	**CBS 478.86**	**Soil from roadway**	**Zimbabwe**	**KF144925**	**KF144970**	**KF145055**	**KF145013**
Apiospora yunnana	**MFLUCC 15-1002**	**Dead culms of *Phyllostachys nigra***	**China**	**KU940147**	**KU863135**	**-**	**-**
Neoarthrinium trachycarpi	**CFCC 53038**	**Dead branches of *Trachycarpus fortune***	**China**	**MK301098**	**-**	**MK303396**	**MK303394**
Neoarthrinium urticae	IMI 326344	Leaf litter	India	AB220245	AB220339	-	AB220292

Notes: Newly generated sequences are indicated in red. Ex-type strains are in bold. Abbreviations: **AMH**, Ajrekar Mycological Herbarium, Pune, Maharashtra, India; **CBS**, Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **CFCC**, China Forestry Culture Collection Center, Beijing, China; **CPC**, Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; **DAOM**, Canadian Collection of Fungal Cultures, Ottawa, Canada; **DDQ**, D.Q. Dai; ICMP: International Collection of Microorganisms from Plants, New Zealand; **IFO**, Institute for Fermentation, Osaka, Japan; **IMI**, Culture collection of CABI Europe UK Centre, Egham, UK; **LC**, personal culture collection of Lei Cai, housed in the Institute of Microbiology, Chinese Academy of Sciences, China; **MFLUCC**, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NFCCI**, National Fungal Culture Collection of India.

Results

Phylogenetic analyses

The ML tree of *Apiospora* species based on ITS-LSU-*tef*1-α-*tub2* dataset comprised 73 sequences from *Apiospora* including isolates from this study. *Neoarthrinium trachycarpi* CFCC 53038 and *N. urticae* IMI 326344 were selected as the outgroup taxa (Fig. 1). The combined alignment of four gene regions was analyzed and the best scoring RAxML tree is shown in Fig. 1 with a final ML optimization likelihood value of -36564.536806. The matrix had 1807 distinct alignment patterns, with 39.41% of undetermined characters or gaps. Estimated base frequencies were as follows;

A = 0.237330, C = 0.258914, G = 0.247102, and T = 0.256653; substitution rates AC = 1.129748, AG = 2.369378, AT = 1.001597, CG = 0.946244, CT = 3.768719, GT = 1.000000, gamma distribution shape parameter α = 0.746790. The MP analysis with combined ITS-LSU-*tef*1-α-*tub2* gene data comprised 3810 total characters including gaps, of which 1912 characters were constant, 1284 characters were parsimony-informative, and 614 variable characters are parsimony-uninformative. In the most parsimonious tree, TL = 6695, CI = 0.468, RI = 0.637, RC = 0.298, and HI = 0.532. The Bayesian analysis resulted in 15,000 trees after 2,000,000 generations. All trees (ML, MP, and BYPP) were similar in topology and did not differ significantly (data not shown). The isolate obtained in this study clustered with a strain IMI 285638b with 97 and 0.99% (ML and BYPP) bootstrap support (Fig. 1).

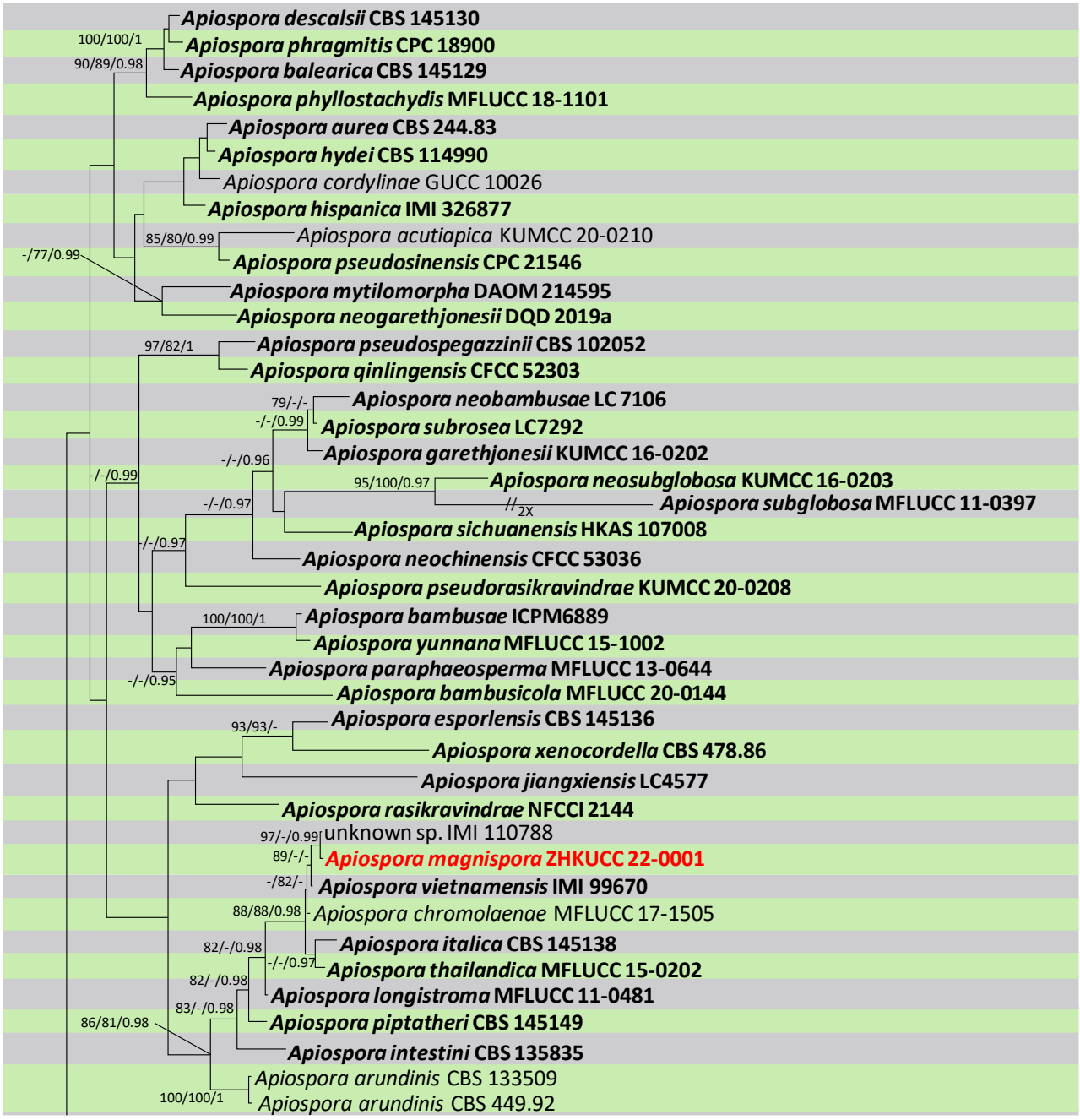

Fig. 1 – Phylogenetic tree generated from maximum likelihood (ML) analysis based on combined ITS, LSU, *tef*1-α and *tub2* sequence data. Bootstrap support values for ML and MP equal to greater than 75%, and posterior probabilities equal to greater than 0.95 are given at the nodes

(ML/MP/BYPP). The tree is rooted to *Neoarthrinium trachycarpi* CFCC 53038 and *N. urticae* IMI 326344. The ex-type strains are indicated in **bold**. The new species is indicated in red.

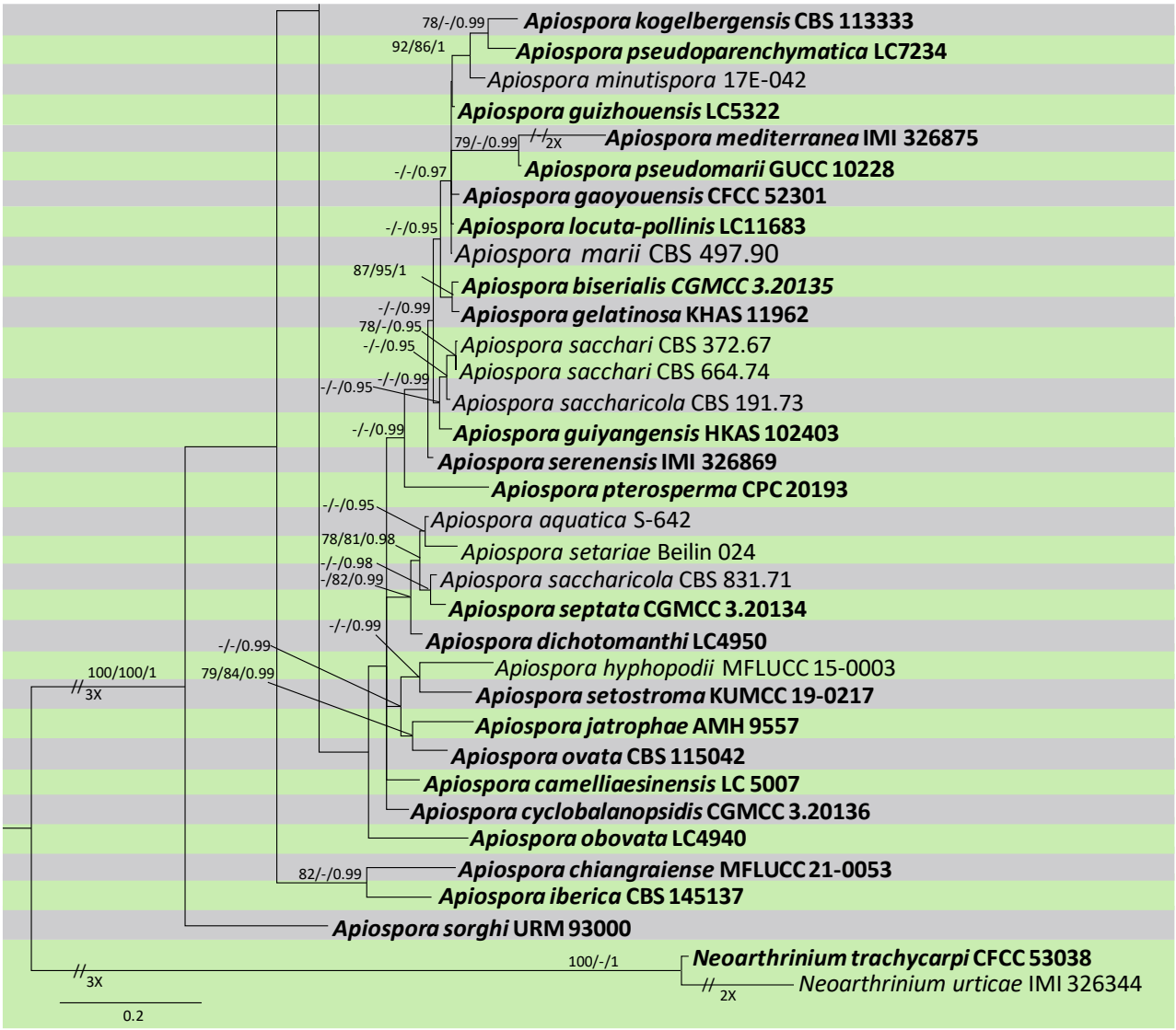

Fig. 1 – Continued.

Taxonomy

Apiospora magnispora H.J. Zhao, Manawas. & W. Dong, sp. nov. Fig. 2

Index Fungorum number: IF557850; Facesoffungi number: FoF12961

Etymology – named in reference to the large conidia.

Diagnosis – Saprobic on dead a dead twig of *Bambusa textilis* McClure (Poaceae). Sexual morph: undetermined. Asexual morph: coelomycetous. *Conidiomata* 700–1000 µm high, 300–500 µm diam., scattered to gregarious, immersed in the substrate, becoming erumpent when mature, dark brown to black, raised, pustulate. *Conidiomatal wall* 20–50 µm thick, composed of dark brown, thin-walled cells of *textura angularis*. *Conidiophores* 20–35 × 4–7 µm ($\bar{x}$ = 28 × 5 µm, n = 20), hyphoid, subcylindrical, brown, barely septate, unbranched, curved, smooth. *Conidiogenus cells* 3–5× 2–5 µm ($\bar{x}$ = 4 × 3 µm, n = 15), holoblastic, monoblastic, integrated, determinate, terminal, doliiform or lageniform, brown, smooth. *Conidia* 20–35 × 15–25 µm ($\bar{x}$ = 26 × 21 µm, n = 40), solitary, irregular-shaped, oval, subglobose, brown to medium brown or reddish brown, smooth, easily become wizened and showing a hollow on the surface.

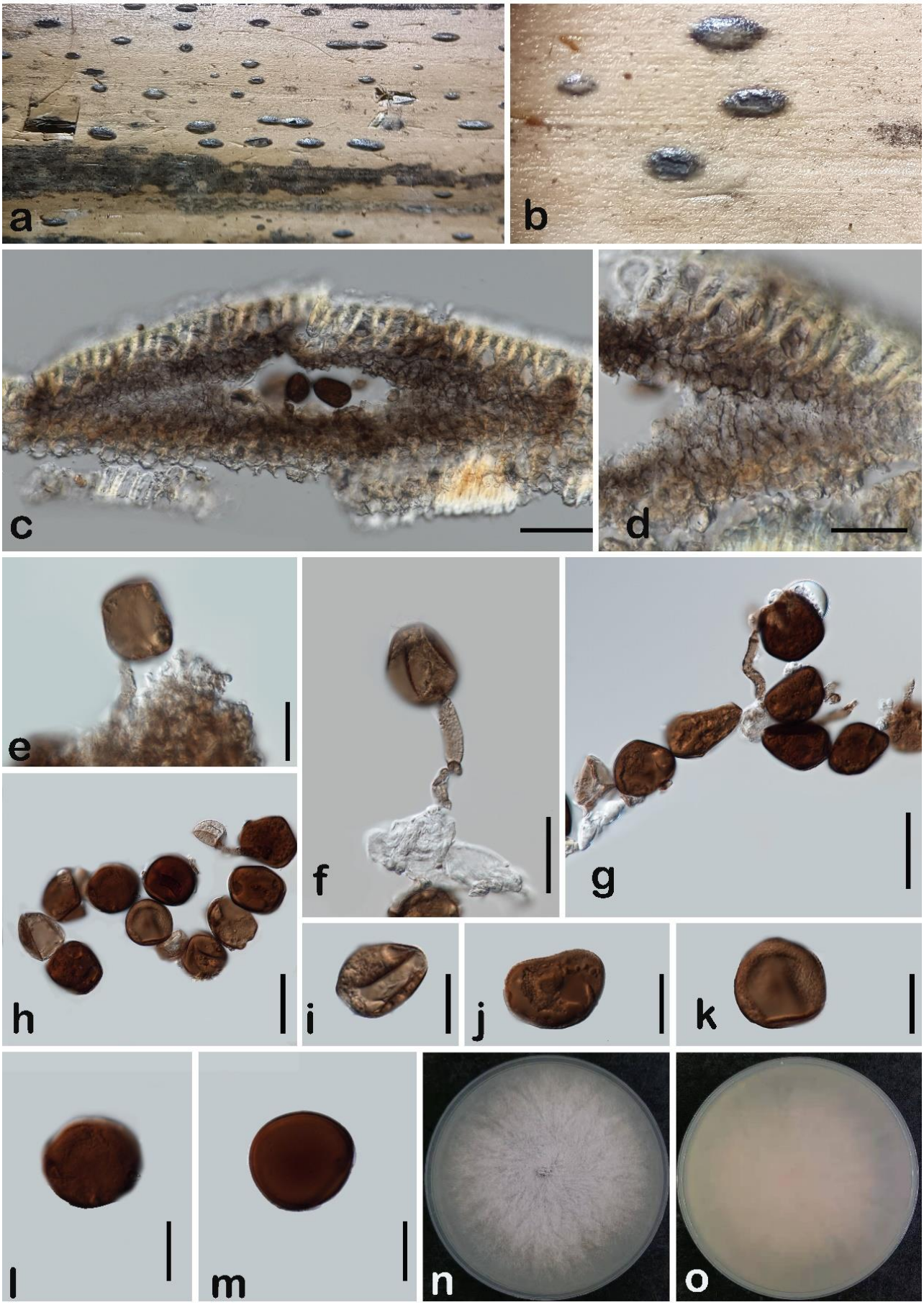

Fig. 2 – *Apiospora magnispora* (ZHKU 22-0001, holotype). a, b Conidiomata on the host substrate. c Section through conidioma. d Conidiomatal wall. e, f Conidiophore bearing conidium.

g–m Conidia. n, o Culture on PDA from the front and below. Scale bars: c, e–m = 10 μm, d = 20 μm.

Culture characteristics – Conidia germinated on PDA within 12 hours at 25°C. Colonies on PDA reach 6–8 cm diam. after 7 days at 25°C. Colonies circular, medium dense, flattened, white radial, margin undulate. Reverse white, becoming tawny from the center.

Material examined – China, Guangdong Province, Shaoguan City, Renhua County, Danxia Mountain, isolated from a dead twig of *Bambusa textilis* McClure (Poaceae), E 113°44′22″, N 25°2′39″, 17 November 2020, H.J. Zhao & Y.R. Xiong ZHJ001 (ZHKU 22-0001, holotype); ex-type living cultures, ZHKUCC 22-0001.

Notes – in our multi-locus phylogeny, the new collection ZHKUCC 22-0001 is affiliated with *A. chromolaenae*, *A. italica*, *A. thailandica* and *A. vietnamensis* (Fig. 1). They share similar characteristics in having pale brown to dark brown and generally subglobose conidia, but they can be easily distinguished by conidial morphology and dimension (Dai et al. 2016, Wang et al. 2017, Pintos et al. 2019, Mapook et al. 2020). The four species are hyphomycetes, while the new collection is a coelomycetes. The four species have subglobose to globose and lenticular conidia with a slit, while the new collection has irregular-shaped conidia which easily become wizened and show a hollow on the surface and lack an obvious slit. In addition, the new collection has larger conidia measured as 20–35 × 15–25 μm, while they are 4–6 × 4.5–6.5 μm in *A. chromolaenae*, 4–6 × 3–4 μm in *A. italica*, 5–9 × 5–8 μm in *A. thailandica* and 5–6 μm diam. in *A. vietnamensis*. Given the above reasons, *Apiospora magnispora* sp. nov. is established for the new collection ZHKUCC 22-0001.

Discussion

Bamboo is a gramineous plant with economic and ornamental value with approximately 1,400 described species in 101–118 genera (Kelchner & Bamboo Phylogeny Group 2013). In this study, we introduce a new species *Apiospora magnispora* which was isolated from a dead bamboo clum of *Bambusa textilis* in Guangdong Province, China. In the multi-locus phylogenetic analysis, *A. magnispora* clustered with a strain IMI 285638b bearing the name "*Apiospora euphorbiae*" with high bootstrap support (Fig. 1). The nucleotide comparison showed that they had 100% similarities in ITS, *tef* and *tub2* loci, respectively. Although the strain IMI 285638b was often used in the previous phylogenetic analyses, it has never been published and the morphological data is unavailable for comparison. Therefore, the type specimen *A. euphorbiae* IMI 110788 was compared with *A. magnispora*.

Apiospora euphorbiae is a hyphomycetes characterized by hyaline conidiophores with numerous brown transverse septa, and lenticular, brown or olivaceous brown conidia with a pale equatorial slit (Ellis 1965). In contrast, *A. magnispora* is coelomycetes having brown conidiophores with quite sparse septa, and irregular-shaped, reddish-brown conidia without a slit but easily become wizened and showing a hollow on the surface. According to the figure illustration in the protologue, *A. euphorbiae* has a long conidiophore with intercalary conidiogenous cells producing a string of conidia, which also significantly differs from *A. magnispora* (Ellis 1965). The above evidence shows that the scientific name of IMI 285638b must not be "*A. euphorbiae*" but it might be another strain of our new species. In this study, IMI 285638b (ITS: AB220241, LSU: AB220335, tub2: AB220288) is treated as an invalid strain and labelled as "unknown sp. IMI 285638b" in the phylogenetic tree (Fig. 1).

Apiospora is a large genus comprising 146 epithets in Index Fungorum 2022. The genus is quite similar to *Arthrinium* and their relationships were debated for a long time (Crous & Groenewald 2013, Pintos & Alvarado 2021). With more fresh collections and molecular data populated in the phylogenetic analyses, some *Arthrinium* species have been transferred to *Apiospora* (Tian et al. 2021). Nevertheless, the morphological and phylogenetic relationships of *Apiospora* and *Arthrinium* have not been well-resolved due to the inconspicuous differences in morphology and insufficient sequence data. In this study, 75 strains were subjected to multi-locus

analysis, of which 15 strains lack LSU sequence data, 21 strains lack *tef* sequence data and 18 strains lack *tub2* sequence data. As a result, many species are not well-separated in the phylogenetic tree, such as the clade comprising *A. locuta-pollinis*, *A. marii* and *A. gaoyouensis*, and the clade comprising *A. chromolaenae* and *A. vietnamensis* (Fig. 1). In recent years, the guidelines of introducing the novel taxa have been reviewed and they highlighted the importance of fungal morphology and enough molecular characters (Jeewon & Hyde 2016, Chethana et al. 2021, Maharachchikumbura et al. 2021, Jayawardena et al. 2021). Wijayawardene et al. (2012) detailed how the taxonomy of coelomycetes has developed and a monograph of coelomycetes was completed by Li et al. (2020) who provided a comprehensive understanding of hyaline-spored coelomycetes. However, the dematiaceous coelomycetes still receive less attention. The fungal number has recently been estimated and the fact showed that a large number of fascinating fungi are yet to be discovered and described (Bhunjun et al. 2022, Calabon et al. 2022, Phukhamsakda et al. 2022, Senanayake et al. 2022, Wijayawardene et al. 2022). To obtain natural and stable phylogenetic relationships of *Apiospora* species as well as its allied genus *Arthrinium*, much taxonomic work is pending in the future.

Acknowledgements

Wei Dong thanks the National Natural Science Foundation of China (Grant no. 32200015) and Talent Program of Zhongkai University of Agricultural and Engineering (KA22016B787). Mingkwan Doilom acknowledges the Talent Program of Zhongkai University of Agricultural and Engineering (KA22016B746). The project was funded by the High-Level Talents in Zhongkai University of Agriculture and Engineering (Grant no. J2201080102). We would like to acknowledge Dr. Shaun Pennycook, Nomenclature Editor, Mycotaxon for helping with new species names.

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