Mycosphere 7 (9): 1332–1345 (2016) www.mycosphere.org ISSN 2077 7019

Article – special issue
Doi 10.5943/mycosphere/7/9/7

Two new species of *Arthrinium* (*Apiosporaceae*, *Xylariales*) associated with bamboo from Yunnan, China

Dai DQ[1,*], Jiang HB[1], Tang LZ[1], Bhat DJ[2,3]

[1] *Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, China*
[2] *Formerly, Department of Botany, Goa University, Goa, India*
[3] *No. 128/1-J, Azad Housing Society, Curca, Goa Velha-403108, India*
Corresponding author: Dong-Qin Dai, email: cicidaidongqin@gmail.com

Dai DQ, Jiang HB, Tang LZ, Bhat DJ 2016 – Two new species of *Arthrinium* (*Apiosporaceae*, *Xylariales*) associated with bamboo from Yunnan, China. Mycosphere 7 (9), 1332–1345, Doi 10.5943/mycosphere/7/9/7

Abstract

Arthrinium, a globally distributed genus, is characterized by basauxic conidiogenesis in its asexual morph, with globose to subglobose conidia, which are usually lenticular in side view and obovioid and brown to dark brown. The sexual morph develops multi-locular perithecial stromata with hyaline apiospores usually surrounded by a thick gelatinous sheath. Four *Arthrinium* species collected in Kunming, China, are described and illustrated in this paper. Based on the morphology and analyses of ITS sequence data, we introduce two new species of *Arthrinium*. *Arthrinium garethjonesii* and *A. neosubglobosa* spp. nov. are provided with descriptions of sexual morphs and compared with phylogenetically close taxa. *Arthrinium hydei* and *A. hyphopodii* are new records on their sexual morphs.

Keywords – bambusicolous fungi – phylogeny – Sordariomycetes – taxonomy

Introduction

Arthrinium Kunze ex Fr. (sexual morph: *Apiospora* Sacc.) is the type genus of *Apiosporaceae* and has a widespread distribution occurring in various habitats (Sharma et al. 2014). Taxa are endophytes, pathogens or saprobes, usually isolated from soil debris, plants, lichens and marine algae (Senanayake et al. 2015), or occasionally from erythematous nodules of human beings (Sharma et al. 2014). Some *Arthrinium* species such as *A. arundinis* (Corda) Dyko & B. Sutton has been utilized in the pharmaceutical industry as antifungal agents. Crous & Groenewald (2013) synonymized *Apiospora* under *Arthrinium*. The genus *Arthrinium* currently contains 64 epithets with 53 names under *Apiospora* in Index Fungorum (2016). However, most of existing taxa lack sequence data and detailed morphological descriptions. Crous & Groenewald (2013) reviewed the genus *Arthrinium* and included 16 species in the phylogenetic analysis. Sequence data from fresh collections are however, needed to better resolve their natural classifications and establish species numbers. We are studying bambusicolous fungi (Hyde et al. 2002, Dai et al. 2007) and collected four new *Arthrinium* species from Yunnan Province. These are introduced in this paper with molecular data.

Submitted 20 October 2016, Accepted 4 December 2016, Published online 28 December 2016

Materials and methods

Collection, isolation and specimen examination

Dead bamboo culms were collected from Yunnan Province in China. The samples were placed in plastic Zip lock bags and brought to the laboratory. The specimens were examined under stereo- and compound microscopes. Single spore isolates were obtained following the method of Chomnunti et al. (2014), and the specimens were incubated in a moist chamber for seven days at room temperature, to induce sporulation. Microscopic observations were made from slides prepared in distilled water. The micro-morphological characters were examined by differential interference contrast (DIC) using a Nikon ECLIPSE 80*i* microscope with a 600D digital camera. Measurements were made using the Tarosoft (R) Image Frame Work program. Type material is deposited at the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). The living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC), Kunming Culture Collection (KUMCC). Facesoffungi (Jayasiri et al. 2015) and Index Fungorum (2016) numbers were provided for newly described taxa.

DNA extraction, PCR amplification and sequencing

Pure cultures were grown on PDA for 30 days at 27˚C and genomic DNA was extracted from fresh mycelia, following the specifications of the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). ITS5 and ITS4, NS1 and NS4 (White et al. 1990) and LROR and LR5 (Vilgalys & Hester 1990) primers were used for the amplification of internal transcribed spacers (ITS), small subunit rDNA (SSU) and large subunit rDNA (LSU) respectively. Polymerase chain reaction (PCR) amplification was carried out following Dai et al. (2017). Amplified PCR fragments were sequenced at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. and BGI Tech Solutions Co., Ltd. (BGI-Tech), P.R. China. The sequences generated from the ITS, LSU and SSU regions are deposited in GenBank.

Phylogenetic analyses

A blast search in GenBank, using ITS gene was carried out to determine the closest *Arthrinium* taxa. Additional sequences (Crous & Groenewald 2013, Dai et al. 2017) were downloaded from GenBank. A single gene sequence alignment was generated with MAFFT v. 7.215 (Katoh & Standley, 2013, http://mafft.cbrc.jp/alignment/server/index.html) and improved when necessary in BioEdit v. 7.0 (Hall 2004) by manual adjustment. In the analyses, gaps were treated as missing data, and all characters were unordered and of equal weight (Dai et al. 2012).

Maximum-likelihood (ML) analysis including 1000 bootstrap replicates was performed in raxmlGUI v.1.0. (Stamatakis 2006; Silvestro & Michalak 2011). Alignments in PHYLIP format were exchanged and loaded (http://sing.ei.uvigo.es/ALTER/), and the online tool Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was used to determine the best nucleotide substitution model for sequence data. GTR+G was chosen as the best model.

Maximum-parsimony (MP) analysis was run using PAUP v. 4.0b10 (Swofford 2002) with 1000 replications. Maxtrees were set to 1000, branches of zero length were collapsed, and all multiple equally most parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated from 1 000 bootstrap replications (Phillips et al. 2013).

Bayesian analyses were performed using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). The model of evolution was performed by using MrModeltest v. 2.3 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4. Six simultaneous Markov chains were run for 5,000,000 generations and trees were sampled every 100th generation. The burn-in was set to 0.25, and the run was automatically stopped when the average standard deviation of split frequencies reached below 0.01 (Maharachchikumbura et al. 2015).

Trees were visualized with TreeView (Page 1996), and additionally layouts were carried out with Adobe Illustrator CS v. 5. Maximum-parsimony bootstrap values (MPBP) and Maximum-likelihood bootstrap values (MLBP) equal or greater than 50 % are given. Bayesian posterior probabilities (BYPP) greater than 0.80 are given. The alignment and trees were submitted in TreeBASE (www.treebase.org, submission ID: 20359)

Table 1 List of strains and sequences used in the phylogenetic tree in this study.

Species name	Strain	substrate	location	collector	ITS
Arthrinium arundinis	CBS 106.12	/	Germany: Bromberg	E. Schaffnit	KF144883
Arthrinium arundinis	CBS 114316	Leaf of *Hordeum vulgare*	Iran: Shabestar	B. Askari	KF144884
Arthrinium arundinis	CBS 124788	Living leaves of *Fagus sylvatica*	Switzerland: Basel	M. Unterseher	KF144885
Arthrinium arundinis	CBS 133509	*Aspergillus flavus* sclerotium buried in sandy field	USA: Kilbourne	/	KF144886
Arthrinium arundinis	CBS 449.92	Culm of cultivated *Sasa*	Canada: Vancouver	R.J. Bandoni	KF144887
Arthrinium arundinis	CBS 464.83	Dead culms of *Phragmites australis*	The Netherlands: Harderbos	W. Gams	KF144888
Arthrinium arundinis	CBS 732.71	Dung	India	B.C. Lodha	KF144889
Arthrinium aureum	CBS 244.83	Dead culms of *Phragmites australis*	Netherlands: Harderbos	W. Gams	KF144888
Arthrinium garethjonesii	KUMCC 16-0202	Dead culms of bamboo	China: Kunming	H.B. Jiang & D.Q. Dai	KY356086
Arthrinium hydei	**CBS 114990**	Culms of *Bambusa tuldoides*	Hong Kong: Tai Po Kau	K.D. Hyde	KF144890
Arthrinium hydei	KUMCC 16-0204	Dead culms of bamboo	China: Kunming	H.B. Jiang & D.Q. Dai	KY356087
Arthrinium hyphopodii	**MFLUCC 15-0003**	Culms of *Bambusa tuldoides*	China: Kunming	D.Q. Dai & D.Q. Dai	KR069110
Arthrinium hyphopodii	KUMCC 16-0201	Culms of Bamboo	China: Kunming	H.B. Jiang & D.Q. Dai	KY356088
Arthrinium kogelbergense	CBS 113332	Dead culms of *Cannomois virgata*	South Africa	S. Lee	KF144891
Arthrinium kogelbergense	**CBS 113333**	Dead culms of *Restionaceae*	South Africa	S. Lee	KF144892
Arthrinium kogelbergense	CBS 113335	Dead culms of *Restio quadratus*	South Africa	S. Lee	KF144893
Arthrinium kogelbergense	CBS 114734	*Juncus gerardi*	Sweden: Börstil par.	K. & L. Holm	KF144894
Arthrinium kogelbergense	CBS 117206	Unknown algae	Croatia	E. Eguereva	KF144895
Arthrinium longistromum	**MFLUCC 11-0479**	Dead culms of bamboo	Thailand: Chiang Rai	D.Q. Dai	KU940142
Arthrinium longistromum	MFLUCC 11-0481	Dead culms of bamboo	Thailand: Chiang Rai	D.Q. Dai	KU940141
Arthrinium malaysianum	**CBS 102053**	*Macaranga hullettii* stem colonised by ants	Malaysia: Gombak	W. Federle	KF144896
Arthrinium malaysianum	CBS 251.29	Stembase of *Cinnamomum camphora*	/	/	KF144897
Arthrinium marii	CBS 113535	Oats	Sweden	C. Svenson	KF144898
Arthrinium marii	CBS 114803	Culm of *Arundinaria hindsi*	Hong Kong: Lung Fu Shan	K.D. Hyde	KF144899
Arthrinium marii	CBS 200.57	Leaf of *Beta vulgaris*	Netherlands	/	KF144900
Arthrinium marii	CBS 497.90	Beach sand	Spain: Barcelona	J.V. Larrondo & A. Calvo	AB220252
Arthrinium marii	CPC 18902	Stems of *Phragmites australis*	Italy: Bomarzo	W. Gams	KF144901
Arthrinium marii	CPC 18904	Stems of *Phragmites australis*	Italy: Bomarzo	W. Gams	KF144902
Arthrinium montagnei	ToD.7.1	Insect: Ips typographus	Sweden	/	FJ824610
Arthrinium montagnei	VL170	Stump of burned *Pinus mugo*	Lithuania	V. Lygis	JF440582
Arthrinium neosubglobosa	JHB006	Dead culms of bamboo	China: Kunming	H.B. Jiang & D.Q. Dai	KY356089
Arthrinium	KUMCC	Dead culms of bamboo	China: Kunming	H.B. Jiang & D.Q.	KY356090

Species name	Strain	substrate	location	collector	ITS
neosubglobosa	16-0203			Dai	
Arthrinium ovatum	**CBS 115042**	*Arundinaria hindsii*	Hong Kong	K.D. Hyde	KF144903
Arthrinium paraphaeospermum	**MFLUCC 13-0644**	Dead culms of bamboo	Thailand: Chiangmai	C. Senanayake	KX822128
Arthrinium phaeospermum	CBS 114314	Leaf of *Hordeum vulgare*	Iran: Marand	B. Askari	KF144904
Arthrinium phaeospermum	CBS 114315	Leaf of *Hordeum vulgare*	Iran: Shabestar	B. Askari	KF144905
Arthrinium phaeospermum	CBS 114317	Leaf of *Hordeum vulgare*	Iran: Marand	B. Askari	KF144906
Arthrinium phaeospermum	CBS 114318	Leaf of *Hordeum vulgare*	Iran: Marand	B. Askari	KF144907
Arthrinium phragmites	**CPC 18900**	Culms of *Phragmites australis*	Italy: Bomarzo	W. Gams	KF144909
Arthrinium pseudosinense	**CPC 21546**	Leaf of bamboo	Netherlands: Utrecht	U. Damm	KF144910
Arthrinium pseudospegazzinii	**CBS 102052**	*Macaranga hullettii* stem colonised by ants	Malaysia: Gombak	W. Federle	KF144911
Arthrinium pterospermum	CBS 123185	Leaf lesion of *Machaerina sinclairii*	New Zealand: Auckland	C.F. Hill	KF144912
Arthrinium pterospermum	CPC 20193	Leaf of *Lepidosperma gladiatum*	Australia: Adelaide	W. Quaedvlieg	KF144913
Arthrinium rasikravindrae	MFLUCC 11-0616	Dead culms of bamboo	Thailand: Krabi	K.D. Hyde	KU940144
Arthrinium rasikravindrae	MFLUCC 15-0203	Dead culms of bamboo	Thailand: Krabi	K.D. Hyde	KU940143
Arthrinium rasikravindrae	CBS 337.61	*Cissus*	Netherlands	H.A. van der Aa	KF144914
Arthrinium rasikravindrae	**NFCCI 2144**	Soil	Norway: Ny-Alesund	S.M.Singh	JF326454
Arthrinium sacchari	CBS 212.30	*Phragmites australis*	UK: Cambridge	E.W. Mason	KF144916
Arthrinium sacchari	CBS 301.49	Bamboo	Indonesia	K.B. Boedijn & J. Reitsma	KF144917
Arthrinium sacchari	CBS 372.67	Air	/	/	KF144918
Arthrinium sacchari	CBS 664.74	Soil under *Calluna vulgaris*	Netherlands	H. Linder	KF144919
Arthrinium saccharicola	CBS 191.73	Air	Netherlands	H.A. van der Aa	KF144920
Arthrinium saccharicola	CBS 463.83	Dead culms of *Phragmites australis*	Netherlands: Harderbos	W. Gams	KF144921
Arthrinium saccharicola	CBS 831.71	/	Netherlands	M. van Schothorst	KF144922
Arthrinium saccharicola	CPC 18977	*Phragmites australis*	Netherlands	P.W. Crous	KF144923
Arthrinium subglobosa	MFLUCC 11-0397	Dead culms of bamboo	Thailand: Chiang Rai	D.Q. Dai	KR069112
Arthrinium thailandicum	MFLUCC 15-0199	Dead culms of bamboo	Thailand: Krabi	K.D. Hyde	KU940146
Arthrinium thailandicum	**MFLUCC 15-0202**	Dead culms of bamboo	Thailand: Krabi	K.D. Hyde	KU940145
Arthrinium xenocordella	**CBS 478.86**	Soil from roadway	Zimbabwe: Matopos	J.C. Krug	KF144925
Arthrinium xenocordella	CBS 595.66	Soil	Austria: Plaseckerjoch	M.A.A. Schipper	KF144926
Arthrinium yunnanum	**MFLU 15-0002**	Dead culms of *Phyllostachys nigra*	China: Kunming	D.Q. Dai	KU940147
Arthrinium yunnanum	DDQ00281	Dead culms of *Phyllostachys nigra*	China: Kunming	D.Q. Dai	KU940148
Seiridium phylicae	CPC 19965	*Phylica arborea*	UK: Inaccessible Island	P. Ryan	KC005787

Ex-type strains are in bold, new sequences are in red. Symbol "/" means the information is lacking. Abbreviations: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS. MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; DDQ: D.Q. Dai; JHB: H.B. Jiang; VL:V. Lygis.

Results

Phylogeny

An ITS dataset was used to determine the taxonomic placement of our strains. The dataset comprised 63 strains including five newly sequenced strains plus one outgroup taxon (Table 1).

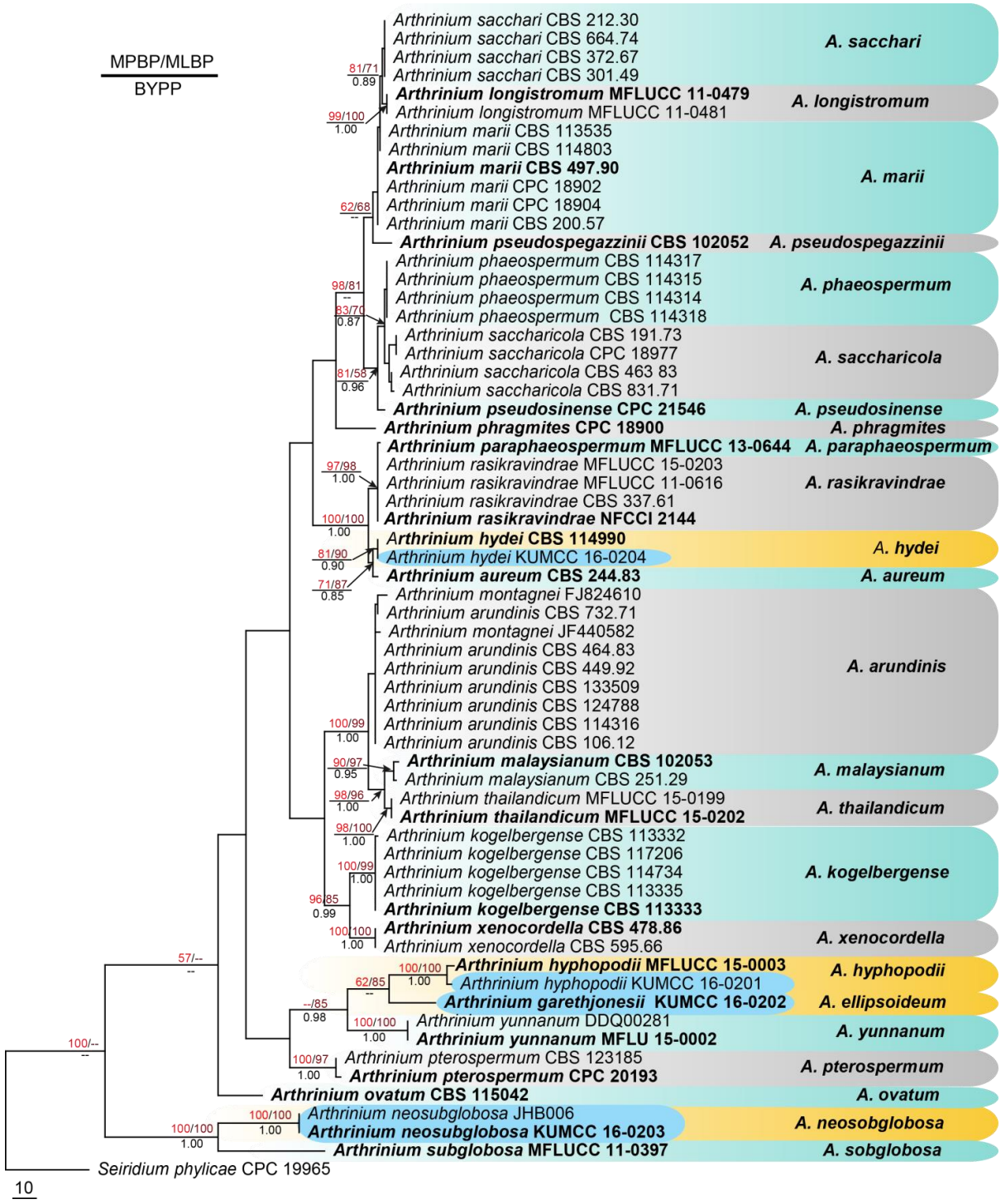

Fig. 1 – One of the parsimonious trees resulting from 1000 bootstrap replicates obtained from the ITS sequence data (CI 0.634, RI 0.876, RC 0.555), The tree is rooted to *Seiridium phylicae* (CPC 19965). Ex-type strains are in bold and newly generated sequences are highlighted with a blue background.

The best tree is shown in Fig. 1. The phylogenetic trees generated by maximum-parsimony (MP), maximum likelihood (ML) and Bayesian analyses using the ITS region demonstrate that new strains clustered within the genus *Arthrinium*, *Apiosporaceae*, *Xylariales*. *Arthrinium hydei* (JHB012) and *A. hyphopodii* (JHB004) grouped with the type strain CBS114990 and MFLUCC 15–0003 with high support (MPBS/MLBS/BYPP 81/90/0.90) and (MPBS/MLBS/BYPP 100/100/1.00) respectively. The new species, *A. garethgonesii* is closest to *A. hyphopodii*, while the branch length can separate these two species. A second novel species *A. neosuboglobosa* groups

with *A. subglobosa* in the same clade, however, they can be phylogenetically distinguished with high bootstrap support (MPBS/MLBS/BYPP 100/100/1.00). Bootstrap support values of MP, ML and the Bayesian posterior probabilities from MCMC analyses are shown in Fig 1. All the strains used in this paper together with their GenBank associate numbers are listed in Table 1. The new sequences of ITS, LSU and SSU regions are deposited in GenBank for further study (Table 2).

Table 2 List of strains sequenced in this study.

Species name	Strain	GenBank accession numbers		
		LSU	SSU	ITS
Arthrinium garethjonesii	KUMCC 16-0202	KY356091	KY356096	KY356086
Arthrinium hydei	KUMCC 16-0204	KY356092	KY356097	KY356087
Arthrinium hyphopodii	KUMCC 16-0201	KY356093	KY356098	KY356088
Arthrinium neosubglobosa	JHB006	KY356094	KY356099	KY356089
Arthrinium neosubglobosa	KUMCC 16-0203	KY356095	KY356100	KY356090

Taxonomy

Arthrinium garethjonesii D.Q. Dai & H.B. Jiang, ***sp. nov.***

Index Fungorum number: IF 552679; Facesoffungi number: FoF 02759, Fig. 2

Etymology – The specific epithet *garethjonesii* refers to the significant contribution of Professor E.B. Gareth Jones made to mycology.

Holotype – HKAS 96289

Saprobic on dead bamboo culms. Sexual morph: *Stromata* 1.3–2 mm long, 0.3–0.5 mm wide, 188–282 μm high, scattered to gregarious, partly immersed, becoming erumpent to superficial, raised, dark brown, in linear rows, with a slit-like opening, multi-loculate. *Ascomata* perithecial, 177–235 μm high, 141–232 μm diam., arranged in rows, clustered, gregarious, with 3–10 perithecia forming groups immersed in stromata to erumpent through host surface, ellipsoidal to subglobose, dark brown, membranous. *Ostiole* raised from centre of ascomata, internally lined with periphyses. *Peridium* 22–35 μm wide, composed of dark brown to reddish brown to hyaline cells of *textura angularis*. *Hamathecium* composed of dense paraphyses, 3.9–6.1 μm wide, filamentous, unbranched and not anastomosed, indistinctly septate, constricted at the septum. *Asci* 125–154 × 35–42 μm ($\bar{x}$ = 139 × 38 μm, n = 20), 8-spored, unitunicate, clavate, apedicellate, apically rounded. *Ascospores* 30–42 × 11–16 μm ($\bar{x}$ = 39 × 13 μm, n = 20), 2-seriate, 1-septate, ellipsoidal, with a small lower cell and a large upper cell, with many guttules, hyaline, smooth-walled, with a faint gelatinous sheath. Asexual morph: Undetermined.

Culture characters – Ascospores germinating on WA within 24 h and germ tubes developing from the upper cell. Colonies fast growing on PDA, reaching 40 mm in 1 week at 23 °C, under 12 h light/12 h dark, cottony, circular, with irregular edge, white from above and below. Mycelium superficial to immersed in media, with branched, septate, smooth hyphae.

Materials examined – CHINA, Yunnan Province, Kunming, Southwest Forest University campus, on dead culms of bamboo, 20 July 2016, Hong-Bo Jiang & Dong-Qin Dai JHB004 (KUN, HKAS 96289, holotype); *Ibid*. (MFLU, isotype), living cultures, MFLUCC, KUMCC 16-0202.

Notes – *Arthrinium garethjonesii* is phylogenetically close to *A. hyphopodii* based on maximum-parsimony (MP) analysis (Fig. 1). Both of these species have dark brown ascostromata in linear rows on bamboo, with a slit-like opening. However, *A. garethjonesii* has shorter stromata (188–282 μm high vs. 369–488 μm high) and smaller 1-septate ascospores (30–42 × 11–16 μm vs. 61–80 × 14–18.5 μm), whereas *A. hyphopodii* has comparatively larger 1-septate ascospores. Furthermore, they are phylogenetically separated by branch length (Fig. 1).

Arthrinium neosubglobosa D.Q. Dai & H.B. Jiang, ***sp. nov.*** Fig. 3

Index Fungorum number: IF552680; Faceoffungi number: FoF02760

Etymology – Refers to the morphological similarity with *Arthrinium subglobosa*.

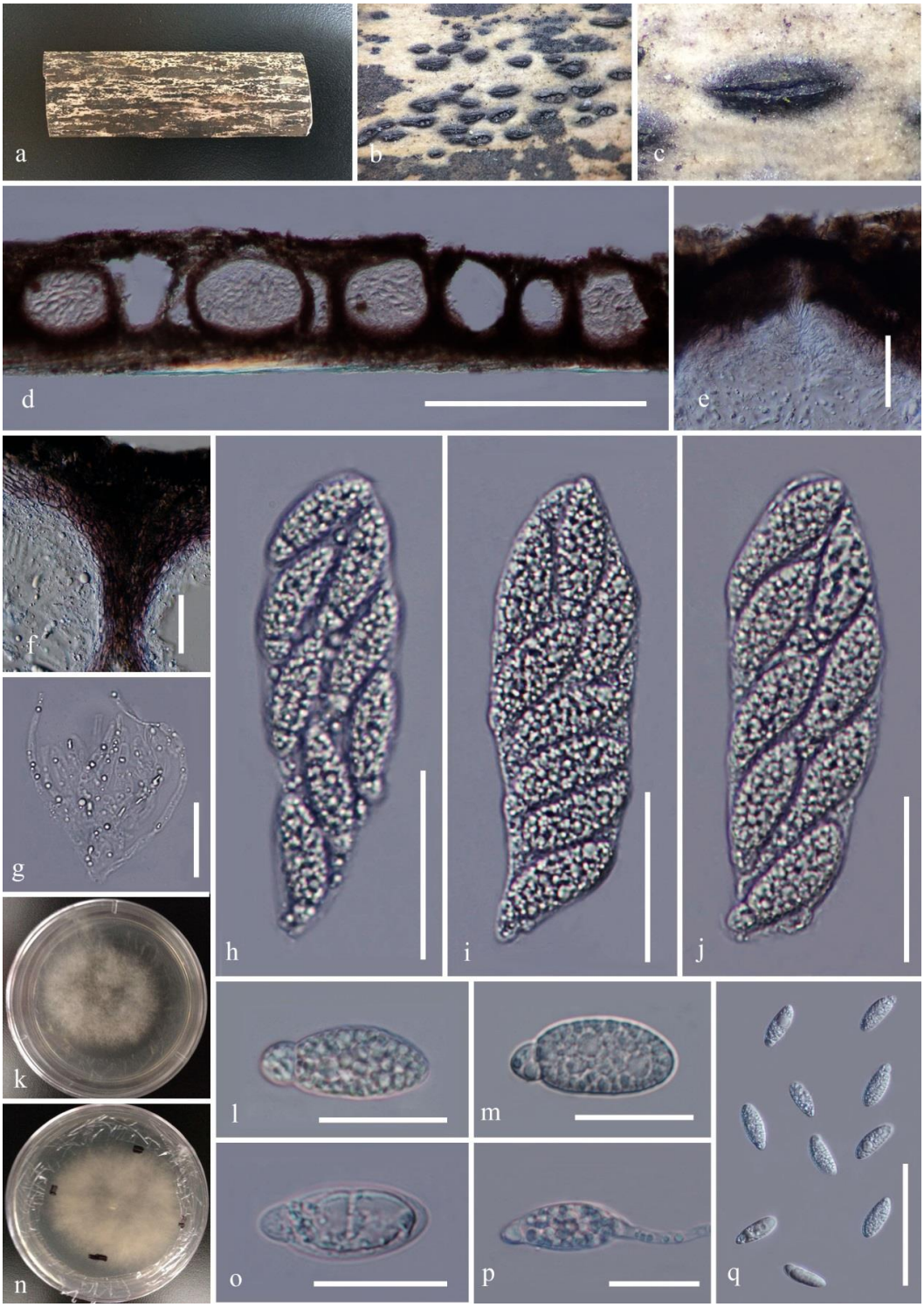

Fig. 2 – *Arthrinium garethjonesii* (HKAS 96289, holotype). a Appearance of stromata on bamboo host. b, c Stromata. d Vertical section of stroma. e Ostiole. f Peridium. g Paraphyses. h–j Asci. l–m, o, q Ascospores. p Germinating ascospore. k, n Colony on PDA. d = 500 μm, q = 100 μm, e–j = 50 μm, l–p = 30 μm.

Holotype – HKAS 96354

Saprobic on dead bamboo culms. Sexual morph: *Stromata* 1.4–2 mm long, 0.3–0.35 mm wide, 300–350 μm high, scattered to gregarious, superficial to raised, with a slit-like opening, dark brown to black, naviculate, with black papillate ostiole, multi-loculate. *Ascomata* perithecial, 278–319 μm high, 196–355 μm diam., arranged in a row, clustered, gregarious, immersed in stromata,

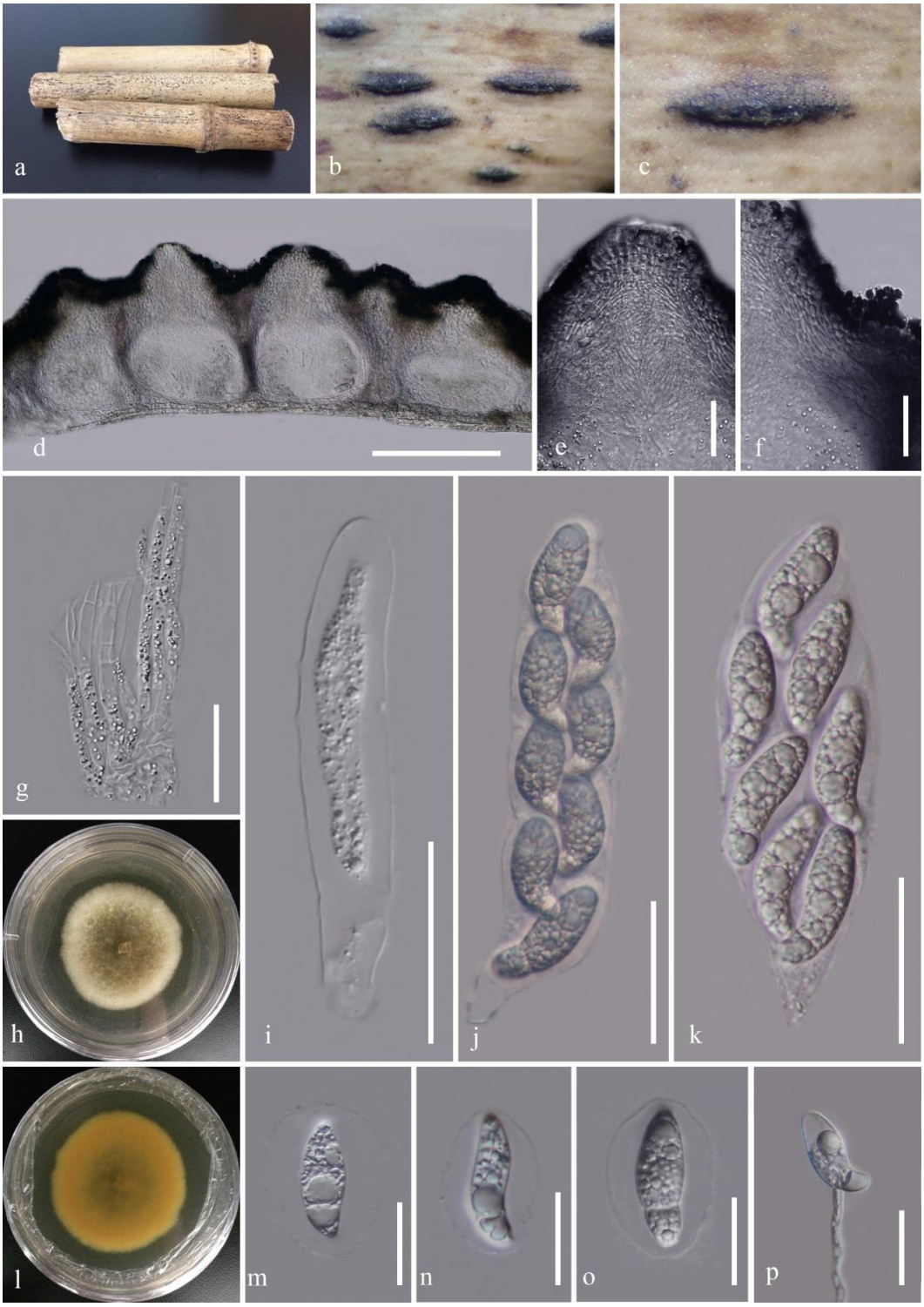

Fig. 3 – *Arthrinium neosubglobosa* (HKAS 96354, holotype). a Appearance of stromata on bamboo host. b, c Stromata. d Vertical section of stroma. e Ostiole. f Peridium. g Paraphyses. i–k Asci. m–o Ascospores surrounded by gelatinous sheath. p Germinating spore. h, l Colony on PDA. d = 250 μm, e–g, i–k = 50 μm, m–p = 30 μm.

later becoming erumpent through host surface to superficial, obpyriform to ampulliform, dark brown, membranous. *Ostiole* raised from centre of ascomata, internally lined with periphyses. *Peridium* 26–44 μm thick, with 2 layers; outer layer composed of dark brown, cells of *textura angularis*; inner layer thin, with hyaline cells of *textura angularis*. *Hamathecium* comprising dense paraphyses, 4.2–6.3 μm wide, indistinctly septate, unbranched, not anastomosing, filamentous-clustered embedded in gelatinous matrix. *Asci* 130–170 × 30–43 μm ($\bar{x}$ = 150 × 37 μm, n = 20), 8-

spored, unitunicate, clavate, with a short pedicel, apically rounded. *Ascospores* 39–47 × 13–16 μm ($\bar{x}$ = 43 × 15 μm, n = 20), 2-seriate, elliptical, hyaline, 1-septate, constricted at the septum, mostly curved at the lower cell, rarely straight, with a large upper cell and a small lower cell, smooth-walled, guttulate, with a shallow 10 μm thick gelatinous sheath. Asexual morph: Undetermined.

Culture characters – Ascospores germinating on WA within 48 h and germ tubes developing from the upper cell. Colonies slow growing on PDA, reaching 20 mm in 1 week at 23 °C, under 12 h light/12 h dark, velvety, circular, with regular edge, yellowish, sparse in the centre, white, dense at the margin from above; yellowish brown from below. Mycelium superficial to immersed in media, with branched, septate, smooth hyphae.

Materials examined – CHINA, Yunnan Province, Kunming, Southwest Forest University campus, on dead culms of bamboo, 20 July 2016, Hong-Bo Jiang & Dong-Qin Dai JHB007 (KUN, HKAS 96354, holotype); *Ibid*. (MFLU, isotype), living cultures, MFLUCC, KUMCC 16-0203; JHB006.

Notes – *Arthrinium neosubglobosa* is similar to *A. subglobosa* in having ascospores with a curved lower cell and yellowish brown culture on PDA (Senanayake et al. 2015). These two species original isolated from bamboo, and form an individual clade in the phylogenetic analysis with high bootstrap support (MPBS/MLBS/BYPP 100/100/1.00) (Fig. 1). However, *A. neosubglobosa* differs by having higher stromata (300–350 μm high vs. 150–300 μm high) and longer asci (130–170 μm long vs. 75–150 μm long) than *A. subglobosa*. In addition, *A. neosubglobosa* has obpyriform to ampulliform ascomata, whereas *A. neosubglobosa* occurs has subglobose ascomata with a flattened base.

Arthrinium hydei Crous, IMA Fungus 4(1): 142 (2013)
Facesoffungi number: FoF02761, Fig 4

Saprobic on rotting bamboo culms, forming black, filiform areas, arranged in a compact way, with stromata breaking through raised cracks at the black centre. Sexual morph: *Stromata* 10–20 mm long, 3.5–4 mm wide, 215–265 μm high, gregarious, initially immersed and raised, later becoming erumpent through host tissue to superficial, shaping a slit-like axis at the top, filiform, dark brown, multi-loculate. *Ascomata* perithecial, 135–230 μm high, 180–215 μm diam., gregarious, immersed in stromata, reddish brown, ampulliform to subglobose usually with flattened base, membranous, with a central, periphysate ostiole. *Peridium* 19–30 μm thick, composed of brown to reddish brown to hyaline cells of *textura angularis*. *Hamathecium* comprising septate, unbranched paraphyses. *Asci* 110–130 × 17–24 μm ($\bar{x}$ = 118 × 21 μm, n = 20), 8-spored, unitunicate, cylindrical to ellipsoidal, no pedicel, apically truncate. *Ascospores* 35–45 × 8.5–11 μm ($\bar{x}$ = 40 × 9.6 μm, n = 20), 2-seriate, broad fusiform to cylindrical, with a large upper cell and a small lower cell, hyaline, 1-septate, constricted at septum, slightly curved, smooth-walled, with many guttules, with a large guttule at the centre of large upper cell, with a distinct gelatinous sheath, 10–20 μm thick. Asexual morph: See Crous et al (2013).

Culture characters – Ascospores germinating on WA within 24 h and germ tubes developing from the upper large part of spore. Colonies growing fast on PDA, reaching 40 mm in 1 week at 23 °C, under 12 h light/12 h dark, cottony, circular, diffuse from centre, with irregular edge, white from above and below. Mycelium superficial to immersed in media, with branched, septate, smooth hyphae.

Materials examined – CHINA, Yunnan Province, Kunming, Southwest Forest University campus, on dead culms of bamboo, 20 July 2016, Hong-Bo Jiang & Dong-Qin Dai JHB0012 (KUN, HKAS 96355); *Ibid*. (MFLU), living cultures, MFLUCC, KUMCC 16-0204.

Notes – *Arthrinium hydei* was originally described by Crous & Groenewald (2013) based on the asexual morph from a culture (CBS 114990) which was isolated from bamboo culms in Hong Kong, China. However, no sexual morph was observed by Crous & Groenewald (2013). A new specimen was collected of sexual morph in Kunming, China and the phylogenetic sequence data indicate that it is *A. hydei* (MPBS/MLBS/BYPP 81/90/0.90, Fig. 1).

Arthrinium hyphopodii D.Q. Dai & K.D. Hyde, in Senanayake et al., Fungal Diversity 73: 112 (2015) Figs 5, 6

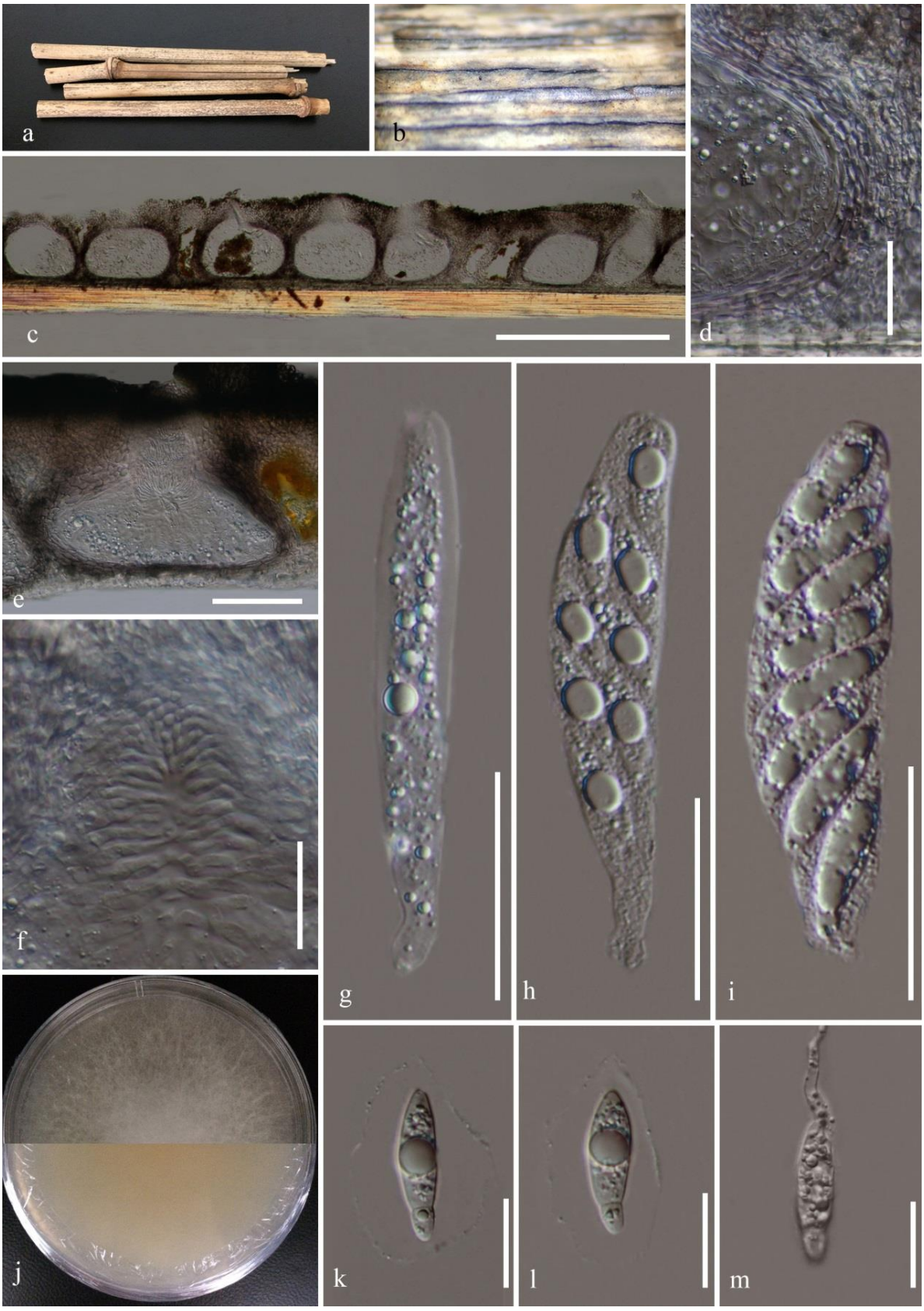

Fig. 4 – *Arthrinium hydei*. a Appearance of stromata on bamboo host. b Stromata. c Vertical section of stroma. d Peridium. e Ascoma. f Ostiole with periphyses. g–i Asci. k, l Ascospores. m Germinating ascospore. j Colony on PDA. c = 500 µm, e = 100 µm, d, g–i = 50 µm, f, k–m = 30 µm.

Saprobic on dead bamboo culms, forming black, lenticular spots on the host surface, with stromata breaking through raised cracks with black centre. Sexual morph: *Stromata* 1.5–2 mm long, 0.35–0.45 mm wide, 370–490 μm high, scattered to gregarious, immersed to erumpent, later becoming superficial, dark brown to black, fusiform to ellipsoid, forming a slit-like opening at the apex, multi-loculate, membranous, with a periphysate ostiole. *Ascomata* perithecial, 295–360 μm high, 185–280 μm diam., arranged in rows, clustered, gregarious, immersed in stromata, ampulliform, dark brown to black. *Peridium* 28–40 μm wide, composed of several layers of dark brown to hyaline cells of *textura angularis*. *Hamathecium* comprising dense, 5–7.8 μm wide, hyaline, septate paraphyses. *Asci* 160–225 × 35–55 μm ($\bar{x}$ = 193.7 × 45.3 μm, n = 20), 8-spored, unitunicate, clavate, apedicellate, apically rounded. *Ascospores* 60–80 × 14–18.5 μm ($\bar{x}$ = 70.5 × 16.3 μm, n = 20), partly overlapping, biseriate, cylindrical to broadly fusiform, slightly rounded both ends, slightly curved, hyaline, septa not observed, smooth-walled, with many guttules, with an indistinct gelatinous sheath. Asexual morph: See Senanayake et al. (2015).

Culture characters – Ascospores germinating on WA within 24 h and germ tubes produced from lower end. Colonies fast growing on PDA, reaching 34 mm in 12 days at 23 °C, under 12 h light/12 h dark, cottony, circular, sparse, raised, with irregular edge, white in centre becoming reddish-brown at the margin from above and below. Mycelium superficial to immersed in media, with branched, septate, smooth hyphae.

Materials examined – CHINA, Yunnan Province, Kunming, Southwest Forest University campus, on dead culms of bamboo, 20 July 2016, Hong-Bo Jiang & Dong-Qin Dai JHB003 (KUN, HKAS 96288); *Ibid*. (MFLU), living cultures, MFLUCC, KUMCC 16-0201; Other material examined: Kunming Institute of Botany, Chinese Academy of Sciences, on dead culm of *Bambusa tuldoides* Munro (*Poaceae*), 7 July 2014, Dong-Qin Dai DDQ00280 (MFLU 15-0383, holotype).

Notes – Senanayake et al. (2015) introduced the species *Arthrinium hyphopodii* based on an asexual morph specimen collected in China, Kunming. We collected a new specimen of sexual morph within a close vicinity, and the phylogenetic analysis showed that they are same species with high bootstrap support (MPBS/MLBS/BYPP 100/100/1.00) (Fig. 1). *Arthrinium hyphopodii* is characterized by brown hyphopodia developing on its conidial germ tubes. Usually *Arthrinium* has apiospores shorter than 50 μm, however, *A. hyphopodii* is unusual in that it has 60–80 μm long ascospores which are acellular, a feature which has not been recorded previously in species of *Arthrinium*.

Discussion

This study treats four *Arthrinium* species, including two new species, *Arthrinium garethjonesii* and *A. neosubglobosa*, two existing species, *Arthrinium hydei* and *A. hyphopodii* which were previously published with only asexual morphs. In the present study, their sexual morphs are confirmed by sequence data. Species of *Arthrinium* are difficult to identify to species level when only the asexual morph are available (Crous & Groenewald 2013). For instance, the *Arthrinium* species can produce hyphomyceteous fruiting bodies in culture or coelomycetous structures on host substrates (Dai et al. 2017). In addition, their conidia are not a useful identifying feature, as most *Arthrinium* species have similar conidia, and their morphology can vary depending on the period of incubation on different habitats (Crous & Groenewald 2013). This study suggests that the identification of *Arthrinium* taxa needs to be carried out based on a combination of sequence data and observation of the sexual-asexual morphs. The linkage of such sexual and asexual morphs should be determined through cultures obtained from single spores and by subsequent molecular analyses.

Fig. 5 – *Arthrinium hyphopodii* (HKAS 96288). a Appearance of stromata on bamboo host. b, c Stromata. d Vertical section of stroma. e Ostiole. f Peridium. g Paraphyses. i–k Asci. m–o Ascospores. p Germinated spore. h, l Colony on PDA. d = 500 μm, f, g, k, p = 100 μm, e, i, j, m–o = 50 μm.

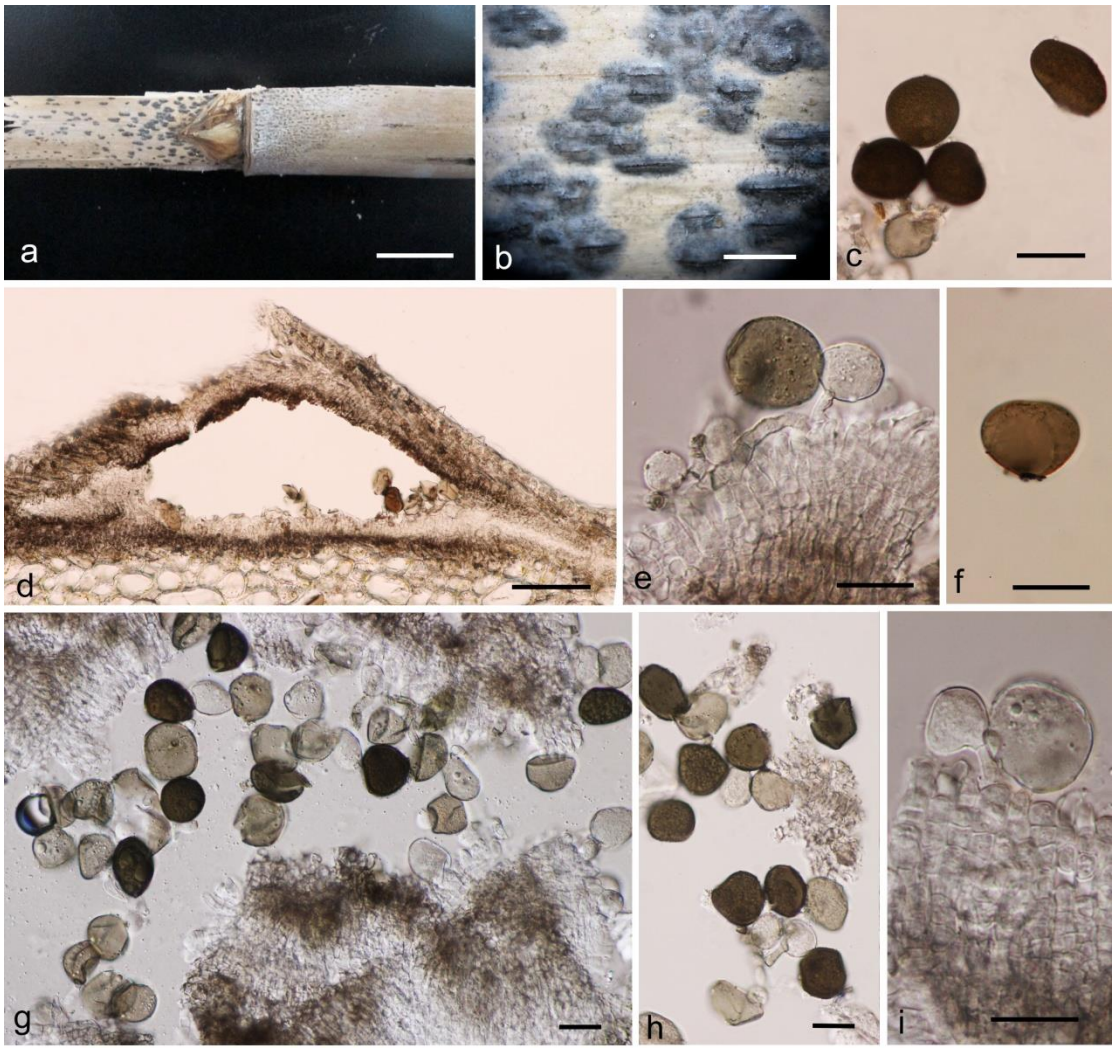

Fig. 6 – *Arthrinium hyphopodii* (holotype). a Appearance of conidiomata on bamboo host. b Conidiomata. d Section of conidioma. e, i Conidiogenous cells. c, f–h Conidia. Scale bars: a = 5 cm, b = 500 μm, d = 100 μm, c, e–i = 5 μm.

Acknowledgements

This work is supported by the Key Laboratory of Yunnan Province Universities of the Diversity and Ecological Adaptive Evolution for Animals and plants on Yun-Gui Plateau and Yunnan Province Universities of the Science and Technology Innovation Team for the exploitation and utilization of endophytes. Hong-Bo Jiang would like to thank Qiu-Ju Shang for help with lab work. The research is supported by the National Natural Science Foundation of China (No. NSFC 31260087, NSFC 31460561). The authors would like to thank J.E. Taylor for suggestions and annotations to the paper.

References

Chomnunti P, Hongsanan S, Aguirre-Hudson B et al. 2014 – The sooty moulds. Fungal Diversity 66, 1–36.

Crous PW, Groenewald JZ. 2013 – A phylogenetic re-evaluation of *Arthrinium*. IMA Fungus 4, 133–154.

Dai DQ, Bhat DJ, Liu JK et al. 2012 – *Bambusicola*, a new genus from bamboo with asexual and sexual morphs. Cryptogamie Mycologie 33, 363–379.

Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ et al. 2017 – Bambusicolous fungi. Fungal Diversity, http://dx.doi.org 10.1007/s13225-016-0367-8.

Hall T 2004 – BioEdit. Ibis Therapeutics, Carlsbad, CA, 92008, USA. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) (accessed 18 Mar 2005).
Hyde KD, Zhou DQ, Dalisay T. 2002 – Bambusicolous fungi: A review. Fungal Diversity 9, 1-14.
Hyde KD, Hongsanan S, Jeewon R et al. 2016 – Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80, 1–270.
Index Fungorum 2016 – http://www.indexfungorum.org/Names/Names.asp.
Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat DJ et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
Katoh K, Standley DM. 2013 – MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30, 772–780.
Maharachchikumbura SS, Hyde KD, Jones EBG, McKenzie EHC et al. 2015 – Towards a natural classification and backbone tree for *Sordariomycetes*. Fungal Diversity 72, 199–301.
Nylander JAA 2004 – MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
Page RDM 1996 – TreeView: an application to display phylogenetic trees on personal computers. Bioinformatics, 12, 357–358.
Phillips AJL, Alves A, Pennycook SR, Johnston PR et al. 2008 – Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the *Botryosphaeriaceae*. Persoonia 21, 29–55.
Rannala B, Yang Z. 1996 – Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. Journal of Molecular Evolution 43, 304–311.
Ronquist F, Huelsenbeck JP. 2003 – MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD et al. 2015 – Towards unraveling relationships in *Xylariomycetidae* (*Sordariomycetes*). Fungal Diversity 73, 73–144.
Sharma R, Kulkarni G, Sonawane MS, Shouche YS. 2014 – A new endophytic species of *Arthrinium* (*Apiosporaceae*) from *Jatropha podagrica*. Mycoscience 55, 118–123.
Silvestro D, Michalak I. 2011 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12, 335–337.
Stamatakis A 2006 – RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Swofford DL 2002 – PAUP: phylogenetic analysis using parsimony, version 4.0 b10. Sinauer Associates, Sunderland.
Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246.
White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: A Guide to Methods and Applications, 315–322.
Zhaxybayeva O, Gogarten JP. 2002 – Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3, 1–15.