Asian Journal of Mycology 2(1): 254–268 (2019) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/2/1/16

Arthrinium setostromum (Apiosporaceae, Xylariales), a novel species associated with dead bamboo from Yunnan, China

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Jiang HB, Hyde KD, Doilom M, Karunarathna SC, Xu JC, Phookamsak R 2019 – *Arthrinium setostromum* (Apiosporaceae, Xylariales), a novel species associated with dead bamboo from Yunnan, China. Asian Journal of Mycology 2(1), 254–268, Doi 10.5943/ajom/2/1/16

Abstract

Arthrinium setostromum sp. nov., collected from dead branches of bamboo in Yunnan Province of China, is described and illustrated with the sexual and asexual connections. The sexual morph of the new taxon is characterized by raised, dark brown to black, setose, lenticular, 1–3-loculate ascostromata, immersed in a clypeus, unitunicate, 8-spored, broadly clavate to cylindric-clavate asci and hyaline apiospores, surrounded by an indistinct mucilaginous sheath. The asexual morph develops holoblastic, monoblastic conidiogenesis with globose to subglobose, dark brown, 0–1-septate conidia. *Arthrinium setostromum* can be distinguished from other phylogenetically closely related species by its setose ascostroma, with setae raising through a split of the clypeus. Maximum likelihood, maximum parsimony and bayesian inference analyses based on a catenated ITS, LSU, TEF1-α and TUB2 DNA sequence dataset demonstrate that *A. setostromum* is a distinct new species phylogenetically closely related to *A. garethjonesii*, and clusters with *A. bambusae*, *A. mytilomorphum* and *A. subroseum*.

Key words – 1 new species – bambusicolous fungi – holomorph – multi-gene phylogeny – Sordariomycetes

Introduction

Arthrinium was introduced by Kunze (1817) with *A. caricicola* Kunze & J.C. Schmidt as the type species. Traditionally, *Arthrinium* (as an asexual morph) was linked to *Apiospora* Sacc. (as a sexual morph) (Ellis 1971, Seifert et al. 2011). *Pteroconium* Sacc. ex Grove was also reported as an asexual morph of *Apiospora*, however, the genus has a large morphological difference from *Arthrinium* in conidial shape (Ellis 1971, Wang et al. 2018). Crous & Groenewald (2013) treated *Pteroconium* and *Cordella* Speg. as the synonym of *Arthrinium* based on molecular phylogenetic data. In general, *Apiospora* was regarded as a synonym of *Arthrinium* based on the one fungus-one name policy (Hawksworth et al. 2011, Crous & Groenewald 2013).

The sexual morph of *Arthrinium* is characterized by immersed to erumpent through a longitudinal split, uni- to multi-loculate, dark brown to black, lenticular, or dome-shaped

Submitted 18 September 2019, Accepted 23 October 2019, Published 11 November 2019
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ascostromata with or without setae growing from perithecia, 8-spored, unitunicate, broadly clavate to cylindric-clavate asci and hyaline, ellipsoidal, inequilaterally, 2–3-seriate, 1-septate near the lower end ascospores with or without sheath (Senanayake et al. 2015, Dai et al. 2016, 2017, Maharachchikumbura et al. 2016, Pintos et al. 2019). The asexual morphs of *Arthrinium* includes coelomycetous and hyphomycetous forms; for example, *A. hyphopodii* D.Q. Dai & K.D. Hyde and *A. qinlingense* C.M. Tian & N. Jiang produced coelomycetous asexual morph (Senanayake et al. 2015, Dai et al. 2016, Jiang et al. 2018), while *A. esporlense* Pintos & P. Alvarado formed hyphomycetous asexual morph (Pintos et al. 2019).

The hyphomycetous asexual morph of *Arthrinium* is described as subhyaline or pale brown, subcylindrical, septate conidiophores reduced to conidiogenous cells or arising from basal cells, forming conidia laterally or terminally, with or without setae, hyaline or subhyaline to pale brown, doliiform to ampulliform or subcylindrical, smooth or finely roughened, holoblastic, monoblastic or polyblastic conidiogenous cells and 0–1-septate, dark brown or brown to pale olivaceous conidia, vary in shape (e.g. lenticular, globose to subglobose, elongated to ellipsoidal, fusiform, spindle-shaped, lemon-shaped, etc.), with or without a truncate base and a germ-slit (Singh et al. 2012, Crous & Groenewald 2013, Crous et al. 2015, Hyde et al. 2016, Dai et al. 2017, Wang et al. 2018, Pintos et al. 2019, Yan et al. 2019).

The coelomycetous asexual morph of *Arthrinium* is characterized by immersed in host tissue or superficial on media, black, globose to subglobose, coriaceous conidiomata, forming subhyaline or pale brown, hyphoid, cylindrical, septate conidiophores reduced to conidiogenous cells or arising from basal cells, subhyaline or pale brown, cylindrical, smooth or with verrucose, holoblastic, monoblastic or polyblastic conidiogenous cells and dark brown, globose to subglobose, smooth, conidia with or without a truncate basal scar and a germ-slit (Senanayake et al. 2015, Dai et al. 2017, Jiang et al. 2018, Yang et al. 2019).

Arthrinium species are widely distributed (Sharma et al. 2014). Species of the genus can be endophytes, pathogens or saprobes, usually isolated from soil debris, plants, lichens and marine algae (Senanayake et al. 2015, Wijayawardene et al. 2017), as well as from gut of insect (Crous et al. 2015) and erythematous nodules of human (Sharma et al. 2014).

In this study, we introduce a novel member of *Arthrinium* with the sexual and asexual morph linkage, collected from Yunnan, China, based on distinct morphological characteristics and multi-gene phylogenetic analyses. The new taxon is illustrated and compared with similar taxa.

Materials & methods

Collection and examination of specimen, fungal isolation and conservation

The fresh sample, collected from Mengla County, Xishuangbanna Dai Autonomous Prefecture of Yunnan Province, China was brought to the laboratory for observation and examination. Ascostromata appeared on the host surface were examined and captured using an Olympus stereo microscope series MoDELSZ2-ILST. Micromorphological features were examined and captured by a Nikon ECLIPSE Ni-U compound microscope connected with a Canon EOS 600D digital camera. The presence of mucilaginous sheath surrounded ascospores was detected by immersing in the Indian ink. The asci were stained by Melzer's reagent for checking the J-/J+ apical ring. Measurements of morphological structures (e.g. asci, ascospores, paraphyses, peridium, ascostromata, conidia, conidiogenous cells and conidiophores) were made in Tarosoft® Image Frame Work software version 0.9.7. Photographic plates were edited and combined in Adobe Photoshop CS6 software (Adobe Systems Inc., United States).

Single spore isolation was performed following the method described by Dai et al. (2017). Germinating ascospores were aseptically transferred to the medium plate of potato dextrose agar (PDA) for incubation under normal light at 20–25°C. Fungal colonies were observed and morphology and size of colonies were recorded at weekly intervals for two months. After two months, the sporulation of the asexual morph of the new taxon was observed and examined by using the same method of the sexual morph examination.

Herbarium materials are deposited in the herbarium of the Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS) and duplicated in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The ex-type living culture was conserved at the Kunming Institute of Botany Culture Collection (KUMCC) and Mae Fah Luang University Culture Collection (MFLUCC). Facesoffungi and Index Fungorum numbers are provided for the newly described taxon (Jayasiri et al. 2015, Index Fungorum 2019). The novel taxon was justified based on the guideline of Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh mycelia grown on PDA media with the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, and P.R. China) following the manufacturer's instructions.

Partial DNA sequences of the internal transcribed spacers (ITS1-5.8S-ITS2), the 28S large subunit rDNA (LSU) and the translation elongation factor 1-alpha (TEF1-α), were amplified by primers ITS5 and ITS4 (White et al. 1990), LR0R and LR5 (Vilgalys & Hester 1990) and EF1-728F and EF-2 (O'Donnell et al. 1998, Carbone & Kohn 1999), respectively.

PCR was performed in a 25 µl total volume containing 2 µl of DNA template, 1 µl of each forward and reverse primers, 12.5 µl of 2 × Power Taq PCR Master Mix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing Bio Teke Corporation (Bio Teke), P.R. China) and 8.5 µl of ddH_2O (double-distilled water). The annealing temperatures were adjusted to 52°C for ITS and LSU, and 55°C for TEF1-α. PCR products were sent to sequence at Sangon Biotech (Shanghai) Co., Ltd, China and TsingKe Biological Technology (Beijing) Co., Ltd, China.

Phylogenetic analyses

Through the BLASTn search tool in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) based on newly generating ITS sequence data, we found out that our taxon related to *Arthrinium* (Apiosporaceae) and the closest taxon is *A. garethjonesii* D.Q. Dai & H.B. Jiang (HKAS 96289) with 96.55% similarity. Further sequence matrices of ITS, LSU, TEF1-α and TUB2 were used to resolve the accurate phylogenetic position of our taxon based on the related sequences of taxa in *Arthrinium* obtained from GenBank (Table 1). Individual gene matrix was aligned with the online version of MAFFT v. 7.427 (Katoh et al. 2017) and improved manually in BioEdit v. 5.0.6 (Hall 2001). Individual gene alignments were analyzed separately by Randomized Axelerated Maximum Likelihood (RAxML) implemented in RAxMLGUI v. 1.0 (Stamatakis 2006, Silvestro & Michalak 2011) for prior comparing of tree topologies. Further, a concatenated ITS, LSU, TEF1-α and TUB2 alignment was analyzed based on maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) criteria.

ML analysis was carried out by RAxML implemented in RAxMLGUI v. 1.0 (Stamatakis 2006, Silvestro & Michalak 2011). BI analysis was performed by MrBayes v. 3.2.1 (Ronquist et al. 2012). Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were evaluated based on Markov Chain Monte Carlo sampling (MCMC). Six simultaneous Markov chains were set up at 3,000,000 generations and trees were sampled every 100th generation (yielded 30,000 total trees). The first 20% trees, which represented the burn-in phase of the analysis, were discarded and the remaining trees were used for calculating PP in the 50% majority rule consensus tree. MP analysis was operated in PAUP v. 4.0b10 (Swofford 2002) based on the heuristic search option with 1,000 random stepwise addition replicates and the tree bisection–reconnection (TBR) as the branch-swapping algorithm. All informative characters were unordered and of equal weight. MaxTrees reset to 1,000 and gaps were treated as missing data. All parsimonious trees were saved. The tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated. Statistical supports for branches of the most parsimonious tree were estimated using maximum parsimony bootstrap (BS) analysis with 1,000 bootstrap replicates (Felsenstein 1985).

Phylogenetic trees were represented by FigTree v. 1.4.0 (Rambaut 2012) and edited in Microsoft Office PowerPoint 2016 (Microsoft Inc., United States). Newly generated sequences in this study were deposited in GenBank (Table 1) and the final matrices used for the phylogenetic analyses were submitted to TreeBASE (www.treebase.org; accession number: 25246).

Table 1 List of fungal taxa and their GenBank accession numbers of the sequences used in this study.

Species	Strain no.	GenBank Accession numbers			
		ITS	**LSU**	**TEF1-α**	**TUB2**
Arthrinium arundinis	CBS 106.12	KF144883	KF144927	KF145015	KF144973
Arthrinium arundinis	CBS 114316	KF144884	KF144928	KF145016	KF144974
Arthrinium arundinis	CBS 124788	KF144885	KF144929	KF145017	KF144975
Arthrinium arundinis	CBS 133509	KF144886	KF144930	KF145018	KF144976
Arthrinium arundinis	CBS 449.92	KF144887	KF144931	KF145019	KF144977
Arthrinium aureum	**CBS 244.83**	AB220251	KF144935	KF145023	KF144981
Arthrinium balearicum	**CBS 145129**	MK014869	MK014836	MK017946	MK017975
Arthrinium bambusae	**LC7106**	KY494718	KY494794	KY806204	KY705186
Arthrinium bambusae	LC7124	KY494727	KY494803	KY806206	KY705195
Arthrinium bambusae	LC7125	KY494728	KY494804	KY705124	KY705196
Arthrinium camelliae-sinensis	**LC5007**	KY494704	KY494780	KY705103	KY705173
Arthrinium camelliae-sinensis	LC8181	KY494761	KY494837	KY705157	KY705229
Arthrinium caricicola	CBS 145127	MK014871	MK014838	MK017948	MK017977
Arthrinium descalsii	**CBS 145130**	MK014870	MK014837	MK017947	MK017976
Arthrinium dichotomanthi	**LC4950**	KY494697	KY494773	KY705096	KY705167
Arthrinium dichotomanthi	LC8175	KY494755	KY494831	KY705151	KY705223
Arthrinium esporlense	**CBS 145136**	MK014878	MK014845	MK017954	MK017983
Arthrinium euphorbiae	IMI 285638b	AB220241	AB220335	/	AB220288
Arthrinium gaoyouense	**CFCC 52301**	MH197124	/	MH236793	MH236789
Arthrinium gaoyouense	CFCC 52302	MH197125	/	MH236794	MH236790
Arthrinium garethjonesii	**KUMCC 16-0202**	KY356086	KY356091	/	/
Arthrinium guizhouense	LC5318	KY494708	KY494784	KY705107	KY705177
Arthrinium guizhouense	**LC5322**	KY494709	KY494785	KY705108	KY705178
Arthrinium gutiae	**CBS 135835**	KR011352	KR149063	KR011351	KR011350
Arthrinium hispanicum	**IMI 326877**	AB220242	AB220336	/	AB220289
Arthrinium hydei	**CBS 114990**	KF144890	KF144936	KF145024	KF144982
Arthrinium hydei	KUMCC 16-0204	KY356087	KY356092	/	/
Arthrinium hyphopodii	**MFLUCC 15-0003**	KR069110	/	/	/
Arthrinium hyphopodii	KUMCC 16-0201	KY356088	KY356093	/	/
Arthrinium hysterinum	**ICMP 6889**	MK014874	MK014841	MK017951	MK017980
Arthrinium ibericum	**CBS 145137**	MK014879	MK014846	MK017955	MK017984
Arthrinium italicum	**CBS 145138**	MK014880	MK014847	MK017956	MK017985
Arthrinium japonicum	IFO 30500	AB220262	AB220356	/	AB220309
Arthrinium japonicum	IFO 31098	AB220264	AB220358	/	AB220311
Arthrinium jatrophae	AMH-9556	HE981191	/	/	/
Arthrinium jatrophae	**AMH-9557**	JQ246355	/	/	/

Table 1 Continued.

Species	Strain no.	GenBank Accession numbers			
		ITS	**LSU**	**TEF1-α**	**TUB2**
Arthrinium jiangxiense	LC4494	KY494690	KY494766	KY705089	KY705160
Arthrinium jiangxiense	**LC4577**	KY494693	KY494769	KY705092	KY705163
Arthrinium kogelbergense	CBS 113332	KF144891	KF144937	KF145025	KF144983
Arthrinium kogelbergense	**CBS 113333**	KF144892	KF144938	KF145026	KF144984
Arthrinium kogelbergense	CBS 113335	KF144893	KF144939	KF145027	KF144985
Arthrinium kogelbergense	CBS 117206	KF144895	KF144941	KF145029	KF144987
Arthrinium locuta-pollinis	**CGMCC 3.18782**	MF939595	/	MF939616	MF939622
Arthrinium longistromum	MFLUCC 11-0479	KU940142	KU863130	/	/
Arthrinium longistromum	**MFLUCC 11-0481**	KU940141	KU863129	/	/
Arthrinium malaysianum	**CBS 102053**	KF144896	KF144942	KF145030	KF144988
Arthrinium marii	**CBS 497.90**	AB220252	KF144947	KF145035	KF144993
Arthrinium mediterranei	**IMI 326875**	AB220243	AB220337	/	AB220290
Arthrinium minus	CBS 145131	MK014872	MK014839	MK017949	MK017978
Arthrinium mytilomorphum	**DAOM 214595**	KY494685	/	/	/
Arthrinium neosubglobosa	JHB006	KY356089	KY356094	/	/
Arthrinium neosubglobosa	**KUMCC 16-0203**	KY356090	KY356095	/	/
Arthrinium obovatum	**LC4940**	KY494696	KY494772	KY705095	KY705166
Arthrinium obovatum	LC8177	KY494757	KY494833	KY705153	KY705225
Arthrinium obovatum	LC8178	KY494758	KY494834	KY705154	KY705226
Arthrinium ovatum	**CBS 115042**	KF144903	KF144950	KF145037	KF144995
Arthrinium paraphaeospermum	**MFLUCC 13-0644**	KX822128	KX822124	/	/
Arthrinium phaeospermum	CBS 114314	KF144904	KF144951	KF145038	KF144996
Arthrinium phaeospermum	CBS 114315	KF144905	KF144952	KF145039	KF144997
Arthrinium phaeospermum	CBS 114317	KF144906	KF144953	KF145040	KF144998
Arthrinium phaeospermum	CBS 114318	KF144907	KF144954	KF145041	KF144999
Arthrinium phragmites	**CPC 18900**	KF144909	KF144956	KF145043	KF145001
Arthrinium phyllostachium	**MFLUCC 18-1101**	MK351842	MH368077	MK340918	MK291949
Arthrinium piptatheri	**CBS 145149**	MK014893	MK014860	MK017969	/
Arthrinium pseudoparenchymaticum	**LC7234**	KY494743	KY494819	KY705139	KY705211
Arthrinium pseudoparenchymaticum	LC8173	KY494753	KY494829	KY705149	KY705221
Arthrinium pseudoparenchymaticum	LC8174	KY494754	KY494830	KY705150	KY705222
Arthrinium pseudosinense	**CPC 21546**	KF144910	KF144957	KF145044	/
Arthrinium pseudospegazzinii	**CBS 102052**	KF144911	KF144958	KF145045	KF145002
Arthrinium pterospermum	CBS 123185	KF144912	KF144959	/	KF145003
Arthrinium pterospermum	**CPC 20193**	KF144913	KF144960	KF145046	KF145004
Arthrinium puccinioides	CBS 549.86	AB220253	AB220347	/	AB220300
Arthrinium qinlingense	**CFCC 52303**	MH197120	/	MH236795	MH236791
Arthrinium qinlingense	CFCC 52304	MH197121	/	MH236796	MH236792
Arthrinium rasikravindrii	MFLUCC 11-0616	KU940144	KU863132	/	/

Table 1 Continued.

Species	Strain no.	GenBank Accession numbers			
		ITS	LSU	TEF1-α	TUB2
Arthrinium rasikravindrii	MFLUCC 15-0203	KU940143	KU863131	/	/
Arthrinium rasikravindrii	**NFCCI 2144**	JF326454	/	/	/
Arthrinium sacchari	CBS 212.30	KF144916	KF144962	KF145047	KF145005
Arthrinium sacchari	CBS 301.49	KF144917	KF144963	KF145048	KF145006
Arthrinium sacchari	CBS 372.67	KF144918	KF144964	KF145049	KF145007
Arthrinium sacchari	CBS 664.74	KF144919	KF144965	KF145050	KF145008
Arthrinium saccharicola	CBS 191.73	KF144920	KF144966	KF145051	KF145009
Arthrinium saccharicola	CBS 463.83	KF144921	KF144968	KF145053	KF145011
Arthrinium saccharicola	CBS 831.71	KF144922	KF144969	KF145054	KF145012
Arthrinium serenense	**IMI 326869**	AB220250	AB220344	/	AB220297
Arthrinium setostromum	**KUMCC 19-0217**	**MN528012**	**MN528011**	**MN527357**	**/**
Arthrinium sporophleum	CBS 145154	MK014898	MK014865	MK017973	MK018001
Arthrinium subglobosum	**MFLUCC 11-0397**	KR069112	KR069113	/	/
Arthrinium subroseum	LC7215	KY494740	KY494816	KY705136	KY705208
Arthrinium subroseum	LC7291	KY494751	KY494827	KY705147	KY705219
Arthrinium subroseum	**LC7292**	KY494752	KY494828	KY705148	KY705220
Arthrinium thailandicum	MFLUCC 15-0199	KU940146	KU863134	/	/
Arthrinium thailandicum	**MFLUCC 15-0202**	KU940145	KU863133	/	/
Arthrinium trachycarpum	**CFCC 53038**	MK301098	/	MK303396	MK303394
Arthrinium urticae	IMI 326344	AB220245	AB220339	/	AB220292
Arthrinium vietnamensis	**IMI 99670**	KX986096	KX986111	/	KY019466
Arthrinium xenocordella	**CBS 478.86**	KF144925	KF144970	KF145055	KF145013
Arthrinium xenocordella	CBS 595.66	KF144926	KF144971	/	/
Arthrinium yunnanum	**MFLUCC 15-0002**	KU940147	KU863135	/	/
Arthrinium yunnanum	DDQ00281	KU940148	KU863136	/	/
Seiridium phylicae	**CPC 19962**	LT853092	NG_042759	LT853189	LT853239
Seiridium phylicae	CPC 19965	LT853093	KC005809	LT853190	LT853240

Notes: Ex-type strains are in black bold, and newly generated sequences are in blue bold.
Abbreviations: AMH: Ajrekar Mycological herbarium, Pune, Maharashtra, India; **CBS:** Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **CFCC:** China Forestry Culture Collection Center, Beijing, China; **CGMCC**: China General Microbiological 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; **JHB:** H.B. Jiang; **KUMCC:** Culture collection of Kunming Institute of Botany, Yunnan, China; **LC:** Working collection of Lei Cai, housed at CAS, China; **MFLUCC:** Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **NFCCI:** National Fungal Culture Collection of India.

Results

Phylogenetic analyses

Phylogenetic analyses of the combined ITS, LSU, TEF1-α and TUB2 sequence dataset comprise 102 strains. The dataset consists of 3179 total characters including gaps (ITS: 1–750 bp, LSU: 751–1,593 bp, TEF1-α: 1,594–2,217 bp, TUB2: 2,218–3,179). The best scoring ML tree was chosen to represent the phylogenetic relationships of the new taxon with other representative taxa in *Arthrinium* (Fig. 1), with the final ML optimization likelihood value of -26,209.134905 (ln). All free model parameters were estimated by GAMMA+P-Invar model, with 1,679 distinct alignment patterns and 35.50% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.236636, C = 0.253087, G = 0.253192, T = 0.257085, with substitution rates AC = 1.324697, AG = 2.935595, AT = 1.165991, CG = 0.959152, CT = 4.739433, GT = 1.000000. The proportion of invariable sites I = 0.316534, the gamma distribution shape parameter alpha = 0.659233 and the Tree-Length = 4.284453. In the MP analysis, 1,689 characters were constant, 283 variable characters were parsimony-uninformative, and 1,207 were (included) parsimony-informative characters. The most parsimonious tree is shown where TL = 4883, CI = 0.520, RI = 0.832, RC = 0.432, HI = 0.480. Bayesian posterior probabilities (BYPP) from MCMC were evaluated with a final average standard deviation of split frequencies of 0.009913.

The phylogenetic tree topology obtained from ML analysis resembled results as previous studies on *Arthrinium* (Crous & Groenewald 2013, Dai et al. 2016, 2017, Jiang et al. 2018, Wang et al. 2018, Yan et al. 2019, Pintos et al. 2019, Yang et al. 2019). Phylogenetic trees carried out by ML, MP and BI analyses were similar in overall topologies. Phylogenetic analysis of a combined ITS, LSU, TEF1-α and TUB2 sequence dataset showed that the new taxon clustered within the genus *Arthrinium* (Apiosporaceae, Xylariales) and has a close relationship with *A. garethgonesii* (HKAS 96289) with high statistic supported values (100% MLBP and 100% MPBP).

Taxonomy

Arthrinium setostromum H.B. Jiang, K.D. Hyde & Phookamsak, sp. nov. Figs 2, 3

Index Fungorum number: IF556844; Facesoffungi number: FoF 06571

Etymology – The specific epithet "*setostromum*" refers to the ascostromata having black, hair-like setae raising from perithecia through a split of the clypeus.

Holotype: KUN-HKAS 106736

Saprobic on dead bamboo branches. Sexual morph: *Ascostromata* 250–600 μm long, 140–180 μm high, solitary to gregarious, scattered, immersed in a clypeus, visible as black, raised, lenticular or dome-shaped, becoming superficial on host surface, 1–3-loculate, ostiolate, with a slit-like axis. *Ascomata* 210–260 μm diam., 100–170 μm high, dark brown to black, perithecial, arranged in a row, clustered, immersed in ascostromata, obpyriform to ampulliform, individually central ostiolate, with pore-like opening, embedded in a clypeus, setose, with dark brown to black, septate, hair-like setae growing from perithecia, raising through a split of the clypeus. *Peridium* 20–25 μm thick, composed several layers of brown to dark brown pseudoparenchymatous cells, paler towards the inner layers, outer layers intermixed with host tissue, arranged in *textura angularis* to *textura prismatica*. *Hamathecium* composed of dense paraphyses, 3–5 μm broad, septate, unbranched, not anastomosing, embedded in gelatinous matrix. *Asci* 82.5–102.5 × 20–30 μm ($\bar{x}$ = 92.5 × 25 μm, n = 20), 8-spored, unitunicate, broadly cylindrical to clavate, sessile to subsessile, apically rounded, with J- subapical ring. *Ascospores* 27–33 × 9.5–12.5 μm ($\bar{x}$ = 30 × 11 μm, n = 20), 2–3-seriate, hyaline, broadly ellipsoidal to clavate, or obovoid, straight to slightly curved near the lower cell, 1-septate, small subglobose at the lower cell and broadly large ellipsoidal at the upper cell, smooth-walled, with multi-guttulate, surrounded by a mucilaginous sheath. Asexual morph: Sporulated on PDA after two months, spore masses visible as dark brown to black on white-grey colonies. *Hyphae* 1.8–2.5 μm diam., hyaline to pale brown, branched, septate. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 42–66 × 1.5–2.7 μm ($\bar{x}$ = 54 ×

2.1 μm, n = 20), micronematous, holoblastic, monoblastic, hyaline, cylindrical, flexible to curved, discrete, aseptate, smooth-walled. *Conidia* 18–20 × 15–19 μm ($\bar{x}$ = 19 × 17 μm, n = 20), acrogenous, brown to dark brown, subglobose to obovoid, 0–1-septate, smooth-walled, multi-guttulate, with a truncate basal scar.

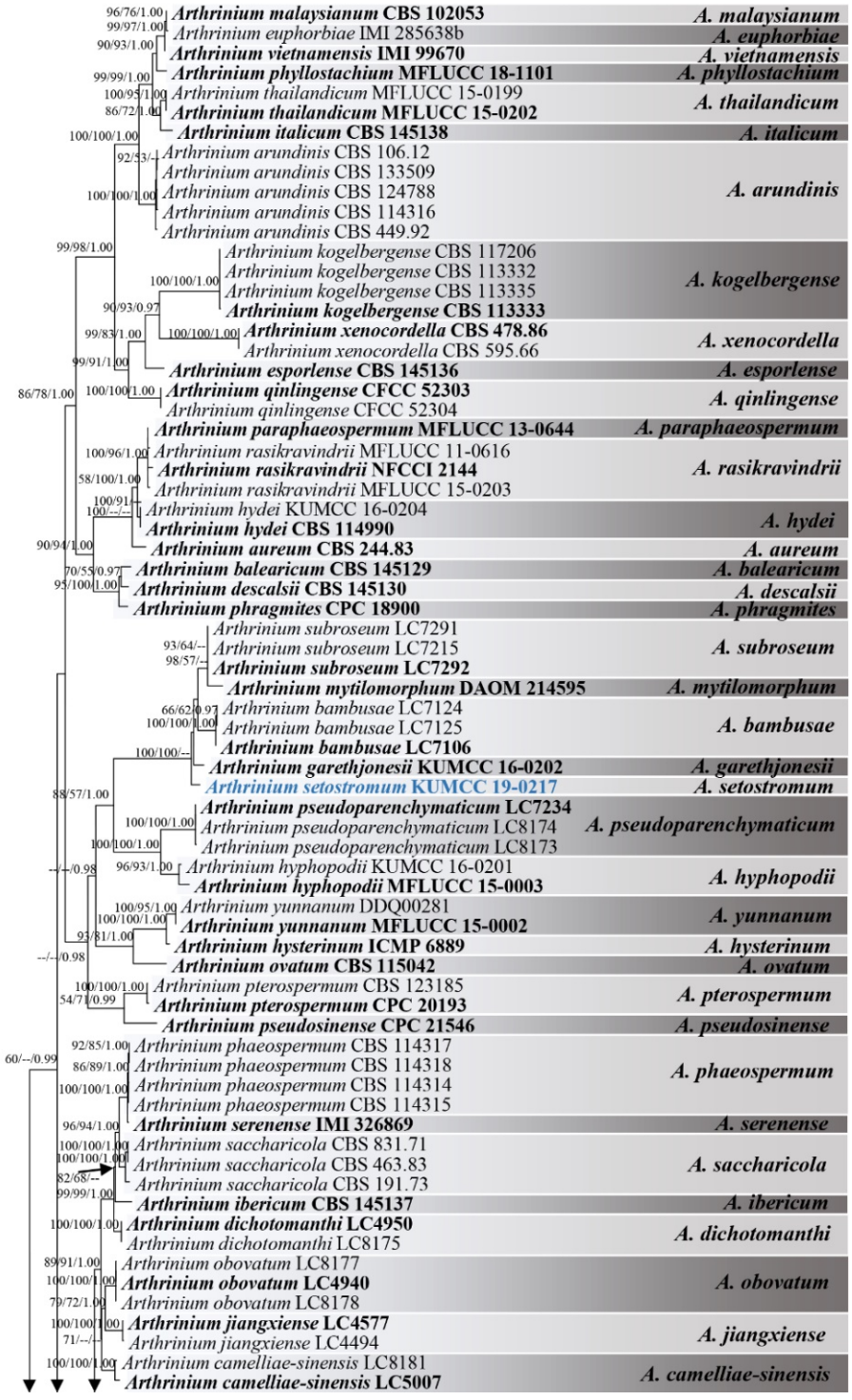

Fig. 1 – Maximum likelihood phylogenetic tree generated by RAxML based on a concatenated ITS, LSU, TEF1-α and TUB2 sequence data. The tree is rooted to *Seiridium phylicae* (CPC 19962 and CPC 19965). Maximum likelihood (ML), and maximum parsimony (MP) bootstrap values equal to or greater than 50% and bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are written above the nodes as ML/MP/PP. Ex-type strains are in black bold and newly generated sequence is in blue bold.

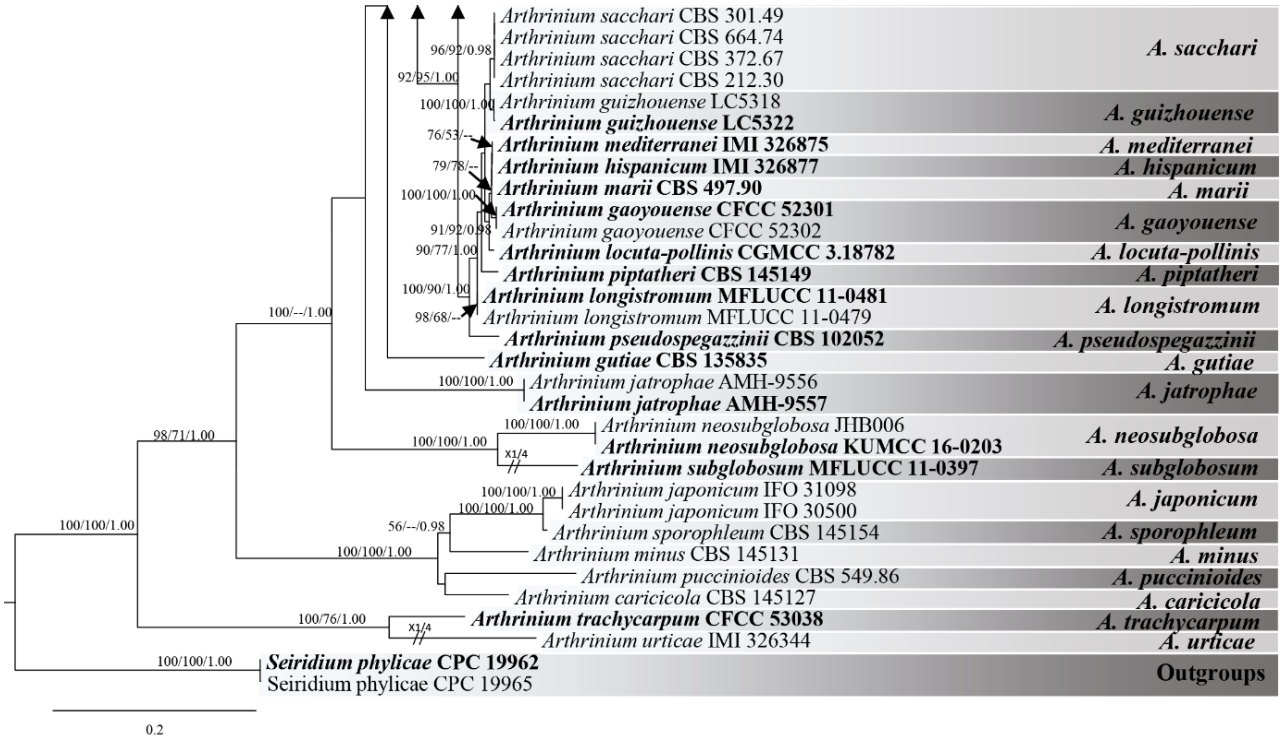

Fig. 1 – Continued.

Culture characteristics – Ascospores germinating on PDA within 24 hrs and germ tubes produced from the upper cell of ascospore. Colonies fast growing on PDA, reaching 35 mm in 1 week at 20–25°C, under the normal light, cottony, circular, raised, initially white, becoming greyish, reverse reddish. Mycelium superficial to immersed in media, with branched, septate, smooth hyphae.

Material examined – China, Yunnan Province, Xishuangbanna, Mengla county, Nearby Huiying River, on dead branches of bamboo, 21 January 2019, H.B. Jiang, BN006 (KUN-HKAS 106736, holotype; MFLU 19-2443, isotype), ex-type living cultures, MFLUCC, KUMCC 19-0217.

Notes – *Arthrinium setostromum* can be distinguished from other phylogenetically related species of *Arthrinium* in having dark brown to black, hair-like setae growing from perithecia, raising through a clypeus. Morphological comparisons of our novel species with other phylogenetically related taxa are provided in table 2. Based on a pairwise nucleotide comparison of ITS (Jeewon & Hyde 2016), *A. setostromum* differs from *A. garethjonesii* in 20/ 580 bp (3.45%) and also differs from *A. mytilomorphum* in 5/325 bp (1.53%). Pairwise nucleotide comparison of ITS and TEF1-α also showed that *A. setostromum* differs from *A. bambusae* in 24/ 549 bp (4.37%) and 10/ 313 bp (3.19%) and differs from *A. subroseum* in 21/ 548 bp (3.83%) and 15/ 400 bp (3.75%).

Discussion

The genus *Arthrinium* currently contains 92 epithets in Index Fungorum (2019). However, only 85 described species are presently accepted in *Arthrinium*, while seven species were transferred to other genera or treated as synonyms of other *Arthrinium* species (Wang et al. 2017, Index Fungorum 2019, Yang et al. 2019). The phylogenetic placement of 58 *Arthrinium* species has been confirmed based on molecular data (Wijayawardene et al. 2017, Jiang et al. 2018, Wang et al. 2018, Zhao et al. 2018, Pintos et al. 2019, Yan et al. 2019, Yang et al. 2019).

It is difficult to distinguish most of *Arthrinium* species based on morphology. Both sexual and asexual morphs of *Arthrinium* species share similar morphological characteristics, except for some species, such as *A. caricicola*, *A. cuspidatum* (Cooke & Harkn.) Tranzschel and *A. luzulae* M.B. Ellis. Some *Arthrinium* species could not be distinguished based on the ITS region, such as *A.*

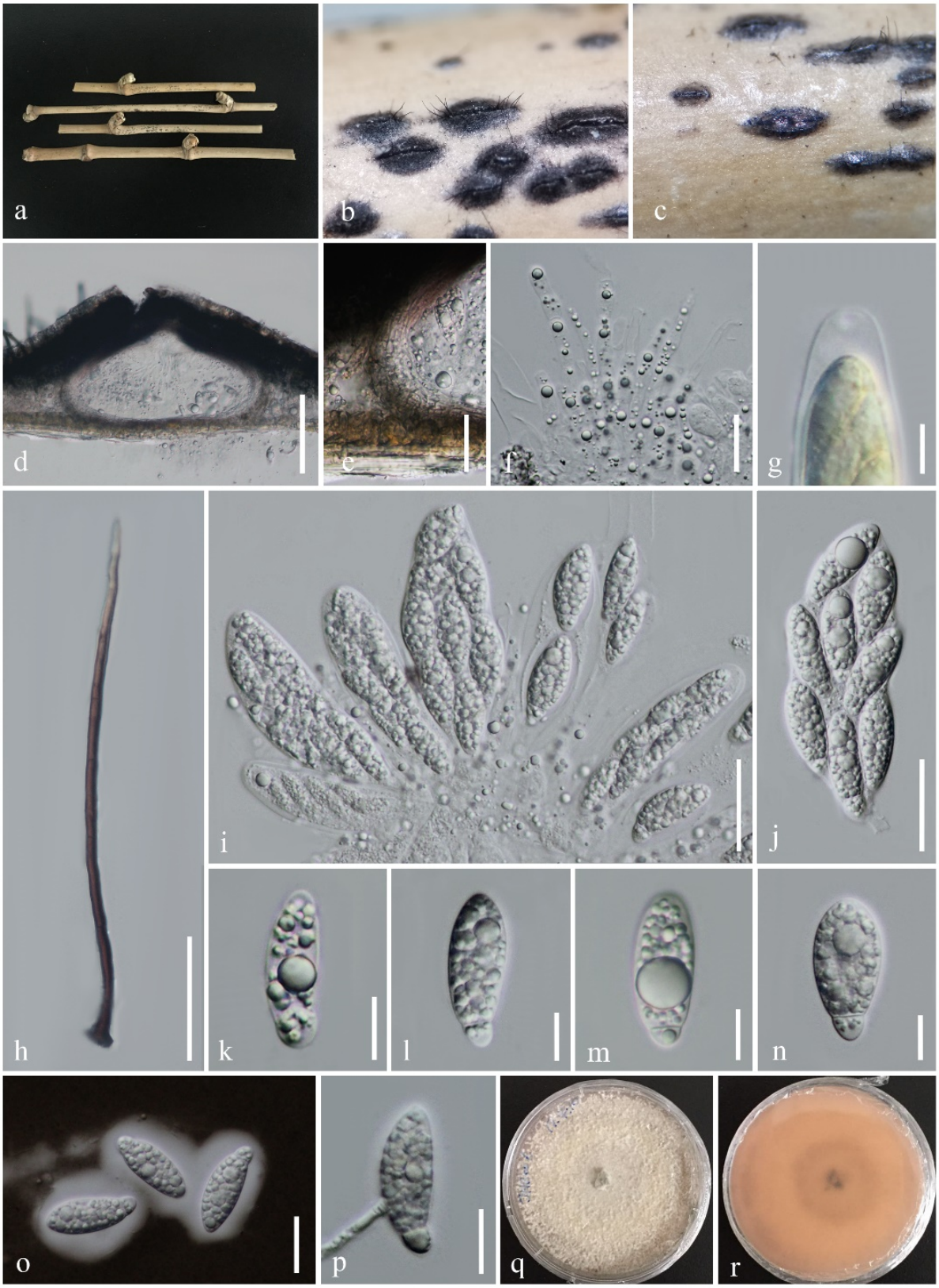

Fig. 2 – Sexual morph of *Arthrinium setostromum* (KUN-HKAS 106736, holotype). a Dead branches of bamboo. b, c Ascostromata on bamboo host. d Vertical section of ascostroma. e Peridium. f Paraphyses. g J-, Subapical ring stained by Melzer's reagent. h Setae growing from perithecia. i, j Asci. k–n Ascospores. o Ascospores with gelatinous sheath immersing in Indian ink. p Germinating ascospore. q, r Culture characteristics on PDA (q = from above, r = from below). Scale bars: d = 100 μm, h = 50 μm, e, i, j = 30 μm, f, o = 20 μm, p = 15 μm, k–n = 10 μm, g = 5 μm.

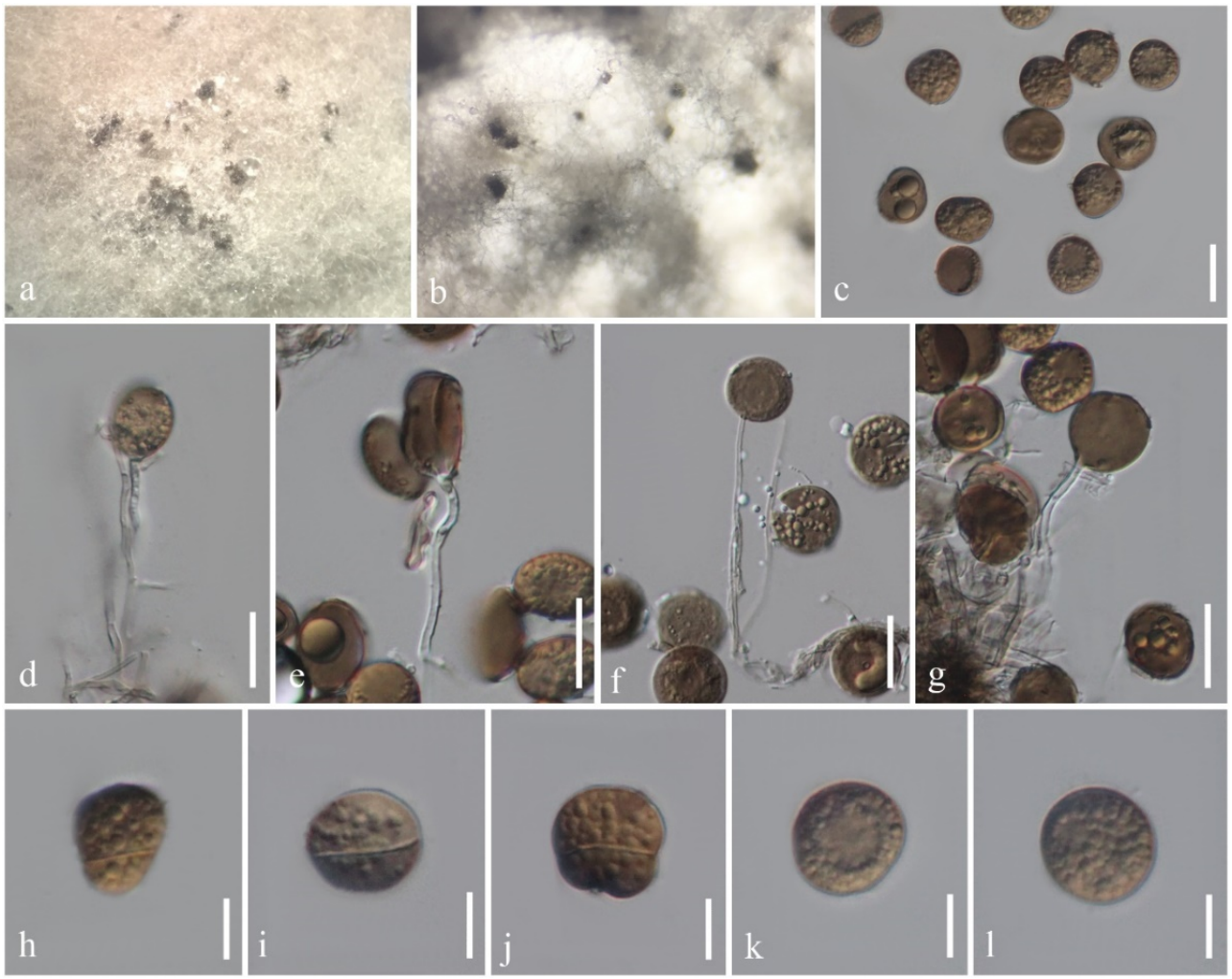

Fig. 3 – Asexual morph of *Arthrinium setostromum* (from ex-type culture). a, b Colonies on PDA sporulated conidial masses. d-g Conidiogenous cells. c, h-l Conidia. Scale bars: c-g = 20 μm, h-l = 10 μm.

phyllostachium C.L. Yang, X.L. Xu & K.D. Hyde and *A. vietnamensis* (Hol.-Jech.) Mei Wang & L. Cai. Their ITS regions have high similarities (>99% similarity). However, these species can be distinguished by the protein-coding genes such as TEF1-α and TUB2 (Wang et al. 2018, Yang et al. 2019). Thus, it suggests that multigene phylogenetic analyses based on protein-coding genes coupled with sexual-asexual morphological characteristics are very important to clarify this complex genus *Arthrinium*.

Arthrinium is widely distributed over the world and lives on various hosts, however, most of them have been found on bamboo plants (Dai et al. 2016, 2017, Jiang et al. 2018, Wang et al. 2018, Farr & Rossman 2019, Yang et al. 2019). To date, there are 31 recorded species of *Arthrinium* in China (Hyde et al. 1998, Wang et al. 2018, Zhao et al. 2018, Farr & Rossman 2019, Yang et al. 2019). Most of the *Arthrinium* species are distributed in southeast China, south China and southwest China (Farr & Rossman 2019). *Arthrinium qinlingense* and *A. trachycarpum* C.M. Tian & H. Yan are known from the highest latitude in Shaanxi Province of China (Jiang et al. 2018, Farr & Rossman 2019, Yan et al. 2019). According to Farr & Rossman (2019), the specific hosts of *Arthrinium* species in China are *Bambusa* and *Phyllostachys*.

Table 2 Synopsis of morphological features of the other phylogenetically closely related species to *Arthrinium setostromum*.

Species name	Sexual morph				Asexual morph			References
	Ascostromata (long × high)	Number of locules	Asci (µm)	Ascospores (µm)	Conidiophores	Conidiogenous cells	Conidia	
Arthrinium bambusae	N/A	N/A	N/A	N/A	Reduced to conidiogenous cells	4–12 × 3–7 µm, aggregated in clusters on hyphae, holoblastic, monoblastic, hyaline to pale brown, doliiform to ampulliform, or lageniform, smooth	11.5–15.5 × 7–14 µm, olivaceous to brown, subglobose to ellipsoid, aseptate, smooth to finely roughened	Wang et al. 2018
A. garethjonesii	1.3–2 mm × 188–282 µm	3–10	125–154 × 35–42	30–42 × 11–16	N/A	N/A	N/A	Dai et al. 2016
A. mytilomorphum	N/A	N/A	N/A	N/A	Up to 80 µm long, 4.5–5 µm wide, arising from conidiophore mother cells	9–10 × 4.5–5 µm, integrated, terminal or intercalary, mono- to polyblastic, minutely denticulate	20–30 × 6–8.5 µm, dark brown, fusiform or navicular, aseptate, smooth	Bhat & Kendrick 1993
A. setostromum	250–600 × 140–180 µm	1–3	82.5–102.5 × 20–30	27–33 × 9.5–12.5	Reduced to conidiogenous cells	42–66 × 1.5–2.7 µm, discrete, holoblastic, monoblastic, hyaline, cylindrical, flexible to curved, smooth.	18–20 × 15–19 µm, brown to dark brown, subglobose to obovoid, 0–1-sepate, smooth	This study
A. subroseum	N/A	N/A	N/A	N/A	Up to 20 µm long, 2–4.5 µm wide, sporodochia	3–6.5 × 2–5 µm, holoblastic, monoblastic, pale brown, doliiform to subcylindrical, smooth	12–17.5 × 9–16 µm, pale brown to dark brown, globose to subglobose or ellipsoidal, aseptate, smooth	Wang et al. 2018

Acknowledgements

The authors are grateful to the Mushroom Research Foundation, Chiang Rai, Thailand supporting this research and also acknowledge the Biology Experimental Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences for providing molecular laboratory facilities to extract DNA and amplify PCR fragments. Shaun Pennycook is thanked for his valuable suggestion on naming the new fungus. Rungtiwa Phookamsak thanks CAS President's International Fellowship Initiative (PIFI) for young staff (grant no. Y9215811Q1), the Yunnan Provincial Department of Human Resources and Social Security (grant no. Y836181261), and National Science Foundation of China (NSFC) project code 31850410489 (grant no. Y81I982211) for financial support. Kevin D. Hyde thanks the Thailand Research Fund entitled Impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion (grant no: RDG6130001), and the Chinese Academy of Sciences, project number 2013T2S0030, for the award of visiting Professorship for Senior International Scientists at Kunming Institute of Botany. Samantha C. Karunarathna thanks CAS President's International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2018PC0006) and the National Science Foundation of China (NSFC) for funding this work under the project code 31750110478. Jianchu Xu thanks the Key Research Program of Frontier Sciences of the Chinese Academy of Sciences (grant no. QYZDY-SSW-SMC014). Mingkwan Doilom would like to thank the 5th batch of Postdoctoral Orientation Training Personnel in Yunnan Province and the 64th batch of China Postdoctoral Science Foundation.

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