

A novel bambusicolous fungus from China, *Arthrinium chinense* (Xylariales)

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Arthrinium (Apiosporaceae, Xylariales) is a globally distributed genus inhabiting various substrates, mostly plant tissues. *Arthrinium* specimens from bamboo culms were characterized on the basis of morphology and phylogenetic inference, which suggested that they are different from all known species. Hence, the new taxon, *Arthrinium chinense*, is proposed. *Arthrinium chinense* can be distinguished from the phylogenetically close species, *A. paraphaeospermum* and *A. rasikravindrae*, by much shorter conidia.

Keywords: Apiosporaceae, bamboo, taxonomy, molecular phylogeny. – 1 new species.

Bambusoideae (bamboo) is an important plant subfamily comprising multiple genera and species widely distributed in China. Taxonomy of bamboo-associated fungi has been studied worldwide in the past two decades, and more than 1000 fungal species have been recorded (Hyde et al. 2002a, b). Recently, additional fungal species from bamboo were described in China on the basis of morphology and molecular evidence (Dai et al. 2016, 2017; Jiang et al. 2018; Wang et al. 2018).

Arthrinium Kunze is a globally distributed genus of Apiosporaceae K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr. Its species have been reported from various substrates, including air, soil debris, plants, lichens, marine algae and human tissues (Agut & Calvo 2004, Crous & Groenewald 2013, Sharma et al. 2014). Morphologically, *Arthrinium* specimens are difficult to identify to species level, because of the wide host range of many species, and scarce differences between asexual morphs. Molecular genetic data helped to clarify the species concepts in this genus, allowing the proposal of additional taxa (Dai et al. 2016, 2017; Jiang et al. 2018; Wang et al. 2018; Zhao et al. 2018; Pintos et al. 2019; Yan et al. 2019).

The aim of present study was to identify the newly collected *Arthrinium* specimens on bamboo from Shaanxi Province (China) using morphology and sequencing. Since the sexual morph of *Arthrin-*

ium qinlingense C.M. Tian & N. Jiang, a species described from *Fargesia qinlingensis* in Qinling mountains (Shaanxi, China; Jiang et al. 2018), we also collected dead and dying culms of *Fargesia qinlingensis* in order to find it.

Materials and methods

Sample collection and isolation

In Qinling mountain in Shaanxi Province (China) dead plant culms with stromata were collected. For isolation, the stromata from the dead culms were cut out, washed in distilled water for 10 seconds, and transferred onto the surface of potato dextrose agar (PDA; 200 g potatoes, 20 g dextrose, 20 g agar per l). Plates were incubated at 25 °C in the dark to obtain a pure culture. Specimens and cultures were deposited in the Museum of Beijing Forestry University (BJFC) and China Forestry Culture Collection Center (CFCC), respectively.

Morphology

Morphological descriptions of the new *Arthrinium* species are based on stromata from natural culms and conidiomata from PDA cultures. Micro-morphological features were observed under a Leica compound microscope (DM 2500). More than 50 ascospores and conidia were selected randomly for

measurement. Cultural characteristics of isolates grown on PDA were observed and recorded, including colony colour and texture.

DNA extraction and phylogenetic analysis

Genomic DNA was extracted from mature mycelium growing on PDA using a modified CTAB method (Doyle & Doyle 1990). Three loci, 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS), part of the translation elongation factor 1-alpha (*tef1*) and beta-tubulin (*tub2*) were amplified with primer pairs ITS1/ITS4 (White et al. 1990), EF1-728F/EF-2 (O'Donnell et al. 1998, Carbone & Kohn 1999), and T1/Bt-2b (O'Donnell & Cigelnik 1997, Glass & Donaldson 1995), respectively. Amplifications of ITS, *tef1* and *tub2* were performed following Wang et al. (2018). The PCR amplification products were estimated visually by electrophoresis in 2 % agarose gels. DNA sequencing was performed using an ABI PRISM® 3730XL DNA Analyzer with BigDye® Terminator Kit v.3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Sequences from this study and reference sequences obtained from GenBank (Tab. 1) were aligned and edited manually using MEGA6 (Tamura et al. 2013). Phylogenetic analyses were performed in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) for Bayesian inference (BI) and PhyML v. 3.0 (Guindon et al. 2010) for maximum likelihood (ML). Sequences were deposited in GenBank (Tab. 1), and the alignment was deposited in TreeBASE (www.treebase.org) with the accession no. S25053. Introduction of the newly described species was based on molecular genetic data following the recommendations of Jeewon & Hyde (2016).

Results and discussion

Phylogeny

The alignment of the combined sequence dataset (ITS, *tef1* and *tub2*) included 86 ingroup taxa and one outgroup taxon (*Nigrospora gorlenkoana* CBS 480.73), comprising 2058 characters in the aligned matrix. Of these, 806 characters were constant, 160 variable characters were parsimony-uninformative and 1092 characters were parsimony informative. The ML analysis supported more or less the same tree topology as the Bayesian analysis (Fig. 1). Two *Arthrinium* strains from *Fargesia qinlingensis* significantly clustered together (ML/BI = 100/1), probably representing a different species from all others

included in the present analysis. A new taxon is therefore proposed below to accommodate them.

Taxonomy

Arthrinium chinense C.M. Tian & N. Jiang, sp. nov.

— Fig. 2.

MycoBank no.: MB 830663

Type. — CHINA. Shaanxi Province, Huoditang forest farm in Qinling mountain range, on culms of *Fargesia qinlingensis*, 16 July 2018, leg. N. Jiang & C. M. Tian (BJFC-S1732 holotype; ex-holotype culture CFCC 53036).

Description. — Stromata 500–1300 × 300–650 µm (n = 20), scattered to gregarious, partially immersed, becoming erumpent to superficial, raised, dark brown, in linear rows, with a slit-like opening, multi-loculate. — Perithecia (100)130–170(200) µm (n = 30), arranged in rows, clustered, gregarious, with 4–9 perithecia forming groups immersed in stromata to erumpent through host surface, ellipsoidal to subglobose, dark brown, membranous. — Peridium 8–15 µm thick, consisting of 4–5 layers of cells arranged in *textura angularis*, externally dark brown, hyaline in the inner part. — Ostiole raised from the centre of the peritheciun, internally lined with periphyses, with a periphysate channel. — Paraphyses intermingled among asci, not very prominent, hyphae-like, hyaline, smooth, septate, sparingly branched, thin-walled. — Asci 85–135 × 20–35 µm (n = 20), 8-spored, unitunicate, clavate, broadly cylindrical, with an inconspicuous pedicel, rounded apex, thin-walled, without an apical apparatus. — Ascospores (18)22–27(32) × (7)9–12(14) µm (x = 25 ± 1.9 × 10.5 ± 1.1 µm, n = 50), 1–2-seriate, 1-septate, ellipsoidal, with a smaller lower cell and a larger upper cell, hyaline, smooth-walled, with a faint gelatinous sheath. — Conidiophore reduced to conidiogenous cells. — Conidiogenous cells 1.5–6.5 × 1–3.5 µm (n = 20), erect, aggregated in clusters on hyphae, hyaline to pale brown, smooth, doliform to clavate or lageniform. — Conidia (8)8.5–11(12) × (5.5)6.5–8(9) µm (x = 10 ± 0.9 × 7.5 ± 0.4 µm, n = 50), brown to dark brown, smooth to finely roughened, subglobose to lenticular, with a longitudinal germ slit.

Culture characteristics. — On PDA colonies fluffy, spreading, with sparse aerial mycelium, white on surface, reverse smoke grey with patches of grey. Conidiomata formed after 25 d at 25 °C.

Etymology. — In reference to China, the country where the type specimen was obtained.

Habitat. — On dead culms of *Fargesia qinlingensis*.

Species	Strains	Substrate	Location	GenBank accession number		
				ITS	tub2	tef1
<i>A. marii</i>	CBS 497.90	Air	Spain	AB220252	KF144993	KF145035
<i>A. marii</i>	CBS 114803	<i>Arundinaria hindsii</i>	China	KF144899	KF144991	KF145033
<i>A. mediterranei</i>	IMI 326875	Air	Spain	AB220243	AB220290	NA
<i>A. neosubglobosa</i>	JHB006	Bamboo	China	KY356089	NA	NA
<i>A. neosubglobosa</i>	KUMCC 16-0203	Bamboo	China	KY356090	NA	NA
<i>A. obovatum</i>	CGMCC 3.18331	<i>Lithocarpus</i> sp.	China	KY494696	KY705166	KY705095
<i>A. obovatum</i>	LC8177	<i>Lithocarpus</i> sp.	China	KY494757	KY705225	KY705153
<i>A. ovatum</i>	CBS 115042	<i>Arundinaria hindsii</i>	China	KF144903	KF144995	KF145037
<i>A. paraphaeospermum</i>	MFLUCC 13-0644	Bamboo	Thailand	KX822128	NA	NA
<i>A. phaeospermum</i>	CBS 114314	<i>Hordeum vulgare</i>	Iran	KF144904	KF144996	KF145038
<i>A. phaeospermum</i>	CBS 114315	<i>H. vulgare</i>	Iran	KF144905	KF144997	KF145039
<i>A. phragmitis</i>	CBS 135458	<i>Phragmites australis</i>	Italy	KF144909	KF145001	KF145043
<i>A. piptatheri</i>	CBS 145149	<i>Piptatherum miliaceum</i>	Spain	MK014893	NA	MK017969
<i>A. pseudoparenchymaticum</i>	CGMCC 3.18336	Bamboo	China	KY494743	KY705211	KY705139
<i>A. pseudoparenchymaticum</i>	LC8173	Bamboo	China	KY494753	KY494753	KY494753
<i>A. pseudosinense</i>	CBS 135459	Bamboo	Netherlands	KF144910	NA	KF145044
<i>A. pseudospegazzinii</i>	CBS 102052	<i>Macaranga hullettii</i>	Malaysia	KF144911	KF145002	KF145045
<i>A. pterospermum</i>	CBS 123185	<i>Machaerina sinclairii</i>	New Zealand	KF144912	KF145003	NA
<i>A. pterospermum</i>	CBS 134000	<i>M. sinclairii</i>	Australia	KF144913	KF145004	KF145046
<i>A. puccinoides</i>	CBS 549.86	<i>Lepidosperma gladiatum</i>	Germany	AB220253	AB220300	NA
<i>A. puccinoides</i>	CBS 145150	<i>Carex arenaria</i>	Germany	MK014894	MK017998	MK017970
<i>A. qinlingense</i>	CFCC 52303	<i>Fargesia qinlingensis</i>	China	MH197120	MH236791	MH236795
<i>A. qinlingense</i>	CFCC 52304	<i>F. qinlingensis</i>	China	MH197121	MH236792	MH236796
<i>A. rasikravindrae</i>	CBS 337.61	<i>Cissus</i> sp.	Netherlands	KF144914	NA	NA
<i>A. rasikravindrae</i>	MFLUCC 11-0616	Bamboo	Thailand	KU940144	NA	NA
<i>A. sacchari</i>	CBS 212.30	<i>Phragmites australis</i>	UK	KF144916	KF145005	KF145047
<i>A. sacchari</i>	CBS 301.49	Bamboo	Indonesia	KF144917	KF145006	KF145048
<i>A. saccharicola</i>	CBS 191.73	Air	Netherlands	KF144920	KF145009	KF145051
<i>A. saccharicola</i>	CBS 463.83	<i>Phragmites australis</i>	Netherlands	KF144921	KF145010	KF145052
<i>A. serenense</i>	IMI 326869	NA	Spain	AB220250	AB220297	NA
<i>A. sporophleum</i>	CBS 145154	<i>Juncus</i> sp.	Spain	MK014898	MK018001	MK017973
<i>A. subroseum</i>	LC7291	Bamboo	China	KY494751	KY705219	KY705147
<i>A. subroseum</i>	CGMCC3.18337	Bamboo	China	KY494752	KY705220	KY705148
<i>A. subglobosum</i>	MFLUCC 11-0397	Bamboo	Thailand	KR069112	NA	NA
<i>A. thailandicum</i>	MFLUCC 15-0199	Bamboo	Thailand	KU940146	NA	NA
<i>A. thailandicum</i>	MFLUCC 15-0202	Bamboo	Thailand	KU940145	NA	NA
<i>A. trachycarpum</i>	CFCC 53038	<i>Trachycarpus fortunei</i>	China	MK301098	MK303394	MK303396
<i>A. trachycarpum</i>	CFCC 53039	<i>T. fortunei</i>	China	MK301099	MK303395	MK303397
<i>A. vietnamensis</i>	IMI 99670	<i>Citrus sinensis</i>	Vietnam	KX986096	KY019466	NA
<i>A. xenocordella</i>	CBS 478.86	Soil	Zimbabwe	KF144925	NA	NA
<i>A. xenocordella</i>	CBS 595.66	Soil	Austria	KF144926	KF145013	KF145055
<i>A. yunnanum</i>	MFLU 15-0002	<i>Phyllostachys nigra</i>	China	KU940147	NA	NA
<i>A. yunnanum</i>	CFCC 52312	Bamboo	China	MH191120	NA	NA
<i>Nigrospora gorlenkoana</i>	CBS 480.73	<i>Vitis vinifera</i>	Kazakhstan	KX986048	KY019456	KY019420

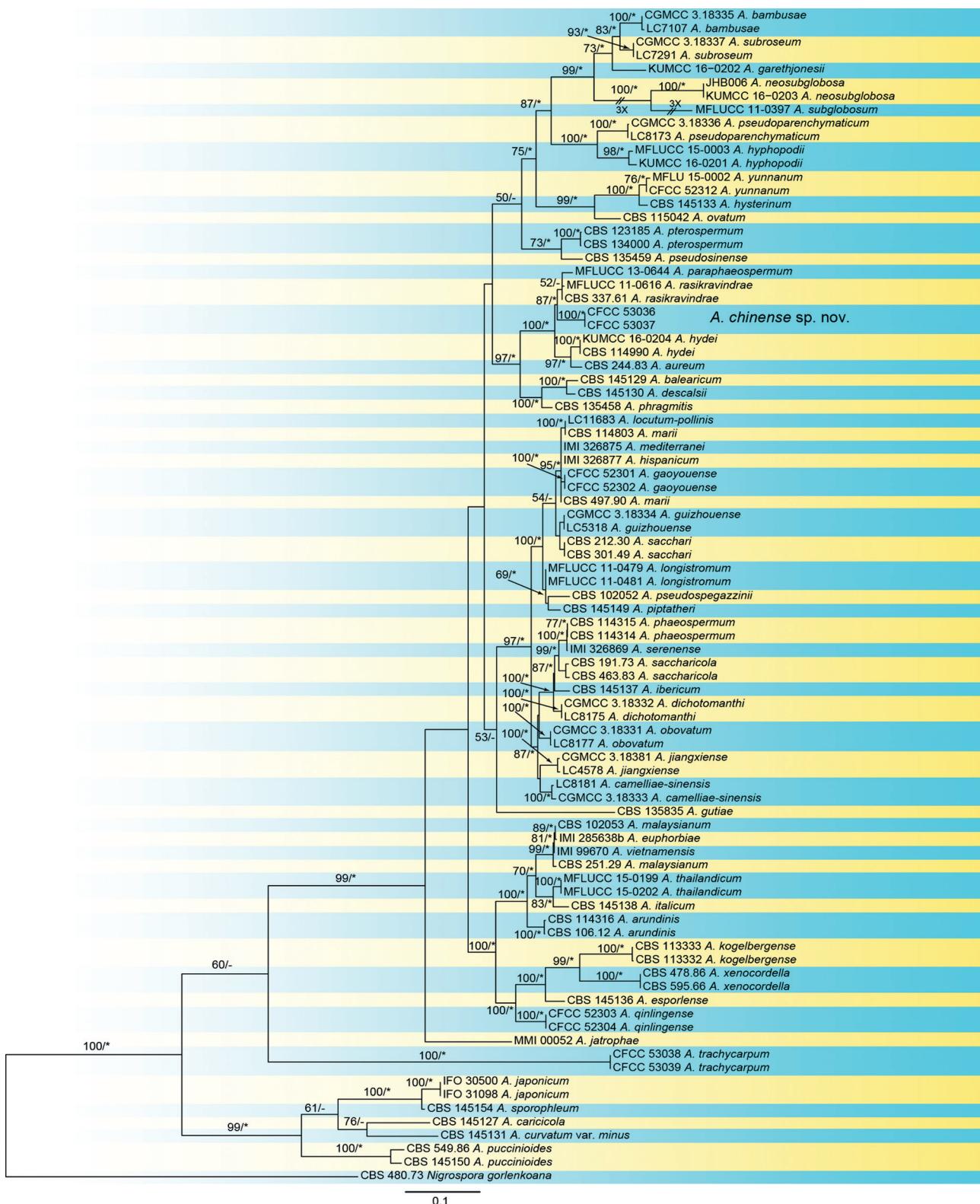


Fig. 1. Phylogram of the best maximum likelihood tree from an analysis of the combined ITS-tub2-tef1 matrix of 86 selected members of *Arthrinium*, with *Nigrospora gorlenkoana* (CBS 480.73) as outgroup. Bootstrap support values (ML BP $\geq 50\%$) and posterior probabilities given at the nodes (ML/PP), * represents posterior probabilities (BI PP ≥ 0.95) from Bayesian inference.

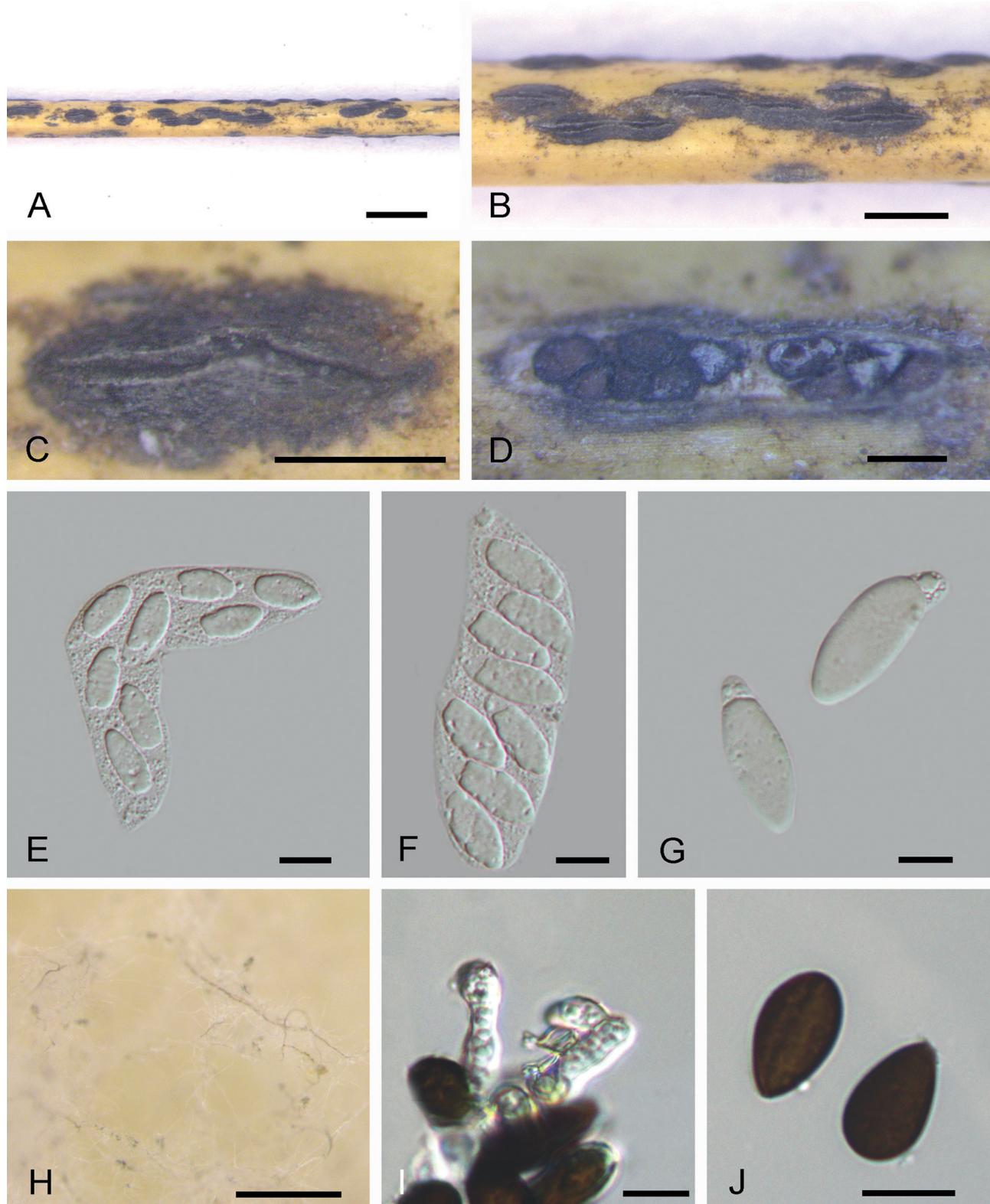


Fig. 2. Morphology of *Arthriniun chinense* on culms of *Fargesia qinlingensis* (BJFC-S1732, CFCC 53036). A–C. Stromata on host; D. Transverse section of a stroma; E, F. Ascii; G. Ascospores; H. Black masses of conidia in culture; I. Conidiogenous cells giving rise to conidia; J. Conidia. Bars: A 2 mm; B 1 mm; C, H 500 μ m; D 200 μ m; E–G, I, J 10 μ m.

Distribution. – Shaanxi Province, China.

Additional specimen examined. – CHINA. Shaanxi Province, Huoditang forest farm in Qinling mountain range, on culms of *Fargesia qinlingensis*, 18 July 2018, leg. N. Jiang & C. M. Tian (BJFC-S1733; living culture CFCC 53037); ditto (BJFC-S1734).

Notes. – Two isolates of *Arthrinium chinense* cluster in a well-supported clade (ML/BI = 100/1) in Fig. 1, which is phylogenetically related to *A. paraphaeospermum* Senan. & K.D. Hyde from *Bambusa* and *A. rasikravindrae* Shiv M. Singh, L.S. Yadav, P.N. Singh, Rah. Sharma & S.K. Singh from soil. However, *A. chinense* has shorter conidia than these species (8–12 µm in *A. chinense* vs. 10–19 µm in *A. paraphaeospermum* and 15–25 µm in *A. rasikravindri*) and differs also in substrate (Singh et al. 2013, Hyde et al. 2016).

Discussion

The primary aim of this study was to obtain the sexual morph of *Arthrinium qinlingense* by collecting dead and dying culms from its host *Fargesia qinlingensis* in Qinling mountain. However, the strains obtained from these samples were phylogenetically different from *Arthrinium qinlingense* (Fig. 1) and had longer conidia (8–12 µm in *A. chinense* versus A. vs. 5–8 µm in *A. qinlingense*) (Jiang et al. 2018). It is therefore evident that there are at least two, maybe even more *Arthrinium* species growing on *Fargesia qinlingensis* and the sexual morph of *A. qinlingensis* is still to be found.

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