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Arthrinium species associated with bamboo and reed plants in China

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Abstract: Arthrinium species are presently recognised based on a combination of morphological characteristics and internal transcribed spacer (ITS) sequence data. In the present study fresh Arthrinium specimens from bamboo and reed plants were collected in China. Morphological comparison and phylogenetic analyses were subsequently performed for species identification. From the results obtained two new species, Arthrinium gaoyouense and A. qinlingense are proposed, and three known species, Arthrinium arundinis, A. paraphaeospermum and A. yunnanum are identified based on morphological characteristics from the host and published DNA sequence data.

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INTRODUCTION

Arthrinium (Kunze 1817) is a globally distributed genus inhabiting a wide range of hosts and substrates, including air, soil debris, plants, lichens, marine algae (Agut & Calvo 2004, Senanayake *et al.* 2015, Dai *et al.* 2016), and even human tissues (Sharma *et al.* 2014). Although Arthrinium species have been commonly reported as saprophytes on different plant substrates (Agut & Calvo 2004, Crous & Groenewald 2013), the genus also includes phytopathogenic species, namely *A. arundinis* causing kernel blight of barley in America, *A. sacchari* causing damping-off of wheat in Canada, and *A. phaeospermum* causing culm rot of bamboos in China (Martínez-Cano *et al.* 1992, Mavragani *et al.* 2007, Li *et al.* 2016).

Bamboo and reed plants are known for their economic and cultural significance in China. They are used as building materials, food sources, and in various raw products. Culm rot is a common disease in bamboo and reed forests, and *Arthrinium* is thought to be the causal agent (Zhang *et al.* 1995, Ma *et al.* 2003, Hu *et al.* 2005). Recent studies indicated that there is a rich species diversity of *Arthrinium* on bamboo plants in China (Dai *et al.* 2016, Dai *et al.* 2017). More than 17 *Arthrinium* species have been reported from these host plants (Crous & Groenewald 2013, Senanayake *et al.* 2015, Dai *et al.* 2016, Dai *et al.* 2017). However, taxonomic work of *Arthrinium* species on bamboo and reeds is still largely lacking in China, because the hosts are widely distributed, and have never been comprehensively surveyed.

The genus Arthrinium was first described in 1817 with numerous generic synonyms, namely Apiospora, Pteroconium and Scyphospora (Kunze 1817, Crous & Groenewald 2013, Réblová et al. 2016). In agreement with Crous & Groenewald (2013) and Réblová et al. (2016), the generic name Arthrinium is recommended for use, as Arthrinium (1817) was proposed earlier than Apiospora (1875), Pteroconium (1892) and Scyphospora (1928), and is the most widely used of these generic names.

The asexual morph of *Arthrinium* species can be easily recognised based on its dark, aseptate, lenticular conidia with a hyaline rim or germ slit (Singh *et al.* 2012). However, identification of *Arthrinium* to species level is not easy with only the asexual morph because of their relatively conserved morphology. Molecular data and phylogenetic analysis have thus in recent years been used to identify *Arthrinium* species (Crous & Groenewald 2013, Dai *et al.* 2016, Dai *et al.* 2017), making it possible to distinguish closely related taxa.

During our *Arthrinium* survey conducted in 2017, 12 fresh specimens were collected from Jiangsu, Shaanxi and Shandong Provinces in China. These specimens were identified to five *Arthrinium* species based on their conidial characteristics and ITS sequence data. Thus, three known species and two new species are described in the present study.

MATERIALS AND METHODS

Isolates and morphology

In our study, 10 fresh specimens of *Arthrinium* spp. were collected from dead culms of bamboo plants, and two from live culms of reeds in China. Single conidial isolates were acquired following the method of Chomnunti *et al.* (2014), by spreading the conidial suspension on the surface of 1.8 % potato dextrose agar (PDA media). After inoculation, agar plates were incubated at 25 °C to induce spore germination, which usually takes 48 h. Single germinating spores or single hyphal stands were transferred to clean plates under a dissecting microscope with a sterile needle. Species identification was based on morphological features of the fruiting bodies produced on infected plant tissues, supplemented by culture characteristics. Hence, cross-sections were prepared by hand using a double-edge blade. More than 20 fruiting bodies were sectioned, and 50 spores were selected randomly for measurement using a Leica compound microscope

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Table 1. Arthrinium species included in the present study (in bold).

Species	Strains	Substrate	Location	ITS	TUB	TEF
A. arundinis	CBS 106.12	N/A	Germany	KF144883	KF144973	KF145015
	CBS 114316	Hordeum vulgare	Iran	KF144884	KF144974	KF145016
	CFCC 52305	Bamboo	China	MH197126	NA	NA
	CFCC 52306	Bamboo	China	MH197127	NA	NA
	CFCC 52307	Bamboo	China	MH197118	NA	NA
	CFCC 52308	Bamboo	China	MH197119	NA	NA
A. aureum	CBS 244.83	Air	Spain	AB220251	KF144981	KF145023
A. gaoyouense	CFCC 52301	Phragmites australis	China	MH197124	MH236789	MH236793
	CFCC 52302	Phragmites australis	China	MH197125	MH236790	MH236794
A. garethjonesii	KUMCC16-0202	Bamboo	China	KY356086	NA	NA
A. hydei	KUMCC 16-0204	Bambusa tuldoides	China	KY356087	NA	NA
	CBS 114990	Bamboo	China	KF144890	KF144982	KF145024
A. hyphopodii	MFLUCC 15-0003	Bambusa tuldoides	China	KR069110	NA	NA
	KUMCC 16-0201	Bamboo	China	KY356088	NA	NA
A. kogelbergense	CBS 113332	Cannomois virgata	South Africa	KF144891	KF144983	KF145025
	CBS 113333	Restionaceae sp.	South Africa	KF144892	KF144984	KF145026
A. longistromum	MFLUCC 11-0479	Bamboo	Thailand	KU940142	NA	NA
	MFLUCC 11-0481	Bamboo	Thailand	KU940141	NA	NA
A. malaysianum	CBS 102053	Macaranga hullettii	Malaysia	KF144896	KF144988	KF145030
	CBS 251.29	Cinnamomum camphora	N/A	KF144897	KF144989	KF145031
A. marii	CBS 113535	Oats	Sweden	KF144898	KF144990	KF145032
	CBS 114803	Arundinaria hindsi	China	KF144899	KF144991	KF145033
A. montagnei	ToD.7.1	Insect: Ips typographus	Sweden	FJ824610	NA	NA
	VL170	Pinus mugo	Lithuania	JF440582	NA	NA
A. neosubglobosa	JHB006	Bamboo	China	KY356089	NA	NA
	KUMCC 16-0203	Bamboo	China	KY356090	NA	NA
A. ovatum	CBS 115042	Arundinaria hindsii	China	KF144903	KF144995	KF145037
A. paraphaeospermum	MFLUCC 13-0644	Bamboo	Thailand	KX822128	NA	NA
	CFCC 52309	Bamboo	China	MH197122	NA	NA
	CFCC 52310	Bamboo	China	MH197123	NA	NA
A. phaeospermum	CBS 114314	Hordeum vulgare	Iran	KF144904	KF144996	KF145038
	CBS 114315	Hordeum vulgare	Iran	KF144905	KF144997	KF145039
A. phragmites	CBS 135458	Phragmites australis	Italy	KF144909	KF145001	KF145043
A. pseudosinense	CBS 135459	Bamboo	Netherlands	KF144910		KF145044
A. pseudospegazzinii	CBS 102052	Macaranga hullettii	Malaysia	KF144911	KF145002	KF145045
A. pterospermum	CBS 123185	Machaerina sinclairii	New Zealand	KF144912	KF145003	
	CBS 134000	Machaerina sinclairii	Australia	KF144913	KF145004	KF145046
A. qinlingense	CFCC 52303	Fargesia qinlingensis	China	MH197120	MH236791	MH236795
	CFCC 52304	Fargesia qinlingensis	China	MH197121	MH236792	MH236796
A. rasikravindrii	CBS 337.61	Cissus sp.	Netherlands	KF144914	NA	NA
	MFLUCC 11-0616	Bamboo	Thailand	KU940144	NA	NA
A. sacchari	CBS 212.30	Phragmites australis	UK	KF144916	KF145005	KF145047
	CBS 301.49	Bamboo	Indonesia	KF144917	KF145006	KF145048
A. saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF145009	KF145051
	CBS 463.83	Phragmites australis	Netherlands	KF144921	KF145010	KF145052
A. subglobosa	MFLUCC 11-0397	Bamboo	Thailand	KR069112	NA	NA
A. thailandicum	MFLUCC 15-0199	Bamboo	Thailand	KU940146	NA	NA
	MFLUCC 15-0202	Bamboo	Thailand	KU940145	NA	NA

Table 1. (Continued).						
Species	Strains	Substrate	Location	ITS	TUB	TEF
A. vietnamensis	IMI 99670	Citrus sinensis	Vietnam	KX986096	KY019466	NA
A. xenocordella	CBS 478.86	Soil	Zimbabwe	KF144925	NA	NA
	CBS 595.66	Soil	Austria	KF144926	KF145013	KF145055
A. yunnanum	MFLU 15-0002	Phyllostachys nigra	China	KU940147	NA	NA
	DDQ00281	Phyllostachys nigra	China	KU940148	NA	NA
	CFCC 52311	Bamboo	China	MH191119	NA	NA
	CFCC 52312	Bamboo	China	MH191120	NA	NA
Seiridium phylicae	CPC 19965	Phylica arborea	UK	KC005787	KC005821	KC005817

(LM, DM 2500). Specimens and isolates are deposited in the Museum of Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC).

DNA amplification, sequencing and phylogeny

Genomic DNA was extracted from 7-d-old mycelium grown on PDA with cellophane using a modified CTAB method (Doyle & Doyle 1990). ITS5 and ITS4 (White *et al.* 1990), EF1-728F (Carbone & Kohn 1999) and EF-2 (O'Donnell *et al.* 1998) and T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) primers were used for the amplification of internal transcribed spacers (ITS), translation elongation factor 1-alpha (*TEF*) and the beta-tubulin gene region (*TUB*) respectively. Polymerase chain reaction (PCR) amplification was carried out following Crous & Groenewald (2013). 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® Terminater Kit v. 3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

DNA sequence analysis

The new sequences generated in this study, and the reference sequences of all *Arthrinium* isolates selected from recent studies, were included in the phylogenetic analyses (Table 1). *Seiridium phylicae* (CPC 19965) was used as outgroup (Dai *et al.* 2016). These sequences were aligned with MAFFT v. 7 (Katoh & Standley 2013) and manually adjusted. Phylogenetic analyses were performed on ITS, *TEF* and *TUB* sequences respectively (Crous & Groenewald 2013) by PAUP v. 4.0b10 (Swofford *et al.* 2003) for maximum parsimony (MP), MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003) for Bayesian inference (BI) and PhyML v. 7.2.8 (Guindon *et al.* 2010) for maximum likelihood (ML). Sequence alignments were deposited at TreeBASE (www. treebase.org) under the accession number S22400. Taxonomic novelties were deposited in MycoBank (Crous *et al.* 2004).

RESULTS

Phylogeny

The ITS alignment contained 56 ITS sequences (including one outgroup) with 716 characters including alignment gaps. Of these, 402 characters were constant, 75 variable characters were parsimony-uninformative and 239 characters were

parsimony informative. The MP analysis resulted in five equally most parsimonious trees, with the first tree (TL = 735, CI = 0.638, RI = 0.866, RC = 0.553) shown in Fig. 1. The phylogenetic tree obtained from ML and BI with the MCMC algorithm was similar to the MP tree. *Arthrinium qinlingense* sp. nov. appeared in a distinct clade with high bootstrap support (Fig. 1). However, *Arthrinium marii, A. gaoyouense* sp. nov., *A. longistromum* and *A. sacchari* were not well-supported in the ITS phylogeny (Fig. 1).

The combined *TEF* and *TUB* alignment contained 26 sequences (including one outgroup) and 1 399 characters including alignment gaps; 518 of these were parsimony-informative, 219 were variable and parsimony-uninformative, and 632 were constant. The MP analysis resulted in a single most parsimonious tree (TL = 1719, CI = 0.678, RI = 0.791, RC = 0.536) shown in Fig. 2.

Taxonomy

Arthrinium gaoyouense C.M. Tian & N. Jiang, sp. nov. MycoBank MB824581. Fig. 3.

Etymology: gaoyouense, named after Gaoyou city, where the extype strain of this fungus was collected.

Sexual morph: Undetermined. Asexual morph: Conidiomata 1–15 mm long, 0.5–5 mm wide, scattered to gregarious, superficial on leaf and culms. Conidiophores reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, smooth, short and wide, 1–2 μ m × 2–3 μ m. Conidia brown, smooth, granular, globose to elongate ellipsoid in surface view, 5–8 μ m diam, lenticular in side view, with pale equatorial slit, 4–8 μ m diam in side view; with central basal scar, 1–2 μ m diam. Brown, elongated cells seldom intermingled among conidia.

Culture characteristics: On PDA, colonies are flat, spreading, with sparse aerial mycelium, olivaceous grey on surface, reverse smoke-grey with patches of olivaceous grey. Conidiomata formed after 20 d at 25 °C.

Materials examined: **China**, Jiangsu Province, Gaoyou City, 32°47′25.10″N, 119°28′11.81″E, 2 m asl, on leaves and culms of *Phragmites australis*, 12 Oct. 2017, *N. Jiang* (holotype BJFC-S1411, extype culture CFCC52301); Jiangsu Province, Gaoyou City, 32°47′25.10″N, 119°28′11.81″E, 2 m asl, on leaves and culms of *P. australis*, 12 Oct. 2017, *N. Jiang* (paratype BJFC-S1412, culture CFCC 52302).

Notes: Two isolates of *Arthrinium gaoyouense* cluster in a wellsupported clade (MP/ML/BI = 99/100/1) in Fig. 1 and (MP/ML/BI FUSE



Fig. 1. Phylogram of *Arthrinium* based on ITS. Values above the branches indicate maximum parsimony bootstrap (MP BP \ge 50 %) and maximum likelihood bootstrap (ML BP \ge 50 %). Values below the branches indicate posterior probabilities above 0.90 from BI. Scale bar = 20 nucleotide changes. The new sequences resulting from the current study are in blue.



Fig. 2. Phylogram of *Arthrinium* based on combined *TEF* and *TUB*. Values above the branches indicate maximum parsimony bootstrap (MP BP \geq 50 %) and maximum likelihood bootstrap (ML BP \geq 50 %). Values below the branches indicate posterior probabilities above 0.90 from BI. Scale bar = 60 nucleotide changes. The new sequences resulting from the current study are in blue.

= 100/100/1) in Fig. 2. Arthrinium gaoyouense is phylogenetically closely related to Arthrinium marii, A. longistromum and A. sacchari in the ITS phylogram (Fig. 1). However, the branch length indicates that they are different species. In addition, Arthrinium gaoyouense differs from A. marii in having much smaller conidia in surface view (5–8 µm in A. gaoyouense vs. 8–13 µm in A. marii) and differs from A. sacchari in the size of its conidiogenous cells (1–2 µm × 2–3 µm in A. gaoyouense vs. 5–12 µm × 2.5–4 µm in A. marii), which is consistent with the results shown in *TEF* and *TUB* phylogram (Fig. 2).

Arthrinium qinlingense C.M. Tian & N. Jiang, sp. nov. MycoBank MB824582. Fig. 4.

Etymology: qinlingense, named after the Qinling mountain range, where the ex-type strain of this fungus was collected.

Sexual morph: Undetermined. Asexual morph: Conidiomata 1–4 mm long, 0.5–3 mm wide, up to 0.3 mm high, scattered, partly immersed, becoming erumpent to superficial, dark brown. Conidiophores reduced to conidiogenous cells. Conidiogenous



Fig. 3. Morphology of *A.* gaoyouense from *Phragmites* australis (BJFC-S1413, holotype). **A–C.** Habit of conidiomata on a culm. **D–E.** Colonies on PDA. **F–G.** Conidiomata in culture. **H–K.** Conidiogenous cells giving rise to conidia. **L–N.** Conidia. Scale bars: A–C = 2 mm; F–G = 1 mm; H–N = 10 μm.

cells aggregated in clusters on hyphae, smooth, short, 1–2 μ m long. Conidia brown, smooth, granular, globose to suborbicular, 5–8 μ m diam; with central basal scar, 1–2 μ m diam.

Culture characteristics: On PDA, colonies are fluffy, spreading, with sparse aerial mycelium, white on surface, reverse smokegrey with patches of olivaceous grey. Conidiomata formed after 30 d at 25 °C.

Materials examined: **China**, Shaanxi Province, Huoditang forest farm in Qinling mountain range, 33°18'22.30"N, 108°35'45.26"E, 1 820 m asl, on culms of *Fargesia qinlingensis*, 27 Jun. 2017, Ning Jiang (holotype BJFC-S1413, ex-type culture CFCC 52303); Shaanxi Province, Huoditang forest farm in Qinling mountain range, 33°18'22.30"N, 108°35'45.26"E, 1 820 m asl, on culms of *Fargesia qinlingensis*, 27 Jun. 2017, *N. Jiang* (paratype BJFC-S1414, living culture CFCC 52304). Notes: Two isolates of Arthrinium qinlingense cluster in a wellsupported clade (MP/ML/BI = 100/100/1) in Fig. 1, and (MP/ ML/BI = 100/100/1) in Fig. 2. The conidial size of A. qinlingense was similar to that of A. arundinis, A. malaysianum and A. thailandicum, so it is not easy to distinguish these four species based on morphology only. However, based on DNA sequence data (ITS, TUB and TEF), they can easily be separated.

DISCUSSION

In the present study we conducted a plant disease survey on bamboo and reed plantations in Jiangsu, Shaanxi and Shandong provinces in China. Culm rot of bamboo and reed was a common but not serious disease observed during the collection trip. In agreement with the previous observations and publications,





Fig. 4. Morphology of *A. qinlingense* from *Fargesia qinlingensis* (BJFC-S1411, holotype). **A–B.** Habit of conidiomata on a culm. **C.** Transverse sections through conidiomata. **D.** Longitudinal sections through conidiomata. **E–F.** Colonies on PDA. **G.** Conidiogenous cells giving rise to conidia. **H–I.** Conidia. Scale bars: A–D = 2 mm; G–I = 10 μ m.

casual agents were assigned to the genus *Arthrinium* (Zhang *et al.* 1995, Ma *et al.* 2003, Hu *et al.* 2005, Dai *et al.* 2016, Li *et al.* 2016, Dai *et al.* 2017).

Based on morphological observations and DNA sequence data, Arthrinium arundinis, A. paraphaeospermum, A. qinlingense and A. yunnanum were considered as the potential causal agents of bamboo culm rot, being associated with typical disease symptoms. Necrotic culms exhibited similar symptoms, but with some variation in detail (Figs 4, 5). Conidiomata of Arthrinium arundinis and A. qinlingense were more gregarious than those of A. paraphaeospermum and A. yunnanum on the culms. The conidiomatal size of A. yunnanum on culms was less than 2 mm, being obviously smaller compared to those of the other three species. Additionally, conidial size proved useful but inconclusive for species identification: 5–7 μ m in A. arundinis vs. 11–15 μ m in A. paraphaeospermum vs. 5–8 μm in A. qinlingense vs. 10–16 μm in A. yunnanum. These morphological characteristics were thus not robust enough to distinguish the species occurring on bamboo, because there was considerable overlap in size. Dai et al. (2016) proposed Arthrinium yunnanum as a new species based on a sexual morph on culms, and asexual morph in cultures. Conidia in culture (15.5–26.5 μm diam) were much larger than the conidia observed on culms in this study (10–16 µm diam). This leads us to conclude that morphology alone should no longer be seen as sufficient for distinguishing species of Arthrinium. This finding is in agreement with the observations of Crous & Groenewald (2013), who stated that species of Arthrinium species are highly variable morphologically, depending on the substrate and period of incubation, and that morphological features exhibited in vitro do not always match those observed in vivo.



Fig. 5. Morphology of *A. arundinis* (A1–A6), *A. paraphaeospermum* (B1–B6), *A. yunnanum* (C1–C6) from bamboo in China. **A1–C2.** Habit of conidiomata on a culm. **A3–C4.** Colonies on PDA. **A5–C5.** Conidiomata in culture. **A6–C6.** Conidia. Scale bars: A1–C2 = 2 mm; A5–C6 = 10 μm.

Crous & Groenewald (2013) used ITS sequence data to perform species identification, and combined *TEF* and *TUB* alignments to resolve species complexes in *Arthrinium*. In this study, the ITS phylogenetic backbone tree separated the four species from bamboo and one from reeds. Additionally, a phylogeny based on combined *TEF* and *TUB* alignments was performed to confirm the monophyly of *Arthrinium gaoyouense* and *A. qinlingense*.

This study showed that the 12 isolates from bamboo and reed plants represent five distinct species of *Arthrinium*,

meaning that different fungal pathogens are associated with culm rot symptoms in China. Further studies are now required, however, to confirm pathogenicity.

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