**Arthrinium** species associated with bamboo and reed plants in China

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taxonomy

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 bamboo in China (Martinez-Can 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, Rèbélová et al. 2016). In agreement with Crous & Groenewald (2013) and Rèbélová 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.
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           |              |
| A. hydei           | KUMCC 16-0204| Bamboosa tuldoides | China | KY356087     | NA           |              |
|                    | CBS 114990| Bamboosa tuldoides | China | KF144890     | KF144982     | KF145024     |
| A. hyphopodii      | MFLUCC 15-0003| Bamboosa tuldoides | China | KR069110     | NA           |              |
|                    | MFLUCC 16-0201| Bamboo   | China    | KY356088     | 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           |              |
| MFLUCC 11-0481     | Bamboo    | Thailand | KU940141 | NA           |              |              |
| A. malaysianum     | CBS 102053| Macaranga hulletii | 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           |              |
|                    | VL170     | Pinus mugo | Lithuania | JF440582     | NA           |              |
| A. neosubglobosa   | JHB006    | Bamboosa tuldoides | China    | KY356089     | NA           |              |
|                    | KUMCC 16-0203| Bamboosa tuldoides | China    | KY356090     | NA           |              |
| A. ovatum          | CBS 115042| Arundinaria hindsi | China   | KF144903     | KF144995     | KF145037     |
| A. paraphaeospermum| MFLUCC 13-0644| Bamboo   | Thailand | KX822128     | NA           |              |
| CFCC 52309         | Bamboo    | China     | MH197122 | NA           |              |              |
| CFCC 52310         | Bamboo    | China     | MH197123 | 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| Bamboosa tuldoides | Netherlands | KF144910     |              | KF145044     |
| A. pseudospegazzinii| CBS 102052| Macaranga hulletti | 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           |              |
|                    | MFLUCC 11-0616| Bamboosa tuldoides | Thailand | KU940144     | NA           |              |
| A. sacchari        | CBS 212.30| Phragmites australis | UK      | KF144916     | KF145005     | KF145047     |
| CBS 301.49         | Bamboosa tuldoides | Indonesia | KF144917     | KF145006     | KF145048     |
| A. saccharicina    | CBS 191.73| Air       | Netherlands | KF144920     | KF145009     | KF145051     |
| CBS 463.83         | Phragmites australis | Netherlands | KF144921     | KF145010     | KF145052     |
| A. subglobosa      | MFLUCC 11-0397| Bamboosa tuldoides | Thailand | KR069112     | NA           |              |
| A. thailandicum    | MFLUCC 15-0199| Bamboosa tuldoides | Thailand | KU940146     | NA           |              |
| MFLUCC 15-0202     | Bamboosa tuldoides | Thailand | KU940145     | NA           |              |
Table 1. (Continued).

| Species          | Strains    | Substrate | Location | ITS    | TUB   | TEF   |
|------------------|------------|-----------|----------|--------|-------|-------|
| A. vietnamensis  | IMI 99670  | Citrus sinensis | Vietnam  | KX986096 | KY019466 | NA    |
| A. xenochordella | CBS 478.86  | Soil       | Zimbabwe | KF144925 | NA    | NA    |
| A. yunnanum      | CBS 595.66  | Soil       | Austria  | KF144926 | KF145013 | KF145055 |
|                  | 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 |

Results

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® Terminator 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. longistomum 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 with cellophane using a modified CTAB method (Doyle & Doyle 1990), 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 ex-type 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 μm × 2–3 μm. Conidia brown, smooth, granular, globose to elongate ellipsoid in surface view, 5–8 μm diam, lenticular in side view, with pale equatorial slit, 4–8 μm diam in side view; with central basal scar, 1–2 μm 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, ex-type 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 CFCC52302).

Notes: Two isolates of Arthrinium gaoyouense cluster in a well-supported clade (MP/ML/BI = 99/100/1) in Fig. 1 and (MP/ML/BI
| **Arthrinium xenocordella** CBS 47886 |
| **Arthrinium pseudospegazzinii** CBS 102052 |
| **Arthrinium hydei** CBS 114316 |
| **Arthrinium qinlingense** CFCC 52304 |
| **Arthrinium arundinis** CFCC 52305 |
| **Arthrinium arundinis** CFCC 52306 |
| **Arthrinium arundinis** CFCC 52307 |
| **Arthrinium arundinis** CFCC 52308 |
| **Arthrinium arundinis** ToD.7.1 |

Fig. 1. Phylogram of *Arthrinium* based on ITS. Values above the branches indicate maximum parsimony bootstrap (MP BP ≥ 50 %) and maximum likelihood bootstrap (ML BP ≥ 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.
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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
cells aggregated in clusters on hyphae, smooth, short, 1–2 μm long. Conidia brown, smooth, granular, globose to suborbicular, 5–8 μm diam; with central basal scar, 1–2 μm diam.

Culture characteristics: On PDA, colonies are fluffy, spreading, with sparse aerial mycelium, white on surface, reverse smoke-grey 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, 1820 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, 1820 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 well-supported 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,
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 μm in *A. arundinis* vs. 11–15 μm 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*. 

![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 μm.](image-url)
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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