A survey of bambusicolous fungi in Bijiashan Mountain Park, Shenzhen, Guangdong Province, China, revealed several *Arthrinium*-like taxa from dead sheaths, twigs, and clumps of *Bambusa* species. Phylogenetic relationships were investigated based on morphology and combined analyses of the internal transcribed spacer region (ITS), large subunit nuclear ribosomal DNA (LSU), beta tubulin (β-tubulin), and translation elongation factor 1-alpha (tef 1-α) gene sequences. Based on morphological characteristics and phylogenetic data, *Arthrinium acutiapicum* sp. nov. and *Arthrinium pseudorasikravindrae* sp. nov. are introduced herein with descriptions and illustrations. Additionally, two new locality records of *Arthrinium bambusae* and *Arthrinium guizhouense* are described and illustrated.

**Keywords:** Apiosporaceae, bamboo, fungal taxonomy, new locality records, novel species

**INTRODUCTION**

*Arthrinium* Kunze is accommodated in Apiosporaceae, Xylariales, which is morphologically different from other xylariaceous genera by the presence of basauxic conidiophores and dark, asceptate, globose to lenticular conidia with a hyaline rim or germ slit (Minter, 1985; Petrini and Müller, 1986; Singh et al., 2012; Jiang et al., 2018; Pintos et al., 2019). Basauxic conidiophores simply mean elongation of conidiogenous cells from the basal growing point after formation of a single, terminal blastic conidium at its apex (Cole, 1986).

*Arthrinium* species are distributed worldwide in various hosts as endophytes, epiphytes, or saprobes, as well as plant pathogens on some commercial crops and ornamentals (Agut and Calvo, 2004; Ramos et al., 2010; Crous and Groenewald, 2013; Sharma et al., 2014; Senanayake et al., 2015; Wijayawardene et al., 2020). Also, species of *Arthrinium* (Schmidt and Kunze, 1817) inhabit a wide range of substrates, i.e., air, soil debris, lichens, marine algae (Agut and Calvo, 2004; Senanayake et al., 2015; Dai et al., 2016; Luo et al., 2019), and even human tissues (Sharma et al., 2014). The genus *Arthrinium* morphologically differs from other xylariaceous anamorphic genera by the presence of basauxic conidiogenous cells which arise from conidiophore mother cells (Schmidt and Kunze, 1817; Minter, 1985).

The commonly reported diseases by *Arthrinium* species are kernel blight of barley and brown culm streak of *Phyllostachys praecox* by *A. arundinis*, damping-off of wheat by *A. sacchari*, and culm rot of bamboos and *Phyllostachys viridis* by *A. phaeospermum* (Martínez-Cano et al., 1992; Mavragani et al., 2007; Chen et al., 2014; Li et al., 2016). Additionally, the causative agent of cutaneous infections of humans has been reported as *A. phaeospermum* (Rai, 1989; Zhao et al., 1990; De Hoog et al., 2000; Crous and Groenewald, 2013). Some *Arthrinium* species produce...
bioactive compounds with pharmacological and medicinal properties (Hong et al., 2015), while some produce industrially important enzymes (Shrestha et al., 2015). Crous and Groenewald (2013) reviewed the genus Arthrinium based on morphology and multigene phylogeny. There are several subsequent studies providing additions to the genus (Singh et al., 2012; Sharma et al., 2014; Crous et al., 2015; Senanayake et al., 2015; Dai et al., 2016, 2017; Hyde et al., 2016; Réblová et al., 2016; Jiang et al., 2018, 2020; Wang et al., 2018; Pintos et al., 2019).

Several studies revealed bambusicolous Arthrinium species from Poaceae and Cyperaceae host plants in China (Dai et al., 2016, 2017; Jiang et al., 2018, 2020; Wang et al., 2018), and several Arthrinium-like taxa on dead leaves, clumps, and twigs of bamboo were collected from Shenzhen (China) during this study. The aims of this study are identifying these Arthrinium-like taxa based on morphology and phylogeny and describe and illustrate them. Phylogenetic relationships were investigated based on DNA sequence data of the internal transcribed spacer region (ITS), large subunit nuclear ribosomal DNA (LSU), beta tubulin (β-tubulin), and translation elongation factor 1 -alpha (tef 1-α), and two new Arthrinium species from bamboo are introduced as Arthrinium pseudorasikravindrae and A. acutiapicum and two locality records, Arthrinium bambusae and Arthrinium guizhouense, are described and illustrated.

MATERIALS AND METHODS

Sample Collection and Fungal Isolation

Fresh specimens of Arthrinium-like taxa were collected from Bijiashan Mountain Park, Shenzhen, Guangdong Province, China, in September–October 2018, and the collection site has a tropical climate with abundant sunshine and rainfall all year round. The yearly average temperature is 22°C and vegetative type is tropical evergreen monsoon forests (Li et al., 2015). Specimens were brought to the laboratory in paper bags and they were examined under a stereomicroscope (Carl Zeiss Discovery V8), and blackish conidial mass and fruit bodies were observed. The fruit bodies were studied and photographed using a stereomicroscope fitted with a camera (ZEISS Axiocam 208). The micromorphological characters were studied and photographed using a compound microscope (Nikon Eclipse 80i) fitted with a digital camera (Canon 450D). All microscopic measurements such as the length of the conidiophores, conidiogenous cells, and conidia were made with Tarosoft image framework (v. 0.9.0.7).

Single conidial isolation was carried out following the method described by Senanayake et al. (2020a). Germinated conidia were aseptically transferred into fresh potato dextrose agar (PDA) plates, incubated at 20°C to obtain pure cultures, and later transferred to PDA slants and stored at 4°C for further study. Colony characteristics were recorded from cultures grown on PDA. Additionally, pure cultures were inoculated in 2% PDA together with sterilized bamboo leaves and incubated at 25°C for sporulation.

Fungarium materials are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS), and all the ex-type living cultures are deposited at the Culture Collection of Kunming Institute of Botany (KUMCC). Index Fungorum numbers1 were obtained for the new strains.

DNA Extraction, PCR Amplification, and Sequencing

Fresh fungal mycelium grown on PDA for 2 weeks at 20°C in the dark was used for DNA extraction using fungal genomic DNA extraction kit (Biospin DNA Extraction Kit, Bior Technology, Co. Ltd., Hangzhou, China) following the manufacturer’s protocols. Polymerase chain reactions (PCR) and sequencing were carried out for the following loci: the complete ITS region with the primer pair of ITS1/ITS4 (White et al., 1990); the LSU ribosomal DNA, amplified and sequenced as a single fragment with primers LR0R/LR5 (Vilgalys and Hester, 1990); the tef 1-α gene with primers EF1-728F/EF2 (Carbone and Kohn, 1999; Rehner, 2001); and the β-tubulin gene with primers bt2a and bt2b (Glass and Donaldson, 1995).

The PCR amplification reactions were carried out with the protocol. The total volume of the PCR reaction was 25 μl reaction containing 1 μl of DNA template, 1 μl of each forward and reverse primer, 12.5 μl of 2 × PCR Master Mix, and 9.5 μl of double-distilled sterilized water (ddH2O). The reaction was conducted by running for 35 cycles following the condition of Senanayake et al. (2020b). The PCR products were observed on 1% agarose electrophoresis gel stained with ethidium bromide. Purification and sequencing of PCR products were carried out at Sunbiotech Company, Beijing, China. Sequence quality was checked and sequences were condensed with DNASTAR Lasergene v.7.1. Sequences derived in this study were deposited in GenBank and accession numbers were obtained (Table 1).

Sequence Alignments and Phylogenetic Analyses

BLASTn searches were made using the newly generated sequences to assist in taxon sampling for phylogenetic analyses. Jiang et al. (2018, 2020), Wang et al. (2018), and Pintos et al. (2019) were followed to obtain sequences from GenBank that are listed in Table 1. The concatenated ITS, LSU, β-tubulin, and tef 1-α sequence dataset comprised 101 strains of Arthrinium, while the outgroup taxon was Pestalotiopsis chamaeeroops (CBS 237.38). DNA sequences of the ITS, LSU, β-tubulin, and tef 1-α were aligned using the online version of MAFFT v. 7.0362 (Katoh et al., 2020) with default settings and manually adjusted using BioEdit 7.1.3 (Hall, 1999) to allow maximum alignment and minimum gaps. Further, single gene alignments were combined to obtain the final multiloci alignment that was containing 2,817 nucleotide characters, viz. 681 of ITS, 875 of LSU, 434 of β-tubulin, and 827 of tef 1-α. Both single and concatenated alignments were used for the analyses.

1http://www.indexfungorum.org
2http://mafft.cbrc.jp/alignment/server/large.html
## Details of the isolates used in the phylogenetic analyses.

| Species | Strains | Substrate | Location | GenBank Accession Number |
|---------|---------|-----------|----------|--------------------------|
| Arthrinium acutipicum | KUMCC 20-0209 | Bambusa bambos | China | MT946342 MT946338 MT947365 MT947359 |
| A. acutipicum | KUMCC 20-0210 | Bambusa bambos | China | MT946343 MT946339 MT947366 MT947360 |
| A. aquaticum | MFLU 18-1628 | Submerged wood | China | MK828608 MK838586 N/A N/A |
| A. arundinis | CBS 450.92 | N/A | N/A | AB202059 N/A AB202036 N/A |
| A. arundinis | CBS 114316 | Hordeum vulgare | Iran | KF144884 KF144928 KF144974 KF145016 |
| A. arundinis | AP11118A | Bambusa sp. | Spain | MK014886 MK014835 MK017974 MK017945 |
| A. aureum | CBS 244.83 | Phragmites australis | | AB202051 KF144905 KF144981 KF145023 |
| A. balearicum | CBS 145129 | Poaceae sp. | Spain | MK014869 MK014836 MK017975 MK017946 |
| A. bambusae | CGMCC 3.18335 | Bamboo | China | KY494718 KY494794 KY705186 KY806204 |
| A. bambusae | KUMCC 20-0207 | Bambusa dolichochlada | China | MT946346 MT946340 MT947370 MT947363 |
| A. bambusae | LC7107 | Bamboo | China | KY494719 KY494795 KY705187 KY705117 |
| A. camelliae-sinensis | CGMCC 3.18333 | Camellia sinensis | China | KY494704 KY494780 KY705173 KY705103 |
| A. camelliae-sinensis | LC8181 | Brassica campestris | China | KY494761 KY494837 KY705229 KY705157 |
| A. caricola | CBS 145127 | Carex ericetorum | Germany | MK014871 MK014838 MK017977 MK017948 |
| A. chinense | CFCC 53036 | Fargesia qinlingensis | China | MK819291 N/A MK818547 MK818545 |
| A. chinense | CFCC 53037 | Fargesia qinlingensis | China | MK819292 N/A MK818548 MK818546 |
| A. curvatum | CBS 145131 | Carex sp. | Germany | MK014872 MK014839 MK017978 MK017949 |
| A. descalsii | CBS 145130 | Ampelodesmos mauritanicus | Spain | MK014870 MK014837 MK017976 MK017947 |
| A. dichotomanthi | CGMCC 3.18332 | Dichotomanthus tristisannaeacarpa | China | KY494697 KY494823 KY705167 KY705096 |
| A. dichotomanthi | LC8175 | Dichotomanthus tristisannaeacarpa | China | KY494755 KY494831 KY705223 KY705151 |
| A. esporlense | CBS 145136 | Phyllostachys aura | Spain | MK014878 MK014845 MK017983 MK017954 |
| A. euphorbiae | IMI 285636b | Bambusa sp. | Bangladesh | AB202041 N/A AB202088 N/A |
| A. gaeyouense | CFCC 52301 | Phragmites australis | China | MH197124 N/A MH236789 MH236793 |
| A. gaeyouense | CFCC 52302 | Phragmites australis | China | MH197125 N/A MH236790 MH236794 |
| A. garethiensis | JHB004 | Bambusa sp. | China | KY356086 KY356091 N/A N/A |
| A. garethiensis | HKAS 96289 | Bambusa sp. | China | NR_154736 NG_057131 N/A N/A |
| A. guizhouense | LCS318 | Air | China | KY494708 KY494784 KY705177 KY705017 |
| A. guizhouense | KUMCC 3.18333 | Bambusa multiplex | China | MT946347 MT946341 MT947369 MT947364 |
| A. guizhouense | LC8175 | Bambusa multiplex | China | KY494709 KY494785 KY705178 KY705108 |
| A. guttiae | CBS 135835 | Gut of a grasshopper | India | KR011352 MH877577 KR011350 KR011351 |
| A. hispanicum | IMI 296677 | Maritime sand | Spain | AB202042 AB202036 AB202038 N/A |
| A. hydei | KUMCC 16-0204 | Bambusa tuloides | China | KY356087 KY356092 N/A N/A |
| A. hydei | CBS 114990 | Bamboo | China | KY494990 KY494836 KY494823 KY494504 |
| A. hyphopodi | MFLUCC 15-0003 | Bambusa tuloides | China | KR069110 N/A N/A N/A |
| A. hyphopodi | KUMCC 16-0201 | Bamboo | China | KY356088 N/A N/A N/A |
| A. hysterinum | IOPM8889 | Bamboo | New Zealand | MK014874 MK014841 MK017980 MK017951 |
| A. hysterinum | AP2410173 | Phyllostachys aurea | Spain | MK014876 MK014845 N/A N/A |
| A. ibericum | CBS 145137 | Arundo donax | Portugal | MK014879 MK014846 MK017984 MK017955 |
| A. italicum | CBS 145138 | Phragmites australis | Spain | MK014880 MK014847 MK017985 MK017956 |
| A. italicum | AP221017 | Phragmites australis | Spain | MK014881 MK014848 MK017986 MK017957 |
| A. japonicum | IFO 30500 | Carex despalata | Japan | AB202062 AB202036 AB202039 N/A |
| A. japonicum | IFO 31098 | Carex despalata | Japan | AB202064 AB202038 AB202031 N/A |
| A. jatrophae | MIM 900051 | Jatropha podagrica | India | JQ246355 N/A N/A N/A |
| A. jiangiensis | CGMCC 3.18381 | Maesa sp. | China | KY494693 N/A KY705163 KY705092 |
| A. jiangiensis | LC4578 | Camellia sinensis | China | KY494694 KY494770 KY705164 KY705093 |
| A. kogelbergense | CBS 113332 | Cannomos virgare | South Africa | KF144891 KF144937 KF144983 KF145025 |
| A. kogelbergense | CBS 113333 | Restionaceae sp. | South Africa | KF144892 KF144938 KF144984 KF145026 |
| A. locutum-pollinis | LC11683 | Brassica carpestris | China | MF939595 N/A MF939622 MF939616 |
| A. longistromum | MFLUCC 11-0479 | Bamboo | Thailand | KU940142 KU863130 N/A N/A |
| A. longistromum | MFLUCC 11-0481 | Bamboo | Thailand | KU940141 KU863129 N/A N/A |
| A. longistromum | MFLU 15-1184 | Bambusa sp. | Thailand | NR_154716 N/A N/A N/A |
| A. malaysiense | CBS 251.29 | Cinnamomum camphora | N/A | KF144897 KF144943 KF144989 KF145031 |

(Continued)
TABLE 1 | Continued

| Species       | Strains   | Substrate | Location       | GenBank Accession Number |
|---------------|-----------|-----------|----------------|----------------------------|
|               |           |           |                | ITS | LSU | α-Tubulin | tef 1-α |
| A. malaysianum| OBS 102053| Macaranga hulitilli | Malaysia       | KF144896 | KF144942 | KF144988 | KF145030 |
| A. manii      | OBS 497.90| Air       | Spain          | AB220252 | KF144947 | KF144993 | KF145035 |
| A. manii      | OBS 114803| Arundinaria hindiensis | China         | KF144899 | KF144945 | KF144991 | KF145033 |
| A. mediterranei| IMI 326875| Air       | Spain          | AB220243 | N/A | AB220290 | NA |
| A. neosubglobosa| JHB006    | Bamboo    | China          | KY356089 | KY356094 | N/A | NA |
| A. neosubglobosa| JHB007    | Bamboo    | China          | KY356090 | KY356095 | N/A | NA |
| A. obovatum   | CGMCC 3.18331| Lithocarpus sp. | China         | KY494696 | KY494834 | KY705166 | KY705095 |
| A. obovatum   | LC8177    | Lithocarpus sp. | China          | KY494757 | KY494833 | KY705225 | KY705153 |
| A. ovatum     | OBS 115042| Arundinaria hindiensis | China         | KF144903 | KF144950 | KF144995 | KF145037 |
| A. paraphaeospermum| MFLUCC 13-0644| Bamboo | Thailand       | KX822128 | KX822124 | N/A | NA |
| A. paeospermum| OBS 114314| Hordeum vulgare | Iran           | KF144904 | KF144951 | KF144996 | KF145038 |
| A. paeospermum| OBS 114315| Hordeum vulgare | Iran           | KF144905 | KF144952 | KF144997 | KF145039 |
| A. phragmitis | CPC 18900 | Phragmites australis | Italy         | KF144909 | N/A | KF145001 | KF145043 |
| A. phragmitis | AP3218    | Phragmites australis | Spain         | MK014891 | MK014858 | MK017996 | MK017967 |
| A. phragmitis | AP2410172A| Phragmites australis | Spain         | MK014890 | MK014857 | MK017995 | MK017966 |
| A. pipthari   | CBS 145149| Piptatherum milicaceum | Spain       | MK014893 | MK014860 | N/A | MK017969 |
| A. pseudoparenchymaticum| CGMCC 3.18336| Bamboo | China          | KY494743 | KY494830 | KY705211 | KY705139 |
| A. pseudoparenchymaticum| LC8173    | Bamboo    | China          | KY494753 | KY494829 | KY705221 | KY705149 |
| A. pseudosinense| OBS 135459| Hordeum vulgare | Iran           | KF144904 | KF144951 | KF144996 | KF145038 |
| A. pseudopogonaezii| OBS 102062| Macaranga hulitilli | Malaysia       | KF144911 | KF144958 | KF145002 | KF145045 |
| A. pterospermum| OBS 123185| Machaerina sinclairi | New Zealand   | KF144912 | KF144959 | KF145003 | NA |
| A. pterospermum| OBS 134000| Machaerina sinclairi | Australia     | KF144913 | KF144960 | KF145004 | KF145046 |
| A. puccinioides| CBS 549.86| Lepidogramma cladatum | Germany | AB220253 | AB220347 | AB220300 | NA |
| A. qinlingense| OFCC 52303| Fargesia qinlingensis | China        | MH197120 | N/A | MH236791 | MH236795 |
| A. qinlingense| OFCC 52304| Fargesia qinlingensis | China        | MH197121 | N/A | MH236792 | MH236796 |
| A. raskrivandrae| NFCCI 2144| Cissus sp. | Netherlands     | KF144914 | N/A | N/A | N/A |
| A. raskrivandrae| MFLUCC 11-0616| Bamboo | Thailand       | KU940144 | KU863132 | N/A | NA |
| A. sacchari   | OBS 212.30 | Phragmites australis | UK           | KF144916 | KF144962 | KF145005 | KF145047 |
| A. sacchari   | OBS 301.49 | Bamboo     | Indonesia       | KF144917 | KF144963 | KF145006 | KF145048 |
| A. saccharicola| OBS 191.73| Air        | Netherlands     | KF144920 | KF144966 | KF145009 | KF145051 |
| A. saccharicola| OBS 463.83| Phragmites australis | Netherlands   | KF144921 | KF144968 | KF145010 | KF145052 |
| A. serenense  | IMI 326869| N/A | Spain          | AB220250 | N/A | AB220297 | NA |
| A. serenense  | ATCC 76309| N/A | N/A            | AB220240 | N/A | AB220287 | NA |
| A. sporophleum| OBS 145154| Juncus sp. | Spain          | MK014898 | MK014865 | MK018001 | MK017973 |
| A. subglobosum| MFLUCC 11-0397| Bamboo | Thailand       | KR069112 | KR069113 | N/A | NA |
| A. subrosum   | LC7291    | Bamboo | China          | KY494751 | KY494827 | KY705219 | KY705147 |
| A. subrosum   | CGMCC 3.18337| Bamboo | China          | KY494752 | KY494828 | KY705220 | KY705148 |
| A. thailandicum| MFLUCC 15-0199| Bamboo | Thailand       | KU940146 | KU863134 | N/A | NA |
| A. thailandicum| MFLUCC 15-0202| Bamboo | Thailand       | KU940145 | KU863134 | N/A | NA |
| A. trininnitus| ICPM 6899 | Bamboo | New Zealand     | K014874 | K014841 | K017980 | K017951 |
| A. trachycarpum| OFCC 53038| Trachycarpus fortune | China    | KF144909 | N/A | KF145099 | KF145036 |
| A. trachycarpum| OFCC 53039| Trachycarpus fortune | China    | KF144909 | N/A | KF145099 | KF145036 |
| A. vietnamense| IMI 99670 | Citrus sinensis | Vietnam      | KO996096 | KO996111 | KO194866 | NA |
| A. xenocordella| OBS 478.86| Soil        | Zimbabwe       | KF144925 | KY494763 | N/A | NA |
| A. xenocordella| OBS 596.86| Soil        | Austria        | KF144926 | KF144971 | KF145013 | KF145055 |
| A. yunnanum   | MFLU 15-0002| Phyllostachys nigra | China | KU940147 | KU863135 | N/A | NA |
| A. yunnanum   | OFCC 52312| Bamboo | China          | MH191120 | N/A | N/A | NA |

Newly obtained sequences are indicated in black. ATCC, The American Type Culture Collection, Virginia, United States; CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CFCF, China Forestry Culture Collection, Beijing, China; CGMCC, China General Microbiological Culture Collection, Beijing, China; CPC, Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; HKAS, Herbarium of Cryptogams, Kunming, China; ICPM, International Collection of Microorganisms from Plants, Auckland, New Zealand; IFO, Institute for Fermentation, Osaka; IMI, Culture collection of GABI Europe UK Centre, UK; KUMCC, Culture Collection of Kunming Institute of Botany, Kunming, China; MFLU, Mae Fah Luang University Herbarium, Chiang Rai, Thailand; 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.
FIGURE 1 | Continued
FIGURE 1 | Continued
Maximum likelihood analyses were performed by RAxML (Stamatakis and Alachiotis, 2010) implemented in raxmlGUI v.1.5 (Silvestro and Michalak, 2012) using the ML + rapid bootstrap setting and the GTR + I + G model of nucleotide substitution with 1,000 replicates. The matrix was partitioned for the different gene regions included in the combined multilocus analyses.

For the Bayesian inference (BI) analyses, the optimal substitution model for the combined dataset was determined to be GTR + I + G using the MrModeltest software v. 2.2 (Nylander, 2004). The BI analyses was computed with MrBayes v. 3.2.6 (Ronquist et al., 2012) with four simultaneous Markov chain Monte Carlo chains from random trees over 10 M generations (trees were sampled every 500th generation).

The distribution of log-likelihood scores was observed to check whether sampling is in stationary phase or not, and Tracer v.1.5 was used to check if further runs were required to reach convergence or not (Rambaut and Drummond, 2007). The Bayesian analyses lasted until the average standard deviation of split frequencies has a value less than 0.01, and the consensus tree and posterior probabilities were calculated after discarding the first 20% of the sampled trees as burn-in. The phylogram was visualized in FigTree v. 1.4 (Rambaut, 2009). All the phylogenetic trees derived from this study were deposited in TreeBase\(^3\) under accession number S27147.

\(^3\)www.treebase.org
RESULTS

Phylogenetic Inferences

All individual trees generated under different criteria and from single gene datasets were essentially similar in topology and not significantly different from the tree generated from the concatenated dataset (not discussed herein). Additionally, this tree topology is similar to previous studies on Arthrinium (Dai et al., 2016; Jiang et al., 2018, 2020; Wang et al., 2018; Pintos et al., 2019).

Maximum likelihood analysis of Arthrinium species in this study with 1,000 bootstrap replicates yielded the best ML tree (Figure 1) with the likelihood value of −29,933.362493 and the following model parameters: estimated base frequencies—A = 0.239654, C = 0.250345, G = 0.255054, and T = 0.254948; substitution rates—AC = 1.275584, AG = 2.530572, AT = 1.397969, CG = 1.184045, CT = 4.063803, and GT = 1.0; proportion of invariable sites—I = 0.203121; gamma distribution shape parameter—α = 0.54383. The alignment contained a total of 1,756 distinct alignment patterns and 28.72% of aligned characters. After discarding the first 20% of generations, 36,000 trees remained from which 50% consensus trees and posterior probabilities (PP) were calculated (generations, 36,000 trees remained from which 50% consensus trees and posterior probabilities (PP) were calculated (Figure 1). Maximum likelihood bootstrap values ≥60% and BI ≥ 0.95 are given at each node. Tree topologies of the ML and Bayesian analyses were similar to each other and there are no significant differences.

There are 101 Arthrinium strains in this study together with a new isolate that is introduced here. All the ex-type strains of Arthrinium species were included if available, and other authentic strains were selected when sequences from ex-type strains are unavailable. Our new isolate KUMCC 20-0206 clustered with the type strain of A. guizhouense (CGMCC 3.18334) and another representative strain (LC5318) with 87% ML and 0.95 PP support. This clade (clade A) has a close phylogenetic affinity to Arthrinium longistromum, A. pipitleri, and A. sacchari with 95% ML and 0.95 PP support. Two strains of A. bambusae (CGMCC 3.18335 and LC7107) and the new isolate KUMCC 20-0207 were grouped in a separate clade with 96% ML and 1.00 PP support. This clade (subclade B1) shares a monophyletic relationship to Arthrinium garethjonesii, A. mytilomophum, A. neogarethjonesii, A. setostromum, and A. subrasum with strong bootstrap supports (100% ML, 1.00 PP, clade B, Figure 1). Two new isolates, KUMCC 20-0208 and KUMCC 20-0211, were monophyletic in subclade C1 (Figure 1) with 90% ML and 0.96 PP support. Subclade C2 is also monophyletic with two novel strains, viz. KUMCC 20-0209 and KUMCC 20-0210, which are sisters to subclade C1 with 90% ML and 0.96 PP support (clade C, Figure 1). With these four new strains, clade C shares a close phylogenetic affiliation to A. paraphacospermum and A. rasikravindrae.

Taxonomy

Arthrinium acutiapicum Senan. and Cheew. sp. nov.

Figure 2

Index Fungorum number: IF557868

Etymology: Species epithet “acuti” refers to pointed and “acu” refers to apex of conidiogenous cells.

Holotype: HKAS 107673

Saprobic on dead twigs of Bambusa bambos (L.) Voss. Hyphae 1.5–2.5 µm diam., hyaline, branched, septate, sparse. Sexual morph: undetermined. Asexual morph: Conidiomata 350–450 µm, pycnidal, immersed, aggregated, scattered, subglobose, ostiolate, black, coriaceous. Conidiophores reduced to conidiogenous cells. Conidiogenous cells 4–7 × 2–3 µm (ξ = 6.3 × 2.1 µm, n = 30), holoblastic, develop from conidiophore mother cells, erect, basauxic, cylindrical to ampulliform, apex pointed and hyaline, smooth and thick-walled, pale brown. Conidia 7.5–10 × 8.5–12 µm (ξ = 9.3 × 11.9 µm, n = 30), globose in surface view, subglobose to oval in side view, apex and base blunt, smooth-walled, brown to dark brown, with a dark equatorial slit.

Culture Characteristics

Colonies grew on PDA at 20°C in the dark attenuated 2 cm diam., within 7 days, flat, circular, entire margin, wooly, with abundant aerial mycelia, white in surface view and off-white to yellow in reverse. Sporulation occurred after 10 days on PDA incubated at 20°C in the dark without any host substrate. Conidia seem black mass and well spread on culture.

Specimen Examined

China, Guangdong Province, Shenzhen City, Futian District, northwest of Futian, Bijiaoshan Park, on dead twigs of B. bambusae (L.) Voss (Poaceae), 23 September 2018, IS, SI 86 (HKAS 107673, holotype), ex-type culture, KUMCC 20-0210; ibid 15 October 2018, IS, SI 86-1 (HKAS 107674, paratype), ex-paratype culture KUMCC 20-0211.

Notes

Arthrinium acutiapicum forms a distinct subclade (subclade C2, Figure 1) with strong bootstrap support values (ML/PP = 90/0.96) in our phylogenetic analysis, which is a sister to the newly introduced species A. pseudorasikravindrae. Additionally, A. acutiapicum shows close phylogenetic affinities to A. paraphacospermum, A. pseudorasikravindrae, and A. rasikravindrae in clade C (Figure 1) and A. chinense. Blast results of ITS, LSU, β-tubulin, and tef 1-α sequences of A. acutiapicum show high similarity to A. hydei, A. paraphacospermum, and A. rasikravindrae. Morphologically, A. acutiapicum is distinct from A. pseudorasikravindrae by the reduction of conidiophores to conidiogenous cells, cylindrical to ampulliform, pale brown conidiogenous cells with pointed, hyaline apex and brown to dark brown, smooth-walled conidia with dark equatorial slit. Additionally, A. acutiapicum is distinct from A. rasikravindrae by the reduction of conidiophores to pale brown conidiogenous cells and dimorphous, acroleuropeogenously arising conidia.
FIGURE 2 | Arthrinium acutiapicum (HKAS 107673). (A) Host. (B) Fungarium specimen. (C) Conidiomata on substrate. (D) Surface view of culture on potato dextrose agar (PDA). (E) Reverse view of culture on PDA. (F) Conidia and conidiogenous cells. (G, H) Conidia. Scale bars: (C) = 500 \( \mu \)m, (F–H) = 10 \( \mu \)m.
**Saprobic** on sheath of *Bambusa dolichoclada* Hayata. *Hyphae* 1–3 µm diam., hyaline, branched, septate, sparse. *Sexual morph*: undetermined. *Asexual morph*: appears as black, spotty patches on host surface. *Conidiomata* immersed, pycnidial, scattered, globose to slightly conical, ostiolate, black, coriaceous. *Conidiomatal wall* thin, comprising several layers of black, large cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells or rarely inconspicuous with cylindrical, thick-walled, hyaline. *Conidiogenous cells* 5–12 × 3–10 µm (\(\bar{x} = 8.6 \times 4 \mu m, n = 30\)), basauxic, holoblastic, develop from conidiophore mother cells, with periclinal thickening, doliiform to ampulliform or lageniform, erect, aggregated in clusters on hyphae, hyaline to pale brown, smooth. *Conidia* 10.5–17.5 × 8–15 µm (\(\bar{x} = 15 \times 12 \mu m, n = 30\)), subglobose to ellipsoid, guttulate, smooth to finely roughened, olivaceous to dark brown.

**Culture Characteristics**
Colonies grew on PDA at 20°C in the dark attenuated 2 cm diam., within 7 days, flat, spreading, margin circular, with abundant aerial mycelia, surface and reverse white to off-white.
Specimen Examined
China, Guangdong Province, Shenzhen City, Futian District, northwest of Futian, Bijiaoshan Park, on sheath of B. dolichoclada Hayata (Poaceae), 23 September 2018, IS, SI 80 (HKAS 107671), living culture, KUMCC 20-0207.

Notes
Arthrinium bambusae was introduced by Wang et al. (2018) from Guangdong Province, China, where our collection also was obtained. However, the exact locality is not mentioned in the original description there. The morphology of our collection was obtained from fungal structures on the host specimen, while Wang et al. (2018) had described the fungus from sporulated cultures. However, the morphology of our observation is similar to the holotype. Phylogenetically, A. bambusae clusters with A. garethjonesii, A. neogarethjonesii, A. mytilomorphum, A. setostromum, and A. subruseum with strong bootstrap value (ML/PP = 100/1), and the A. bambusae isolate (KUMCC 20-0207) clustered well with the ex-type culture (ML/PP = 96/1).

Arthrinium guizhouense M. Wang and L. Cai, in Wang et al. (2018) Figure 4
Index Fungorum number: IF824909

Saprobic on dead twigs of Bambusa multiplex (Lour.) Raeusch. ex Schult. f. saprobic, ex type culture, KUMCC 20-0209, living culture, KUMCC 20-0211.

Saprobic: Arthrinium pseudorasikravindrae Senan., and Cheew. sp. nov. Figure 5
Notes

Arthrinium pseudorasikravindrae Senan., and Cheew. sp. nov. Figure 5
Index Fungorum number: IF 557870
Etymology: Species epithet the morphological similarity of this collection to Arthrinium rasikravindrae.

Holotype: HKAS 107669

Saprobic on sheaths of B. dolichoclada Hayata. Mycelium 1.5–3 μm in diam., consisting of smooth, hyaline, septate, branched, hyphae. Sexual morph: undetermined. Asexual morph: Conidiophores 10–15 × 3–7 μm (T = 12.3 × 5.2 μm, n = 30), basauxic, straight or flexuous, cylindrical, hyaline, thick, smooth-walled, aseptate. Conidiogenous cells 4–10 × 1.2–5 μm (T = 8.6 × 4.2 μm, n = 30), holoblastic, develop from conidiophore mother cells, ampulliform, cylindrical or doliform, hyaline to olivaceous. Conidia 5–10 × 5.5–11 μm (T = 9.3 × 10.1 μm, n = 30), globose in face view, lenticular in side view, with a pale longitudinal slit, dark brown, thick-walled, finely roughened with one or two concentric pale rings.

Culture Characteristics
Colonies grew on PDA at 20°C in the dark attenuated 2 cm diam., within 5 days, flat, widely spreading, circular, margin filiform with abundant aerial mycelia, surface initially white, becoming grayish white and reverse yellowish white.

Specimen Examined
China, Guangdong Province, Shenzhen City, Futian District, northwest of Futian, Bijiaoshan Park, on twigs of B. multiplex (Lour.) Raeusch. ex Schult. f. (Poaceae), 23 September 2018, I.C. Senanayake, SI 84 (HKAS 107672), living culture, KUMCC 20-0206.

Notes
NCBI blast result for β-tubulin sequences of this isolate gives high sequence similarities to A. guizhouense (99.55%), A. sacchari (98.25%), A. aruninis (98.25%), and A. marii (95.62%) while A. guizhouense (93.41%) and A. marii (92.94%) for tef-1α. Additionally, high blast similarities for ITS loci are A. marii (99.54%), A. sacchari (99.22%), A. phaeospermum (99.20%), A. pseudospegazzinii (98.13%), A. longistromum (98.72%), and A. guizhouense (99.83%) while A. marii (100%), A. sacchari (100%), A. guizhouense (100%), and Apiospora montagnei (100%) for LSU. In the phylogenetic analysis, this isolate (KUMCC 20-0206) clusters with the ex-holotype strain of A. guizhouense (CGMCC3.18334) with moderate support value (ML/PP = 87/0.95). Morphologically, this collection is closely similar to the holotype specimen of A. guizhouense having brown to black, smooth to finely roughened, globose or subglobose, conidia with pale brown, subglobose, ampulliform or doliform conidiogenous cells. However, the holotype of A. guizhouense has been collected from the air in karst cave in Guizhou Province, China, and this collection was obtained from bamboo twigs in Guangdong Province. Hence, HKAS 107672 is identified as A. guizhouense based on morphology and phylogeny. This is the first record of A. guizhouense in Guangdong Province and on bamboo.

Arthrinium pseudorasikravindrae Senan., and Cheew. sp. nov. Figure 5

Index Fungorum number: IF 557870
Etymology: Species epithet the morphological similarity of this collection to Arthrinium rasikravindrae.

Holotype: HKAS 107669

Saprobic on sheaths of B. dolichoclada Hayata. Mycelium 1.5–3 μm in diam., consisting of smooth, hyaline, septate, branched, hyphae. Sexual morph: undetermined. Asexual morph: Conidiophores 10–15 × 3–7 μm (T = 12.3 × 5.2 μm, n = 30), basauxic, straight or flexuous, cylindrical, hyaline, thick, smooth-walled, aseptate. Conidiogenous cells 4–10 × 1.2–5 μm (T = 8.6 × 4.2 μm, n = 30), holoblastic, develop from conidiophore mother cells, ampulliform, cylindrical or doliform, hyaline to olivaceous. Conidia 5–10 × 5.5–11 μm (T = 9.3 × 10.1 μm, n = 30), globose in face view, lenticular in side view, with a pale longitudinal slit, dark brown, thick-walled, finely roughened with one or two concentric pale rings.

Culture Characteristics
Colonies grew on PDA at 20°C in the dark attenuated 2 cm diam., within 5 days, flat, spreading, circular, margin filiform with abundant aerial mycelia, surface white to off-white and reverse pale yellow, sporulation occurs on 2% PDA incubated at 25°C after 2 weeks, black, conidial mass concentrated at colony margins. Sporulation occurred after 10 days on PDA incubated at 20°C in the dark without any host substrate. Conidia seem black mass and spread mostly in colony margins.

Specimen Examined
China, Guangdong Province, Shenzhen City, Futian District, northwest of Futian, Bijiaoshan Park, on sheath of B. dolichoclada Hayata (Poaceae), 23 September 2018, IS, SI 73 (HKAS 107669, holotype), ex-type culture, KUMCC 20-0208; ibid October 15, 2018, IS, SI 73-1 (HKAS 107670, paratype), ex-paratype culture KUMCC 20-0211.

Notes
Blast results of ITS, LSU, β-tubulin, and tef-1α sequences of A. pseudorasikravindrae (KUMCC 20-0208, KUMCC 20-0211) show high similarity to A. hydei, A. paraphaeospermum, and A. rasikravindrae. In our phylogenetic analysis, A. pseudorasikravindrae forms a subclade (subclade C1, Figure 1)
FIGURE 4 | *Arthrinium guizhouense* (HKAS 107672). (A) Host. (B) Fungarium specimen. (C) Conidiomata on substrate. (D) Surface view of culture on potato dextrose agar (PDA). (E) Reverse view of culture on PDA. (F–H) Conidia and conidiogenous cells. (I) Conidia. Scale bars: (C) = 1,000 µm, (F–I) = 5 µm.
FIGURE 5 | Arthrinium pseudorasikravindrae (HKAS 107669). (A) Host. (B) Fungarium specimen. (C) Surface view of culture on potato dextrose agar (PDA). (D) Reverse view of culture on PDA. (E) Conidial mass on cultures. (F–J) Conidia, conidiogenous cells, and conidiophores. (K–N) Conidia (concentric pale rings are arrowed in I). Scale bars: (F–N) = 10 µm.
with strong bootstrap support values (ML/PP = 90/0.96), which is a sister to the newly introduced species *A. acutiapicum*. Additionally, *A. pseudorasikravindrae* shows close phylogenetic affinities to *A. chinense*, *A. paraphaeospermum*, and *A. rasikravindrae* (*Figure 1*).

*Arthrinium pseudorasikravindrae* is morphologically distinct from the above species (*Table 2*) by its thick-walled, finely roughened conidia with pale, equatorial slit and ampulliform, cylindrical or doliiform, basauxic conidiogenous cells. The morphology of *A. pseudorasikravindrae* is compared with other closely related species (*Table 2*). Therefore, considering morphological and molecular uniqueness, these isolates are introduced here as belonging to a new species, *A. pseudorasikravindrae*. HKAS 107669 and HKAS 107670 represent a distinct clade (clade A, *Figure 1*) which was not known before in the phylogenetic analysis, and hence, these collections are introduced here as a new species based on their morphology and phylogeny.

**DISCUSSION**

Bamboo is an important group of flowering plants that helps to conserve and manage forest ecosystems and reduce soil erosion and it is also important for panda conservation and many more commercial applications such as making fishing rod, flute, paper, flooring material, etc. and as food for humans and livestock (Chapman and Peat, 1992). Members of bamboo belong to the family Poaceae comprising more than 115 genera with 1,450 species (Gratani et al., 2008; Kelchner and Group, 2013), and bamboo occurs in all tropical, subtropical, and temperate regions as herbaceous or woody plants. Microfungi associate with bamboo in many ways and phytopathogenic or endophytic microfungi form diseases while saprobic microfungi help to decompose plant debris (Zhang and Wang, 1999; Hyde et al., 2002a,b).

The first monograph on bambusicolous fungi was published with 258 fungal species by Hino and Katumoto (1960), and 63 new species were introduced by Petrini et al. (1989). Eriksson and Yue (1998) provided a checklist of the ascomycetes on bamboo, while Zhang and Wang (1999) recorded 213 species described with 258 fungal species by Hino and Katumoto (1960), and 63 new species were introduced by Petrini et al. (1989). Eriksson and Yue (1998) provided a checklist of the ascomycetes on bamboo, while Zhang and Wang (1999) recorded 213 species described from bamboo in China. Kuai (1996) listed phytopathogenic bambusicolous fungi in China and Taiwan. Hyde et al. (2002a) reviewed bambusicolous fungi that grow on all bamboo substrates including the leaves, culms, branches, rhizomes, and roots and enlisted more than 1,100 species, which belong to 228 genera. Dai et al. (2018) have reviewed the taxonomy of bambusicolous fungi. This study is one of the articles in the series on bambusicolous microfungi in Guangdong Province. Herein, we collected *Arthrinium*-like taxa from bamboo plant samples from Shenzhen, Guangdong Province, China. Currently, there are 81 species in the *Arthrinium* (Species Fungorum, 2020) and only 61 have molecular data. More than 30% of holotypes of *Arthrinium* species have been collected in China (*Table 1*). Therefore, the aims of this paper were to study *Arthrinium*-like fungi in Guangdong Province and to introduce several putative new species by comparing them morphologically and genetically with existing taxa.

According to morphology and phylogeny, two novel *Arthrinium* species were obtained with two new locality records. Most phylogenetic studies on *Arthrinium* used ITS, β-tubulin, and tef-1-α; however, LSU has been added to the analyses
here. Negligible variations occur in tree topology in spite of adding LSU. *A. guizhouense* (HKAS 107672) is the first record in Guangdong Province and also from bamboo. The holotype of *A. guizhouense* was collected from the air in karst caves in Guizhou Province, China (Wang et al., 2018). This suggests that fungal conidia in plant hosts release the conidia and conidia can survive in the air for a sufficiently long time. Our strain of *A. bambusae* is identical to the holotype which was collected from Guangdong Province on bamboo (Wang et al., 2018). Hence, this specimen can be used as an epitype if the holotype cannot be used for taxonomic purpose. The morphological differences between these two *Arthrinium* species are listed in Table 2. However, the life mode, host, and colony characters of these two species are not significantly different.

**DATA AVAILABILITY STATEMENT**

The datasets generated in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

**AUTHOR CONTRIBUTIONS**

IS designed the study, performed the morphological study and phylogenetic analyses, and wrote the manuscript. JB, NX, and RC reviewed and edited the manuscript. All authors approved the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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