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Research Article

# Three new endophytic *Apiospora* species (Apiosporaceae, Amphisphaeriales) from China

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## Abstract

*Apiospora* species are widely distributed fungi with diverse lifestyles, primarily functioning as plant pathogens, as well as exhibiting saprophytic and endophytic behaviors. This study reports the discovery of three new species of *Apiospora*, namely *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, isolated from healthy Poaceae plants in China. These novel species were identified through a multi-gene phylogenetic analysis. The phylogenetic analysis of the combined ITS, LSU, *tef1*, and *tub2* sequence data revealed that the three new species formed a robustly supported clade with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Detailed descriptions of the newly discovered species are provided and compared with closely related species to enhance our understanding of the genus *Apiospora*.

**Key words:** *Apiospora*, Ascomycota, endophyte, phylogeny, taxonomy

## Introduction

*Apiospora* is an important genus of fungal Sordariomycetes, that produces a basauxic, arthrinium-like conidiogenesis (Hyde et al. 2020). The family Apiosporaceae was established to accommodate the genus *Apiospora* with the special conidiogenesis (Hyde et al. 1998). Over time, the membership of Apiosporaceae has undergone several revisions. It presently comprises several genera of fungi with similar morphology, including *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* (Wang et al. 2017; Pintos and Alvarado 2021; Jiang et al. 2022).

Within the family Apiosporaceae, *Apiospora* is closely related to *Arthrinium* and they were once considered as two life stages of a single taxon (Ellis 1965; Crous and Groenewald 2013; Réblová et al. 2016; Jiang et al. 2019). Morphologically, *Apiospora* and *Arthrinium* lack clear diagnostic features, although species of *Arthrinium* often produce conidia of various shapes (Minter and Cannon 2018; Pintos and Alvarado 2021), while most species of *Apiospora* have rounded lenticular conidia (Li et al. 2023; Liao et al. 2023). Ecologically, most sequenced collections of *Arthrinium* were found on Cyperaceae or Juncaceae in temperate, cold, or alpine habitats, while those of *Apiospora* were mainly collected on Poaceae, as well as various other plant host families, in a wide range of habitats, including tropical and subtropical regions (Dai et al. 2016; Jiang et

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al. 2018; Wang et al. 2018; Feng et al. 2021; Tian et al. 2021; Kwon et al. 2022; Monkai et al. 2022). With the addition of molecular evidence and the expansion of the sample, the latest phylogenetic analysis suggests that *Arthrinium* s. str. and *Apiospora* represent independent lineages within Apiosporaceae (Pintos and Alvarado 2021). Consequently, most species of *Arthrinium* have been reclassified under *Apiospora*. Furthermore, Pintos and Alvarado defined the exact identity of *Apiospora montagnei* (the type species of *Apiospora*) and delineated the phylogenetic boundaries of *Apiospora* (Pintos and Alvarado 2022).

Currently, there are 176 records in *Apiospora* (Index Fungorum; http://www.indexfungorum.org/; accessed on 8 Mar 2024). These fungi primarily act as plant pathogens, causing diseases in a wide range of host plants. For example, *A. arundinis* is the causal agent for several important plant diseases, such as kernel blight of barley (Martínez-Cano et al. 1992), brown culm streak of *Phyllostachys praecox* (Chen et al. 2014), moldy sugarcane (Liao et al. 2022), and leaf spot on *Polygonatum cyrtonema* (Gong et al. 2023). *A. marii* causes dieback of olive trees (Gerin et al. 2020), while *A. kogelbergense* leads to blight of *Bambusa intermedi* (Yin et al. 2020). Whereas, many *Apiospora* species are saprophytes, such as *A. acutiapica* (Senanayake et al. 2020), *A. garethjonesii* (Dai et al. 2016), *A. magnispora* (Zhao et al. 2023), *A. sasae* (Crous et al. 2021), and *A. thailandicum* (Dai et al. 2017). In addition, certain *Apiospora* species are reported as endophytes with wide host range, including bamboo (Wang et al. 2018), *Camellia sinensis* (Wang et al. 2018), *Wurfbainia villosa* (Liao et al. 2023), and even hive-stored pollen (Zhao et al. 2018).

Endophytic fungi exhibit rich diversity and play a significant role in the ecosystem. In a previous study, we collected and isolated endophytic fungi from healthy Poaceae plants in China (Liu et al. 2021). In this study, three new endophytic species of *Apiospora* were identified and described based on morphological characteristics and a multi-gene phylogenetic analysis, utilizing a dataset comprising the combined nuclear ribosomal DNA internal transcribed spacer (ITS), nuclear ribosomal DNA large subunit (LSU), the translation elongation factor 1-alpha (*tef1*), and β-tubulin (*tub2*) sequences.

## Materials and methods

### Fungal isolation

In the present work, Poaceae plant samples were collected from three locations in China: Xilingol Grassland National Nature Reserve in Inner Mongolia, Xishuangbanna, Naban River Watershed National Nature Reserve in Yunnan province, and Baishanzu National Nature Reserve in Zhejiang province (Liu et al. 2021). To isolate endophytic *Apiospora* strains, healthy tissues of asymptomatic plants were first disinfected for 3 min in 75% ethanol and 10 min in 1% sodium hypochlorite, followed by three washes in sterile distilled water. The disinfected tissues were excised, and then incubated on malt extract agar (MEA) medium at 25 °C. Subsequently, the growing hyphae were transferred to potato dextrose agar (PDA) medium to obtain pure cultures.

All strains of *Apiospora* were stored in the Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou, China. In addition, the holotype and ex-type culture were deposited in the Guangdong Microbial Culture Collection Center (GDMCC). Fungal names were registered in the Fungal Names, one of the recognised repositories of fungal taxonomy (https://nmdc.cn/fungalnames/).

### Morphological study

Morphological descriptions were recorded on PDA and MEA. The morphological characteristics of the colonies were captured with a digital camera (Canon EOS700D). The fungal structures were observed and photographed using a stereomicroscope (Leica S9D) and a Leica DM2500 microscope equipped with differential interference contrast (DIC). Measurements of conidiogenous cells and conidia were reported as follows: a-b × c-d (mean, n), where “a” and “c” represent the minimum values, “b” and “d” represent the maximum values, and the mean value and number of measurements (n) are shown in parentheses (Wang et al. 2018).

### DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia from pure cultures grown on PDA at 25 °C for 5–7 d were used for DNA extraction. Genomic DNA was extracted following the method as described in Chi et al. (2009).

Polymerase chain reaction (PCR) amplification was applied to amplify four gene fragments, including ITS, LSU, *tef1*, and *tub2*. The primer pairs were used: ITS1/ITS4 for ITS (White et al. 1990), LR0R/LR5 for LSU (Rehner and Samuels 1995), EF1-728F/EF2 for *tef1* (O’Donnell et al, 1998; Carbone and Kohn 1999), and T1/Bt2b for *tub2* (Glass and Donaldson 1995; O’Donnell and Cigelnik 1997). PCR program for ITS amplification was conducted with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The annealing temperatures were adjusted to 56 °C for LSU, *tef1*, and *tub2*.

PCR was performed using a Veriti Thermal Cycler (Waltham, MA, USA). Amplification reactions contained 10 μL of 2× Taq Plus Master Mix II (Vazyme, Nanjing, China), 0.8 μL of each primer (10 μM) (Sunya, Hangzhou, China), 0.8 μL of DNA template, and double-distilled water to reach a total volume of 20 μL. Purification and sequencing of PCR products were performed by Sunya Biotechnology Company (Hangzhou, China). All sequences generated in this study were deposited in GenBank (Table 1).

### Phylogenetic analyses

The quality of obtained sequences was assessed using Chromas v.2.6.6 and the sequences were assembled using SeqMan v.7.1.0. The reference sequences were retrieved from GenBank. All sequences, including the reference sequences, were aligned in batches with MAFFT (Katoh and Standley 2013), manually correcting the resulting alignment by MEGA v.11.0.13 where necessary. A single alignment was made using ITS, LSU, *tef1* region including partial exon 4 and partial exon 5 (the largest exon), *tub2* region including exon 2, exon 3, and partial exon 4. Then phylogenetic analyses were conducted using partial sequences of the above four loci.

**Table 1.** Species of Apiosporaceae used in the phylogenetic analyses. Notes: Strains in this study are marked in bold. "T" indicates a type culture. NA = not available.

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | ***tef1*** | ***tub2*** |
| *Apiospora acutiapica* | KUMCC 20-0209 | *Bambusa bambos* | China | MT946342 | MT946338 | MT947359 | MT947365 |
| *Apiospora acutiapica* | KUMCC 20-0210 $^{T}$ | *Bambusa bambos* | China | MT946343 | MT946339 | MT947360 | MT947366 |
| *Apiospora adinandrae* | SAUCC 1282B-1 $^{T}$ | Diseased leaves of *Adinandra glischroloma* | China | OR739431 | OR739572 | OR753448 | OR757128 |
| *Apiospora adinandrae* | SAUCC 1282B-2 | Diseased leaves of *Adinandra glischroloma* | China | OR739432 | OR739573 | OR753449 | OR757129 |
| *Apiospora agari* | KUC21333, SFC20161014-M18 $^{T}$ | *Agarum cribrosum* | South Korea | MH498520 | MH498440 | MH544663 | MH498478 |
| *Apiospora aquatic* | MFLU 18-1628, S-642 $^{T}$ | Submerged wood | China | MK828608 | MK835806 | NA | NA |
| *Apiospora arctoscopi* | KUC21331, SFC20200506-M05 $^{T}$ | Eggs of *Arctoscopus japonicus* | South Korea | MH498529 | MH498449 | MN868918 | MH498487 |
| *Apiospora arundinis* | CBS 124788 | Living leaves of *Fagus sylvatica* | Switzerland | KF144885 | KF144929 | KF145017 | KF144975 |
| *Apiospora arundinis* | LC4951 | *Dichotomanthes tristaniicarpa* | China | KY494698 | KY494774 | KY705097 | KY705168 |
| *Apiospora aseptata* | KUNCC 23-14169 $^{T}$ | Living roots of *Dicranopteris pedata* | China | OR590341 | OR590335 | OR634949 | OR634943 |
| *Apiospora aurea* | CBS 244.83 $^{T}$ | Air | Spain | AB220251 | KF144935 | KF145023 | KF144981 |
| *Apiospora balearica* | CBS 145129, AP24118 $^{T}$ | Poaceae plant | Spain | MK014869 | MK014836 | MK017946 | MK017975 |
| *Apiospora bambusicola* | MFLUCC 20-0144 $^{T}$ | *Schizostachyum brachycladum* | Thailand | MW173030 | MW173087 | MW183262 | NA |
| *Apiospora bawanglingensis* | SAUCC BW0444 $^{T}$ | Leaves of *Indocalamus longiauritus* | China | OR739429 | OR739570 | OR753446 | OR757126 |
| *Apiospora biserialis* | CGMCC 3.20135 $^{T}$ | Bamboo | China | MW481708 | MW478885 | MW522938 | MW522955 |
| *Apiospora camelliae-sinensis* | CGMCC 3.18333, LC5007 $^{T}$ | *Camellia sinensis* | China | KY494704 | KY494780 | KY705103 | KY705173 |
| *Apiospora camelliae-sinensis* | LC8181 | *Brassica rapa* | China | KY494761 | KY494837 | KY705157 | KY705229 |
| *Apiospora cannae* | ZHKUCC 22-0139 | Leaves of *Canna* sp. | China | OR164902 | OR164949 | OR166286 | OR166322 |
| *Apiospora cannae* | ZHKUCC 22-0127 $^{T}$ | Leaves of *Canna* sp. | China | OR164901 | OR164948 | OR166285 | OR166321 |
| *Apiospora chiangraiense* | MFLUCC 21-0053 $^{T}$ | Dead culms of bamboo | Thailand | MZ542520 | MZ542524 | NA | MZ546409 |
| *Apiospora chromolaenae* | MFLUCC 17-1505 $^{T}$ | *Chromolaena odorata* | Thailand | MT214342 | MT214436 | MT235802 | NA |
| *Apiospora cordylinae* | GUCC 10026 | *Cordyline fruticosa* | China | MT040105 | NA | MT040126 | MT040147 |
| *Apiospora cordylinae* | GUCC 10027 $^{T}$ | *Cordyline fruticosa* | China | MT040106 | NA | MT040127 | MT040148 |
| *Apiospora coryli* | CFCC 58978 $^{T}$ | Dead plant culms of *Corylus yunnanensis* | China | OR125564 | OR133586 | OR139974 | OR139978 |
| *Apiospora coryli* | CFCC 58979 $^{T}$ | Dead plant culms of *Corylus yunnanensis* | China | OR125565 | OR133587 | OR139975 | OR139979 |
| *Apiospora cyclobalanopsidis* | CGMCC 3.20136 $^{T}$ | *Cyclobalanopsidis glauca* | China | MW481713 | MW478892 | MW522945 | MW522962 |
| *Apiospora cyclobalanopsidis* | GZCC 20-0103 | *Cyclobalanopsidis glauca* | China | MW481714 | MW478893 | MW522946 | MW522963 |
| *Apiospora dendrobii* | MFLUCC 14-0152 $^{T}$ | Roots of *Dendrobium harveyanum* | Thailand | MZ463151 | MZ463192 | NA | NA |
| *Apiospora dematiacea* | KUNCC 23-14202 $^{T}$ | Living stems of *Dicranopteris ampla* | China | OR590346 | OR590339 | OR634953 | OR634948 |
| *Apiospora descalsii* | CBS 145130 $^{T}$ | *Ampelodesmos mauritanicus* | Spain | MK014870 | MK014837 | MK017947 | MK017976 |
| *Apiospora dichotomanthi* | CGMCC 3.18332, LC4950 $^{T}$ | *Dichotomanthes tristaniicarpa* | China | KY494697 | KY494773 | KY705096 | KY705167 |
| *Apiospora dichotomanthi* | LC8175 | *Dichotomanthes tristaniicarpa* | China | KY494755 | KY494831 | KY705151 | KY705223 |
| *Apiospora dicranopteridis* | KUNCC23-14171 $^{T}$ | Living stems of *Dicranopteris pedata* | China | OR590342 | OR590336 | OR634950 | OR634944 |
| *Apiospora dicranopteridis* | KUNCC23-14177 | Roots of *Dicranopteris pedata* | China | OR590343 | OR590337 | OR634951 | OR634945 |
| *Apiospora dongyingensis* | SAUCC 0302 $^{T}$ | Leaves of bamboo | China | OP563375 | OP572424 | OP573264 | OP573270 |

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | ***tef1*** | ***tub2*** |
| *Apiospora dongyingensis* | SAUCC 0303 | Leaves of bamboo | China | OP563374 | OP572423 | OP573263 | OP573269 |
| *Apiospora elliptica* | ZHKUCC 22-0131 $^{T}$ | Dead stems of unknown plant | China | OR164905 | OR164952 | OR166284 | OR166323 |
| *Apiospora elliptica* | ZHKUCC 22-0140 | Dead stems of unknown plant | China | OR164906 | OR164953 | NA | OR166324 |
| *Apiospora endophytica* | ZHKUCC 23-0006 $^{T}$ | Living leaves of *Wurfbainia villosa* | China | OQ587996 | OQ587984 | OQ586062 | OQ586075 |
| *Apiospora endophytica* | ZHKUCC 23-0007 | Living leaves of *Wurfbainia villosa* | China | OQ587997 | OQ587985 | OQ586063 | OQ586076 |
| *Apiospora esporlensis* | CBS 145136 $^{T}$ | *Phyllostachys aurea* | Spain | MK014878 | MK014845 | MK017954 | MK017983 |
| *Apiospora esporlensis* | UNIPAMPA010 | Living leaves of the Antarctic Hairgrass *Deschampsia antarctica* | Antarctica | MN947641 | genome | genome | genome |
| *Apiospora euphorbiae* | IMI 285638b | *Bambusa* sp. | Bangladesh | AB220241 | AB220335 | NA | AB220288 |
| *Apiospora fermenti* | KUC21288, SFC20140423-M86 | Seaweeds | South Korea | MF615230 | NA | MH544668 | MF615235 |
| *Apiospora fermenti* | KUC21289 $^{T}$ | Seaweeds | South Korea | MF615226 | MF615213 | MH544667 | MF615231 |
| *Apiospora gaoyouensis* | CFCC 52301$^{T}$ | *Phragmites australis* | China | MH197124 | NA | MH236793 | MH236789 |
| *Apiospora gaoyouensis* | CFCC 52302 | *Phragmites australis* | China | MH197125 | NA | MH236794 | MH236790 |
| *Apiospora garethjonesii* | GZCC 20-0115 | Dead culms of bamboo | China | MW481715 | MW478894 | MW522947 | NA |
| *Apiospora garethjonesii* | KUMCC 16-0202, JHB004, HKAS 96289 $^{T}$ | Dead culms of bamboo | China | KY356086 | KY356091 | NA | NA |
| *Apiospora garethjonesii* | SICAUCC 22-0027 | Bamboo | China | ON228603 | ON228659 | NA | ON237651 |
| *Apiospora garethjonesii* | SICAUCC 22-0028 | Bamboo | China | ON228606 | ON228662 | NA | ON237654 |
| *Apiospora gelatinosa* | GZAAS 20-0107 | Bamboo | China | MW481707 | MW478889 | MW522942 | MW522959 |
| *Apiospora gelatinosa* | HKAS 11962 $^{T}$ | Bamboo | China | MW481706 | MW478888 | MW522941 | MW522958 |
| *Apiospora globosa* | KUNCC 23-14210 $^{T}$ | Living stems of *Dicranopteris linearis* | China | OR590347 | OR590340 | OR634954 | NA |
| ***Apiospora gongcheniae*** | **GDMCC 3.1045, YNE00465 $^{T}$** | **Living stems of *Oryza meyeriana* subsp. *granulata*** | **China** | **PP033259** | **PP033102** | **PP034683** | **PP034691** |
| ***Apiospora gongcheniae*** | **YNE00565** | **Living stems of *Oryza meyeriana* subsp. *granulata*** | **China** | **PP033260** | **PP033103** | **PP034684** | **PP034692** |
| *Apiospora guangdongensis* | ZHKUCC 23-0004 $^{T}$ | Living leaves of *Wurfbainia villosa* | China | OQ587994 | OQ587982 | OQ586060 | OQ586073 |
| *Apiospora guangdongensis* | ZHKUCC 23-0005 | Living leaves of *Wurfbainia villosa* | China | OQ587995 | OQ587983 | OQ586061 | OQ586074 |
| *Apiospora guiyangensis* | HKAS 102403 $^{T}$ | Dead culms of Poaceae | China | MW240647 | MW240577 | MW759535 | MW775604 |
| *Apiospora guiyangensis* | KUNCC 22-12539 | Poaceae plant | China | OQ029540 | OQ029613 | OQ186444 | OQ186446 |
| *Apiospora guizhouensis* | CGMCC 3.18334, LC5322 $^{T}$ | Air in karst cave | China | KY494709 | KY494785 | KY705108 | KY705178 |
| *Apiospora guizhouensis* | LC5318 | Air in karst cave | China | KY494708 | KY494784 | KY705107 | KY705177 |
| *Apiospora hainanensis* | SAUCC 1681 $^{T}$ | Leaves of bamboo | China | OP563373 | OP572422 | OP573262 | OP573268 |
| *Apiospora hainanensis* | SAUCC 1682 | Leaves of bamboo | China | OP563372 | OP572421 | OP573261 | OP573267 |
| *Apiospora hispanica* | IMI 326877 $^{T}$ | Beach sands | Spain | AB220242 | AB220336 | NA | AB220289 |
| *Apiospora hydei* | CBS 114990 $^{T}$ | Culms of *Bambusa tuldoides* | China | KF144890 | KF144936 | KF145024 | KF144982 |
| *Apiospora hydei* | LC7103 | Leaves of bamboo | China | KY494715 | KY494791 | KY705114 | KY705183 |
| *Apiospora hyphopodii* | JHB003, HKAS 96288 | Bamboo | China | KY356088 | KY356093 | NA | NA |
| *Apiospora hyphopodii* | MFLUCC 15-003 $^{T}$ | *Bambusa tuldoides* | Thailand | KR069110 | NA | NA | NA |
| *Apiospora hyphopodii* | SICAUCC 22-0034 | Bamboo | China | ON228605 | ON228661 | NA | ON237653 |
| *Apiospora hysterina* | AP12118 | *Phyllostachys aurea* | Spain | MK014877 | KM014844 | MK017953 | MK017982 |
| *Apiospora hysterina* | AP29717 | *Phyllostachys aurea* | Spain | MK014875 | MK014842 | MK017952 | MK017981 |
| *Apiospora hysterina* | ICPM 6889 $^{T}$ | Bamboo | New Zealand | MK014874 | MK014841 | MK017951 | MK017980 |
| *Apiospora iberica* | CBS 145137, AP10118 $^{T}$ | *Arundo donax* | Portugal | MK014879 | MK014846 | MK017955 | MK017984 |
| *Apiospora intestine* | CBS 135835 | Gut of grasshopper | India | KR011352 | MH877577 | KR011351 | KR011350 |
| *Apiospora intestine* | MFLUCC 21-0052 $^{T}$ | Dead culms of bamboo | Thailand | MZ542521 | MZ542525 | MZ546406 | MZ546410 |

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *tef1* | *tub2* |
| *Apiospora italic* | CBS 145138, AP221017 $^{T}$ | *Arundo donax* | Italy | MK014880 | MK014847 | MK017956 | MK017985 |
| *Apiospora italic* | CBS 145139 | *Phragmites australis* | Spain | MK014881 | MK014848 | NA | MK017986 |
| *Apiospora jatrophae* | CBS 134262, MMI00052 $^{T}$ | Living *Jatropha podagrica* | India | JQ246355 | NA | NA | NA |
| *Apiospora jiangxiensis* | CGMCC 3.18381, LC4577 $^{T}$ | *Maesa* sp. | China | KY494693 | KY494769 | KY705092 | KY705163 |
| *Apiospora jiangxiensis* | LC4578 | *Camellia sinensis* | China | KY494694 | KY494770 | KY705093 | KY705164 |
| *Apiospora kogelbergensis* | CBS 113332 | *Cannomois virgata* | South Africa | KF144891 | KF144937 | KF145025 | KF144983 |
| *Apiospora kogelbergensis* | CBS 113333 $^{T}$ | Dead culms of Restionaceae | South Africa | KF144892 | KF144938 | KF145026 | KF144984 |
| *Apiospora koreanum* | KUC21332, SFC20200506-M06 $^{T}$ | Eggs of *Arctoscopus japonicus* | South Korea | MH498524 | MH498444 | MH544664 | MH498482 |
| *Apiospora koreanum* | KUC21348 | Eggs of *Arctoscopus japonicus* | South Korea | MH498523 | NA | MN868927 | MH498481 |
| *Apiospora lageniformis* | KUC21686 $^{T}$ | Culms of *Phyllostachys nigra* | Korea | ON764022 | ON787761 | ON806626 | ON806636 |
| *Apiospora lageniformis* | KUC21687 | Culms of *Phyllostachys nigra* | Korea | ON764023 | ON787764 | ON806627 | ON806637 |
| *Apiospora locuta-pollinis* | LC11683 $^{T}$ | *Brassica campestris* | China | MF939595 | NA | MF939616 | MF939622 |
| *Apiospora longistroma* | MFLUCC 11-0479 | Dead culms of bamboo | Thailand | KU940142 | KU863130 | NA | NA |
| *Apiospora longistroma* | MFLUCC11-0481 $^{T}$ | Dead culms of bamboo | Thailand | KU940141 | KU863129 | NA | NA |
| *Apiospora lophatheri* | CFCC 58975 $^{T}$ | Diseased leaves of *Lophatherum gracile* | China | OR125566 | OR133588 | OR139970 | OR139980 |
| *Apiospora lophatheri* | CFCC 58976 $^{T}$ | Diseased leaves of *Lophatherum gracile* | China | OR125567 | OR133589 | OR139971 | OR139981 |
| *Apiospora machili* | SAUCC 1175A-4 $^{T}$ | Diseased leaves of *Machilus nanmu*<br>of Machilus nanmu | China | OR739433 | OR739574 | OR753450 | OR757130 |
| *Apiospora machili* | SAUCC 1175 | Diseased leaves of *Machilus nanmu*<br>of Machilus nanmu | China | OQ592560 | OQ615289 | OQ613333 | OQ613307 |
| *Apiospora magnispora* | ZHKUCC 22-0001 $^{T}$ | Dead stems of *Bambusa textilis* | China | OM728647 | OM486971 | OM543543 | OM543544 |
| *Apiospora malaysiana* | CBS 102053 $^{T}$ | *Macaranga hullettii* | Malaysia | KF144896 | KF144942 | KF145030 | KF144988 |
| *Apiospora marianiae* | AP18219 $^{T}$ | Dead stems of *Phleum pratense* | Spain | ON692406 | ON692422 | ON677180 | ON677186 |
| *Apiospora marii* | CBS 497.90 $^{T}$ | Beach sands | Spain | AB220252 | KF144947 | KF145035 | KF144993 |
| *Apiospora marinum* | KUC21328, SFC20140423-M02 $^{T}$ | Seaweeds | South Korea | MH498538 | MH498458 | MH544669 | MH498496 |
| *Apiospora mediterranea* | IMI 326875 $^{T}$ | Air | Spain | AB220243 | AB220337 | NA | AB220290 |
| *Apiospora minutispora* | 1.70E-042 $^{T}$ | Mountain soils | South Korea | LC517882 | NA | LC518889 | LC518888 |
| *Apiospora montagnei* | AP19421 | *Arundo micrantha* | Spain | ON692418 | ON692425 | ON677183 | ON677189 |
| *Apiospora montagnei* | AP301120, CBS 148707, PC:0125164 $^{T}$ | *Arundo micrantha* | Spain | ON692408 | ON692424 | ON677182 | ON677188 |
| *Apiospora mori* | MFLUCC 20-0181 $^{T}$ | Dead leaves of *Morus australis* | China | MW114313 | MW114393 | NA | NA |
| *Apiospora mori* | NCYUCC 19-0340 | Dead leaves of *Morus australis* | China | MW114314 | MW114394 | NA | NA |
| *Apiospora mukdahanensis* | MFLUCC 22-0056 $^{T}$ | Dead leaves of bamboo | Thailand | OP377735 | OP377742 | NA | NA |
| *Apiospora multiloculata* | MFLUCC 21-0023 $^{T}$ | Dead culms of Bambusae | Thailand | OL873137 | OL873138 | NA | OL874718 |
| *Apiospora mytilomorpha* | DAOM 214595 $^{T}$ | Dead blades of *Andropogon* sp. | India | KY494685 | NA | NA | NA |
| *Apiospora neobambusae* | CGMCC 3.18335, LC7106 $^{T}$ | Leaves of bamboo | China | KY494718 | KY494794 | KY806204 | KY705186 |
| *Apiospora neobambusae* | LC7107 | Leaves of bamboo | China | KY494719 | KY494795 | KY705117 | KY705187 |
| *Apiospora neobambusae* | LC7124 | Leaves of bamboo | China | KY494727 | KY494803 | KY806206 | KY705195 |
| *Apiospora neochinensis* | CFCC 53036 $^{T}$ | *Fargesia qinlingensis* | China | MK819291 | NA | MK818545 | MK818547 |
| *Apiospora neochinensis* | CFCC 53037 | *Fargesia qinlingensis* | China | MK819292 | NA | MK818546 | MK818548 |
| *Apiospora neogarethjonesii* | KUMCC 18-0192, HKAS 102408 $^{T}$ | Dead culms of Bambusae | China | MK070897 | MK070898 | NA | NA |
| ***Apiospora neogongcheniae*** | **GDMCC 3.1047, YNE01248** $^{T}$ | **Living stems of Poaceae plant** | **China** | **PP033263** | **PP033106** | **PP034687** | **PP034695** |

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *tef1* | *tub2* |
| ***Apiospora neogongcheniae*** | **YNE01260** | **Living stems of Poaceae plant** | **China** | **PP033264** | **PP033107** | **PP034688** | **PP034696** |
| *Apiospora neosubglobosa* | JHB 006 | Bamboo | China | KY356089 | KY356094 | NA | NA |
| *Apiospora neosubglobosa* | JHB 007$^{T}$ | Bamboo | China | KY356090 | KY356095 | NA | NA |
| *Apiospora obovata* | CGMCC 3.18331, LC4940$^{T}$ | *Lithocarpus* sp. | China | KY494696 | KY494772 | KY705095 | KY705166 |
| *Apiospora obovata* | LC8177 | *Lithocarpus* sp. | China | KY494757 | KY494833 | KY705153 | KY705225 |
| *Apiospora oenotherae* | CFCC 58972 | Diseased leaves of *Oenothera biennis* | China | OR125568 | OR133590 | OR139972 | OR139982 |
| *Apiospora oenotherae* | LS 395 | Diseased leaves of *Oenothera biennis* | China | OR125569 | OR133591 | OR139973 | OR139983 |
| *Apiospora ovate* | CBS 115042$^{T}$ | *Arundinaria hindsii* | China | KF144903 | KF144950 | KF145037 | KF144995 |
| *Apiospora pallidesporae* | ZHKUCC 22-0129$^{T}$ | Dead wood of unknown host | China | OR164903 | OR164950 | NA | NA |
| *Apiospora pallidesporae* | ZHKUCC 22-0142 | Dead wood of unknown host | China | OR164904 | OR164951 | NA | NA |
| ***Apiospora paragongcheniae*** | **GDMCC 3.1046, YNE00992$^{T}$** | **Living stems of Poaceae plant** | **China** | **PP033261** | **PP033104** | **PP034685** | **PP034693** |
| ***Apiospora paragongcheniae*** | **YNE01259** | **Living stems of Poaceae plant** | **China** | **PP033262** | **PP033105** | **PP034686** | **PP034694** |
| *Apiospora paraphaeosperma* | MFLUCC 13-0644$^{T}$ | Dead culms of bamboo | Thailand | KX822128 | KX822124 | NA | NA |
| *Apiospora paraphaeosperma* | KUC21488 | Culms of bamboo | Korea | ON764024 | ON787763 | ON806628 | ON806638 |
| *Apiospora phragmitis* | CPC 18900$^{T}$ | *Phragmites australis* | Italy | KF144909 | KF144956 | KF145043 | KF145001 |
| *Apiospora phyllostachydis* | MFLUCC 18-1101$^{T}$ | *Phyllostachys heteroclada* | China | MK351842 | MH368077 | MK340918 | MK291949 |
| *Apiospora piptatheri* | CBS 145149, AP4817A$^{T}$ | *Piptatherum miliaceum* | Spain | MK014893 | MK014860 | MK017969 | NA |
| *Apiospora piptatheri* | SAUCC BW0455 | Diseased leaves of *Indocalamus longiauritus* | China | OR739430 | OR739571 | OR753447 | OR757127 |
| *Apiospora pseudomarii* | GUCC 10228$^{T}$ | Leaves of *Aristolochia debilis* | China | MT040124 | NA | MT040145 | MT040166 |
| *Apiospora pseudohyphopodii* | KUC21680$^{T}$ | Culms of *Phyllostachys pubescens* | Korea | ON764026 | ON787765 | ON806630 | ON806640 |
| *Apiospora pseudohyphopodii* | KUC21684 | Culms of *Phyllostachys pubescens* | Korea | ON764027 | ON787766 | ON806631 | ON806641 |
| *Apiospora pseudoparenchymatica* | CGMCC 3.18336, LC7234$^{T}$ | Leaves of bamboo | China | KY494743 | KY494819 | KY705139 | KY705211 |
| *Apiospora pseudoparenchymatica* | LC8173 | Leaves of bamboo | China | KY494753 | KY494829 | KY705149 | KY705221 |
| *Apiospora pseudorasikravindrae* | KUMCC 20-0208$^{T}$ | *Bambusa dolichoclada* | China | MT946344 | NA | MT947361 | MT947367 |
| *Apiospora pseudosinensis* | CPC 21546$^{T}$ | Leaves of bamboo | Netherlands | KF144910 | KF144957 | KF145044 | MN868936 |
| *Apiospora pseudosinensis* | SAUCC 0221 | Leaves of bamboo | China | OP563377 | OP572426 | OP573266 | OP573272 |
| *Apiospora pseudospegazzinii* | CBS 102052$^{T}$ | *Macaranga hullettii* | Malaysia | KF144911 | KF144958 | KF145045 | KF145002 |
| *Apiospora pterosperma* | CBS 123185 | *Machaerina sinclairii* | New Zealand | KF144912 | KF144959 | NA | KF145003 |
| *Apiospora pterosperma* | CPC 20193, CBS 134000$^{T}$ | *Lepidosperma gladiatum* | Australia | KF144913 | KF144960 | KF145046 | KF145004 |
| *Apiospora pusillispermum* | KUC21321$^{T}$ | Seaweeds | South Korea | MH498533 | MH498453 | MN868930 | MH498491 |
| *Apiospora pusillispermum* | KUC21357 | Seaweeds | South Korea | MH498532 | NA | MN868931 | MH498490 |
| *Apiospora qinlingensis* | CFCC 52303$^{T}$ | *Fargesia qinlingensis* | China | MH197120 | NA | MH236795 | MH236791 |
| *Apiospora qinlingensis* | CFCC 52304 | *Fargesia qinlingensis* | China | MH197121 | NA | MH236796 | MH236792 |
| *Apiospora rasikravindrae* | LC8179 | *Brassica rapa* | China | KY494759 | KY494835 | KY705155 | KY705227 |
| *Apiospora rasikravindrae* | MFLUCC 21-0051 | Dead culms of bamboo | Thailand | MZ542523 | MZ542527 | MZ546408 | MZ546412 |

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | ***tef1*** | ***tub2*** |
| *Apiospora sacchari* | CBS 372.67 | Air | Not mentioned | KF144918 | KF144964 | KF145049 | KF145007 |
| *Apiospora sacchari* | CBS 664.74 | Soils under *Calluna vulgaris* | Netherlands | KF144919 | KF144965 | KF145050 | KF145008 |
| *Apiospora saccharicola* | CBS 191.73 | Air | Netherlands | KF144920 | KF144966 | KF145051 | KF145009 |
| *Apiospora saccharicola* | CBS 831.71 | Not mentioned | Netherlands | KF144922 | KF144969 | KF145054 | KF145012 |
| *Apiospora sargassi* | KUC21228 $^{T}$ | *Sargassum fulvellum* | South Korea | KT207746 | KT207696 | MH544677 | KT207644 |
| *Apiospora sargassi* | KUC21232 | Seaweeds | South Korea | KT207750 | NA | MH544676 | KT207648 |
| *Apiospora sasae* | CPC 38165, CBS 146808 $^{T}$ | Dead culms of *Sasa veitchii* | Netherlands | MW883402 | MW883797 | MW890104 | MW890120 |
| *Apiospora septata* | CGMCC 3.20134, CS19-8 $^{T}$ | Bamboo | China | MW481711 | MW478890 | MW522943 | MW522960 |
| *Apiospora septata* | GZCC 20-0109 | Bamboo Food | China | MW481712 | MW478891 | MW522944 | MW522961 |
| *Apiospora serenensis* | IMI 326869 $^{T}$ | Excipients, atmosphere and home dust | Spain | AB220250 | AB220344 | NA | AB220297 |
| *Apiospora setariae* | CFCC 54041 $^{T}$ | Decaying culms of *Setaria viridis* | China | MT492004 | NA | MW118456 | MT497466 |
| *Apiospora setariae* | MT492005 | *Setaria viridis* | China | MT492005 | NA | MW118457 | MT497467 |
| *Apiospora setostroma* | KUMCC 19-0217 | Dead branches of bamboo | China | MN528012 | MN528011 | MN527357 | NA |
| *Apiospora sichuanensis* | HKAS 107008 $^{T}$ | Dead culms of Poaceae | China | MW240648 | MW240578 | MW759536 | MW775605 |
| *Apiospora sorghi* | URM 93000, URM 7417 $^{T}$ | *Sorghum bicolor* | Brazil | MK371706 | NA | NA | MK348526 |
| *Apiospora sphaerosperma* | CBS 114314 | Leaves of *Hordeum vulgare* | Iran | KF144904 | KF144951 | KF145038 | KF144996 |
| *Apiospora sphaerosperma* | CBS 114315 | Leaves of *Hordeum vulgare* | Iran | KF144905 | KF144952 | KF145039 | KF144997 |
| *Apiospora stipae* | CPC 38101, CBS 146804 $^{T}$ | Dead culms of *Stipa gigantea* | Spain | MW883403 | MW883798 | MW890082 | MW890121 |
| *Apiospora subglobosa* | MFLUCC 11-0397 $^{T}$ | Dead culms of bamboo | Thailand | KR069112 | KR069113 | NA | NA |
| *Apiospora subrosea* | CGMCC 3.18337, LC7292 $^{T}$ | Leaves of bamboo | China | KY494752 | KY494828 | KY705148 | KY705220 |
| *Apiospora subrosea* | LC7291 | Leaves of bamboo | China | KY494751 | KY494827 | KY705147 | KY705219 |
| *Apiospora taeanense* | KUC21322$^{T}$ | Seaweeds | South Korea | MH498515 | NA | MH544662 | MH498473 |
| *Apiospora taeanense* | KUC21359 | Seaweeds | South Korea | MH498513 | NA | MN868935 | MH498471 |
| *Apiospora thailandica* | MFLUCC 15-0199 | Dead culms of bamboo | Thailand | KU940146 | KU863134 | NA | NA |
| *Apiospora thailandica* | MFLUCC 15-0202 $^{T}$ | Dead culms of bamboo | Thailand | KU940145 | KU863133 | NA | NA |
| *Apiospora tropica* | MFLUCC 21-0056 | Dead culms of Bambusoideae | Thailand | OK491657 | OK491653 | NA | OK560922 |
| *Apiospora wurfbainiae* | ZHKUCC 23-0008 $^{T}$ | *Wurfbainia villosa* | China | OQ587998 | OQ587986 | OQ586064 | OQ586077 |
| *Apiospora wurfbainiae* | ZHKUCC 23-0009 | *Wurfbainia villosa* | China | OQ587999 | OQ587987 | OQ586065 | OQ586078 |
| *Apiospora vietnamensis* | IMI 99670 $^{T}$ | *Citrus sinensis* | Vietnam | KX986096 | KX986111 | NA | KY019466 |
| *Apiospora xenocordella* | CBS 478.86 $^{T}$ | Soils from roadway | Zimbabwe | KF144925 | KF144970 | KF145055 | KF145013 |
| *Apiospora xenocordella* | CBS 595.66 | Soils | Austria | KF144926 | KF144971 | NA | NA |
| *Apiospora xishuangbannaensis* | KUMCC 21-0695 $^{T}$ | *Rhinolophus pusillus* | China | ON426832 | OP363248 | OR025969 | OR025930 |
| *Apiospora xishuangbannaensis* | KUMCC 21-0696 | *Rhinolophus pusillus* | China | ON426833 | OP363249 | OR025970 | OR025931 |
| *Apiospora yunnana* | DDQ 00281 | *Phyllostachys nigra* | China | KU940148 | KU863136 | NA | NA |
| *Apiospora yunnana* | MFLUCC 15-1002 $^{T}$ | *Phyllostachys nigra* | China | KU940147 | KU863135 | NA | NA |
| *Apiospora yunnanensis* | ZHKUCC 23-0014 $^{T}$ | Dead stems of grass | China | OQ588004 | OQ587992 | OQ586070 | OQ586083 |
| *Apiospora yunnanensis* | ZHKUCC 23-0015 | Dead stems of grass | China | OQ588005 | OQ587993 | OQ586071 | OQ586084 |
| *Arthrinium austriacum* | GZU 345004 | *Carex pendula* | Austria | MW208928 | NA | NA | NA |
| *Arthrinium austriacum* | GZU 345006 | *Carex pendula* | Austria | MW208929 | MW208860 | NA | NA |
| *Arthrinium caricicola* | CBS 145127, AP23518 | *Carex ericetorum* | China | MK014871 | MK014838 | MK017948 | MK017977 |
| *Arthrinium caricicola* | CBS 145903, CPC33297 $^{T}$ | Dead and attached leaves | Germany | MN313782 | MN317266 | NA | MN313861 |
| *Arthrinium crenatum* | AG19066, CBS 146353 $^{T}$ | *Carex* sp. | France | MW208931 | MW208861 | MW221917 | MW221923 |
| *Arthrinium curvatum* | AP25418 | Leaves of *Carex* sp. | China | MK014872 | MK014839 | MK017949 | NA |
| *Arthrinium japonicum* | IFO 30500 | *Carex despalata* | Japan | AB220262 | AB220356 | NA | AB220309 |
| *Arthrinium japonicum* | IFO 31098 | Leaves of *Carex despalata* | Japan | AB220264 | AB220358 | NA | AB220311 |
| *Arthrinium luzulae* | AP7619-3 | *Luzula sylvatica* | Spain | MW208937 | MW208863 | MW221919 | MW221925 |
| *Arthrinium morthieri* | GZU 345043 | *Cyperaceae carex* | Austria | MW208938 | MW208864 | MW221920 | MW221926 |
| *Arthrinium phaeospermum* | AP25619, CBS 146355 | Poaceae plant | Norway | MW208943 | MW208865 | NA | NA |

| Species | Strain Numbers | Host and Substrates | Locality | GenBank accession numbers | | | |
|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *tef1* | *tub2* |
| *Arthrinium puccinioides* | CBS 549.86 | *Lepidosperma gladiatum* | Germany | AB220253 | AB220347 | NA | AB220300 |
| *Arthrinium sporophleoides* | GZU 345102 | *Carex firma* | Austria | MW208944 | MW208866 | NA | MW221927 |
| *Arthrinium sporophleum* | AP21118, CBS 145154 | Dead leaves of *Juncus* sp. | Spain | MK014898 | MK014865 | MK017973 | MK018001 |
| *Nigrospora guilinensis* | CGMCC 3.18124, LC 3481 $^{T}$ | *Camellia sinensis* | China | KX985983 | KX986113 | KY019292 | KY019459 |
| *Nigrospora guilinensis* | LC 7301 | Stems of *Nelumbo* sp. | China | KX986063 | NA | KY019404 | KY019608 |
| *Nigrospora hainanensis* | CGMCC 3.18129, LC 7030 $^{T}$ | Leaves of *Musa paradisiaca* | China | KX986091 | KX986112 | KY019415 | KY019464 |
| *Nigrospora hainanensis* | LC 6979 | Leaves of *Musa paradisiaca* | China | KX986079 | NA | KY019416 | KY019586 |
| *Nigrospora pyriformis* | CGMCC 3.18122, LC 2045 $^{T}$ | *Citrus sinensis* | China | KX985940 | KX986100 | KY019290 | KY019457 |
| *Nigrospora pyriformis* | LC 2688 | *Lindera aggregata* | China | KX985941 | NA | KY019297 | KY019468 |
| *Nigrospora vesicularis* | CGMCC 3.18128, LC 7010 $^{T}$ | Leaves of *Musa paradisiaca* | China | KX986088 | KX986099 | KY019294 | KY019463 |
| *Nigrospora vesicularis* | LC 0322 | Unknown host plant | Thailand | KX985939 | NA | KY019296 | KY019467 |
| *Neoarthrinium lithocarpicola* | CFCC 54456 $^{T}$ | *Lithocarpus glaber* | China | ON427580 | ON427582 | NA | ON456914 |
| *Neoarthrinium lithocarpicola* | CFCC 55883 | *Lithocarpus glaber* | China | ON427581 | ON427583 | NA | ON456915 |
| *Neoarthrinium trachycarpi* | CFCC 53038 | *Trachycarpus fortune* | China | MK301098 | NA | MK303396 | MK303394 |
| *Neoarthrinium trachycarpi* | CFCC 53039 | *Trachycarpus fortune* | China | MK301099 | NA | MK303397 | MK303395 |
| *Sporocadus trimorphus* | CFCC 55171 | Rose | China | OK655798 | OK560389 | OL814555 | OM401677 |
| *Sporocadus trimorphus* | ROC 113 | Rose | China | OK655799 | OK560390 | OL814556 | OM401678 |

The sequences were trimmed and concatenated, and subsequent phylogenetic analyses were performed in PhyloSuite platform (Zhang et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-unlinked) using the BIC criterion. Maximum likelihood (ML) phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under Edge-linked partition model for 5000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model, in which the initial 27% of sampled data were discarded as burn-in. The resulting phylogenetic tree was visualized in FigTree v1.4.3. (http:/tree.bio.ed.ac.uk/software/figtree/) with maximum likelihood bootstrap proportions (MLBP) greater than 70% and Bayesian inference posterior probabilities (BIPP) greater than 0.90, as shown at the nodes. The phylogram was edited in Adobe Illustrator v.27.5 (Adobe Systems Inc., USA). All GenBank accession numbers of sequences used in this study are provided in Table 1.

## Results

### Phylogeny

The combined ITS, LSU, *tef1*, and *tub2* dataset encompassed 215 strains, including six newly sequenced strains, with *Sporocadus trimorphus* CFCC 55171 and ROC 113 serving as the outgroup taxa, and representative species of *Arthrinium*, *Nigrospora*, and *Neoarthrinium* as the sister groups. The multi-locus sequence dataset comprised 2,081 characters, including gaps, with the following character ranges: ITS (1-352), LSU (353-1149), *tef1* (1150-1775), and *tub2* (1776-2081). The topologies of phylogenetic trees generated by ML and BI analyses were congruent, and the BI tree with MLBP and BIPP is presented in Fig. 1.

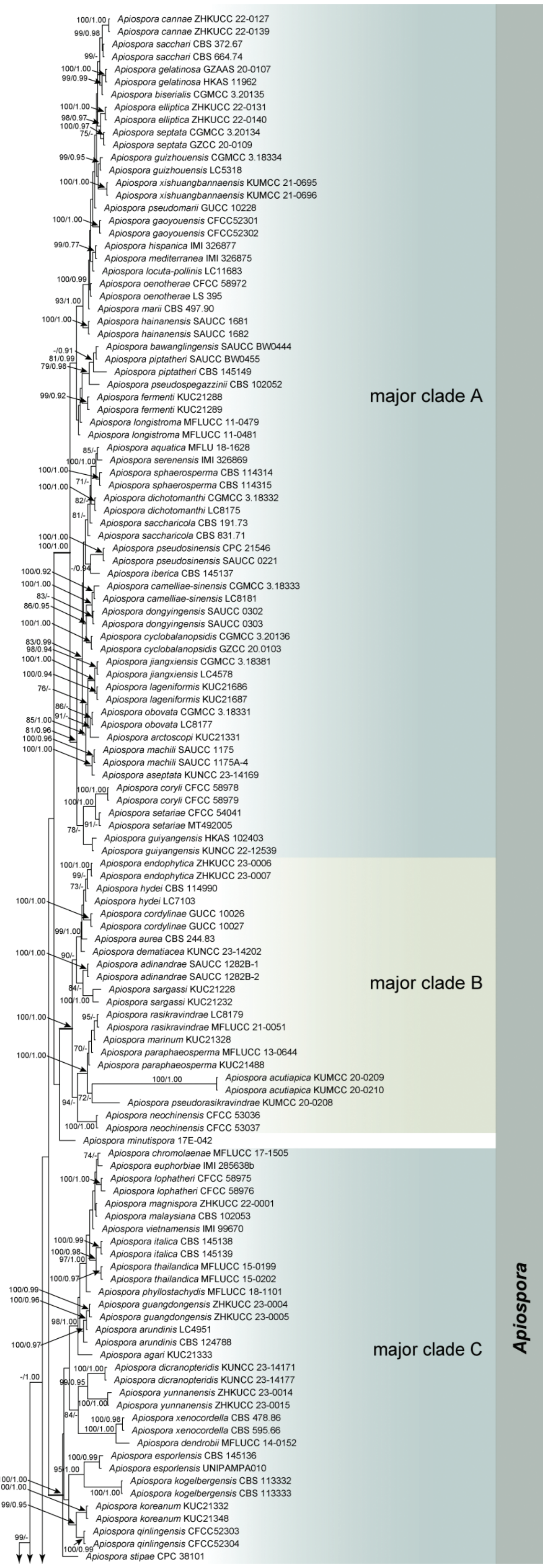

**Figure 1.** Phylogenetic tree of *Apiospora* based on the combined ITS, LSU, *tef1*, and *tub2* sequences alignment. Maximum likelihood bootstrap proportions ≥70% (left) and Bayesian inference posterior probability ≥0.90 (right) are indicated at nodes (MLBP/BIPP). *Sporocadus trimorphus* (CFCC 55171 and ROC 113) are chosen as the outgroup taxa. The novel species from this study are highlighted in red.

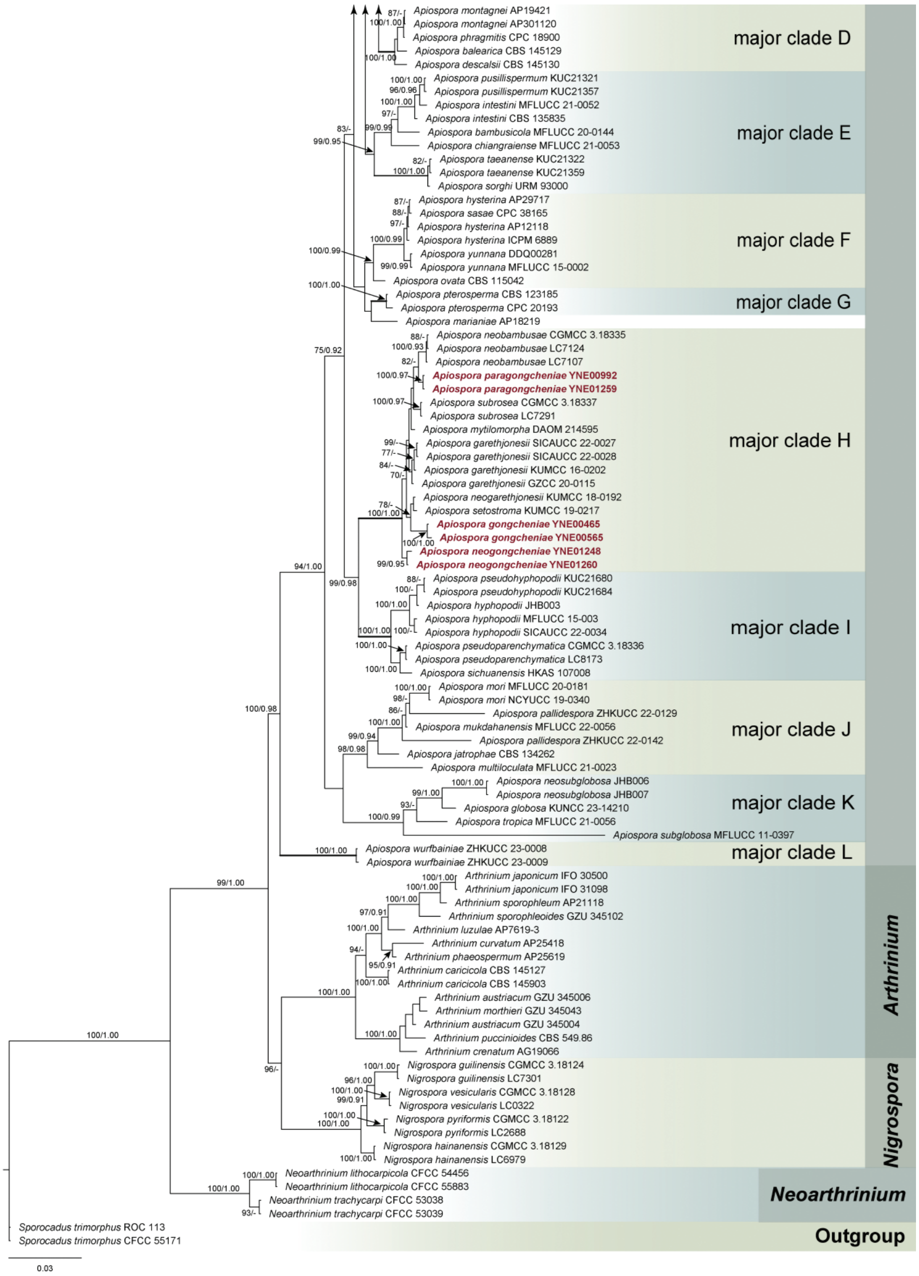

**Figure 1.** Continued.

The phylogenetic analysis revealed that the species of *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* formed four well-supported distinct lineages. Within the genus *Apiospora*, the 187 strains, encompassing six newly sequenced strains, formed twelve well-supported major clades. The six endophytic strains clustered within one of the major clades H, along with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Concurrently, the six endophytic strains segregated into three independent clades with robust supported values, indicating the presence of three novel species. These novel taxa are formally described herein and assigned the new names *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*.

## Taxonomy

***Apiospora gongcheniae*** **C. L. Zhang, sp. nov.**
Fungal Names: FN 571885
Fig. 2

**Etymology.** Named after Prof. Gongchen Wang in recognition of her significant contribution to the fields of mycology and plant pathology in China.

**Type.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00465 (holotype GDMCC 3.1045, stored in a metabolically inactive state); ex-type culture YNE00465.

**Description.** ***Asexual morph*:** Hyphae hyaline, branched, septate, smooth, 1.1–2.6 μm diameter (mean = 1.7 μm, n = 30). Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline to pale brown, erect, verrucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, 3.5–9.4 × 1.9–5.2 μm (mean = 5.6 × 3.1 μm, n = 30). Conidia pale brown to dark brown, smooth, granular, globose to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, 8.0–17.0 × 6.8–16.1 μm (mean = 13.6 × 11.6 μm, n = 50). ***Sexual morph*:** Undetermined.

**Culture characteristics.** On PDA, colonies flat, cottony, dense, margin circular, greyish, reverse light orange, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies dusty pink, dense, covering the 90 mm plate after 7 days at 25 °C. Conidiomata black, globose, abundant, attach to surface of substrate, forming on PDA and MEA after 7–10 days.

**Additional specimens examined.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00565.

**Note.** Phylogenetic analyses confirmed that *A. gongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the GenBank database, it was found that *A. paragongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (93.76% in ITS, 99.81% in LSU), strain GZCC 20-0115 (93.76% in ITS, 99.24% in LSU, 94.06% in *tef1*), strain SICAUCC 22-0027 (93.76% in ITS, 99.81% in LSU, 94.51% in *tub2*), strain SICAUCC 22-0028 (93.76% in ITS, 99.81% in LSU, 93.63% in *tub2*); *A. subrosea* strain CGMCC 3.18337 (96.94% in ITS, 99.42% in LSU, 93.47% in *tef1*, 91.87% in *tub2*), strain LC7291 (90.09% in ITS, 99.41% in LSU, 93.47% in *tef1*, 91.87% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (92.86% in ITS, 99.82% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. gongcheniae*.

As a synopsis of the morphological characteristics presented in Table 2, *A. gongcheniae* differs from *A. garethjonesii* and *A. neogarethjonesii* in having smaller conidia (8.0–17.0 × 6.8–16.1 μm, mean = 13.6 × 11.6 μm) compared to *A. garethjonesii* (surface view: 16–19 μm diam, side view: 17–22 μm diam) and *A. neogarethjonesii* (20–35 × 15–30 μm, mean = 28.5 × 25.6 μm). Additionally, *A. gongcheniae* exhibits shorter conidiogenous cells (3.5–9.4 × 1.9–5.2 μm, mean = 5.6 × 3.1 μm) in contrast to *A. garethjonesii* (6–19 × 3–5 μm,

**Table 2.** Synopsis of morphological characteristics of related *Apiospora* species. Notes: ND = Not determined.

| **Strains** | ***Apiospora garethjonesii*** **(D.Q. Dai & H.B. Jiang) Pintos & P. Alvarado (2021)** | ***A. neogarethjonesii*** **(D.Q. Dai & K.D. Hyde) Pintos & P. Alvarado (2021)** | ***A. subrosea*** **(M. Wang & L. Cai) Pintos & P. Alvarado (2021)** | ***A. neobambusae*** **Pintos & P. Alvarado (2021) (=*Arthrinium bambusae* M. Wang & L. Cai (2018))** | ***A. gongcheniae*** | ***A. paragongcheniae*** | ***A. neogongcheniae*** |
|---|---|---|---|---|---|---|---|
| **Host / Substrate** | Dead culms of bamboo | Dead culms of bamboo | Leaves of bamboo | Leaves of bamboo | Stems of *Oryza meyeriana* subsp. *granulata* | Stems of unidentified Poaceae plant | Stems of unidentified Poaceae plant |
| **Known lifestyle** | Saprobe | Saprobe | Endophyte | Endophyte | Endophyte | Endophyte | Endophyte |
| **Asci** | 125–154 × 35–42 μm ($\bar{x}$ = 139 × 38 μm, n = 20), 8-spored | 95–125 × 20–25 μm ($\bar{x}$ = 97.6 × 21.3 μm, n = 20), 8-spored | ND | ND | ND | ND | ND |
| **Ascospores** | 30–42 × 11–16 μm ($\bar{x}$ = 39 × 13 μm, n = 20), 2-seriate, 1-septate, ellipsoidal | 25–30 × 9.5–11 μm ($\bar{x}$ = 29.1 × 10.3 μm, n = 20), 2-seriate, overlapping, 1-septate, ellipsoidal, 3–10 μm wide | ND | ND | ND | ND | ND |
| **Conidiomata** | Black, with hair-like setae | Black, ellipsoid to irregular, coriaceous | Black, irregular | Black, irregular | Black, globose, abundant, attach to the surface of the substrate | Black, globose to irregular shape, sparse, semi-immersed in the substrate | ND |
| **Conidiophores** | Reduced to conidiogenous cells | 4.5–6 × 3.5–4.5 μm ($\bar{x}$ = 5.4 × 4.3 μm, n = 20), cylindrical, aseptate | Hyaline to pale brown, smooth, erect or ascending, simple, flexuous, subcylindrical, clustered in groups, aggregated in brown sporodochia, up to 20 μm long, 2–4.5 μm width | Reduced to conidiogenous cells | Reduced to conidiogenous cells | Hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, 12.2–35.1 × 2.1–8.8 μm ($\bar{x}$ = 24.5 × 4.3 μm, n = 30) | ND |
| **Conidiogenous cells** | Hyaline to pale brown, smooth, ampulliform, aggregated in black sporodochia, (5–) 6–19 (–20) μm × (2–) 3–5 (–7) μm ($\bar{x}$ = 11 μm × 4 μm, n = 20) | Basauxic, cylindrical, discrete, smooth-walled, 10–48 × 4–5.5 μm ($\bar{x}$ = 35.4 × 4.3 μm, n = 20) | Pale brown, smooth, doliiform to subcylindrical, 3.0–6.5 × 2.0–5.0 μm ($\bar{x}$ = 4.7 ± 1.2 × 3.7 ± 0.9, n = 30) | Hyaline to pale brown, erect, aggregated in clusters on hyphae, smooth, doliiform to ampulliform, or lageniform, 4.0–12.0 × 3.0–7.0 μm ($\bar{x}$ = 6.6 ± 1.8 × 4.8 ± 0.9, n = 30) | Hyaline to pale brown, erect, verrucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, 3.5–9.4 × 1.9–5.2 μm ($\bar{x}$ = 5.6 × 3.1 μm, n = 30) | Hyaline, ampulliform, doliiform to clavate, verrucose, 5.0–13.1 × 2.1–6.0 μm ($\bar{x}$ = 8.2 × 3.9 μm, n = 30) | ND |
| **Conidia** | (14–)16–19 (–20) μm diam, brown, smooth, granular, globose to subglobose in surface view, and (16–) 17–22 (–23) μm diam, with pale equatorial slit in side view | Dark brown, globose to subglobose, smooth-walled, with a truncate basal scar, 20–35 × 15–30 μm ($\bar{x}$ = 28.5 × 25.6 μm, n = 20) | Pale brown to dark brown, smooth, globose to subglobose or ellipsoidal, 12.0–17.5 × 9.0–16.0 μm ($\bar{x}$ = 14.9 ± 1.4 × 11.8 ± 1.8, n = 50) | Olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 μm ($\bar{x}$ = 13.2 ± 0.8 × 11.4 ± 1.2, n = 50) | Pale brown to dark brown, smooth, granular, globose to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, 8.0–17.0 × 6.8–16.1 μm ($\bar{x}$ = 13.6 × 11.6 μm, n = 50) | Pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, 8.2–18.7 × 6.4–13.4 μm ($\bar{x}$ = 12.4 × 10.0 μm, n = 50) | ND |
| **References** | (Dai et al. 2016; Feng et al. 2021) | (Hyde et al. 2020) | (Wang et al. 2018) | (Wang et al. 2018) | This study | This study | This study |

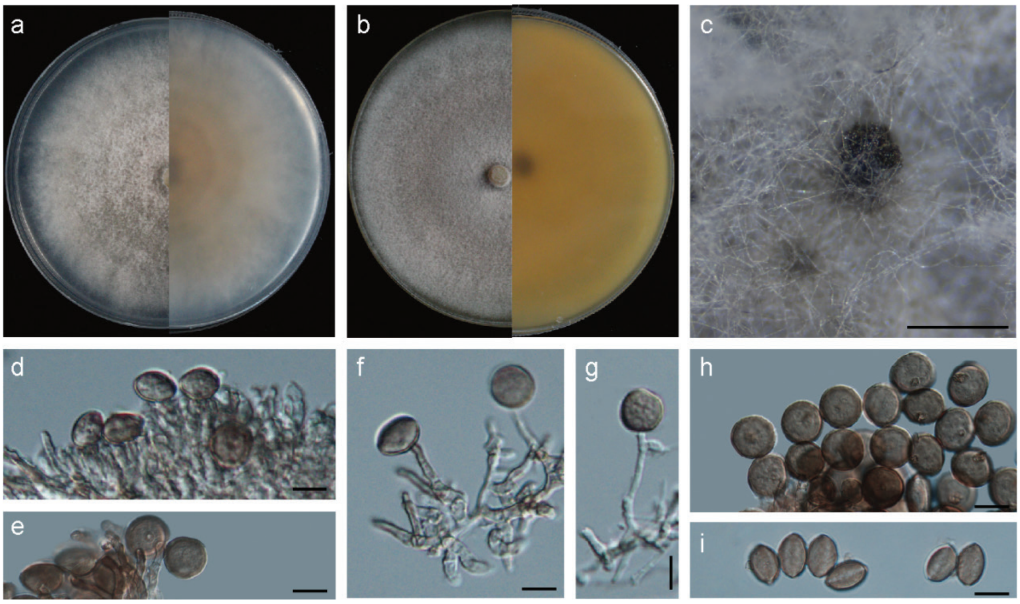

**Figure 2.** *Apiospora gongcheniae* (YNE00465, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** conidiomata on MEA **d-g** conidiogenous cells giving rise to conidia **h–i** conidia with pale germ slit. Scale bars: 500 µm (**e**); 10 µm (**f–k**).

mean = 11 × 4 µm) and *A. neogarethjonesii* (10–48 × 4–5.5 µm, mean = 35.4 × 4.3 µm). While *A. gongcheniae* shares a similar size range for conidia and conidiogenous cells with *A. subrosea*, it is distinguished by *A. gongcheniae* having conidia featuring a central basal scar and cylindrical conidiogenous cells with tiny denticles. Based on molecular and morphological evidence, we propose *A. gongcheniae* as a new species.

### ***Apiospora paragongcheniae*** **C. L. Zhang, sp. nov.**

Fungal Names: FN 571886

Fig. 3

**Etymology.** Named after its phylogenetic close related to *A. gongcheniae*.

**Type.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of unidentified Poaceae plant, Sep 2016, J.J. Chen, YNE00992 (Holotype GDMCC 3.1046, stored in a metabolically inactive state); ex-type culture YNE00992.

**Description.** ***Asexual morph*****:** Hyphae hyaline, branched, septate, smooth, 1.1–2.2 µm diameter (mean = 1.6 µm, n = 30). Conidiophores hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, 12.2–35.1 × 2.1–8.8 µm (mean = 24.5 × 4.3 µm, n = 30). Conidiogenous cells hyaline, ampulliform, doliiform to clavate, verrucose, 5.0–13.1 × 2.1–6.0 µm (mean = 8.2 × 3.9 µm, n = 30). Conidia pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, 8.2–18.7 × 6.4–13.4 µm (mean = 12.4 × 10.0 µm, n = 50). ***Sexual morph*****:** Undetermined.

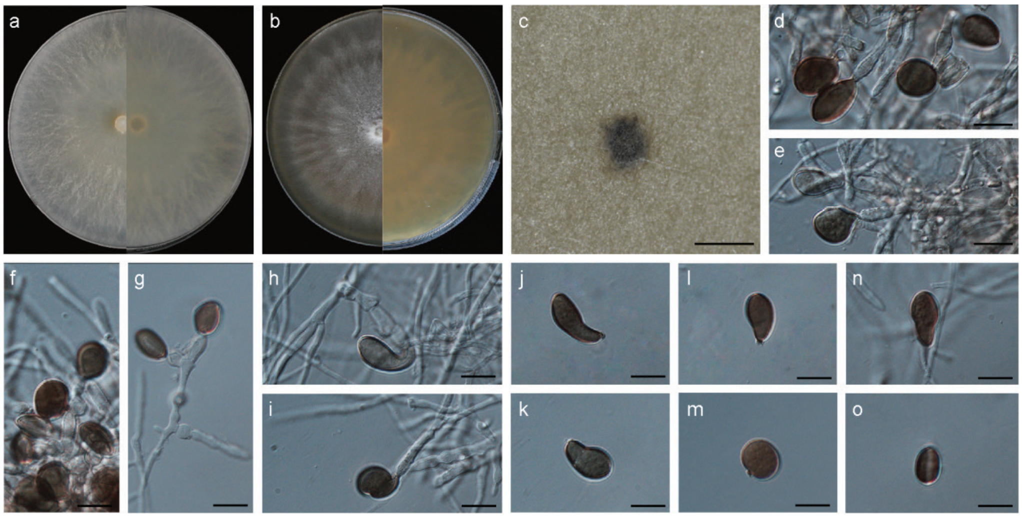

**Figure 3.** *Apiospora paragongcheniae* (YNE00992, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 6 d at 25 °C on MEA **c** conidioma on MEA **d–i** conidiogenous cells giving rise to conidia **j–o** conidia. Scale bars: 500 μm (**c**); 10 μm (**d–o**).

**Culture characteristics.** On PDA, colonies flat, rounded, initially white, becoming yellowish-white, with sparse aerial mycelia, mycelium partly immersed in the medium, covering the 90 mm plate after 6 days at 25 °C. On MEA, colonies white, more abundant aerial mycelia, covering the 90 mm plate after 6 days at 25 °C. Conidiomata black, globose to irregular shape, sparse, solitary, semi-immersed in the substrate, observed on MEA after 21–30 days.

**Additional specimens examined.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001259.

**Note.** Phylogenetic analyses confirmed that *A. paragongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. subrosea*, *A. neobambusae* and *A. neogarethjonesii*. Based on a BLASTN search of the GenBank database, it was found that *A. paragongcheniae* shares high similarities to the following strains: *A. subrosea* strain CGMCC 3.18337 (98.05% in ITS, 99.23% in LSU, 95.93% in *tef1*, 93.63% in *tub2*), strain LC7291 (98.05% in ITS, 99.22% in LSU, 95.93% in *tef1*, 93.63% in *tub2*); *A. neobambusae* strain CGMCC 3.18335 (98.05% in ITS, 100% in LSU, 97.13% in *tef1*, 93.48% in *tub2*), strain LC7107 (98.03% in ITS, 100% in LSU, 94.44% in *tef1*, 93.48% in *tub2*), strain LC7124 (98.05% in ITS, 100% in LSU, 96.82% in *tef1*, 93.47% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (95.43% in ITS, 99.63% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. paragongcheniae*.

As a synopsis of morphological characteristics presented in Table 2, *A. paragongcheniae* distinguishes itself from *A. neobambusae*, *A. neogarethjonesii*, and *A. subrosea* in the shapes and sizes of its conidia. The conidia of *A. paragongcheniae* range from subglobose to oval, occasionally swollen into pyriform to reniform shapes, measuring 8.2–18.7 × 6.4–13.4 μm. This contrasts with *A. neobambusae* (subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 μm), *A. neogarethjonesii* (globose to subglobose, 20–35 × 15–30 μm), and *A. subrosea* (globose to subglobose or ellipsoidal, 12.0–17.5 × 9.0–16.0 μm). Furthermore, *A. paragongcheniae* exhibits elongated conidiogenous cells (5.0–13.1 × 2.1–6.0 µm, mean = 8.2 × 3.9 µm) compared to *A. neobambusae* (4.0–12.0 × 3.0–7.0 µm, mean = 6.6 × 4.8 µm) and *A. subrosea* (3.0–6.5 × 2.0–5.0 µm, mean = 4.7 × 3.7 µm). Additionally, *A. paragongcheniae* exhibits shorter conidiogenous cells (5.0–13.1 × 2.1–6.0 µm) compared to *A. neogarethjonesii* (10–48 × 4–5.5 µm). Moreover, these species differ in the morphology of their conidiophores. *A. paragongcheniae* displays hyaline, basauxic, doliiform, subspherical to barrel-shaped conidiophores, whereas *A. neogarethjonesii* has shorter conidiophores, and *A. subrosea* has hyaline to pale brown, simple, subcylindrical conidiophores. Notably, the conidiophores of *A. neobambusae* have reduced to conidiogenous cells.

## ***Apiospora neogongcheniae*** **C. L. Zhang, sp. nov.**

Fungal Names: FN 571887

Fig. 4

**Etymology.** Named after its phylogenetic close related to *A. gongcheniae*.

**Type.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE01248 (holotype GDMCC 3.1047, stored in a metabolically inactive state); ex-type culture YNE01248.

**Description.** ***Asexual morph*****:** Hyphae hyaline, branched, septate, smooth, 1.0–2.5 µm diameter (mean = 1.5 µm, n = 30). Conidia not observed. Chlamydospores single, terminal, globose, rare. ***Sexual morph*****:** Undetermined.

**Culture characteristics.** On PDA, colonies flat, rounded, initially white, becoming yellowish-white, cottony, with moderate aerial mycelia, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies white, dense aerial mycelia, forming multiple circles around the center, covering the 90 mm plate after 7 days at 25 °C. Conidiomata were not observed.

**Additional specimens examined.** China, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001260.

**Note.** Phylogenetic analyses confirmed that *A. neogongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the GenBank database, it was found that *A. neogongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (94.88% in ITS, 100% in LSU), strain GZCC 20-0115 (94.88% in ITS, 99.41% in LSU, 96.67% in *tef1*), strain SICAUCC 22-0027 (94.88% in ITS, 100% in LSU, 96.69% in *tub2*), strain SICAUCC 22-0028 (94.88% in ITS, 100% in LSU; 96.79% in *tub2*); *A. subrosea* strain CGMCC 3.18337 (98.35% in ITS, 99.80% in LSU, 94.61% in *tef1*, 94.99% in *tub2*), strain LC7291 (91.41% in ITS, 99.80% in LSU, 94.38% in *tef1*, 94.99% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (93.97% in ITS, 100% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. neogongcheniae*.

Due to the absence of sexual and asexual sporulation characters in *A. neogongcheniae*, a comparison of its culture characteristics with those of *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea* was conducted. On PDA,

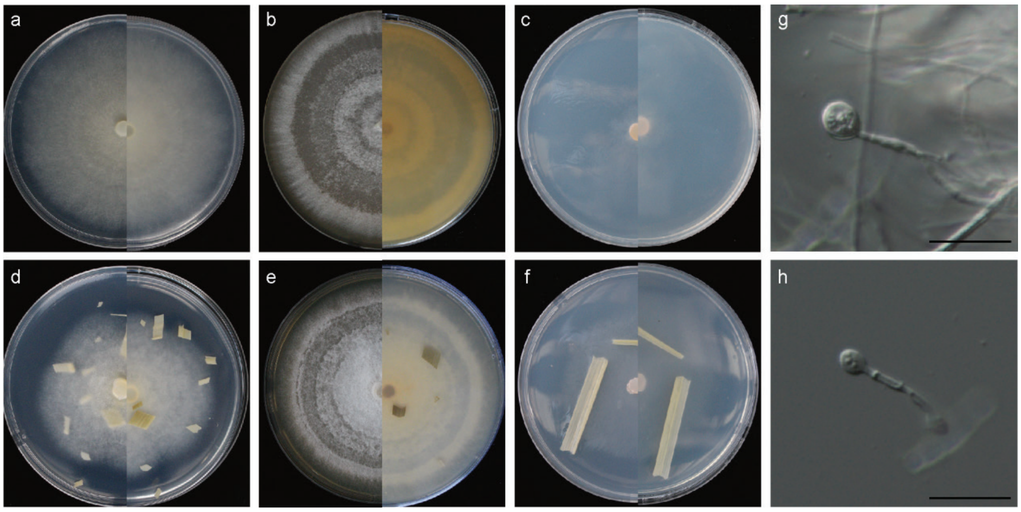

**Figure 4.** *Apiospora neogongcheniae* (YNE01248, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** colonies after 7 d at 25 °C on SNA **d** colonies after 7 d at 25 °C on PDA with rice leaves **e** colonies after 7 d at 25 °C on MEA with rice leaves **f** colonies after 7 d at 25 °C on SNA with rice leaves **g–h** chlamydospores. Scale bars: 20 μm.

*A. neogongcheniae* exhibits a yellowish-white surface and reverse color, whereas *A. garethjonesii* displays a white surface with a reddish reverse, *A. neogarethjonesii* shows a white to black surface coloration, and *A. subrosea* presents a light pink surface with a peach-puff reverse. Phylogenetically, *A. neogongcheniae* strains YNE01248 and YNE01260 form a distinct branch with 99% MLBP and 0.95 BIPP. Therefore, we propose *A. neogongcheniae* as a novel species.

## Discussion

In the present study, three new species of endophytic *Apiospora* were examined: *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, all of them isolated from the stems of Poaceae plants in Yunnan province of China. According to morphological and molecular identification, the taxonomic position of the three new species was verified.

The generic circumscription of *Apiospora* was primarily defined through phylogenetic analysis, given the limited morphological characteristics of *Apiospora* and *Arthrinium*. The results of a multi-locus phylogenetic analysis in this study, utilizing a combined dataset of ITS, LSU, *tef1*, and *tub2* sequences, supported the previous classification that *Apiospora* and *Arthrinium* are distinct lineages rather than synonyms (Pintos and Alvarado 2021). Unlike the six major clades identified in a previous study (Pintos and Alvarado 2022), the current study revealed twelve major clades with robust support through the phylogenetic analysis of 114 *Apiospora* species, including all known species with available sequences. *Apiospora minutispora* (Das et al. 2020) and *Apiospora marianiae* AP18219 (Pintos and Alvarado 2022) were not classified within these twelve major clades due to their representation by a single record. The delineation of most *Apiospora* species into major clades remained consistent across both studies. Notably, *A. garethjonesii*, *A.neogarethjonesii*, *A. neobambusae*, *A. mytilomorpha*, *A. subrosea*, and *A.setostroma* clustered together in a strongly supported major clade H, aligning with findings from previous studies (Crous et al. 2021; Monkai et al. 2022; Pintos and Alvarado 2022; Liao et al. 2023; Liu et al. 2024). Within this major clade, three distinct clades representing three new species were identified (Fig. 1). *A. gongcheniae* is distinguished from *A. garethjonesii* by 34/545 nucleotides in the ITS sequences, from *A. neogarethjonesii* by 39/546, and from *A. subrosea* by 13/425. *A. paragongcheniae* is distinguished from *A. subrosea* by 10/512, from *A. neobambusae* by 10/512, and from *A. neogarethjonesii* by 24/525 nucleotides in the ITS sequences. *A. neogongcheniae* is distinguished from *A. garethjonesii* by 28/547, from *A. neogarethjonesii* by 34/547, and from *A. subrosea* by 7/425 nucleotides in the ITS sequences.

*Apiospora* exhibits ecological diversity, as evidenced by its wide host ranges. Most reported *Apiospora* species show a host preference within the Poaceae family, as noted by Monkai et al. (Monkai et al. 2022). Our new species were also found growing on plant hosts of the Poaceae family. Specifically, *A. gongcheniae* was discovered on the stems of *Oryza meyeriana* subsp. *granulata*, a member of the plant family Poaceae. The other two new species, *A. paragongcheniae* and *A. neogongcheniae*, were found on the stems of unidentified Poaceae plants. Their close relatives, *A. garethjonesii*, *A. neogarethjonesii*, *A. neobambusae*, and *A. subrosea*, were found on bamboo plants. Most *Apiospora* species exhibit saprobic and endophytic lifestyles, which are likely associated with the prevalence of *Apiospora* (Liao et al. 2023). Our new species occurred as endophytic fungi. Further investigation into endophytic *Apiospora* species will significantly enhance the diversity within the *Apiospora* genus.

Morphological characteristics, including asexual and sexual structures, serve as a fundamental basis for fungal systematics and phylogenetic studies, playing a vital role in the comprehensive examination of fungi. However, many endophytes do not form distinct asexual and sexual structures, as observed in *A. neogongcheniae* in this study, posing challenges in determining their taxonomic status based on morphological features. Recent advances in fungal taxonomy and phylogeny have provided new insights into many species with limited morphological features. Future taxonomic efforts necessitate the integration of morphological traits with molecular evidence to elucidate the natural and stable phylogenetic relationships among *Apiospora* species and their related *Arthrinium* species.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Xiao-Ni Yan: Investigation, data curation, formal analysis and writing-original draft. Chu-Long Zhang: Conceptualization, methodology, validaiton, formal analysis, supervision, writing-review & editing, funding acquistition.

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## Data availability

All of the data that support the findings of this study are available in the main text.

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