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Insight into the Taxonomic Resolution of *Apiospora*: Introducing Novel Species and Records from Bamboo in China and Thailand

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Abstract: Taxonomic studies of bambusicolous fungi in China and Thailand have resulted in the collection of three fascinating saprobic coelomycetes strains. Morphology coupled with combined gene analysis of ITS, LSU, *TUB2*, and *TEF1-α* DNA sequence data showed that they belong to the genus *Apiospora*, family *Apiosporaceae*. A new species from Thailand, *Apiospora mukdahanensis*, and new records of *A. locuta-pollinis* from China are herein described. In addition, based on both morphological data coupled with phylogenetics and nomenclatural analyses, *A. mori* is proposed as a new combination. Maximum likelihood, maximum parsimony and Bayesian analyses were performed to clarify the phylogenetic affinities of the species obtained in this study. Newly obtained strains are compared with morphologically- and phylogenetically-related taxa. The comprehensive descriptions, illustrations, and updated phylogeny are provided and discussed for intra-and intergeneric relationships within *Apiospora* species.

Keywords: *Apiosporaceae*; fungal diversity; fungus–host distribution; phylogeny; taxonomy

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1. Introduction

Apiospora is a large genus in the family *Apiosporaceae* (Amphisphaeriales, Sordariomycetes, Ascomycota) [1,2], which is ecologically diverse and distributed worldwide [2–5]. Most species have been identified as saprobes and endophytes of a range of plant hosts, mainly occurring on the family *Poaceae* [2–12]. In addition, some species have been reported as plant pathogens. For instance, *A. kogelbergensis* causes the blight disease of *Schizostachyum* [13], *A. sacchari* causes the damping-off of durum wheat (*Triticum durum*) [14], and *A. xenocordella* causes fruit blight on pistachio (*Pistacia vera*) [15]. *Apiospora* shows a cosmopolitan distribution in diverse substrates, including air [4,16], soil [4,16–18], freshwater [19], marine environments [20–25], lichens [26], insect guts [27], and human tissues [3,28–30]. Interestingly, some species (e.g., *A. arundinis*, *A. sacchari*) have been

reported as a source of useful bioactive compounds, such as antifungal agents and enzymes [21,22,31], possessing great potential for their commercial applications in the pharmaceutical industries.

Apiospora is classified by asexual morph characteristics that produce basauxic conidiophores and unicellular globose to obovoid conidia, usually rounded in face view and lenticular in side view, with a longitudinal germ slit [2,7,10,11,32]. The sexual morph is characterized as having multi-locular perithecial stromata, clavate to broadly cylindrical asci and hyaline ascospores surrounded by a thick gelatinous sheath [2,7,8,11]. *Apiospora* was previously known as the sexual morph of the genus *Arthrinium* [33,34]. According to the International Code of Nomenclature for Algae, Fungi, and Plants (ICN) [35], *Apiospora* was a synonym of *Arthrinium* due to the early introduction of *Arthinium* and is more commonly used in the literature [3]. Crous and Groenewald [3] and Wang et al. [4] provided the upgraded phylogenetic trees of *Arthrinium* species (=*Apiospora*) using combined ITS, *TEF1-α*, and *TUB2* sequence data with additional strains (collected from various hosts, substrates, and locations) and indicated that *Arthrinium* seems to be a species complex which needs further taxonomic revision and epitypification. Multi-gene phylogeny of ITS, LSU, *TEF1-α*, and *TUB2* sequences conducted by Pintos et al. [5] revealed that *Arthrinium caricicola*, the type species, and other species of *Arthrinium* mostly found in *Carex* sp. formed independent lineages unrelated to other species of *Arthrinium*, and reported that *Apiospora* occurred on other hosts. However, the taxonomic placement of both genera was uncertain until Pintos and Alvarado [2] resolved this issue and presented *Arthrinium* and *Apiospora* as well-supported distinct clades suggesting they are separate genera.

The morphological identification of *Apiospora* species is challenging because most species share similar morphological characteristics (e.g., conidia). In addition, their morphological features can vary depending on incubation periods and different substrates [3]. Thus, morphological characteristics integrated with molecular phylogeny have been widely accepted to distinguish *Apiospora* species [3–9,12,17,36–39]. Presently, 117 epithets are recognized in *Apiospora* [40], comprising 76 *Arthrinium* species, which were synonymized under *Apiospora* [2,12,25]. The taxonomic position of other taxa, which lack sequencing information and comprehensive morphological descriptions, remain uncertain and require further study. In this study, we isolated apiospora-like taxa from bamboo in China and Thailand. The morphological characteristics and molecular analyses of ITS, LSU, *TUB2*, and *TEF1-α* were applied to determine a new species, *Apiospora mukdahanensis*, and one new record of *A. locuta-pollinis*. Furthermore, *Arthrinium mori* is also transferred to *Apiospora* as a new combination on the basis of phylogenetic evidence. The host association, geographical distribution, and species diversity of *Apiospora* are also discussed.

2. Materials and Methods

2.1. Sample Collection, Fungal Isolation and Morphological Examination

Dead and decaying bamboo specimens were collected during a series of field trips conducted in China and Thailand from the year 2019–2021. The specimens were packed in zip-lock plastic bags prior for further study. Fungal colonies on the host substrate were observed using a stereo microscope (Nikon SMZ800N, Tokyo, Japan). The micromorphological characteristics were documented and photographed with a compound microscope (Nikon Eclipse Ni U, Tokyo, Japan) equipped with Nikon DS-Ri2 camera. The measurements of fungal structures (i.e., conidiomata, conidiophore mother cells, conidiophores, conidiogenous cells, and conidia) were made using the Tarosoft (R) Image Frame Work program. Images used for figures were combined and edited using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, San Jose, CA, USA). Single-spore isolation was conducted to isolate the fungus as detailed in Senanayake et al. [41]. The germinating conidia were inoculated on potato dextrose agar (PDA) and incubated at 28 °C for two weeks. Culture characteristics were observed and described after four weeks. Axenic cultures were kept in 2 mL sterilized screw-cap tubes containing PDA for short-term storage and duplicated in 10% glycerol for long-term storage. The type specimen and living

culture of *Apiospora mukdahanensis* were deposited in the herbarium of Mae Fah Luang University (MFLU) and the Mae Fah Luang University Culture Collection (MFLUCC), respectively. The specimens of *A. locuta-pollinis* and cultures were deposited in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), and the Kunming Institute of Botany Culture Collection, Kunming, China (KUNCC), respectively. Index Fungorum numbers and Faces of Fungi numbers were obtained for the new taxa as detailed in Index Fungorum [42] and Jayasiri et al. [43], respectively.

2.2. DNA Extraction, PCR Amplification and Sequencing

The total genomic DNA was extracted from mature mycelium grown on PDA at 28 °C for two weeks using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, China). Polymerase chain reaction (PCR) amplification was applied to amplify DNA fragments with three phylogenetic markers, including the internal transcribed spacers region of ribosomal DNA (ITS; ITS1-5.8s-ITS2) using primers ITS5 and ITS4 [44]; the partial 28S large subunit nuclear ribosomal DNA (LSU) using primers LR0R and LR5 [45]; and the translation elongation factor 1-α (*TEF1-α*) using primers EF1-728F and EF-2 [46,47]. The PCR reaction was carried out in the final volume of 25 µL, containing 2 µL template DNA (50 ng/µL), 12.5 µL of PCR Master Mix (0.5 mM of each primer, 50 U *Taq* DNA polymerase 400 mM of each dNTP, and 3 mM $MgCl_2$), 1 µL of each forward and reward primer and 8.5 µL of the sterilized double-distilled water (ddH_2O). The PCR thermal cycling programs for ITS, LSU and *TEF1-α* were adjusted by an initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 50 s, extension at 72 °C for 1 min and a final extension step at 72 °C for 10 min. The PCR products were processed for purification and sequencing by TsingKe Biological Technology, Kunming City, Yunnan Province, China. Newly generated sequences in this study were deposited in Genbank (Table 1).

Table 1. List of the taxa used in phylogenetic reconstruction and their corresponding GenBank numbers.

Taxa	Culture Accession No.	Genbank Accession No.			
		ITS	LSU	*TUB2*	*TEF1-α*
Apiospora acutiapica	**KUMCC 20-0210**	**MT946343**	**MT946339**	**MT947366**	**MT947360**
Apiospora agari	**KUC 21333**	**MH498520**	**N/A**	**MH498478**	**MH544663**
Apiospora aquatica	S-642	MK828608	MK835806	N/A	N/A
Apiospora arctoscopi	**KUC 21331**	**MH498529**	**N/A**	**MH498487**	**MN868918**
Apiospora arundinis	CBS 133509	KF144886	KF144930	KF144976	KF145018
Apiospora arundinis	CBS 449.92	KF144887	KF144931	KF144977	KF145019
Apiospora aurea	**CBS 244.83**	**AB220251**	**KF144935**	**KF144981**	**KF145023**
Apiospora balearica	**CBS 145129**	**MK014869**	**MK014836**	**MK017975**	**MK017946**
Apiospora bambusae	CBS 145133	MK014875	MK014842	MK017981	MK017952
Apiospora bambusae	**ICMP 6889**	**MK014874**	**MK014841**	**MK017980**	**MK017951**
Apiospora bambusicola	**MFLUCC 20-0144**	**MW173030**	**MW173087**	**N/A**	**MW183262**
Apiospora biserialis	**CGMCC 3.20135 ***	**MW481708**	**MW478885**	**MW522955**	**MW522938**
Apiospora biserialis	GZCC 20_0099 *	MW481709	MW478886	MW522956	MW522939
Apiospora camelliae-sinensis	**LC 5007**	**KY494704**	**KY494780**	**KY705173**	**KY705103**
Apiospora camelliae-sinensis	LC 8181	KY494761	KY494837	KY705229	KY705157
Apiospora chiangraiense	**MFLUCC 21-0053**	**MZ542520**	**MZ542524**	**MZ546409**	**N/A**
Apiospora chromolaenae	**MFLUCC 17-1505**	**MT214342**	**MT214436**	**N/A**	**MT235802**
Apiospora cordylinae	GUCC 10026	MT040105	N/A	MT040147	MT040126
Apiospora cyclobalanopsidis	**CGMCC 3.20136**	**MW481713**	**MW478892**	**MW522962**	**MW522945**
Apiospora descalsii	**CBS 145130**	**MK014870**	**MK014837**	**MK017976**	**MK017947**
Apiospora dichotomanthi	**LC 4950**	**KY494697**	**KY494773**	**KY705167**	**KY705096**
Apiospora dichotomanthi	LC 8175	KY494755	KY494831	KY705223	KY705151
Apiospora esporlensis	**CBS 145136**	**MK014878**	**MK014845**	**MK017983**	**MK017954**
Apiospora euphorbiae	IMI 285638b	AB220241	AB220335	AB220288	N/A
Apiospora fermenti	**KUC 21289 ***	**MF615226**	**N/A**	**MF615231**	**MH544667**
Apiospora gaoyouensis	**CFCC 52301 ***	**MH197124**	**N/A**	**MH236789**	**MH236793**

Table 1. *Cont.*

Taxa	Culture Accession No.	Genbank Accession No.			
		ITS	LSU	*TUB2*	*TEF1-α*
Apiospora gaoyouensis	CFCC 52302 *	MH197125	N/A	MH236790	MH236794
Apiospora garethjonesii	**KUMCC 16-0202**	**KY356086**	**KY356091**	**N/A**	**N/A**
Apiospora gelatinosa	**KHAS 11962 ***	**MW481706**	**MW478888**	**MW522958**	**MW522941**
Apiospora guiyangensis	**HKAS 102403**	**MW240647**	**MW240577**	**MW775604**	**MW759535**
Apiospora guizhouensis	LC 5318 *	KY494708	KY494784	KY705177	KY705107
Apiospora guizhouensis	**LC 5322 ***	**KY494709**	**KY494785**	**KY705178**	**KY705108**
Apiospora hispanica	**IMI 326877 ***	**AB220242**	**AB220336**	**AB220289**	**N/A**
Apiospora hydei	**CBS 114990**	**KF144890**	**KF144936**	**KF144982**	**KF145024**
Apiospora hydei	LC 7103	KY494715	KY494791	KY705183	KY705114
Apiospora hyphopodii	**MFLUCC 15-0003**	**KR069110**	**N/A**	**N/A**	**N/A**
Apiospora hyphopodii	KUMCC 16-0201	KY356088	KY356093	N/A	N/A
Apiospora ibericum	**CBS 145137**	**MK014879**	**MK014846**	**MK017984**	**MK017955**
Apiospora intestini	**CBS 135835**	**KR011352**	**KR149063**	**KR011350**	**KR011351**
Apiospora intestini	MFLUCC 21-0052	MZ542521	MZ542525	MZ546410	MZ546406
Apiospora italica	**CBS 145138**	**MK014880**	**MK014847**	**MK017985**	**MK017956**
Apiospora italica	CBS 145139	MK014881	MK014848	MK017986	MK017957
Apiospora jatrophae	AMH-9556	HE981191	N/A	N/A	N/A
Apiospora jatrophae	**AMH-9557**	**JQ246355**	**N/A**	**N/A**	**N/A**
Apiospora jiangxiense	LC 4494	KY494690	KY494766	KY705160	KY705089
Apiospora jiangxiense	**LC 4577**	**KY494693**	**KY494769**	**KY705163**	**KY705092**
Apiospora kogelbergense	CBS 113332	KF144891	KF144937	KF144983	KF145025
Apiospora kogelbergense	**CBS 113333**	**KF144892**	**KF144938**	**KF144984**	**KF145026**
Apiospora koreana	**KUC 21332**	**MH498524**	**N/A**	**MH498482**	**MH544664**
Apiospora locuta-pollinis	GUCC 10228 *	MT040124	N/A	MT040166	MT040145
Apiospora locuta-pollinis	KUNCC 22-12408 *	OP377736	OP377743	N/A	OP381090
Apiospora locuta-pollinis	KUNCC 22-12409 *	OP377737	OP377744	N/A	OP381091
Apiospora locuta-pollinis	**LC 11683 ***	**MF939595**	**N/A**	**MF939622**	**MF939616**
Apiospora locuta-pollinis	LC 11688 *	MF939596	N/A	MF939623	MF939618
Apiospora longistromum	MFLUCC 11-0479 *	KU940142	KU863130	N/A	N/A
Apiospora longistromum	**MFLUCC 11-0481 ***	**KU940141**	**KU863129**	**N/A**	**N/A**
Apiospora malaysiana	**CBS 102053**	**KF144896**	**KF144942**	**KF144988**	**KF145030**
Apiospora marii	CBS 113535 *	KF144898	KF144944	KF144990	KF145032
Apiospora marii	CBS 114803 *	KF144899	KF144945	KF144991	KF145033
Apiospora marii	CPC 18902 *	KF144901	KF144948		
Apiospora marii	CPC 18904 *	KF144902	KF144949	KF144994	KF145036
Apiospora marii	CBS 200.57 *	KF144900	KF144946	KF144992	KF145034
Apiospora marii	**CBS 497.90 ***	**AB220252**	**KF144947**	**KF144993**	**KF145035**
Apiospora marii	KUC 21338 *	MH498549	N/A	MH498507	MH544681
Apiospora marii	MFLUCC 16-0282 *	MH109526	N/A	N/A	MH206166
Apiospora marii	MFLUCC 16-0283 *	MH109527	N/A	N/A	MH220419
Apiospora marina	**KUC 21328**	**MH498538**	**N/A**	**MH498496**	**MH544669**
Apiospora mediterranea	**IMI 326875 ***	**AB220243**	**AB220337**	**AB220290**	**N/A**
Apiospora minutispora	**17E-042**	**LC517882**	**N/A**	**LC518888**	**LC518889**
Apiospora mori	NCYUCC 19-0340	MW114314	MW114394	N/A	N/A
Apiospora mori	**MFLUCC 20-0181**	**MW114313**	**MW114393**	**N/A**	**N/A**
Apiospora mukdahanensis	**MFLUCC 22-0056**	**OP377735**	**OP377742**	**N/A**	**OP381089**
Apiospora multiloculata	**MFLUCC 21-0023**	**OL873137**	**OL873138**	**OL874718**	**N/A**
Apiospora mytilomorpha	**DAOM 214595**	**KY494685**	**N/A**	**N/A**	**N/A**
Apiospora neobambusae	**LC 7106**	**KY494718**	**KY494794**	**KY705186**	**KY806204**
Apiospora neobambusae	LC 7124	KY494727	KY494803	KY705195	KY806206
Apiospora neochinensis	**CFCC 53036**	**MK819291**	**N/A**	**MK818547**	**MK818545**
Apiospora neochinensis	CFCC 53037	MK819292	N/A	MK818548	MK818546
Apiospora neogarethjonesii	**KUMCC 18-0192**	**MK070897**	**MK070898**	**N/A**	**N/A**
Apiospora neosubglobosa	JHB006	KY356089	KY356094	N/A	N/A
Apiospora neosubglobosa	**KUMCC 16-0203**	**KY356090**	**KY356095**	**N/A**	**N/A**
Apiospora obovata	**LC 4940**	**KY494696**	**KY494772**	**KY705166**	**KY705095**

Table 1. *Cont.*

Taxa	Culture Accession No.	Genbank Accession No.			
		ITS	LSU	*TUB2*	*TEF1-α*
Apiospora obovata	LC 8177	KY494757	KY494833	KY705225	KY705153
Apiospora ovata	**CBS 115042**	**KF144903**	**KF144950**	**KF144995**	**KF145037**
Apiospora paraphaeospermum	**MFLUCC 13-0644**	**KX822128**	**KX822124**	**N/A**	**N/A**
Apiospora phragmitis	**CPC 18900**	**KF144909**	**KF144956**	**KF145001**	**KF145043**
Apiospora phyllostachydis	**MFLUCC 18-1101**	**MK351842**	**MH368077**	**MK291949**	**MK340918**
Apiospora piptatheri	**CBS 145149 ***	**MK014893**	**MK014860**	**N/A**	**MK017969**
Apiospora pseudoparenchymatica	**LC 7234**	**KY494743**	**KY494819**	**KY705211**	**KY705139**
Apiospora pseudoparenchymatica	LC 8173	KY494753	KY494829	KY705221	KY705149
Apiospora pseudorasikravindrae	**KUMCC 20-0208**	**MT946344**	**N/A**	**MT947367**	**MT947361**
Apiospora pseudosinensis	**CPC 21546**	**KF144910**	**KF144957**	**N/A**	**KF145044**
Apiospora pseudospegazzinii	**CBS 102052 ***	**KF144911**	**KF144958**	**KF145002**	**KF145045**
Apiospora pterosperma	CBS 123185	KF144912	KF144959	KF145003	N/A
Apiospora pterosperma	**CPC 20193**	**KF144913**	**KF144960**	**KF145004**	**KF145046**
Apiospora pusillisperma	**KUC 21321**	**MH498533**	**N/A**	**MH498491**	**MN868930**
Apiospora qinlingense	**CFCC 52303**	**MH197120**	**N/A**	**MH236791**	**MH236795**
Apiospora qinlingense	CFCC 52304	MH197121	N/A	MH236792	MH236796
Apiospora rasikravindrae	**NFCCI 2144**	**JF326454**	**N/A**	**N/A**	**N/A**
Apiospora rasikravindrae	LC 8179	KY494759	KY494835	KY705227	KY705155
Apiospora sacchari	CBS 372.67 *	KF144918	KF144964	KF145007	KF145049
Apiospora sacchari	CBS 664.74 *	KF144919	KF144965	KF145008	KF145050
Apiospora saccharicola	CBS 191.73	KF144920	KF144966	KF145009	KF145051
Apiospora saccharicola	CBS 831.71	KF144922	KF144969	KF145012	KF145054
Apiospora sargassi	**KUC 21228**	**KT207746**	**N/A**	**KT207644**	**MH544677**
Apiospora septata	**CGMCC 3.20134 ***	**MW481711**	**MW478890**	**MW522960**	**MW522943**
Apiospora serenensis	**IMI 326869**	**AB220250**	**AB220344**	**AB220297**	**N/A**
Apiospora setariae	**CFCC 54041**	**MT492004**	**N/A**	**MT497466**	**MW118456**
Apiospora setostroma	**KUMCC 19-217**	**MN528012**	**MN528011**	**N/A**	**MN527357**
Apiospora sichuanensis	**HKAS 107008**	**MW240648**	**MW240578**	**MW775605**	**MW759536**
Apiospora sorghi	**URM 93000**	**MK371706**	**N/A**	**MK348526**	**N/A**
Apiospora subglobosa	**MFLUCC 11-0397**	**KR069112**	**KR069113**	**N/A**	**N/A**
Apiospora subrosea	LC 7291	KY494751	KY494827	KY705219	KY705147
Apiospora subrosea	**LC 7292**	**KY494752**	**KY494828**	**KY705220**	**KY705148**
Apiospora taeanense	**KUC 21322**	**MH498515**	**N/A**	**MH498473**	**MH544662**
Apiospora thailandica	MFLUCC 15-1999	KU940146	KU863134	N/A	N/A
Apiospora thailandica	**MFLUCC 15-0202**	**KU940145**	**KU863133**	**N/A**	**N/A**
Apiospora tropica	**MFLUCC 21–0056**	**OK491657**	**OK491653**	**OK560922**	**N/A**
Apiospora vietnamense	**IMI 99670**	**KX986096**	**KX986111**	**KY019466**	**N/A**
Apiospora xenocordella	**CBS 478.86**	**KF144925**	**KF144970**	**KF145013**	**KF145055**
Apiospora xenocordella	CBS 595.66	KF144926	KF144971	N/A	N/A
Apiospora yunnana	**MFLUCC 15-1002**	**KU940147**	**KU863135**	**N/A**	**N/A**
Apiospora yunnana	DDQ00281	KU940148	KU863136	N/A	N/A
Arthrinium austriacum	GZU 345004	MW208928	N/A	N/A	N/A
Arthrinium austriacum	GZU 345006	MW208929	MW208860	N/A	N/A
Arthrinium caricicola	CBS 145127	MK014871	MK014838	MK017977	MK017948
Arthrinium cf. *sporophleoides*	GZU 345102	MW208944	MW208866	N/A	N/A
Arthrinium crenatum	**CBS 146353**	**MW208931**	**MW208861**	**MW221923**	**MW221917**
Arthrinium curvatum	AP 25418	MK014872	MK014839	MK017978	MK017949
Arthrinium japonicum	IFO 30500	AB220262	AB220356	AB220309	N/A
Arthrinium japonicum	IFO 31098	AB220264	AB220358	AB220311	N/A
Arthrinium luzulae	**AP 7619-3**	**MW208937**	**MW208863**	**N/A**	**N/A**
Arthrinium morthieri	GZU 345043	MW208938	MW208864	N/A	N/A
Arthrinium phaeospermum	CBS 114317	KF144906	KF144953	KF144998	KF145040
Arthrinium phaeospermum	CBS 114318	KF144907	KF144954	KF144999	KF145041
Arthrinium puccinioides	CBS 549.86	AB220253	AB220347	AB220300	N/A
Arthrinium sphaerospermum	AP 25619	MW208943	MW208865	N/A	N/A

Table 1. *Cont.*

Taxa	Culture Accession No.	Genbank Accession No.			
		ITS	**LSU**	***TUB2***	***TEF1-α***
Arthrinium sporophleum	CBS 145154	MK014898	MK014865	MK018001	MK017973
Arthrinium trachycarpum	**CFCC 53038**	**MK301098**	**N/A**	**MK303394**	**MK303396**
Arthrinium trachycarpum	CFCC 53039	MK301099	N/A	MK303395	MK303397
Arthrinium urticae	IMI 326344	AB220245	AB220339	AB220292	N/A
Nigrospora aurantiaca	**CGMCC 3.18130**	**KX986064**	**KX986098**	**KY019465**	**KY019295**
Nigrospora camelliae-sinensis	**CGMCC 3.18125**	**KX985986**	**KX986103**	**KY019460**	**KY019293**
Nigrospora chinensis	**CGMCC 3.18127**	**KX986023**	**KX986107**	**KY019462**	**KY019422**
Nigrospora gorlenkoana	CBS 480.73	KX986048	KX986109	KY019456	KY019420
Nigrospora guilinensis	**CGMCC 3.18124**	**KX985983**	**KX986113**	**KY019459**	**KY019292**
Nigrospora hainanensis	**CGMCC 3.18129**	**KX986091**	**KX986112**	**KY019464**	**KY019415**
Nigrospora lacticolonia	**CGMCC 3.18123**	**KX985978**	**KX986105**	**KY019458**	**KY019291**
Nigrospora musae	CBS 319.34	MH855545	KX986110	KY019455	KY019419
Nigrospora oryzae	LC 2693	KX985944	KX986101	KY019471	KY019299
Nigrospora osmanthi	**CGMCC 3.18126**	**KX986010**	**KX986106**	**KY019461**	**KY019421**
Nigrospora pyriformis	**CGMCC 3.18122**	**KX985940**	**KX986100**	**KY019457**	**KY019290**
Nigrospora rubi	**LC 2698**	**KX985948**	**KX986102**	**KY019475**	**KY019302**
Nigrospora sphaerica	LC 7298	KX985937	KX986097	KY019606	KY019401
Nigrospora vesicularis	**CGMCC 3.18128**	**KX986088**	**KX986099**	**KY019463**	**KY019294**
Sporocadus trimorphus	**CBS 114203**	**MH553977**	**MH554196**	**MH554636**	**MH554395**

The ex-type cultures are indicated in bold and newly generated sequences are indicated in red. The taxa related to the Apiospora locuta-pollinis/marii clade are marked as an asterisk (*), "N/A" indicates sequence is unavailable.

2.3. Phylogenetic Analyses

The sequences generated by this study were supplemented with the related taxa resulting from the nucleotide blast search in GenBank (www.ncbi.nlm.nih.gov/blast/, accessed on 1 September 2022) and recent publications [2,12,25,39,48,49] (Table 1). The matrix of consensus sequences was aligned using MAFFT v. 7.475 on the web portal (http://mafft.cbrc.jp/alignment/server/index.html) [50] and then the ambiguous sites were manually trimmed using BioEdit 7.1.3.0 [51]. Phylogenetic trees based on the concatenated ITS, LSU, *TUB2*, and *TEF1-α* sequence data (analysis 1) and ITS, LSU, and *TEF1-α* sequence data (analysis 2) were inferred to clarify the phylogenetic relationships of *Apiospora* species using maximum likelihood (ML) and Bayesian inference (BI) analyses. In order to clarify the phylogenetic placements of new strains and related strains in *A. locuta-pollinis/marii* clade, ML, maximum parsimony (MP), and BI were analyzed based on the concatenated ITS, LSU, *TUB2*, and *TEF1-α* sequence data (analysis 3). Phylogenetic trees of these combined gene datasets were further compared to check the congruence of the tree topologies.

ML analyses were implemented using RAxML-HPC2 (v.8.2.12) on the CIPRES web portal (http://www.phylo.org/portal2/) [52]. The GTRGAMMAI model of nucleotide substitution with 1000 rapid bootstrap replicates was used. BI analyses were performed using MrBayes v.3.2.7a via the CIPRES web portal (http://www.phylo.org/portal2/). The optimal substitution model of nucleotide evolution was determined using MrModeltest v. 2.3 [53]. In the first and second analyses, GTR + I + G was chosen as the best-fit model for the ITS, LSU, and *TEF1-α* datasets, and HKY + I + G for the *TUB2* dataset. For the third analysis, the best-fit model for the ITS, LSU, *TUB2*, and *TEF1-α* datasets were as follows: SYM + I, GTR, GTR + G, and K80 + I. Ten million Markov chain Monte Carlo sampling (MCMC) generations were run with six simultaneous Markov chains to calculate Bayesian posterior probabilities [54–56]. Trees were sampled every 100th generation. When the average standard deviation of split frequencies was constantly below 0.01, the runs were automatically stopped and the first 25% of the generated trees were discarded. The remaining trees were used to evaluate the posterior probabilities (PP) of the majority rule consensus tree. MP analyses were conducted with the heuristic search option, as implemented in PAUP v. 4.0b10 [57]. Clade stability was determined using a

bootstrap analysis with 1000 replicates, random sequence additions with maxtrees set to 1000 [58]. The MP tree was described for descriptive tree statistics including Tree Length (TL), Consistency Index (CI), Retention Index (RI), Relative Consistency Index (RC), and Homoplasy Index (HI) under different optimality criteria. Phylogenetic trees were viewed in FigTree v1.4.0 [59] and formatted using Adobe Photoshop CS6 software (Adobe Systems, San Jose, CA, USA).

2.4. Host and Geographical Distribution of Apiospora Species

To investigate geographical distribution and host-substratum diversity of the *Apiospora* species, the data were summarized based on the USDA fungal database (https://nt.ars-grin.gov/fungaldatabases/, accessed on 1 September 2022), academic literature, and this study).

3. Results

3.1. Phylogenetic Analyses

Analysis 1: The combination of ITS, LSU, *TUB2*, and *TEF1-α* sequence dataset comprised 156 taxa of *Apiospora*, and other related taxa in the family *Apiosporaceae*. *Sporocadus trimorphus* (CBS 114203) was selected as the outgroup taxon (Figure 1). The final alignment consisted of 3264 total characters, including gaps (ITS: 1–641 bp, LSU: 642–1524 bp, *TUB2*: 1525–2366 bp, *TEF1-α*: 2367–3264 bp). The RAxML analysis of the integrated dataset yielded a best scoring tree with a final ML optimization likelihood value of −39,292.041642. The aligned sequence matrix contained 1874 distinct alignment patterns, with 38.58% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237643, C = 0.255831, G = 0.254014, T = 0.252512; substitution rates AC = 1.214378, AG = 2.729624, AT = 1.181789, CG = 1.027511, CT = 4.357413, and GT = 1.000000; gamma distribution shape parameter α = 0.294728. Bayesian analysis resulted in the average standard deviation of split frequencies as 0.009934.

Analysis 2: The combination of ITS, LSU, and *TEF1-α* sequence dataset comprised 156 taxa of *Apiospora*, and other related taxa in *Apiosporaceae*. *Sporocadus trimorphus* (CBS 114203) were selected as the outgroup taxon (Figure S1). The final alignment consisted of 2422 total characters, including gaps (ITS: 1–641 bp, LSU: 642–1524 bp, *TEF1-α*: 1525–2422 bp). The RAxML analysis of the integrated dataset yielded a best scoring tree with a final ML optimization likelihood value of −24,247.334486. The aligned sequence matrix contained 1182 distinct alignment patterns, with 37.22% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.245462, C = 0.240858, G = 0.258736, T = 0.254944; substitution rates AC = 1.136736, AG = 2.122936, AT = 1.134884, CG = 1.008342, CT = 4.360445, and GT = 1.000000; gamma distribution shape parameter α = 0.245115. Bayesian analysis resulted in the average standard deviation of split frequencies as 0.009872.

Analysis 3: The combination of ITS, LSU, *TUB2*, and *TEF1-α* sequence dataset comprised 31 taxa in *Apiospora locuta-pollinis/marii* clade. *Apiospora fermenti* KUC 2189 and *A. pseudospegazzinii* CBS 102052 were selected as the outgroup taxa (Figure 2). The final alignment consisted of 2735 total characters, including gaps (ITS: 1–626 bp, LSU: 627–1470 bp, *TUB2*: 1471–2296 bp, *TEF1-α*: 2297–2735 bp). The RAxML analysis of the integrated dataset yielded a best scoring tree with a final ML optimization likelihood value of −5544.668718. The aligned sequence matrix contained 363 distinct alignment patterns, with 25.58% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237367, C = 0.256117, G = 0.247967, T = 0.258550; substitution rates AC = 1.390352, AG = 3.604609, AT = 1.633776, CG = 0.564937, CT = 4.605733, and GT = 1.000000; gamma distribution shape parameter α = 0.020000. The maximum parsimonious dataset consisted of 2744 characters of which 2523 were constant, 119 parsimony-informative and 102 parsimony-uninformative. The parsimony analysis of the data matrix resulted in a single most parsimonious tree (TL = 266, CI = 0.883, RI = 0.914, RC = 0.808, HI = 0.117). Bayesian analysis resulted in the average standard deviation of split frequencies as 0.009680.

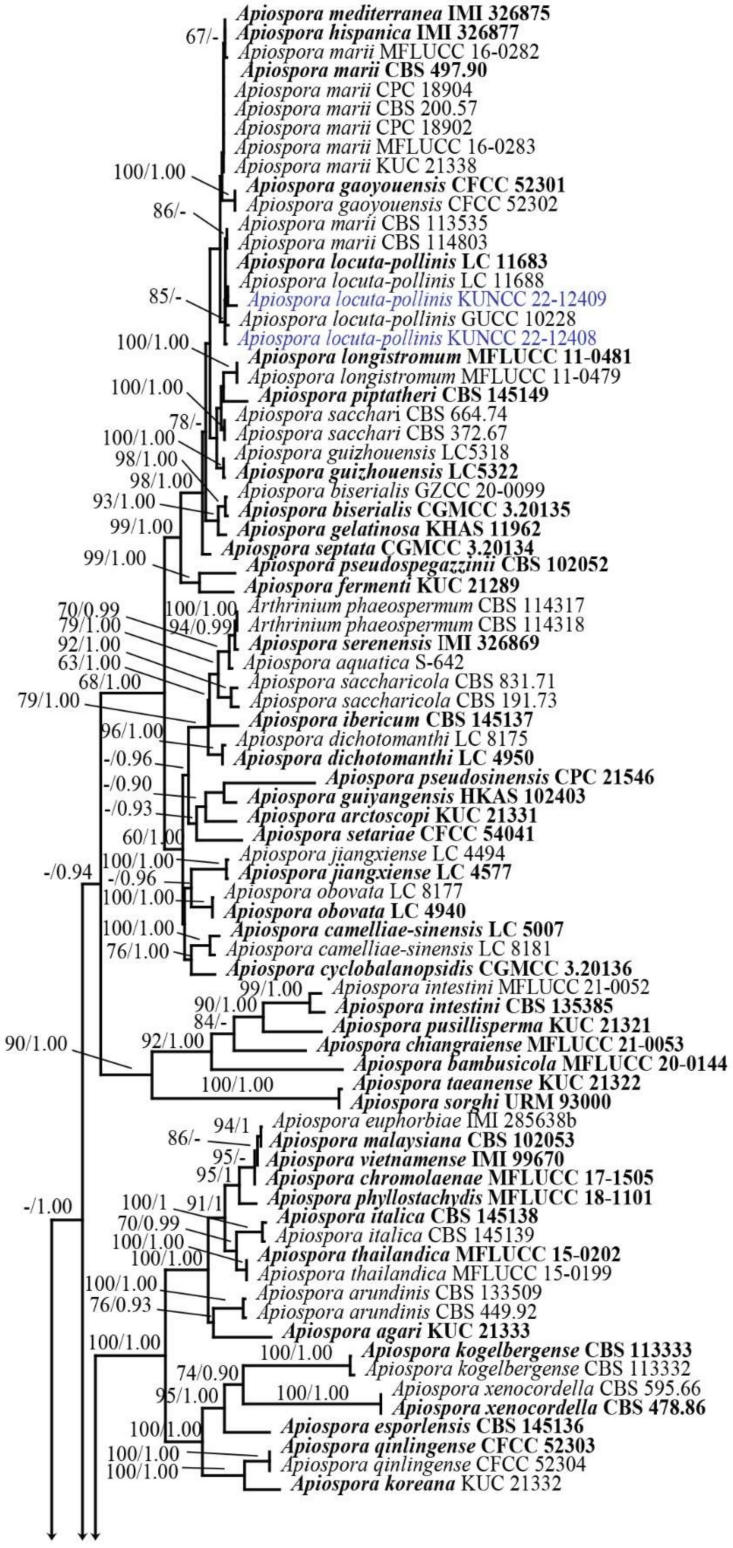

Figure 1. *Cont*.

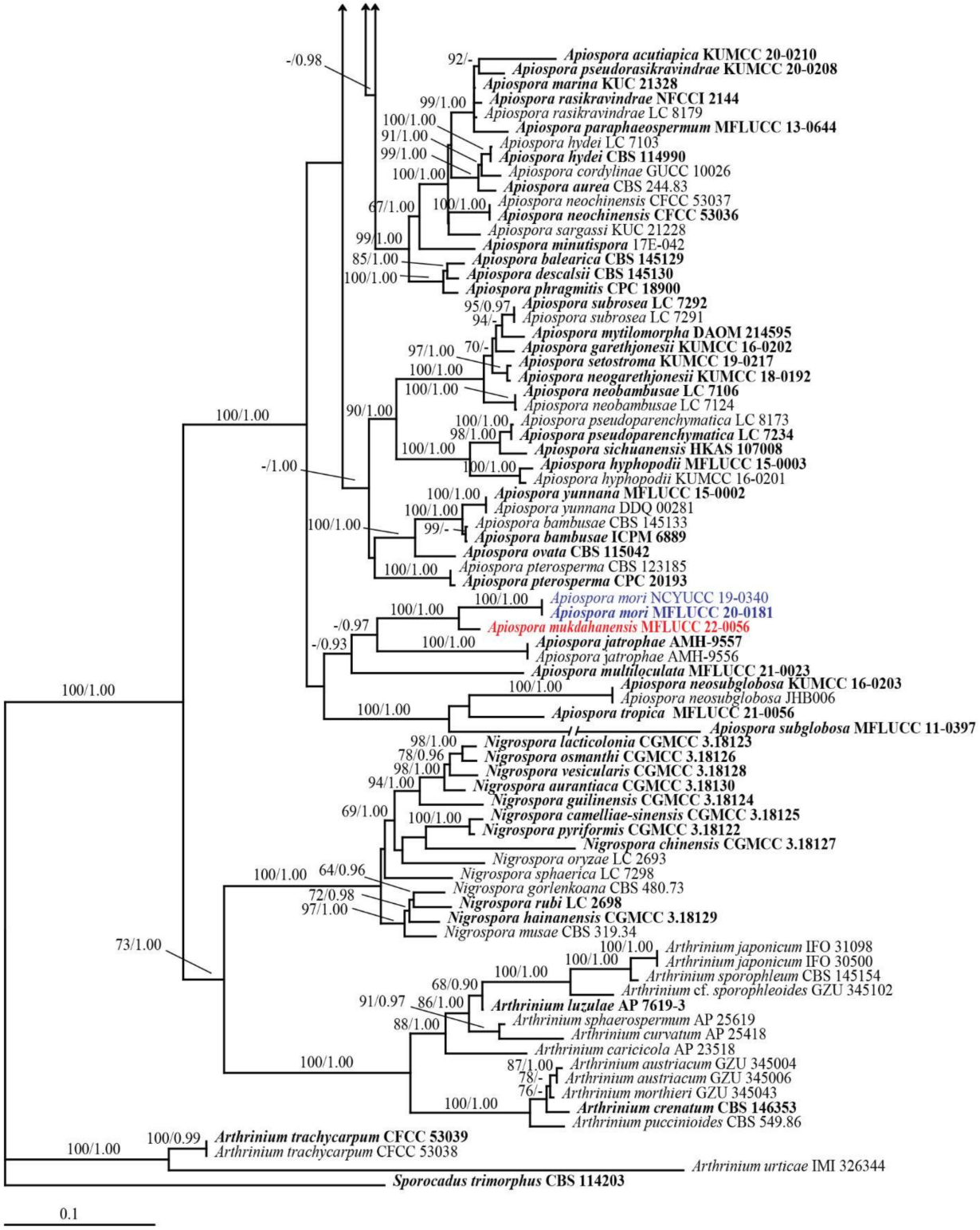

Figure 1. Phylogenetic tree retrieved from RAxML analyses of a combined ITS, LSU, *TUB2*, and *TEF1-α* data sequence of *Apiospora*, and other related taxa in the family *Apiosporaceae*. Bootstrap support values for ML equal or greater than 60% and Bayesian posterior probabilities greater than 0.90 are indicated at the nodes as ML/PP. The ex-type strains are in bold. The new species are in red and new record and new combination species are in blue. The tree is rooted to *Sporocadus trimorphus* (CBS 114203).

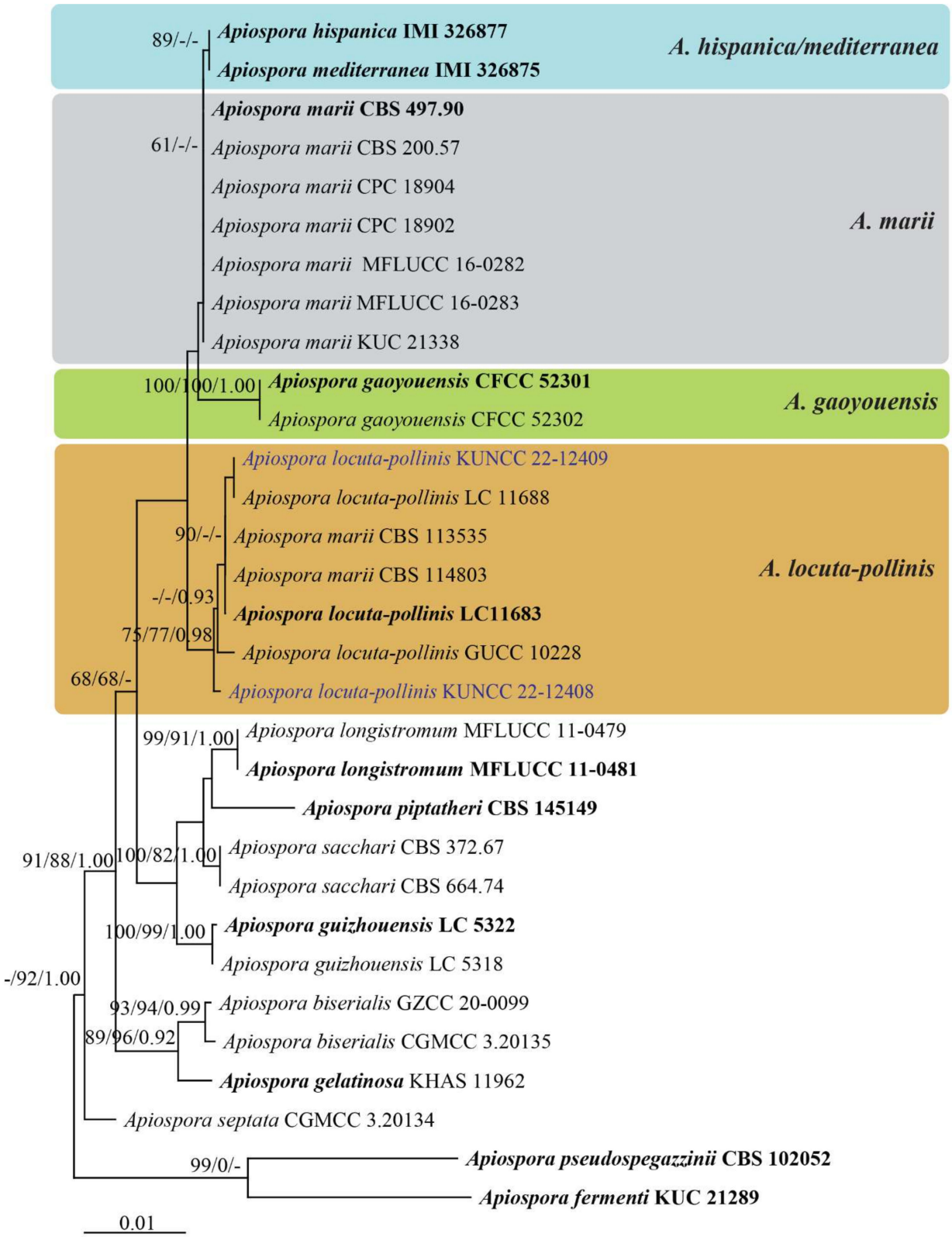

Figure 2. Phylogenetic tree retrieved from RAxML analyses of a combined ITS, LSU, *TUB2*, and *TEF1-α* data sequence of taxa in *Apiospora locuta-pollinis/marii* clade. Bootstrap support values for ML and MP equal or greater than 60% and Bayesian posterior probabilities greater than 0.90 are indicated at the nodes as ML/MP/PP. The ex-type strains are in bold. The new record strains are in blue. The tree is rooted to *Apiospora fermenti* KUC 2189 and *A. pseudospegazzinii* CBS 102052.

Phylogenetic analyses inferred from ML and BI analyses were not significantly different and showed congruent topologies. The overall tree topologies of the concatenated ITS-LSU-*TUB2*-*TEF1*-*α* sequence dataset (Figure 1) were also congruent with the tree topologies of a concatenated ITS-LSU-*TEF1*-*α* sequence matrix (Figure S1). However, the first analysis (Figure 1) revealed higher statistical support than that from the second analysis (Figure S1). Therefore, the phylogenetic results of the concatenated ITS-LSU-*TUB2*-*TEF1*-*α* sequence matrix was selected for discussion in this study. Phylogenetic results showed that the new species, *Apiospora mukdahanensis* formed a well-resolved clade sister to *A. mori* with significant support (100% ML/1.00 PP, Figure 1). The new strains, KUNCC 22-12408 and KUNCC 22-12409 clustered in the same clade with *A. locuta-pollinis* including the ex-type strain (LC 11683) with 85% ML support (Figure 1).

The multigene phylogeny based on the ITS-LSU-*TUB2*-*TEF1*-*α* sequence data of the species in the first clade (*Apiospora locuta-pollinis*/*marii* clade) revealed a similar result between ML, MP, and BI analyses. The results indicated that *A. locuta-pollinis* formed a well-supported clade with 75% ML/77% MP/0.98 PP support including the new strain KUNCC 22-12408 which formed a separated branch basal to other *A. locuta-pollinis* strains and the strain KUNCC 22-12409 shared the same branch length with *A. locuta-pollinis* (LC 11688) (Figure 2), whereas the type of *A. hispanica*, *A. mediterranea*, and *A. marii* were not well separated and clustered together with low support (Figure 2). *Apiospora gaoyouensis* formed an independent clade basal to *A. marii* with significant support (100% ML/100% MP/1.00 PP, Figure 2). The two strains of *A. marii* (CBS 113535 and CBS 114803) grouped within *A. locuta-pollinis* clade (Figure 2).

3.2. Taxonomy

3.2.1. *Apiospora mori* (Tennakoon, C.H. Kuo and K.D. Hyde) Monkai and Phookamsak, comb. nov.

Index Fungorum number: IF559913; Facesoffungi number: FoF 12871

Basionym: *Arthrinium mori* Tennakoon, C.H. Kuo and K.D. Hyde, Fungal diversity 108: 215 (2021).

Notes: *Arthrinium mori* was introduced by Tennakoon et al. [60] from dead leaves of *Morus australis* in Taiwan. Tennakoon et al. [60] noted that *Ar. mori* forms a well-supported branch sister to *Ar. jatrophae* with 86% ML/1.00 PP support, but differs from *Ar. jatrophae* in having smaller conidia (4.5–5.5 × 4–5 vs. 6.5–9.7 × 3.2–6.5 µm) [6]. In our phylogenetic analyses, *Ar. mori* constitutes an independent clade sister to *Apiospora mukdahanensis* with high support (100% ML/1.00 PP, Figure 1). Based on the phylogenetic analysis, 55 *Arthrinium* species were proposed as new combinations of *Apiospora*, but they did not include *Ar. mori* [2]. Thus, we transferred *Ar. mori* under the new combination *A. mori*, on the basis of morphological and molecular data.

3.2.2. *Apiospora mukdahanensis* Monkai and Phookamsak, sp. nov.

Index Fungorum number: IF559912; Facesoffungi number: FoF 12853; Figure 3.

Etymology: Referring to the locality, Mukdahan Province, Thailand, where the holotype was collected.

Holotype: MFLU 22-0104

Saprobic on dead bamboo leaves. Sexual morph: Undetermined. Asexual morph: *Conidiomata* sporodochia, rounded to ovoid, pulvinate, dark brown to black, 150–400 µm diam. *Mycelium* basal, consists of smooth, brown to dark brown, branched septate hyphae. *Conidiophore mother cells* arising from the mycelial mat, obpyriform to lageniform, dark brown, smooth, (6–)7–8.5(–10.5) × (2.5–)3.5–4(–5.5) µm (x = 8.5 × 4 µm, n = 12). *Conidiophores* basauxic, arising from conidiophore mother cells, cylindrical, pale brown, septate, with brown transversal septa, straight or flexuous. *Conidiogenous cells* polyblastic, smooth, hyaline to pale brown, ampulliform, cylindrical or lageniform, (6–)8.5–14(–20) × 1.5–2(–3) µm (x = 11 × 2 µm, n = 15). *Conidia* brown to dark brown, smooth to slightly roughened, thick-walled, globose to subglobose, or irregularly round in face view, lenticular in side

view (6–)–7.5(–8) × (5–)–6.5(–7) μm (x = 7 × 6 μm, n = 30), with a pale equatorial slit, and a central scar.

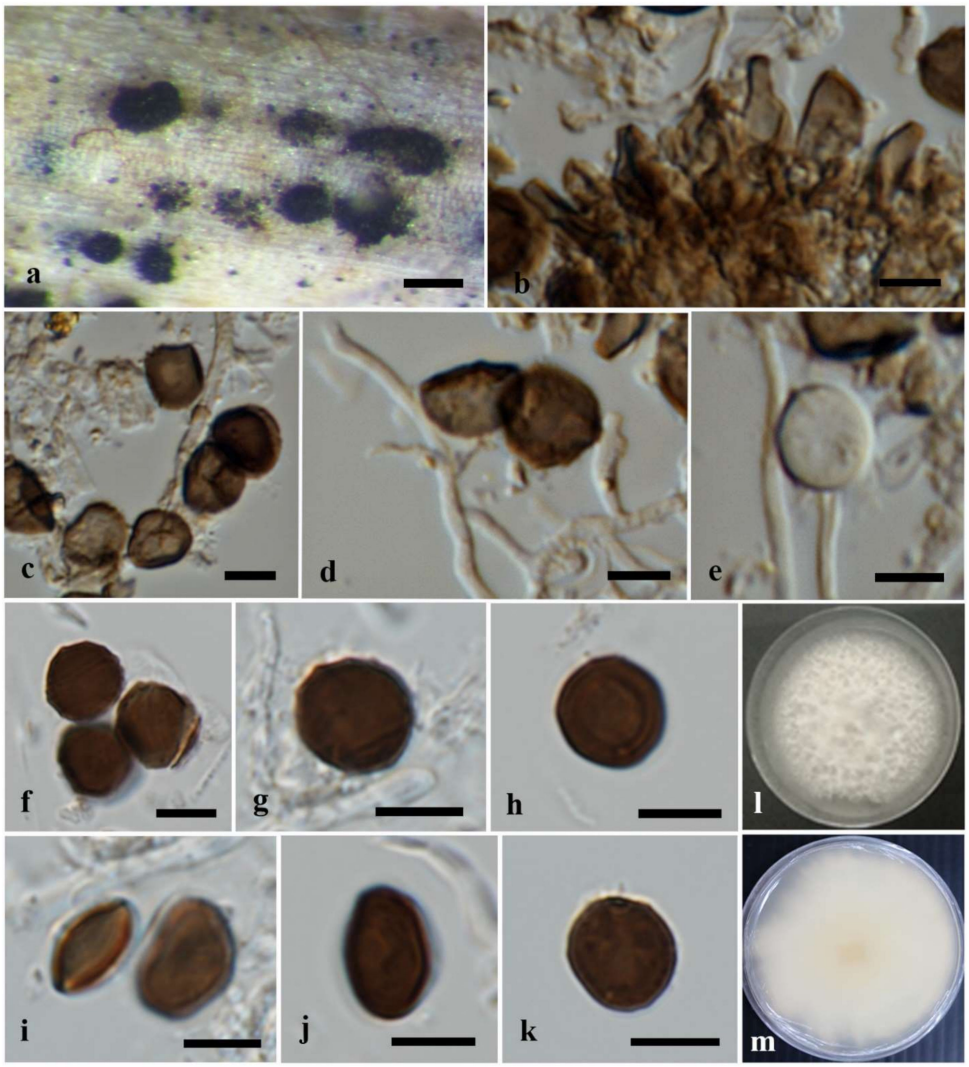

Figure 3. *Apiospora mukdahanensis* (MFLU 22-0104, holotype). (**a**) Conidiomata on host substrate. (**b**) Conidiophore mother cells. (**c**–**e**) Conidiophores, conidiogenous cells and conidia. (**f**–**k**) Conidia. (**l**,**m**) Colonies on PDA ((**l**) from above, (**m**) from reverse). Scale bars: (**a**) = 200 μm, (**b**–**k**) = 5 μm.

Culture characteristics: Colonies on PDA reached at 9 cm diam. in 2 weeks at 28 °C, flat, fluffy, spreading, with abundant aerial mycelium, irregular margin, white to cream, in reverse, white and pale yellowish in the center.

Material examined: THAILAND, Mukdahan Province, Tambon Phang Daeng, Amphoe Dong Luang, on dead bamboo leaves, 24 July 2019, E. Yasanthika, E2B-4 (MFLU 22-0104, holotype), ex-type living culture, MFLUCC 22-0056.

Notes: The nucleotide BLAST search of ITS region showed that *Apiospora mukdahanensis* (MFLUCC 22-0056) has high similarity with *Arthrinium* sp. strain 4–13 (99.19%), *Arthrinium* sp. strain NF34_TK10 and *A. mori* strain MFLU 18-2514 (98.47%). The nucleotide BLAST search of LSU region showed that *A. mukdahanensis* (MFLUCC 22-0056) has high similarity with *Apiospora* sp. strain NF34_TK10 (99.03%), *Apiospora* sp. strain JYZ-2021a (98.95%) and *A. mori* MFLU 18-2514 (98.83%). The nucleotide BLAST search of *TEF1-α* region showed that *A. mukdahanensis* (MFLUCC 22-0056) has high similarity with *A. phragmitis* strain MFLUCC 18-0099 (95.96%), *A. locuta-pollinis* strain LC 11689, LC 11688 (95.92%), and *Arthrinium* sp. strain MFLU 18-2333 (95.64%).

Based on phylogenetic analysis, *Apiospora mukdahanensis* formed an independent lineage sister to *A. mori* with 100% ML and 1.00 PP support (Figure 1). Morphologically, *Apiospora mukdahanensis* is distinct from *A. mori* in having larger conidia (6–8.1 × 5.1–6.9 vs. 4.5–5.5 × 4–5 µm) with slightly roughened wall, whereas *A. mori* has smooth-walled conidia [60]. The new species resembles *A. jatrophae* in having overlapping size range of conidia (6–8.1 × 5.1–6.9 vs. 6.5–9.7 × 3.2–6.5 µm) [6]. However, *A. jatrophae* differs from *A. mukdahanensis* in having thick multi-septate conidiophores with brown transverse septa and two types of conidia including smooth-walled, lenticular conidia and anomalous conidia [6]. The base-pair comparison of ITS gene region showed 4.1% base pair differences (21/508 bp) between *A. mukdahanensis* and *A. mori* (MFLUCC 20-0181 and NCYUCC 19-0340) and 9.6% base pair differences (48/498 bp) between *A. mukdahanensis* and *A. jatrophae* (AMH-9556, AMH-9557). However, the *TEF1-α* sequence data are not available for *A. mori* and *A. jatrophae* to compare with our new species.

3.2.3. *Apiospora locuta-pollinis* (F. Liu and L. Cai) Pintos and P. Alvarado, Fungal Systematics and Evolution 7: 206 (2021)

Index Fungorum number: IF837763; Facesoffungi number: FoF 05221, Figure 4.

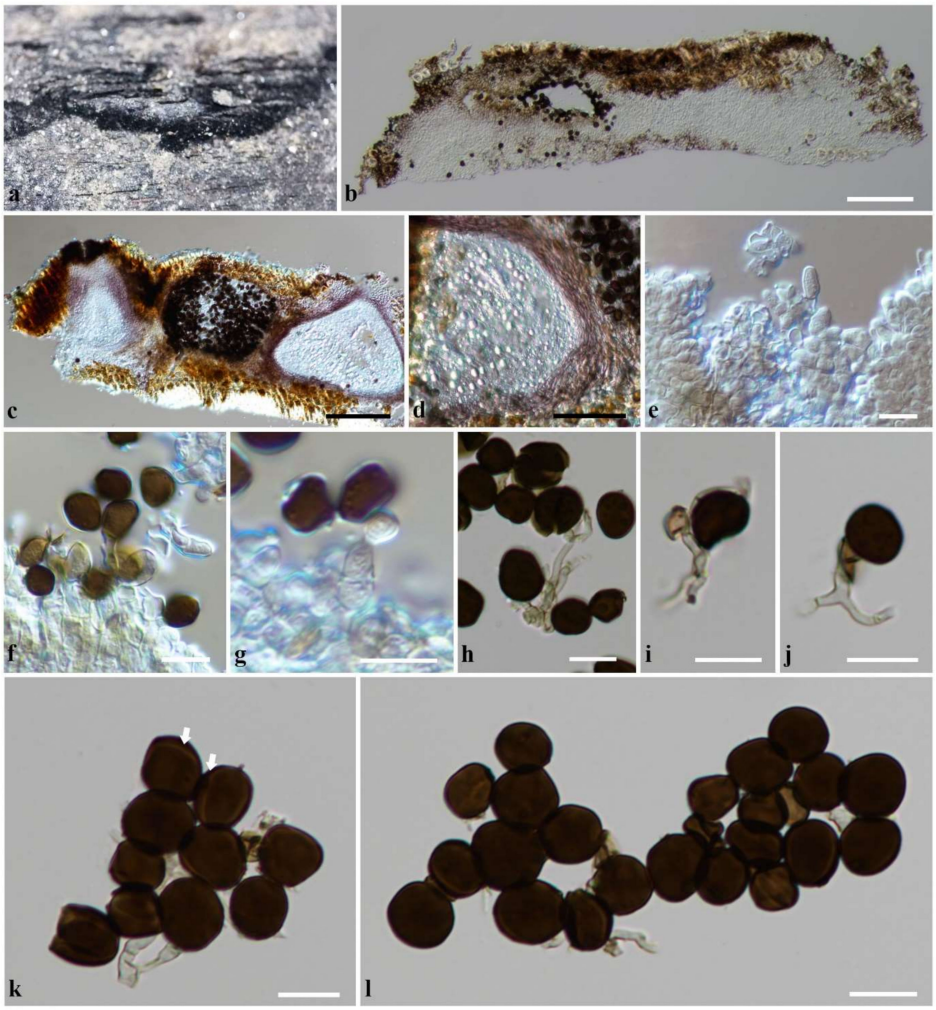

Figure 4. *Apiospora locuta-pollinis* (KUN-HKAS 124566). (**a**) Conidiomata on host substrate. (**b**,**c**) Section through the stromata. (**d**) Section through pycnidial wall. (**e**) Conidiophore mother cells. (**f**,**g**) Conidiogenous cells giving rise conidia. (**h**–**j**) Conidiophores (sterile cells attached to the conidiophore in (**i**,**j**)). (**k**,**l**) Conidia (equatorial slit is indicated by arrows in (**k**)). Scale bars: (**b**,**c**) = 100 µm, (**d**) = 50 µm, (**e**–**l**) = 10 µm.

Basionym: *Arthrinium locuta-pollinis* F. Liu and L. Cai, Mycosphere 9(6): 1094 (2018).

Saprobic on decaying stems of bamboo. Sexual morph: Immature state found associated with asexual morph on host. Asexual morph: *Conidiomata* associated with the sexual morph, pycnidial, raised, stromatic at base, 150–230 µm high, 450–830 µm diam., covered by black conidial masses on host surface, laying parallel to the longitudinal axis of the stem, clustered, solitary to gregarious, subepidermal to erumpent, globose to subconical, or hemispherical, uni- to multi-loculate; individual pycnidium 200–260 µm diam., 150–210 µm high, glabrous, ostiolate, opening by longitudinal splitting of the epidermis. *Pycnidial wall* (15–)20–40(–50) µm wide, of unequal thickness, slightly thick at sides towards apex, thinner at base, composed of several cell layers of reddish-brown to dark brown pseudoparenchymatous cells of *textura angularis* to *textura prismatica*, with outer layers intermixed with the host's tissues. *Conidiophore mother cells* (3.5–)4.5–9(–12) × 3.5–5.5 µm (x = 7.8 × 4.8 µm, n = 30), arising in dense packs from hyaline to light brown, irregularly angled palisade-like cells (4–9 × (2.8–)3–4.5 µm) in the stroma, subhyaline to pale brown, ampulliform to cylindrical, tapering towards rounded apex with small granules. *Conidiophores* (4–)5–10(–20) × (1.5–)2–3.5 µm (x = 8.9 × 2.2 µm, n = 30), basauxic, arising from conidiophore mother cells, conspicuous, short, septate, branched, smooth, pale brown, flexuous. *Conidiogenous cells* (3.5–)4.5–6.5(–9) × 2.5–4.5 µm (x = 6 × 3.5 µm, n = 30), polyblastic, straight or flexuous, cylindrical to subcylindrical or ampulliform, hyaline to light brown, smooth or with small granules, moderately brown, denticulate, sometimes flattened. *Conidia* (8–)9–13 × (7–)9–12 µm (x = 10.5 × 10 µm, n = 50), globose to obovoid, or ellipsoidal, dark brown, smooth-walled, with a paler equatorial slit, sometimes small piece of the denticle remains attached to the base of the conidium, (1–)2–5(–10) × 1.5–3 µm ($\overline{x}$ = 3.8 × 1.9 µm, n = 20). *Sterile cells* are brown, leaf-like, attached to the conidiophore.

Material examined: CHINA, Yunnan Province, Honghe Autonomous Prefecture, Honghe County, Honghe Hani Rice Terraces, on decaying stem of bamboo, 26 January 2021, R. Phookamsak, bn01 (KUN-HKAS 124566), living culture = KUNCC 22-12408; Honghe Hani Rice Terraces, on dead stem of bamboo, 26 January 2021, R. Phookamsak, bn11 (KUN-HKAS 124567), living culture = KUNCC 22-12409.

Notes: The nucleotide BLAST search of ITS region showed that *Apiospora locuta-pollinis* (KUNCC 22-12408 and KUNCC 22-12409) has high similarity with *A. marii* strain CBS 497.90, isolate A4, CPC 18904, CPC 18902, CBS 200.57, CBS 113535, *Arthrinium* sp. strain MFLUCC 16-0282, Fungal sp. BMP3011 (100%). The nucleotide BLAST search of LSU region showed that *A. locuta-pollinis* (KUNCC 22-12408 and KUNCC 22-12409) has high similarity with *A. guizhouensis* strain LC5318, *A. locuta-pollinis* isolate SICAUCC 22-0037, *A. sacchari* strain CBS 664.74, CBS 372.67, CBS 301.49, CBS 212.30, *A. marii* strain CBS 113535, *A. biserialis* isolate CS19-17 and *A. guizhouensis* strain KUMCC 20-0206 (100%). The nucleotide BLAST search of *TEF1-α* region showed that *A. locuta-pollinis* (KUNCC 22-12408) has high similarity with *A. marii* isolate GUCC 10214 (100%) and *A. locuta-pollinis* (KUNCC 22-12409) has high similarity with *A. locuta-pollinis* strain LC 11683, *A. marii* culture-collection CBS 113535, CBS 114803, GUCC 10254, GUCC 10227, *A. locuta-pollinis* strain LC 11688, LC 11689 (100%).

In our multigene phylogeny, two new strains (KUNCC 22-12408 and KUNCC 22-12409) shared a close relationship with *Apiospora locuta-pollinis* based on ML, MP, and BI analyses (Figures 1 and 2). The base-pair comparison of ITS gene regions indicated that strain KUNCC 22-12408 is identical to strain KUNCC 22-12409 and other *A. locuta-pollinis* strains, except for a 1 bp difference with GUCC 10228. In the base-pair comparison of *TEF1-α* gene regions, strain KUNCC 22-12409 had no base pair differences with all the *A. locuta-pollinis* strains and strain KUNCC 22-12408 had a 2 bp difference (0.5%) with KUNCC 22-12409 and other strains of *A. locuta-pollinis*.

Morphological characteristics of our new strains (KUNCC 22-12408 and KUNCC 22-12409) were compared with the type strain of *Apiospora locuta-pollinis* (LC 11683) (Table 2). Both new strains are similar to the type strain of *A. locuta-pollinis* in having globose to subglobose conidia with hyaline equatorial rim, however they have larger conidia (10.5 × 10

and 9.6 × 8 vs. 7.1 × 6.4 µm) (Table 2). The conidiogenous cells of strain KUNCC 22-12409 are more similar to the type strain (LC 11683) in being subglobose to ampulliform to doliiform, but with smaller size (3.2 × 2.2 vs. 4.9 × 3.8 µm) (Table 2)., whereas the strain KUNCC 22-12408 has cylindrical to subcylindrical or ampulliform conidiogenous cells with larger size compared to other strains (6 × 3.5 µm) (Table 2). The morphological description of *A. locuta-pollinis* was based on cultures and its conidiophores were reduced to conidiogenous cells [61]. Thus, the comparisons of conidiomata and conidiophores characteristics between these strains were not possible. In addition, we found some morphological differences between two new strains including strain KUNCC 22-12408 which had longer conidiophore mother cells and conidiogenous cells, but shorter conidiophores compared to strain KUNCC 22-12409 (Table 2).

Table 2. Morphological comparison among *Apiospora locuta-pollinis* strains.

Characteristics	*Apiospora locuta-pollinis* Strains		
	Type Strain (LC 11683)	**KUN-HKAS 124566**	**KUN-HKAS 124567**
Host/substrate	On PDA, MEA isolated from hive-stored pollen	Decaying stem of bamboo	Dead stem of bamboo
Conidiomata	ND	150–230 high × 450–830 µm long	177–243 high × 446–682 µm long
Pycnidial wall	ND	(15–)20–40(–50) µm wide	(12–)17–40(–84) µm wide
Conidiophore mother cell	ND	(3.5–)4.5–9(–12) × 3.5–5.5 µm ($\bar{x}$ = 7.8 × 4.8 µm)	(4.5–)5.5–8 × (3.5–)5.5–8 µm ($\bar{x}$ = 5.7 × 5.5 µm)
Conidiophores	Reduced to conidiogenous cells	Pale brown, septate, branched, flexuous, (4–)5–10(–20) × (1.5–)2–3.5 µm ($\bar{x}$ = 8.9 × 2.2 µm)	Hyaline to light brown, septate, branched, flexuous, (8–)11–15(–24) × (1.5–)2.5–4.5 µm ($\bar{x}$ = 13.8 × 2.9 µm)
Conidiogenous cells	Pale brown, subglobose to ampulliform to doliiform, 3–7.5 × 3–6 µm ($\bar{x}$ = 4.9 × 3.8 µm)	Hyaline to light brown, cylindrical to subcylindrical or ampulliform, (3.5–)4.5–6.5(–9) × 2.5–4.5 µm ($\bar{x}$ = 6 × 3.5 µm)	Hyaline to light brown, subglobose to ampulliform or doliiform, (2–)3–6 × (1–)2–4.5 µm ($\bar{x}$ = 3.2 × 2.2 µm)
Conidia	Pale brown to brown with hyaline equatorial rim, globose to subglobose, 5.5–9 × 4.5–8 µm ($\bar{x}$ = 7.1 × 6.4 µm), or ellipsoidal, 8–15 × 5–9.5 µm ($\bar{x}$ = 10.7 × 7.1 µm)	Dark brown with a paler equatorial slit, globose to obovoid, or ellipsoidal, (8–)9–13 × (7–)9–12 µm ($\bar{x}$ = 10.5 × 10 µm)	Dark brown with a paler equatorial slit, globose to obovoid, or ellipsoidal, (8–)9–11 × (5.5–)8–11 µm ($\bar{x}$ = 9.6 × 8 µm)
Sterile cells	Pale brown or brown, ellipsoidal to clavate, 11.5–21 × 3.5–8 µm ($\bar{x}$ = 15.7 × 5.7 µm)	brown, leaf-like, attached to the conidiophore	ND
References	Zhao et al. [61]	This study	This study

ND = Not determined.

Apiospora locuta-pollinis was previously isolated from hive-stored pollen of *Brassica campestris* in Hubei province, China [61], whereas the new strains KUNCC 22-12408 were isolated from decaying bamboo and KUNCC 22-12409 was isolated from dead bamboo in Yunnan province, China. Although there were some morphological variations among the new strains and the type strain of *A. locuta-pollinis*, the multigene phylogeny and DNA sequence comparisons (ITS and *TEF1-α* gene regions) did not provide the necessary support to delineate them as a distinct species. Therefore, we treated these strains as new records of *A. locuta-pollinis*. It is possible that the strain KUNCC 22-12408 is a new species due to the significant morphological and phylogenetic differences, which caused it to form a

separated branch to other *A. locuta-pollinis* strains. Further taxonomic studies are needed to resolve their conspecific status.

3.3. Host and Geographical Distribution of *Apiospora* Species

Based on species distribution data, *Apiospora* is widely distributed in temperate, subtropical, and tropical areas, including Africa, America, Asia, Australia, and Europe (Figure 5). The highest species diversity of *Apiospora* was found in Asia (e.g., China, India, Japan, Thailand) (Figure 5). However, the data reflect areas in which there have been reports of *Apiospora* species and may thus reflect hotspots of research, and not just species hotspots. Areas shown to be devoid of *Apiospora* species may just be areas that require further study. The host-substratum diversity of *Apiospora* species (Figure 6) showed that most species have been found exclusively associated with *Poaceae* (63%), including bamboo (31%) and non-bamboo (32%). The most common bamboo genera associated with *Apiospora* are *Bambusa*, *Phyllostachys*, and *Arundinaria* and the most common non-bamboo genera are *Saccharum*, *Phragmites*, and *Arundo*.

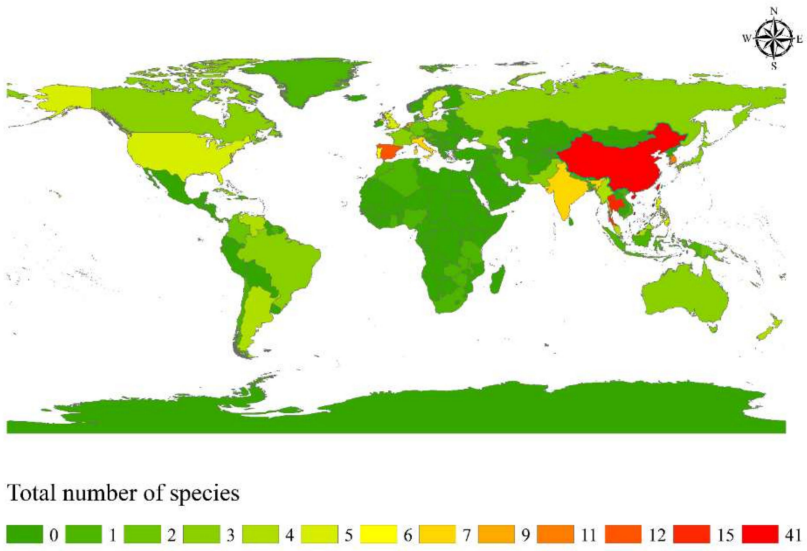

Figure 5. Species diversity hotspot countries of *Apiospora*.

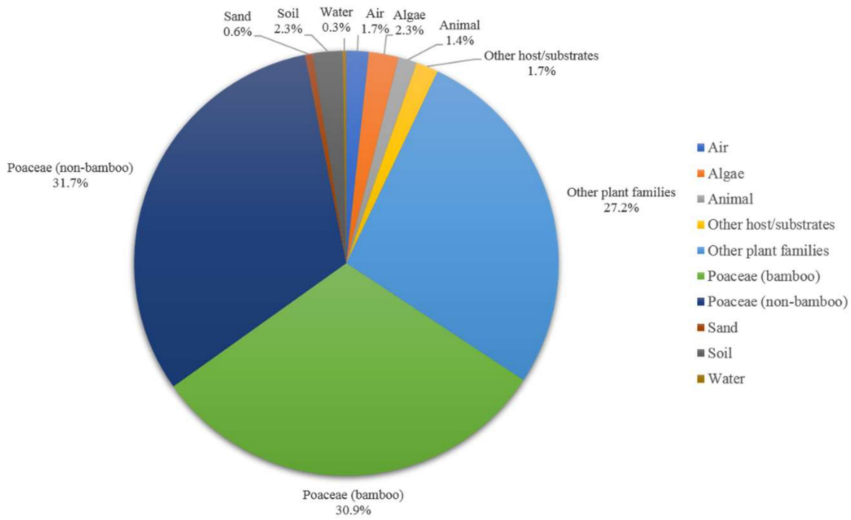

Figure 6. Host-substratum diversity of *Apiospora* species.

4. Discussion

Our study provides better insight into interspecific and intraspecific variation in *Apiospora*, particularly in the *A. locuta-pollinis/marii* clade. Phylogenetic analyses of four gene markers (ITS, LSU, *TUB2*, and *TEF1-α*) revealed the distinct relationships between *A. marii*, *A. locuta-pollinis*, and *A. gaoyouensis* (Figures 1 and 2). Morphologically, they have similar conidia characteristics (globose to elongate ellipsoid in surface, lenticular in side view) with an overlapping size range: 7.2–7.5 µm diam. in *Apiospora marii*, 5.5–9 µm diam. in *A. locuta-pollinis*, and 5–8 µm diam. in *A. gaoyouensis* (Table 3).

In the *Apiospora marii* clade, *A. hispanica* and *A. mediterranea* are not well-resolved (Figures 1 and 2). Morphologically, *A. marii* and *A. hispanica* have overlapping sizes of conidia (7.2–7.5 × 6.1–6.5 vs. 7.5–8.5 × 6.2–7.6 µm) (Table 3), whereas *A. mediterranea* has larger conidia (9–9.5 × 7.5–9 µm) (Table 3). The base-pair difference of ITS and *TUB2* sequence data indicated that they are consistent. However, the LSU sequence data of *A. hispanica* and *A. mediterranea* are in short length (320 base pairs) and their *TEF1-α* sequence data were lacking. The morphological reexamination and molecular data of the type specimens of *A. hispanica* and *A. mediterranea* are required to confirm a putative synonymy.

In the *Apiospora locuta-pollinis* clade, two strains of *A. marii* CBS 113535 and CBS 114803 clustered together (Figure 2). Pintos et al. [5] and Garin et al. [62] also reported that *A. marii* CBS 114803 had a well-supported lineage distant from the *A. marii* clade. Tian et al. [12] synonymized *A. pseudomarii* GUCC 10228 as *A. locuta-pollinis* based on morphology and phylogeny. Crous and Groenewald [3] provided the sequence data of these strains in which CBS 113535 was isolated from oats in Sweden and CBS 114803 was isolated from the culm of *Arundinaria hindsi* in Hongkong. Thus, we treated these strains as *A. locuta-pollinis* based on the phylogenetic evidence.

TUB2 and *TEF1-α* gene regions are essential phylogenetic markers for accurate identification of *Apiospora* species [3–5,9,38]. Most of the recent studies have used multigene phylogenetic analyses of integrated ITS, LSU, *TUB2*, and *TEF1-α* sequence data for *Apiospora* species delineation [2,5,10–12,24,25,38]. However, our phylogenetic analysis based on the integrated ITS, LSU, and *TEF1-α* sequence data also provided the necessary resolution to distinguish species of *Apiospora* (Figure S1). In addition, the *TEF1-α* gene region could support the species delineation between *A. marii* and *A. locuta-pollinis*. As they have a 10 bp difference (2.3%) in the *TEF1-α* gene region, whereas no difference was found in *TUB2* gene region. It seems that the *TUB2* gene region is uninformative for the separation of species in the *A. locuta-pollinis/marii* clade. Nevertheless, with the lack of *TUB2* sequences in our new strains, the *TEF1-α* gene region was not enough to resolve their placements within *A. locuta-pollinis* lineage. We suggest that *TUB2* gene and other protein-coding genes, such as *RPB2*, should be included for further phylogenetic analysis to confirm their actual identity and placements.

Our study also revealed significant morphological variation among *Apiospora locuta-pollinis* strains. This result was also observed for other *Apiospora* species. For instance, *A. yunnana*, introduced by Dai et al. [8], based on a sexual morph on bamboo culms, had larger conidia in cultures (15.5–26.5 µm diam.), compared to the strains CFCC 52311, CFCC 5231 which were isolated and described directly from bamboo culms (10–16 µm diam.) [9]. *Apiospora pseudoparenchymatica*, introduced by Wang et al. [4], and was isolated from living leaves of bamboo and described by its asexual morph. Feng et al. [11] found a new record of *A. pseudoparenchymatica* from decaying bamboo culms and noted the significant difference in the characteristics of conidiophores and conidiogenous cells of the new strain, GZCC 20–0117 (on WA) compared to the type specimen, LC 8173 (on PDA and MEA) [4]. The significant variation in morphology might be due to the differences in substrates (natural substrates or cultures), growth conditions, hosts, and habitats. This observation makes our finding all the more valuable, we found both new strains from the same host substrate and habitat. The only difference is strain KUNCC 22-12408, which was from the decaying

state and strain KUNCC 22-12409 was from the dead state of bamboo. Therefore, our study highlighted the great impact of environmental factors on morphological variation.

Geographically, Asia was found to be home to greatest diversity of *Apiospora* (Figure 5) and is likely the result of the rich diversity of bamboo genera/species, especially those in China [63]. More extensive sampling of these hosts will surely result in the discovery of additional new species. Although the host preference of *Apiospora* is the family *Poaceae*, there remains a number of species which have been found on other plant families (Figure 6), such as *A. euphorbiae* (on *Euphorbiaceae*, *Lauraceae*, *Pinaceae*, *Zingiberaceae*) [64], and *A. jiangxiensis* (on *Lauraceae*, *Primulaceae*, *Theaceae*) [4]., On the contrary, some species seem to be host-specific, such as *A. pterosperma* which has only been found on *Cyperaceae* (*Lepidosperma* and *Machaerina*) [3], and *A. rhododendri* has only been reported from Ericaceae (*Rhododendron*) [64]. Some species are ubiquitous, with a diverse range of hosts and habitats (e.g., *A. arundinis*, *A. marii*, *A. rasikravindrae*, *A. serenensis*) [2–5,8,12,64]. It is noteworthy that the asexual morph is more frequently discovered from different ecological habitats, and it is possible they are the cause of certain plant diseases [2,62]. For example, *A. arundinis* which has been isolated from soil, water, and numerous plant hosts [4,64], is also reported as causing some plant diseases, such as brown culm streak of *Phyllostachys praecox* [65] and the leaf spot of rosemary (*Salvia rosmarinus*) [66]. *Apiospora marii* has been isolated from air, sand, and different plant hosts [3,5], also reported as causing die-back of olives (*Olea europaea*) [62].

Table 3. Morphological comparison among *Apiospora* species in the *A. locuta-pollinis*/*marii* clade.

Characters	*Apiospora* Species				
	A. marii	***A. mediterranea***	***A. hispanica***	***A. gaoyouensis***	***A. locuta-pollinis***
Host/substrate	On MEA	On MEA, isolated from Pharmaceutical excipient, atmosphere and grass	On MEA, isolated from Beach sand	On leaves and culms of *Phragmites australis*	On PDA, MEA isolated from hive-stored pollen
Conidiomata	NR	NR	NR	Scattered to gregarious, superficial on leaf and culms, 1–15 long × 0.5–5 mm wide	NR
Conidiophore mother cell	Ampulliform	Ampulliform	Globose to subglobose	NR	NR
Conidiophores	Basauxic, mononematous, macronematous, brownish with transverse septa of the same color	Basauxic, macronematous, mononematous, nonseptate, colorless	Basauxic, mononematous, macronematous, brownish, without transverse septa	Reduced to conidiogenous cells	Reduced to conidiogenous cells
Conidiogenous cells	NR	Integrated and terminal	NR	Aggregated in clusters on hyphae, smooth	Pale brown, smooth, subglobose to ampulliform to doliiform, 3–7.5 × 3–6 µm
Conidia	Lateral or terminal, dark brown with hyaline rim, 7.2–7.5 × 6.1–6.5 µm	Lateral or terminal, brown with hyaline rim, smooth, lenticular in shape, 9–9.5 × 7.5–9 µm	Lateral or terminal, dark brown with hyaline rim, 7.5–8.5 × 6.2–7.6 µm	Brown with pale equatorial slit, smooth, granular, globose to elongate ellipsoid in surface view, 5–8 µm diam., lenticular in side view, 4–8 µm diam., with central basal scar	Pale brown to brown with hyaline equatorial rim, smooth, globose to subglobose, 5.5–9 × 4.5–8 µm, or ellipsoidal, 8–15× 5–9.5 µm
Sterile cells	NR	Pale brown, 7–7.5 × 6.5–7 µm	Irregularly shaped, much smaller than conidia, occasionally globose	Brown, elongated cells seldom intermingled among conidia	Pale brown or brown, smooth, ellipsoidal to clavate, 11.5–21 × 3.5–8 µm
References	Larrondo and Calvo [67]	Larrondo and Calvo [68]	Larrondo and Calvo [68]	Jiang and Tian [9]	Zhao et al. [61]

NR = Not reported.

5. Conclusions

Based on the rate of discovery, their diverse host ranges and different life-styles, the species number of *Apiospora* is likely to continue to increase in the future [39,48]. There are still a number of regions that have remained unstudied in terms of *Apiospora*, which will likely further add to the current list of species within this genus. A comprehensive survey of these unknown regions along with a polyphasic taxonomic study of *Apiospora* is necessary, especially focusing on *Poaceae* species, will enable a better understanding of host relationships and the ecological significance of this group of fungi.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14110918/s1, Figure S1: Phylogenetic tree retrieved from RAxML analyses of a combined ITS, LSU and TEF1-α sequence data of *Apiospora*, and other related taxa in the family *Apiosporaceae*. Bootstrap support values for ML equal or greater than 60% and Bayesian posterior probabilities greater than 0.90 are indicated at the nodes as ML/PP. The ex-type strains are in bold. The new species are in red and new record and new combination species are in blue. The tree is rooted to *Sporocadus trimorphus* (CBS 114203).

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References

1. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refined families of Sordariomycetes. *Mycosphere* **2020**, *11*, 305–1059. [CrossRef]
2. Pintos, Á.; Alvarado, P. Phylogenetic delimitation of *Apiospora* and *Arthrinium*. *Fungal Syst. Evol.* **2021**, *7*, 197–221. [CrossRef] [PubMed]
3. Crous, P.W.; Groenewald, J.Z. A phylogenetic re-evaluation of *Arthrinium*. *IMA Fungus* **2013**, *4*, 133–154. [CrossRef]
4. Wang, M.; Tan, X.M.; Liu, F.; Cai, L. Eight new *Arthrinium* species from China. *MycoKeys* **2018**, *1*, 1–24. [CrossRef]
5. Pintos, Á.; Alvarado, P.; Planas, J.; Jarling, R. Six new species of *Arthrinium* from Europe and notes about *A. caricicola* and other species found in *Carex* spp. hosts. *MycoKeys* **2019**, *49*, 15. [CrossRef] [PubMed]
6. Sharma, R.; Kulkarni, G.; Sonawane, M.S.; Shouche, Y.S. A new endophytic species of *Arthrinium* (*Apiosporaceae*) from *Jatropha podagrica*. *Mycoscience* **2014**, *55*, 118–123. [CrossRef]
7. Dai, D.Q.; Jiang, H.B.; Tang, L.Z.; Bhat, D.J. Two new species of *Arthrinium* (*Apiosporaceae*, Xylariales) associated with bamboo from Yunnan, China. *Mycosphere* **2016**, *7*, 1332–1345. [CrossRef]

8. Dai, D.Q.; Phookamsak, R.; Wijayawardene, N.N.; Li, W.J.; Bhat, D.J.; Xu, J.C.; Taylor, J.E.; Hyde, K.D.; Chukeatirote, E. Bambusicolous fungi. *Fungal Divers.* **2017**, *82*, 1–105. [CrossRef]
9. Jiang, N.; Li, J.; Tian, C.M. *Arthrinium* species associated with bamboo and reed plants in China. *Fungal Syst. Evol.* **2018**, *2*, 1–9. [CrossRef]
10. Senanayake, I.C.; Bhat, J.D.; Cheewangkoon, R.; Xie, N. Bambusicolous *Arthrinium* Species in Guangdong Province, China. *Front. Microbiol.* **2020**, *11*, 602773. [CrossRef]
11. Feng, Y.; Liu, J.K.J.; Lin, C.G.; Chen, Y.Y.; Xiang, M.M.; Liu, Z.Y. Additions to the genus *Arthrinium* (*Apiosporaceae*) from bamboos in China. *Front. Microbiol.* **2021**, *12*, 661281. [CrossRef] [PubMed]
12. Tian, X.G.; Karunarathna, S.C.; Mapook, A.; Promputtha, I.; Xu, J.C.; Bao, D.F.; Tibpromma, S. One new species and two new host records of *Apiospora* from bamboo and maize in Northern Thailand with thirteen new combinations. *Life* **2021**, *11*, 1071. [CrossRef] [PubMed]
13. Yin, C.; Luo, F.; Zhang, H.; Fang, X.; Zhu, T.; Li, S. First report of *Arthrinium kogelbergense* causing blight disease of *Bambusa intermedia* in Sichuan Province, China. *Plant Dis.* **2021**, *105*, 214. [CrossRef] [PubMed]
14. Mavragani, D.C.; Abdellatif, L.; McConkey, B.; Hamel, C.; Vujanovic, V. First report of damping-off of durum wheat caused by *Arthrinium sacchari* in the semi-arid Saskatchewan fields. *Plant Dis.* **2007**, *91*, 469. [CrossRef]
15. Aiello, D.; Gulisano, S.; Gusella, G.; Polizzi, G.; Guarnaccia, V. First report of fruit blight caused by *Arthrinium xenocordella* on *Pistacia vera* in Italy. *Plant Dis.* **2018**, *102*, 1853. [CrossRef]
16. Zhang, Z.F.; Liu, F.; Zhou, X.; Liu, X.Z.; Liu, S.J.; Cai, L. Culturable mycobiota from Karst caves in China, with descriptions of 20 new species. *Persoonia* **2017**, *39*, 1–31. [CrossRef]
17. Singh, S.M.; Yadav, L.S.; Singh, P.N.; Hepat, R.; Sharma, R.; Singh, S.K. *Arthrinium rasikravindrii* sp. nov. from Svalbard, Norway. *Mycotaxon* **2013**, *122*, 449–460. [CrossRef]
18. Das, K.; Lee, S.Y.; Choi, H.W.; Eom, A.H.; Cho, Y.J.; Jung, H.Y. Taxonomy of *Arthrinium minutisporum* sp. nov., *Pezicula neosporulosa*, and *Acrocalymma pterocarpi*: New records from soil in Korea. *Mycobiology* **2020**, *48*, 450–463. [CrossRef]
19. Luo, Z.L.; Hyde, K.D.; Liu, J.K.; Maharachchikumbura, S.S.N.; Jeewon, R.; Bao, D.F.; Bhat, D.J.; Lin, C.G.; Li, W.L.; Yang, J.; et al. Freshwater Sordariomycetes. *Fungal Divers.* **2019**, *99*, 451–660. [CrossRef]
20. Suryanarayanan, T.S. Fungal endosymbionts of seaweeds. In *Biology of Marine Fungi*; Raghukumar, C., Ed.; Springer: Dordrecht, The Netherlands, 2012; pp. 53–70.
21. Hong, J.-H.; Jang, S.; Heo, Y.M.; Min, M.; Lee, H.; Lee, Y.; Lee, H.; Kim, J.J. Investigation of marine-derived fungal diversity and their exploitable biological activities. *Mar. Drugs* **2015**, *13*, 4137–4155. [CrossRef]
22. Heo, Y.M.; Kim, K.; Ryu, S.M.; Kwon, S.L.; Park, M.Y.; Kang, J.E.; Hong, J.H.; Lim, Y.W.; Kim, C.; Kim, B.S.; et al. Diversity and ecology of marine Algicolous *Arthrinium* species as a source of bioactive natural products. *Mar. Drugs* **2018**, *16*, 508. [CrossRef] [PubMed]
23. Park, M.S.; Oh, S.-Y.; Lee, S.; Eimes, J.A.; Lim, Y.W. Fungal diversity and enzyme activity associated with sailfin sandfish egg masses in Korea. *Fungal Ecol.* **2018**, *34*, 1–9. [CrossRef]
24. Kwon, S.L.; Park, M.S.; Jang, S.; Lee, Y.M.; Heo, Y.M.; Hong, J.H.; Lee, H.; Jang, Y.; Park, J.H.; Kim, C.; et al. The genus *Arthrinium* (Ascomycota, Sordariomycetes, *Apiosporaceae*) from marine habitats from Korea, with eight new species. *IMA Fungus* **2021**, *12*, 13. [CrossRef] [PubMed]
25. Kwon, S.L.; Cho, M.; Lee, Y.M.; Kim, C.; Lee, S.M.; Ahn, B.J.; Lee, H.; Kim, J.J. Two unrecorded *Apiospora* species isolated from marine substrates in Korea with eight new combinations (*A. piptatheri* and *A. rasikravindrae*). *Mycobiology* **2022**, *50*, 46–54. [CrossRef]
26. He, Y.; Zhang, Z. Diversity of organism in the *Usnea longissima* lichen. *Afr. J. Microbiol. Res.* **2012**, *6*, 4797–4804.
27. Crous, P.W.; Wingfield, M.J.; Le Roux, J.J.; Richardson, D.M.; Strasberg, D.; Shivas, R.G.; Alvarado, P.; Edwards, J.; Moreno, G.; Sharma, R. Fungal Planet description sheets: 371–399. *Pers. Mol. Phylogeny Evol. Fungi* **2015**, *35*, 264. [CrossRef]
28. Rai, M.K. Mycosis in man due to *Arthrinium phaeospermum* var. *indicum*. First case report. *Mycoses* **1989**, *32*, 472–475.
29. Zhao, Y.M.; Deng, C.R.; Chen, X. *Arthrinium phaeospermum* causing dermatomycosis, a new record of China. *Acta Mycol. Sin.* **1990**, *9*, 232–235.
30. De Hoog, G.S.; Guarro, J.; Gené, J.; Figueras, M.J. *Atlas of Clinical Fungi*, 2nd ed.; CBS: Utrecht, The Netherlands, 2000; 1126p.
31. Shrestha, P.; Ibáñez, A.B.; Bauer, S.; Glassman, S.I.; Szaro, T.M.; Bruns, T.D.; Taylor, J.W. Fungi isolated from *Miscanthus* and sugarcane: Biomass conversion, fungal enzymes, and hydrolysis of plant cell wall polymers. *Biotechnol. Biofuels* **2015**, *8*, 1. [CrossRef]
32. Kunze, G. Zehn neue Pilzgattungen. *Mykol. Hefte* **1817**, *1*, 1–18.
33. Ellis, M.B. Dematiaceous Hyphomycetes: IV. *Mycol. Pap.* **1963**, *29*, 1–33.
34. Seifert, K.; Morgan-Jones, G.; Gams, W.; Kendrick, B. *The Genera of Hyphomycetes. [CBS Biodiversity Series 9]*; CBSKNAW Fungal Biodiversity Centre: Utrecht, The Netherlands, 2011; pp. 1–1997.
35. McNeill, J.; Barrie, F.R.; Buck, W.R.; Demoulin, V.; Greuter, W.; Hawksworths, D.L.; Herendeen, P.S.; Knapp, S.; Marhold, K.; Prado, J.; et al. International code of nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. *Regnum Veg.* **2012**, *154*, 1–140.

36. Senanayake, I.C.; Maharachchikumbura, S.S.N.; Hyde, K.D.; Bhat, J.D.; Jones, E.B.G.; McKenzie, E.H.C.; Dai, D.Q.; Daranagama, D.A.; Dayarathne, M.C.; Goonasekara, I.D.; et al. Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). *Fungal Divers.* **2015**, *73*, 73–144. [CrossRef]
37. Réblová, M.; Miller, A.N.; Rossman, A.Y.; Seifert, K.; Crous, P.; Hawksworth, D.; Adel-Wahab, M.A.; Cannon, P.F.; Daranagama, D.A.; De Beer, Z.W.; et al. Recommendations for competing sexual-asexually typified generic names in Sordariomycetes (except Diaporthales, Hypocreales, and Magnaporthales). *IMA Fungus* **2016**, *7*, 131–153. [CrossRef] [PubMed]
38. Jiang, H.B.; Hyde, K.D.; Doilom, M.; Karunarathna, S.C.; Xu, J.C.; Phookamsak, R. *Arthrinium setostromum* (*Apiosporaceae*, Xylariales), a novel species associated with dead bamboo from Yunnan, China. *Asian J. Mycol.* **2019**, *2*, 254–268. [CrossRef]
39. Phukhamsakda, C.; Nilsson, R.H.; Bhunjun, C.S.; de Farias, A.R.G.; Sun, Y.R.; Wijesinghe, S.N.; Raza, M.; Bao, D.-F.; Lu, L.; Tibpromma, S.; et al. The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. *Fungal Divers.* **2022**, *114*, 327–386. [CrossRef]
40. Species Fungorum. Available online: http://www.speciesfungorum.org (accessed on 1 September 2022).
41. Senanayake, I.; Rathnayaka, A.; Marasinghe, D.; Calabon, M.; Gentekaki, E.; Lee, H.; Hurdeal, V.; Pem, D.; Dissanayake, L.; Wijesinghe, S.; et al. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere* **2020**, *11*, 2678–2754. [CrossRef]
42. Index Fungorum. Available online: http://www.indexfungorum.org (accessed on 1 September 2022).
43. Jayasiri, S.C.; Hyde, K.D.; Ariyawansa, H.A.; Bhat, J.; Buyck, B.; Cai, L.; Dai, Y.C.; Abd-Elsalam, K.A.; Ertz, D.; Hidayat, I.; et al. The Faces of Fungi database: Fungal names linked with morphology, phylogeny and human impacts. *Fungal Divers.* **2015**, *74*, 3–18. [CrossRef]
44. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: Cambridge, MA, USA, 1990; Volume 18, p. 7.
45. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *J. Bacteriol.* **1990**, *172*, 4238–4246. [CrossRef]
46. O'Donnell, K.; Kistler, H.C.; Cigelnik, E.; Ploetz, R.C. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 2044–2049. [CrossRef] [PubMed]
47. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, *91*, 553–556. [CrossRef]
48. Bhunjun, C.S.; Niskanen, T.; Suwannarach, N.; Wannathes, N.; Chen, Y.J.; McKenzie, E.H.; Maharachchikumbura, S.S.N.; Buyck, B.; Zhao, C.-L.; Fan, Y.-G.; et al. The numbers of fungi: Are the most speciose genera truly diverse? *Fungal Divers.* **2022**, *114*, 387–462. [CrossRef]
49. Samarakoon, M.C.; Hyde, K.D.; Maharachchikumbura, S.S.N.; Stadler, M.; Jones, E.B.G.; Promputtha, I.; Suwannarach, N.; Camporesi, E.; Bulgakov, T.S.; Liu, J.K. Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of Xylariomycetidae (Sordariomycetes). *Fungal Divers.* **2022**, *112*, 1–88. [CrossRef]
50. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2017**, *20*, 1160–1166. [CrossRef] [PubMed]
51. Hall, T. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Proceedings of the Nucleic Acids Symposium Series, London, UK, 2–6 September 1999; pp. 95–98.
52. Miller, M.A.; Pfeiffer, W.; Schwartz, T. Creating the CIPRES science gateway for inference of large phylogenetic trees. In Proceedings of the 2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010; IEEE: New Orleans, LA, USA, 2010; pp. 1–8.
53. Nylander, J.A. *MrModeltest 2. Program Distributed by the Author*; Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University: Uppsala, Sweden, 2004.
54. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef] [PubMed]
55. Zhaxybayeva, O.; Gogarten, J.P. Bootstrap, Bayesian probability and maximum likelihood mapping: Exploring new tools for comparative genome analyses. *BMC Genom.* **2002**, *3*, 4. [CrossRef] [PubMed]
56. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Hoehna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
57. Swofford, D.L. *PAUP* Phylogenetic Analysis Using Parsimony * (and Other Methods)*; Version 4.0.; Sinauer Associates: Sunderland, UK, 2002.
58. Hillis, D.M.; Bull, J.J. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **1993**, *42*, 182–192. [CrossRef]
59. Rambaut, A.; Drummond, A.J. *FigTree: Tree Figure Drawing Tool*; Institute of Evolutionary Biology, University of Edinburgh: Edinburgh, Scotland, 2012.

60. Tennakoon, D.S.; Kuo, C.H.; Maharachchikumbura, S.S.N.; Thambugala, K.M.; Gentekaki, E.; Phillips, A.J.L.; Bhat, D.J.; Wanasinghe, D.N.; de Silva, N.I.; Promputtha, I.; et al. Taxonomic and phylogenetic contributions to *Celtis formosana*, *Ficus ampelas*, *F. septica*, *Macaranga tanarius* and *Morus australis* leaf litter inhabiting microfungi. *Fungal Divers.* **2021**, *108*, 1–215. [CrossRef]
61. Zhao, Y.Z.; Zhang, Z.F.; Cai, L.; Peng, W.J.; Liu, F. Four new filamentous fungal species from newly-collected and hive-stored bee pollen. *Mycosphere* **2018**, *9*, 1089–1116. [CrossRef]
62. Gerin, D.; Nigro, F.; Faretra, F.; Pollastro, S. Identification of *Arthrinium marii* as causal agent of olive tree dieback in *Apulia* (southern Italy). *Plant Dis.* **2020**, *104*, 694–701. [CrossRef] [PubMed]
63. Lobovikov, M.; Paudel, S.; Ball, L.; Piazza, M.; Guardia, M.; Ren, H.; Russo, L.; Wu, J.Q. *World Bamboo Resources: A Thematic Study Prepared in the Framework of the Global Forest Resources Assessment 2005*; Food and Agriculture Organization of The United Nations: Rome, Italy, 2007; Volume 18.
64. Farr, D.F.; Rossman, A.Y. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Available online: https://nt.ars-grin.gov/fungaldatabases/ (accessed on 1 September 2022).
65. Chen, K.; Wu, X.Q.; Huang, M.X.; Han, Y.Y. First report of brown culm streak of *Phyllostachys praecox* caused by *Arthrinium arundinis* in Nanjing, China. *Plant Dis.* **2014**, *98*, 1274. [CrossRef]
66. Bagherabadi, S.; Zafari, D.; Anvar, F.G. First report of leaf spot caused by *Arthrinium arundinis* on rosemary in Iran. *J. Plant Pathol.* **2014**, *96*, 4–126.
67. Larrondo, J.V.; Calvo, M.A. Two new species of *Arthrinium* from Spain. *Mycologia* **1990**, *82*, 396–398. [CrossRef]
68. Larrondo, J.V.; Calvo, M.A. New contributions to the study of the genus *Arthrinium*. *Mycologia* **1992**, *84*, 475–478. [CrossRef]