



Sexual and asexual morphs of a new *Apiospora* (*Apiosporaceae*) species on bamboo from northern Thailand

Tun ZL^{1,2,3}, Bhunjun CS^{1,2*}, Maharachchikumbura SSN⁴, Alotibi F⁵, Hyde KD^{1,2,5,6,7*}

¹Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

²School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

³Mushroom Research Foundation, 128 M.3 Ban Pa Deng T. Pa Pae, A. Mae Taeng, Chiang Mai 50150, Thailand

⁴School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 611731, P.R. China

⁵Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 22452, 11495 Riyadh, Saudi Arabia

⁶CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming, Yunnan 650201, P.R. China

⁷Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, P.R. China

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Abstract

Species of *Apiospora* are distributed globally and can be found in diverse ecological roles, including as endophytes, pathogens, and saprobes. In this study, we identified a novel species, *Apiospora pseudogarethjonesii*, through a multi-locus phylogenetic analysis using combined ITS, LSU, *tef-1α*, and *β-tub* loci, supported by morphological characteristics. This fungus was isolated from the dead bamboo branches at the Mushroom Research Center in northern Thailand. *Apiospora pseudogarethjonesii* differs from its phylogenetically related sister taxon *A. garethjonesii*, *A. mytilomorpha* and *A. subrosea* by having larger ascomata, smaller asci, and ascospores with thick, gelatinous sheaths, and larger conidia.

Keywords – 1 new species – bamboo – phylogeny – taxonomy

Introduction

Saccardo (1875) established the genus *Apiospora* (*Apiosporaceae*) with *A. montagnei* as the type species. *Apiospora* can be found in a wide range of habitats, hosts and geographic distribution either as endophytes, pathogens, or saprobes (Pintos et al. 2019, 2021, Hyde et al. 2020, Jayawardena et al. 2022, Monkai et al. 2022, Ai et al. 2024). Most species are saprobes and endophytes on various plant hosts, primarily *Poaceae* (Pintos 2019, Tian et al. 2021, Bhunjun et al. 2023, Liu et al. 2023, Ai et al. 2024). Certain *Apiospora* species act as plant pathogens, including *A. arundinis*, which is responsible for brown culm streak in bamboo, chestnut leaf spot, and barley kernel blight (Martínez-Cano et al. 1992, Mavragani et al. 2007, Chen et al. 2014, Aiello et al. 2018, Jiang et al. 2021, Yin et al. 2021, Agustí-Brisach et al. 2023), and have also been found on have been identified as an important source of bioactive chemicals with significant economic

potential in the pharmaceutical and biotechnology sectors (Hong et al. 2015, Shrestha et al. 2015, Heo et al. 2018, Mapook et al. et al. 2022). *Apiospora* has been found on a variety of substrates, including air, soil, freshwater, marine habitats, lichens, insect guts, and human tissues (Rai 1989, De et al. 2000, Suryanarayanan et al. 2011, He et al. 2012, Crous et al. 2013, 2015, Zhang et al. 2017, Wang et al. 2018, Luo et al. 2019, Das et al. 2020, Kwon et al. 2022).

The sexual morph of *Apiospora* includes multi-locular perithecial stromata with hyaline ascospores enclosed by a thick gelatinous sheath (Dai et al. 2016, 2017, Pintos et al. 2021, Liu et al. 2023). The asexual forms of *Apiospora* are distinguished by their basauxic conidiogenesis and spherical conidia (Hyde et al. 1998). The conidia typically appear lenticular or egg-shaped when viewed from the side, ranging from light brown to brown (Kunze 1817, Hyde et al. 1998, Dai et al. 2016, Liu et al. 2023). Most species of *Apiospora* are similar in morphology; thus, it is difficult to distinguish them without molecular data (Liu et al. 2023).

In this study, we introduce a new species, *Apiospora pseudogarethjonesii*, collected from dead bamboo branches in northern Thailand. Phylogenetic studies of combined ITS, LSU, *tef-1a*, and *β-tub* data confirm its phylogenetic placement within *Apiosporaceae*. The new species was identified and described using morpho-molecular analysis.

Materials & Methods

Sample collection, isolation and morphology

Dead branches of bamboo were collected from the Mushroom Research Center and brought to the laboratory in plastic bags. The single spore isolation technique was carried out to obtain pure cultures, following the methods outlined in Senanayake et al. (2020). The pure cultures were incubated at 25°C. A Motic SMZ 168 series stereo-microscope was used to observe the morphological characters. Digital images of micro-morphological features were captured with a Cannon 750D camera (Canon, Tokyo, Japan) attached to a Nikon ECLIPSE E600 compound microscope (Nikon, Tokyo, Japan). The photo plates were prepared using Adobe Photoshop CS6 version 2020 (Adobe Systems, USA) and the measurements were carried out using Tarosoft® Image Frame Work software version 0.97.

The holotype specimens and ex-type living cultures were deposited in the Mae Fah Luang University Herbarium (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), respectively. The new taxon was linked with Faces of Fungi and Index Fungorum numbers (Jayasiri et al. 2015, Index Fungorum 2024). The species descriptions and illustrations were submitted to the GMS microfungi (Chaiwan et al. 2021) and Fungalpedia webpage (Hyde et al. 2023).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from pure mycelia using the Omega Biotek DNA extraction kit following the manufacturer's guidelines. The polymerase chain reaction (PCR) comprised 12.5 µL of TaqPCR Master Mix (PROMEGA GoTaq®, Green), 1 µL of forward and reverse primer each, 2 µL of genomic DNA, and 8.5 µL of distilled water (total volume 25 µL). The PCR was carried out in an Applied Biosystems C1000 Touch™ Thermal cycler, with details of amplification listed in Table 1. Agarose gel electrophoresis was performed to check the quality of PCR products before sequencing at SolGent Co., South Korea.

Table 1. Primers and PCR conditions used in this study

Gene region	Primer pairs	PCR conditions	References
Internal transcribed spacer (ITS)	ITS5/ITS4	94°C/30 s, 56 C/50 s, 72°C/60 s	White et al. (1990)
partial 28S large subunit rDNA (LSU)	LR0R/LR5	94°C/30 s, 55°C/50 s, 72°C/60 s	Rehner & Samuels (1994)

Table 1 Continued

Gene region	Primer pairs	PCR conditions	References
Translation elongation factor 1-alpha gene (<i>tef-1α</i>)	EF983F/EF2218R	94°C/30 s, 55 C/50 s, 72°C/60 s	Rehner & Samuels (1994)
Beta-tubulin (<i>β-tub</i>)	T1/T22	95°C/60 s, 54°C/110 s, 72°C/120 s	O'Donnell & Cigelnik (1997)

Phylogenetic Analysis

Sequences were subjected to BLAST search in NCBI (<https://blast.ncbi.nlm.nih.gov/>). The reference sequences were downloaded from previous publications (Kwon et al. 2022, Li et al. 2023). The ITS, LSU, *tef-1 α* and *β -tub* sequences of related taxa were retrieved from GenBank (Tables 2). Each locus was aligned using MAFFT version 7 by applying the default settings (<https://mafft.cbrc.jp/alignment/server/>) (Kato et al. 2019). TrimAl was used to trim uneven ends (Capella-Gutiérrez et al. 2009). Single genes were concatenated using BioEdit version 7.0.5.2 (Hall et al. 1999). ALTER (<http://www.sing-group.org/ALTER/>) was used to convert Fasta files either to Nexus or Phylip format. The phylogenetic placement of the new taxa was confirmed based on single and multi-gene trees. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI) methods.

MrBayes version 3.1.2 was used to perform BI analysis to estimate posterior probabilities (PP) by using Markov chain Monte Carlo sampling (MCMC) (Huelsenbeck et al. 2001, Ronquist & Huelsenbeck 2003). Markov chains were run for 1,000,000 generations, with trees sampled every 100th generation. The first 25% of the sampled trees were discarded in the burn in phase. FigTree version 1.4 was used to visualize the phylograms (Rambaut & Drummond 2012) and edited using Microsoft PowerPoint (2016).

Table 2. GenBank accession numbers of isolates used in the phylogenetic analyses.

Species	Isolate/Strain	Accession numbers			
		ITS	LSU	<i>tef-1α</i>	<i>β-tub</i>
<i>Apiospora acutiapica</i>	KUMCC 20-0210 ^T	MT946343	MT946339	MT947360	MT947366
<i>A. agari</i>	KUC21333 ^T	MH498520	MH498440	MH544663	MH498478
<i>A. aquatica</i>	S-642	MK828608	MK835806	–	–
<i>A. arctoscopi</i>	KUC21331 ^T	MH498529	MH498449	MN868918	MH498487
<i>A. armeniaca</i>	SAUCC DL1831 ^T	OQ592540	OQ615269	OQ613313	OQ613285
<i>A. arundinis</i>	CBS 124788 ^T	KF144885	KF144929	KF145017	KF144975
<i>A. aseptata</i>	HKAS 129875	OR590341	OR590335	OR634949	OR634943
<i>A. aurea</i>	CBS 244.83 ^T	AB220251	KF144935	KF145023	KF144981
<i>A. babylonica</i>	SAUCC DL1841 ^T	OQ592538	OQ615267	OQ613311	OQ613283
<i>A. adinandrae</i>	SAUCC 1282B-1 ^T	OR739431	OR739572	OR753448	OR757128
<i>A. balearica</i>	CBS 145129 ^T	MK014869	MK014836	MK017946	MK017975
<i>A. bawanglingensis</i>	SAUCC BW0444 ^T	OR739429	OR739570	OR753446	OR757126
<i>A. biserialis</i>	CGMCC 3.20135 ^T	MW481708	MW478885	MW522938	MW522955
<i>A. camelliae-sinensis</i>	LC5007 ^T	KY494704	KY494780	KY705103	KY705173
<i>A. Chiangraiense</i>	MFLUCC21-0053 ^T	MZ542520	MZ542524	–	MZ546409

Table 2 Continued

Species	Isolate/Strain	Accession numbers			
		ITS	LSU	<i>tef-1a</i>	β - <i>tub</i>
<i>A. chromolaenae</i>	MFLUCC 17-1505 ^T	MT214342	MT214436	–	–
<i>A. cordylines</i>	GUCC 10027 ^T	MT040106	–	MT040127	MT040148
<i>A. cyclobalanopsidis</i>	CGMCC 3.20136 ^T	MW481713	MW478892	MW522945	MW522962
<i>A. dematiacea</i>	HKAS 129910 ^T	OR590346	OR590339	OR634953	OR634948
<i>A. descalsii</i>	CBS 145130 ^T	MK014870	MK014837	MK017947	MK017976
<i>A. dichotomanthi</i>	LC4950	KY494697	KY494773	KY705096	KY705167
<i>A. dicranopteridis</i>	HKAS 129877 ^T	OR590342	OR590336	OR634950	OR634944
<i>A. dongyingensis</i>	SAUCC 0302 ^T	OP563375	OP572424	OP573264	OP573270
<i>A. endophytica</i>	ZHKUCC:23-0007 ^T	OQ587997	OQ587984	OQ586063	OQ586076
<i>A. esporlensis</i>	CBS 145136 ^T	MK014878	MK014845	MK017954	MK017983
<i>A. euphorbiae</i>	IMI 285638b	AB220241	AB220335	–	AB220288
<i>A. fermenti</i>	KUC21289 ^T	MF615226	MF615213	MH544667	MF615231
<i>A. gaoyouensis</i>	CFCC 52301 ^T	MH197124	–	MH236793	MH236789
<i>A. garethjonesii</i>	NR_154736 ^T	NG_057131	–	–	–
<i>A. garethjonesii</i>	JHB004	KY356086	KY356091	–	–
<i>A. gelatinosa</i>	HKAS 111962 ^T	MW481706	MW478888	MW522941	MW522958
<i>A. globosa</i>	HKAS 129921 ^T	OR590347	OR590340	OR634954	–
<i>A. guangdongensis</i>	ZHKUCC 23-0005 ^T	OQ587995	OQ587983	OQ586061	OQ586074
<i>A. guiyangensis</i>	HKAS 102403 ^T	MW240647	MW240577	MW759535	MW775604
<i>A. guizhouensis</i>	LC5322	KY494709	KY494785	KY705108	KY705178
<i>A. hainanensis</i>	SAUCC 1681 ^T	OP563373	OP572422	OP573262	OP573268
<i>A. hainanensis</i>	SAUCC 1682	OP563372	OP572421	OP573261	OP573267
<i>A. hispanica</i>	IMI 326877 ^T	AB220242	AB220336	–	AB220289
<i>A. hydei</i>	CBS 114990 ^T	KF144890	KF144936	KF145024	KF144982
<i>A. hyphopodii</i>	MFLUCC 15-0003	KR069110	–	–	–
<i>A. hysterina</i>	ICPM 6889 ^T	MK014874	MK014841	MK017951	MK017980
<i>A. iberica</i>	AP10118 ^T	MK014879	MK014846	MK017955	MK017984
<i>A. intestini</i>	CBS 135835 ^T	KR011352	KR149063	KR011351	KR011350
<i>A. italica</i>	CBS 145138 ^T	MK014880	MK014847	MK017956	MK017985
<i>A. jatrophae</i>	CBS 134262 ^T	JQ246355	–	–	–
<i>A. jiangxiensis</i>	LC4577 ^T	KY494693	KY494769	KY705092	KY705163
<i>A. jinanensis</i>	SAUCC DL1981 ^T	OQ592544	OQ615273	OQ613317	OQ613289
<i>A. kogelbergensis</i>	CBS 113333 ^T	KF144892	KF144938	KF145026	KF144984
<i>A. koreana</i>	KUC21332 ^T	MH498524	MH498444	MH544664	MH498482
<i>A. lageniformis</i>	KUC21686 ^T	ON764022	ON787761	ON806626	ON806636
<i>A. locuta-pollinis</i>	LC11683 ^T	MF939595	–	MF939616	MF939622
<i>A. longistroma</i>	MFLUCC 11-0481	KU940141	KU863129	–	–
<i>A. malaysiana</i>	CBS 102053 ^T	KF144896	KF144942	KF145030	KF144988
<i>A. marianiae</i>	AP18219 ^T	ON692406	ON692422	ON677180	ON677186
<i>A. marii</i>	CBS 497.90	MH873913	KF144947	KF145035	KF144993

Table 2 Continued

Species	Isolate/Strain	Accession numbers			
		ITS	LSU	<i>tef-1a</i>	<i>β-tub</i>
<i>A. marina</i>	KUC21328 ^T	MH498538	MH498458	MH544669	MH498496
<i>A. machili</i>	SAUCC 1175A-4 ^T	OR739433	OR739574	OR753450	OR757130
<i>A. mediterranea</i>	IMI 326875 ^T	AB220243	AB220337	–	AB220290
<i>A. minutispora</i>	1.70E-39 ^T	LC517882	–	LC518889	LC518888
<i>A. montagnei</i>	AP301120 ^T	ON692408	ON692424	ON677182	ON677188
<i>A. montagnei</i>	AP19421	ON692418	ON692425	ON677183	ON677189
<i>A. montagnei</i>	CPC 18900	KF144909	KF144956	KF145043	KF145001
<i>A. mori</i>	MFLU 18-2514 ^T	MW114313	MW114393	–	–
<i>A. multiloculata</i>	MFLUCC 21-0023 ^T	OL873137	OL873138	–	OL874718
<i>A. mytilomorpha</i>	DAOM 214595 ^T	KY494685	–	–	–
<i>A. neobambusae</i>	LC7106	KY494718	KY494794	KY806204	KY705186
<i>A. neochinense</i>	CFCC 53036 ^T	MK819291	–	MK818545	MK818547
<i>A. neogarethjonesii</i>	HKAS 102408 ^T	MK070897	MK070898	–	–
<i>A. neosubglobosa</i>	JHB007 ^T	KY356090	KY356095	–	–
<i>A. obovata</i>	LC4940 ^T	KY494696	KY494772	KY705095	KY705166
<i>A. ovata</i>	CBS 115042 ^T	KF144903	KF144950	KF145037	KF144995
<i>A. paraphaeosperma</i>	MFLUCC 13-0644 ^T	KX822128	KX822124	–	–
<i>A. phyllostachydis</i>	MFLUCC 18-1101 ^T	MK351842	MH368077	MK340918	MK291949
<i>A. piptatheri</i>	CBS 145149 ^T	MK014893	MK014860	MK017969	–
<i>A. pseudohyphopodii</i>	KUC21680 ^T	ON764026	ON787765	ON806630	ON806640
<i>A. pseudomarii</i>	GUCC 10228 ^T	MT040124	–	MT040145	MT040166
<i>A. pseudoparenchymatica</i>	LC7234 ^T	KY494743	KY494819	KY705139	KY705211
<i>A. pseudorasikravindrae</i>	KUMCC 20-0208 ^T	MT946344	–	MT947361	MT947367
<i>A. pseudosinensis</i>	CPC 21546 ^T	KF144910	KF144957	KF145044	MN868936
<i>A. pseudosinensis</i>	SAUCC 0221	OP563377	OP572426	OP573266	OP573272
<i>A. pseudosinensis</i>	SAUCC 0222	OP563376	OP572425	OP573265	OP573271
<i>A. pseudospegazzinii</i>	CBS 102052 ^T	KF144911	KF144958	KF145045	KF145002
<i>A. pseudogarethjonesii</i>	MFLU 24-0011 ^T	PP317293	PP316704	PP320230	PP297920
<i>A. pterosperma</i>	CPC 20193 ^T	KF144913	KF144960	KF145046	KF145004
<i>A. pusillisperma</i>	KUC21321 ^T	MH498533	MH498453	MN868930	MH498491
<i>A. qinlingensis</i>	CFCC 52303 ^T	MH197120	–	MH236795	MH236791
<i>A. rasikravindrae</i>	LC5449	KY494713	KY494789	KY705112	KY705182
<i>A. sacchari</i>	CBS 212.30	KF144916	KF144962	KF145047	KF145005
<i>A. saccharicola</i>	CBS191.73	KF144920	KF144966	KF145051	KF145009
<i>A. sargassi</i>	KUC21228 ^T	KT207746	KT207696	MH544677	KT207644
<i>A. sasae</i>	CBS 146808 ^T	MW883402	MW883797	MW890104	MW890120
<i>A. septata</i>	CGMCC 3.20134 ^T	MW481711	MW478890	MW522943	MW522960
<i>A. serenensis</i>	IMI 326869 ^T	AB220250	AB220344	–	AB220297
<i>A. setariae</i>	CFCC 54041 ^T	MT492004	–	–	–

Table 2 Continued

Species	Isolate/Strain	Accession numbers			
		ITS	LSU	<i>tef-1α</i>	<i>β-tub</i>
<i>A. sichuanensis</i>	HKAS 107008 ^T	MW240648	MW240578	MW759536	MW775605
<i>A. sorghi</i>	URM 93000 ^T	MK371706	–	–	MK348526
<i>A. sphaerosperma</i>	CBS114314	KF144904	KF144951	KF145038	KF144996
<i>A. stipae</i>	CBS 146804	MW883403	MW883798	MW890082	MW890121
<i>A. subglobosa</i>	MFLUCC 11-0397 ^T	KR069112	KR069113	–	–
<i>A. subrosea</i>	LC7292 ^T	KY494752	KY494828	KY705148	KY705220
<i>A. thailandica</i>	LC5630	KY494714	KF144970	KY705113	KY806200
<i>A. vietnamensis</i>	IMI 99670 ^T	KX986096	KX986111	–	KY019466
<i>A. wurfbainiae</i>	ZHKUCC 23-0009 ^T	OQ587999	OQ587987	OQ586065	OQ586077
<i>A. xenocordella</i>	CBS 478.86 ^T	KF144925	KF144970	KF145055	KF145013
<i>A. xishuangbannaensis</i>	KUMCC 21-0695 ^T	ON426832	OP363248	OR025969	OR025930
<i>A. yunnana</i>	MFLUCC 15-0002 ^T	KU940147	KU863135	–	–
<i>A. yunnanensis</i>	ZHKUCC 23-0014 ^T	OQ588004	OQ587992	OQ586070	OQ586083
<i>Arthrinium caricicola</i>	CBS 145127 ^T	MK014871	MK014838	MK017948	MK017977

Note: Type strains are denoted with ^T, the newly generated sequence is denoted in red and “–” data not available.

Results

Sequence alignment and phylogenetic analyses

The concatenated ITS, LSU, *tef-1α* and *β-tub* sequence data comprised 108 strains representing 99 *Apiospora* species. *Anthrinum caricicola* was used as the outgroup. The combined alignment consisted of 2,461 characters, including gaps (ITS: 1–669 bp; LSU: 670–1406 bp; *tef-1α*: 1407–2091 bp and *β-tub*: 2092–2461 bp) with 25.05% undetermined characters or gaps. The ML phylogram generated was used as the backbone tree (Fig. 1). The best-scoring ML tree had an optimization likelihood value of -30442.542292. Base frequencies were A = 0.238243, C = 0.246624, G = 0.259151, and T = 0.255981; substitution rates were AC = 1.384020, AG = 3.446683, AT = 1.244146, CG = 0.958882, CT = 5.457046, and GT = 1.000000, with a tree length of 2.832374 and α shape parameter of 0.649838. The best-fit models generated from MrModeltest under the Akaike information criterion (AIC) were HKY+I+G for *rpb2* and TrNef+I+G for ITS, LSU, *tef-1α* and *β-tub* sequences.

Taxonomy

Apiospora pseudogarethjonesii Z.L. Tun, sp. nov.

Fig. 2

Index Fungorum number: IF 902649, Facesoffungi number: FoF 16742

Etymology: Refers to the morphological similarity with *Apiospora garethjonesii*

Holotype: MFLU24-0011

Saprobic on dead bamboo branch. **Sexual morph:** *Stromata* 400–678 μ m high, scattered to gregarious, partly immersed, becoming erumpent to superficial, raised, dark brown, in linear rows, with a slit-like opening, multi-loculate. *Ascomata* 210–235 μ m high, 282–310 μ m diam (\bar{x} = 222.5 \times 297.7 μ m, n = 5), perithecial, arranged in rows, clustered, gregarious, with 3-5 perithecia forming

groups immersed in stromata to erumpent through host surface, ellipsoidal to subglobose, dark brown, membranous. *Ostiole* present. *Peridium* 2–7 μm wide, with two layers; outer layer composed of dark brown, thick cells of *textura angularis*; inner layer white, thin, with *textura angularis*. *Hamathecium* composed of dense paraphyses, 4–7 μm wide, filamentous, longer than asci. *Asci* 52–70 \times 19–16 μm (\bar{x} = 58.50 \times 16.26 μm , n = 20), 8-spored, unitunicate, clavate, with a short pedicel, apically rounded. *Ascospores* 20–25 \times 8–10 μm (\bar{x} = 24.48 \times 8.8 μm , n = 20) 1-septate, ellipsoidal, curved at the lower cell, with many guttules, hyaline, smooth-walled, thick gelatinous sheath 8–7 μm wide. **Asexual morph:** On PDA, *Hyphae* 2.5–4 μm in diameter, hyaline, septate, branched. *Conidiophores* reduced to the conidiogenous cells. Conidiogenous cells 4–6 μm \times 3–5 μm (\bar{x} = 5.05 μm \times 3.95 μm , n = 20), aggregated in clusters on hypha, pale brown, ampuliform or cylindrical. *Conidia* 15–20 μm long (n = 20), brown, smooth, globose to subglobose in surface view, and 15–21 μm long (n = 20), lenticular, with a paler equatorial slit in side view.

Culture characteristics – Colonies on PDA reaching 6 cm diam., after 6 days at 27°C, from above white to white yellow radiating outwards, dense, circular, flattened with smooth surface; reverse yellow-white.

Material examined – Thailand, Chiang Mai Province, forests around the Mushroom Research Center, on dead branches bamboo, 14 November 2022, ZL Tun, M2 (MFLU 24-0011, **holotype**); ex-type living culture (MFLUCC 24-0320).

Notes – *Apiospora pseudogarethjonesii* (MFLUCC 24-0320) clustered with *A. garethjonesii* (NR_154736, JHB004), *A. mytilomorpha* (DAOM 214595) and *A. subrosea* (LC7292) (Fig. 1). We compared the morphology and genetic distances of *A. pseudogarethjonesii* (MFLUCC 24-0320) with *A. garethjonesii* (NR_154736, JHB004), *A. mytilomorpha* (DAOM 214595) and *A. subrosea* (LC7292). *Apiospora pseudogarethjonesii* have larger ascospores (210–235 \times 282–310 μm) compared to *A. garethjonesii* (177–235 \times 141–232 μm) (Dai et al. 2016). The asci of *A. pseudogarethjonesii* are smaller (52–70 \times 19–16 μm) compared to *A. garethjonesii* (125–154 \times 35–42 μm). In addition, *A. pseudogarethjonesii* has smaller ascospores (20–25 \times 8–10 μm) compared to *A. garethjonesii* (30–42 \times 11–16 μm). *Apiospora pseudogarethjonesii* has a thick gelatinous sheath; however, *A. garethjonesii* has a faint gelatinous sheath (Dai et al. 2016). We also compared the asexual morphology of the two species. *Apiospora pseudogarethjonesii* has shorter conidiogenous cells (4–6 \times 3–5 μm) compared to *A. garethjonesii* (6–20 \times 3–7 μm) (Feng et al. 2021). The sexual morphs of *A. pseudogarethjonesii* and *A. mytilomorpha* have not been reported; therefore, we only compared the asexual morphs. The conidia of *A. mytilomorphum* are dark brown, fusiform or navicular, while the conidia of *A. pseudogarethjonesii* are brown, smooth, globose to subglobose (Wang et al. 2018). *Apiospora mytilomorphum* and *A. pseudogarethjonesii* also differ in the size of their conidia (15–20 \times 15–21 μm vs. 20–30 \times 6–8.5 μm , respectively). The sexual morphs of *Apiospora pseudogarethjonesii* and *A. subrosea* have not been reported; therefore, we only compared the asexual morphs. *Apiospora pseudogarethjonesii* has larger conidia (15–20 \times 15–21 μm) compared to *A. subrosea* (12.0–17.5 \times 9.0–16.0 μm) (Wang et al. 2018).

The inter-species genetic distances between *Apiospora pseudogarethjonesii* (MFLUCC 24-0320) and *A. garethjonesii* (HKAS 96289) were 2.24% different across ITS (579 bp) and no difference in the LSU sequences. *Apiospora garethjonesii* lacks *β -tub* and *tef-1 α* sequences and could not be compared. *Apiospora pseudogarethjonesii* (MFLUCC 24-0320) and *A. mytilomorpha* (DAOM 214595) differ by 2.1% across ITS (7/323bp), and *A. mytilomorpha* lack LSU, *β -tub* and *tef-1 α* to compare. *Apiospora pseudogarethjonesii* (MFLUCC 24-0320) and *A. subrosea* (LC7292) were 1.58% different across ITS (569 bp), 1.57% across *tef-1 α* (570 bp), 3% across *tef-1 α* (401 bp) and were similar based on LSU sequences. As a result of the phylogenetic and morphological comparisons, *A. pseudogarethjonesii* is described as a new species.

Discussion

Hawksworth et al. (2011) synonymized *Apiospora* with *Arthrinium* based on the one fungus-one name approach. Crous and Groenewald (2013) validated the genetic identity of many *Arthrinium* (= *Apiospora*) species based on the one fungus, one name principle. Pintos and

Alvarado (2021) subsequently distinguished *Apiospora* and *Arthrimum* by examining the type species of both genera and using multigene phylogeny. Most *Apiospora* species are found in *Poaceae*, while some are found in various plant host families (Lu et al. 2023, Ai et al. 2024). However, the majority of *Arthrimum* species are located in *Juncaceae* and *Cyperaceae* (Pintos & Alvarado 2021). Several studies have shown that it is challenging to identify *Arthrimum* species solely based on morphology. Multi-gene phylogenetic analyses based on ITS, LSU, *tef-1a*, and *tub2* sequences are required in the identification and classification of *Arthrimum* (Crous et al. 2013, Dai et al. 2016, 2017, Wang et al. 2018, Yang et al. 2019, Li et al. 2023). In recent years, the amount of DNA sequencing data available for *Apiospora* species has steadily increased, resulting in the identification of 93 *Apiospora* species (Crous et al. 2013, Wang et al. 2018, Pintos et al. 2019, Ai et al. 2024). In this study, our novel species was identified based on ITS, LSU, *tef-1a*, and *tub2* sequences.

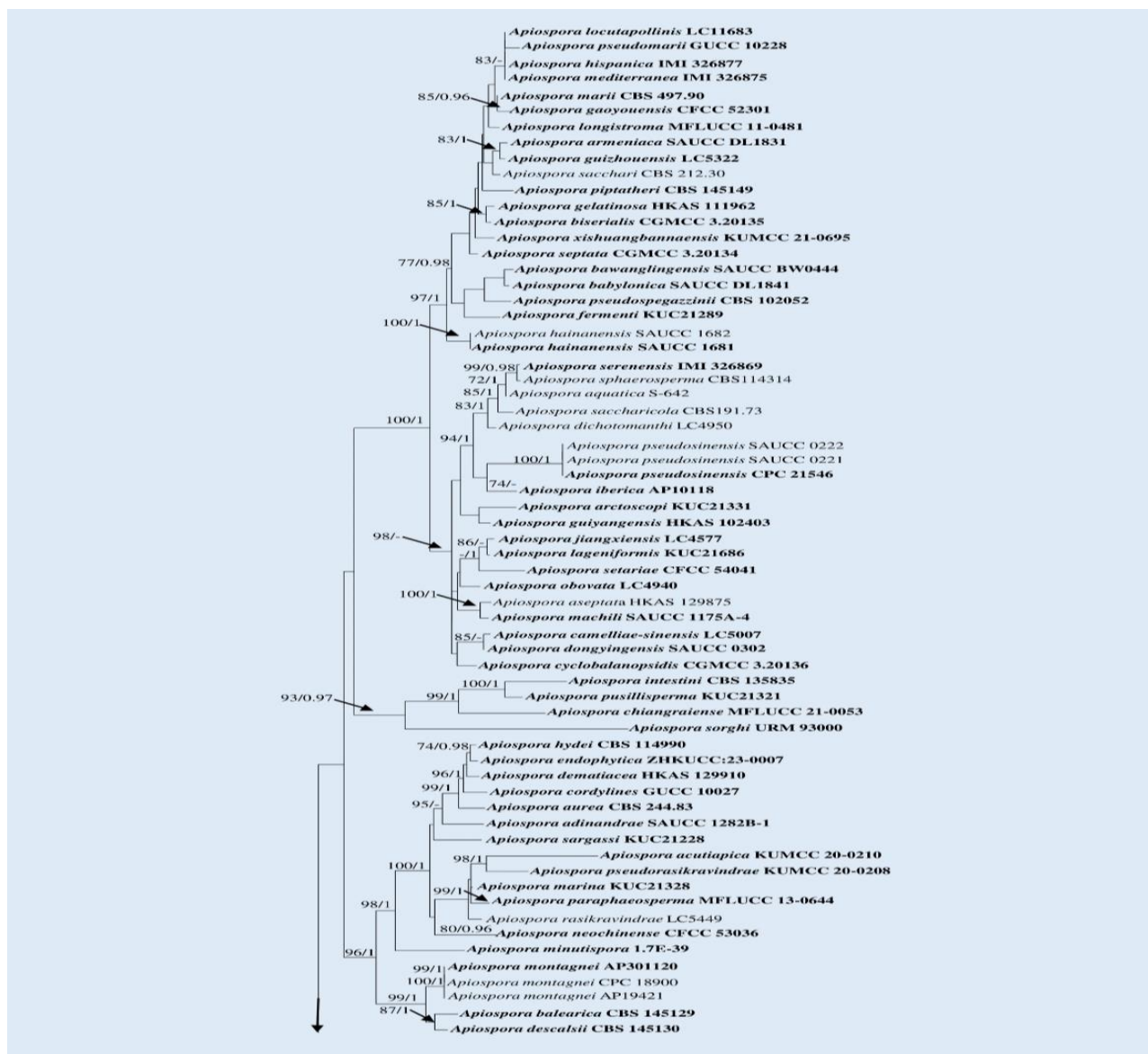


Fig. 1 – Phylogram generated from ML based on the combined ITS, LSU, *tef-1a* and *β-tub* sequences of representative *Apiospora* taxa. *Arthrimum caricicola* (CBS 145127) was used as the outgroup. Bootstrap support values (ML ≥69%) and Bayesian posterior probabilities (PP ≥0.95) are given at respective nodes. Hyphens (-) represent support values below 69% (ML) and 0.95 (PP). The type strains are bolded, and the new isolate is red.

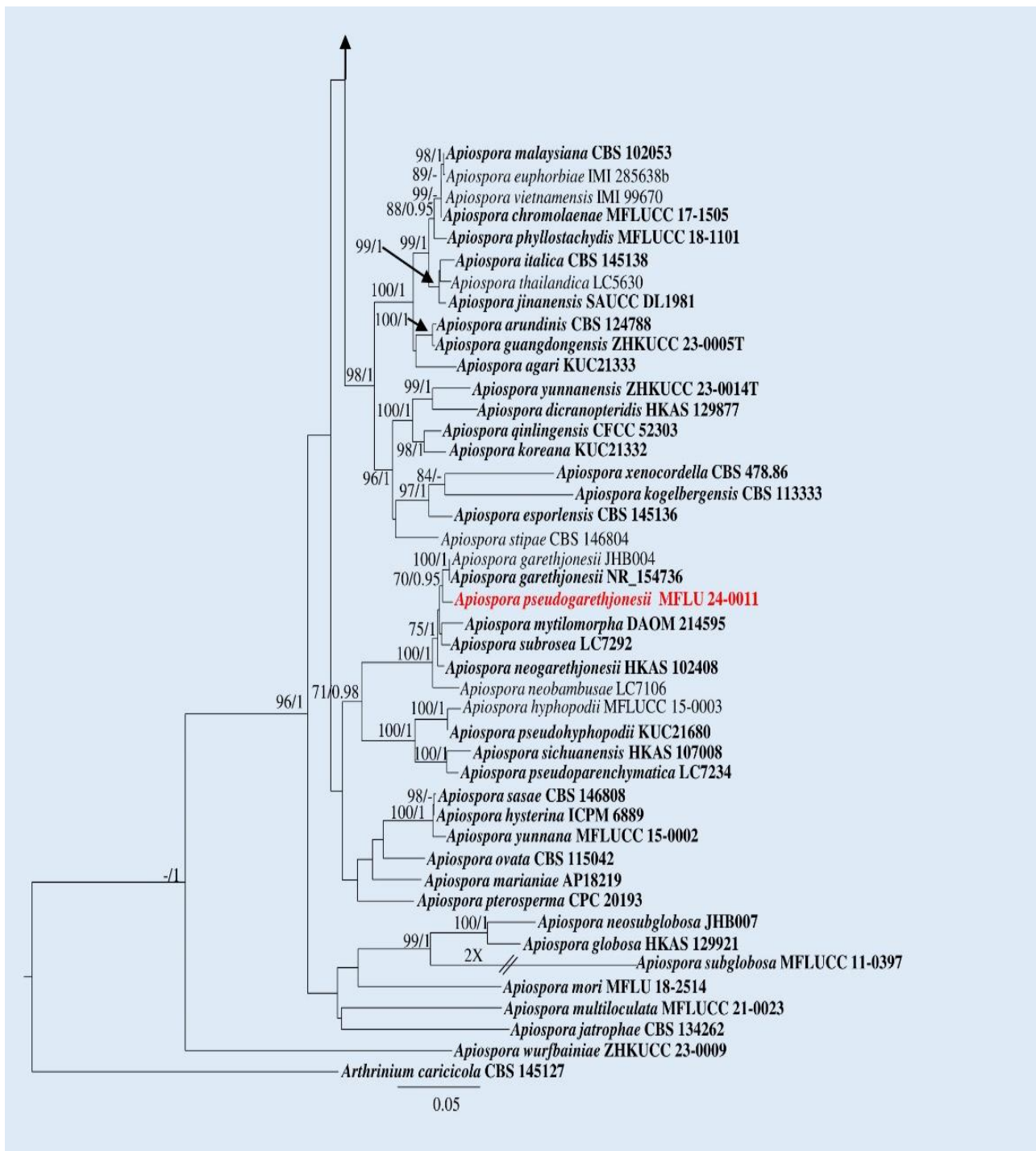


Fig. 1 – Continued.

The majority of *Apiospora* species are associated with *Poaceae* (63%), including bamboo (31%), non-bamboo (32%), and other plant families (27%) (Liao et al. 2023). Diverse fungal groups have been reported on bamboo, with approximately 150 basidiomycetes and 1,150 ascomycetes, including 350 asexual taxa, 240 hyphomycetes and 110 coelomycetes (Hyde et al. 2002, Dai et al. 2018, Bhunjun et al. 2022, Phukhamsakda et al. 2022). More than 50 novel bambusicolous ascomycetes were introduced between 2017 and 2022, with the majority reported from China and Thailand, which are both biodiversity hotspots (Hyde et al. 2018, Bhunjun et al. 2021, Jayawardena et al. 2022, Samarakoon et al. 2022, Jiang et al. 2022). In this study, we also found our novel species on bamboo, which suggests a large diversity of novel taxa are yet to be discovered on *Poaceae*.

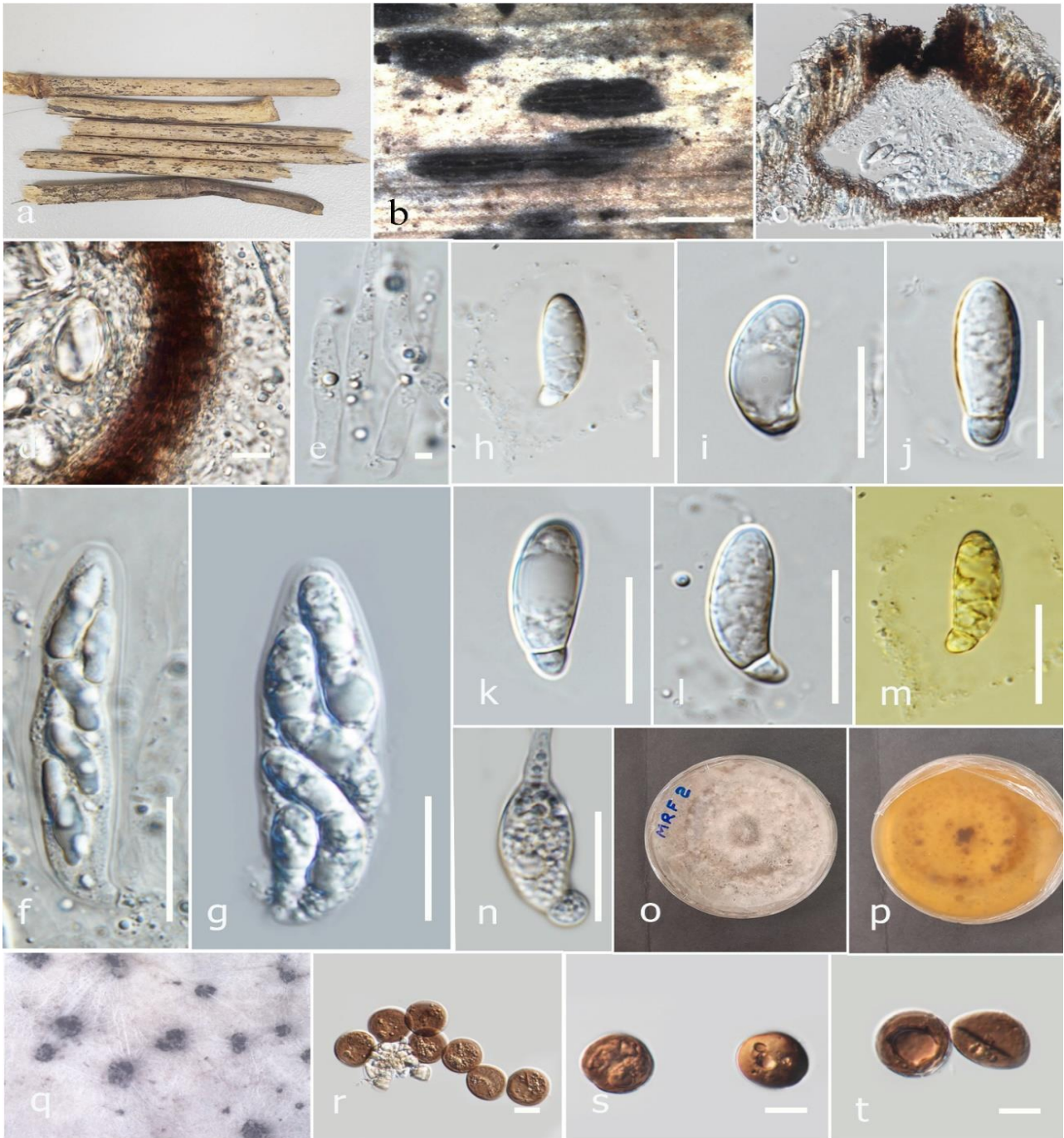


Fig. 2 – *Apiospora pseudogarethjonesii* (MFLU 24-0011, holotype). a Substrate. b Appearance of stromata on bamboo. c Vertical section of stroma. d Peridium wall. e Paraphyses. f, g Asci. h–l Ascospores. m Ascospore with thick gelatinous sheath in Melzer’s reagent. n Germinated ascospore. o, p Front and reverse colony on PDA. q Colony on PDA-producing conidia masses. r Conidiogenous cells give rise to conidia. s, t Dentate conidia. Scale bars: b, c = 200 μm . d, e = 4 μm . f, g = 20 μm . h–n = 20 μm . r–t = 20 μm .

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Availability of data and materials

All data generated and analyzed during this study are included in this article.

Conflicts of interest

The authors declare no conflict of interest.

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