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RESEARCH ARTICLE

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Diversity of the Bambusicolous Fungus *Apiospora* in Korea: Discovery of New *Apiospora* Species

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ABSTRACT

Many *Apiospora* species have been isolated from bamboo plants – to date, 34 bambusicolous *Apiospora* species have been recorded. They are known as saprophytes, endophytes, and plant pathogens. In this study, 242 bambusicolous *Apiospora* were isolated from various bamboo materials (branches, culms, leaves, roots, and shoots) and examined using DNA sequence similarity based on the internal transcribed spacer, 28S large subunit ribosomal RNA gene, translation elongation factor 1-alpha, and beta-tubulin regions. Nine *Apiospora* species (*Ap. arundinis*, *Ap. camelliae-sinensis*, *Ap. hysterina*, *Ap. lageniformis* sp. nov., *Ap. paraphaeosperma*, *Ap. pseudohyphopodii* sp. nov., *Ap. rasikravindrae*, *Ap. saccharicola*, and *Ap. sargassi*) were identified *via* molecular analysis. Moreover, the highest diversity of *Apiospora* was found in culms, and the most abundant species was *Ap. arundinis*. Among the nine *Apiospora* species, two (*Ap. hysterina* and *Ap. paraphaeosperma*) were unrecorded in Korea, and the other two species (*Ap. lageniformis* sp. nov. and *Ap. pseudohyphopodii* sp. nov.) were potentially novel species. Here, we describe the diversity of bambusicolous *Apiospora* species in bamboo organs, construct a multi-locus phylogenetic tree, and delineate morphological features of new bambusicolous *Apiospora* in Korea.

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Bambusicolous *Apiospora*; diversity; multi-locus phylogeny; morphology; novel species

1. Introduction

Apiospora Sacc. (Apiosporaceae, Sordariomycetes, Ascomycota) was recognized and established with *Ap. montagnei* by Saccardo (1875), and 145 epithets of *Apiospora* have been listed in Index Fungorum (2022) [1,2]. *Apiospora* is a cosmopolitan fungus, reported from various sources such as plants, soil, air, and marine samples in tropical, subtropical, Mediterranean, temperate, and even cold regions [3]. Moreover, they have been characterized as endophytes, saprobes, and plant pathogens (especially in Poaceae) [4–7]. Morphologically, *Apiospora* is characterized by globose, subglobose to ellipsoid, oval, and obovoid conidia when observed in face view, lenticular in side view, and basauxic conidiogenous cells [3]. The genus *Apiospora* has been observed to have *Arthrinium*-like morphs in the asexual state and is thus synonymized under *Arthrinium* species [4,8,9]. However, differences in genetic, morphological, and ecological characteristics between the two genera were found by Pintos et al. [3]; 76 species of *Arthrinium* have been synonymized under *Apiospora*, and the two genera have been completely separated [3,6,10].

Bamboo plays a crucial role in global carbon cycling. It absorbs wastewater, and it is used in human economic activities, such as construction, furniture, food, and even medicine [11]. Bamboo is also known as a good host, and more than 1300 bamboo ascomycetes (more than 120 families and 400 genera) have been described or recorded [12]. Most bambusicolous fungi have been reported in bamboo organs, such as culms (665 species), leaves (216 species), sheaths (19 species), and branches (14 species), and the least number of fungi have been recorded in shoots, roots, and inflorescences [12,13]. According to previous research, the most commonly detected endophytic fungus in bamboo (*Yushania brevipaniculata*) is *Arthrinium* species (now including the genus *Apiospora*), comprising almost 50% of isolates, and it is also found in healthy bamboo leaves [14]. Kim et al. [15] isolated fungi (93 ascomycetes and 14 basidiomycetes) from bamboo chips with decayed parts and used them for the fungal decay test against bamboo [15]. In the study,

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Ap. arundinis (=*Ar. arundinis*) was isolated as the second dominant species comprising 19.7% of the ascomycetes, and it contributed to the highest rate of weight loss (17.9%) against giant bamboo (*Phyllostachys bambusoides*) [15]. However, a study of *Apiospora* diversity according to bamboo organs has not been conducted.

Approximately 70 bamboo species are distributed naturally or artificially in Korea, and the distribution area is estimated to occupy approximately 22,067 ha [16]. However, studies on the diversity of bambusicolous fungi (including the bambusicolous *Apiospora*) in Korea are lacking. Currently, 17 *Apiospora* species have been reported in Korea. Among these, 14 *Apiospora* species were collected from marine environments (*Ap. agari*, *Ap. arctoscopi*, *Ap. arundinis*, *Ap. fermenti*, *Ap. koreana*, *Ap. marii*, *Ap. marina*, *Ap. piptatheri*, *Ap. pusillisperma*, *Ap. rasikravindrae*, *Ap. sacchari*, *Ap. saccharicola*, *Ap. sargassi*, and *Ap. taeanensis*). Three *Apiospora* species (*Apiospora arundinis*, *Ap. camelliae-sinensis*, and *Ap. minutispora*) have been collected from terrestrial environments, and only two *Apiospora* species have been reported in bamboo (*Ap. arundinis* and *Ap. camelliae-sinensis*) [5,6,15,17,18].

This study aimed to investigate the bambusicolous *Apiospora* diversity in Korea with bamboo organ specificity and to report new *Apiospora* species (with unrecorded *Apiospora*) in Korea. To accurately identify the *Apiospora* species, four DNA molecular datasets of the internal transcribed spacer (ITS), 28S large subunit ribosomal RNA gene (LSU), translation elongation factor 1-alpha (TEF), and beta-tubulin (TUB) were used for phylogenetic analysis. Furthermore, a detailed analysis of cultural and microscopic characteristics was conducted.

2. Material and methods

2.1. Sampling and isolation

Bamboo materials (branches, culms, leaves, roots, and shoots) were collected from various bamboo forests in Korea (Figure 1S). A small piece of bamboo material was placed on a 2% malt extract agar (MEA) medium containing 0.01% streptomycin. *Apiospora*-like hyphae and spores were isolated continuously until they were pure isolates. The pure strains were stocked in glycerol 10% stock and stored at −20 °C in the Korea University Fungus Collection (KUC), Seoul, Korea. The strains examined in this study, including the type strains of novel *Apiospora* species candidates, were deposited at the National Institute of Biological Resources, Incheon, Korea (NIBR).

2.2. DNA extraction, polymerase chain reaction (PCR), and sequencing

Bambusicolous *Apiospora* strains were used for molecular identification. Genomic DNA was extracted from fungal mycelia using an AccuPrep Genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's protocol. The AccuPower® PCR PreMix Kit (Bioneer) was used for PCR. PCR targeting ITS, LSU, TEF, and TUB regions. For the ITS region, ITS1F (or ITS5)/LR3 (or ITS4) primer sets were used [19,20]. For the LSU region, we used the LR0R/LR7 primer [21]. To amplify the TEF region, 728F (or 983F)/1567R primer sets were used [22,23]. For TUB region, Bt2a (or T1)/Bt2b (or T2) primer sets were used [24,25]. All PCR products were checked by electrophoresis on a 1% agarose gel and purified using the AccuPrep DNA Purification Kit (Bioneer). DNA sequencing was conducted by Cosmo Genetech (Seoul, Korea). All new sequences have been deposited in GenBank.

2.3. Phylogenetic analysis

All obtained sequences were assembled, proofread, and edited using Geneious Prime 2022.1.1 (Biomatter, Ltd., Auckland, New Zealand). The edited sequences were aligned with reference sequences of *Apiospora*, *Arthrinium*, and related genera downloaded from the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using MAFFT 7.450 [26,27]. The ambiguous alignments were manually adjusted, and maximum likelihood (ML) analysis was performed using RAxML v. 8 with the GTR + G model with 1000 bootstrap replicates [28]. MrBayes (MB) analysis was carried out using MrBayes v. 3.2.6, with the best model selected for each ITS, LSU, TEF, and TUB dataset using jModeltest v. 2.1.10 [29,30]. To achieve stationary equilibrium, five million trees were generated, and the trees were sampled every 1000th generation. Posterior probabilities (PP) were calculated in the majority rule consensus tree after discarding the first 25% of the trees as burn-in. All analyses were performed using Geneious Prime software 2022.1.1 (https://www.geneious.com/prime/).

2.4. Morphological observation

The culture characteristics and growth rates of *Apiospora* were observed on the potato dextrose agar (PDA, Difco, Detroit, USA), MEA, and oatmeal agar (OA, Difco) media at 15 °C, 20 °C, and 25 °C in darkness for 2 weeks. The colony form, elevation, margin, presence of aerial mycelia, the color of mycelia and medium, and sporulation were recorded. Color-corresponding codes were determined according to the Munsell color chart (Munsell Color, 2009). Growth rates were measured every 24 h, and each measurement was performed in triplicates. Microscopic characteristics were observed on water agar medium (WA, Bacto agar (Difco) 15 g, distilled water 1000 mL) using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) with a DP20 microscope camera (Olympus). The shape, size, and color of the conidiophores, conidiogenous cells, conidia, and hyphae were observed and recorded. Ultra-high-resolution scanning electron microscopy (UHR SEM, Hitachi SU-70, Hitachi, Tokyo, Japan) was used to observe the detailed morphological characteristics.

3. Results

3.1. Diversity of bambusicolous Apiospora in Korea

A total of 108 bamboo samples were collected from 20 bamboo forests in Korea (Figure 1S). The collected bamboo materials were composed of 33 branches, 44 culms, 14 leaves, 13 roots, and four shoots, and were used as fungal isolation sources. As a result, 242 bambusicolous *Apiospora* strains were isolated and identified based on the DNA sequence similarity of ITS, LSU, TEF, and TUB regions against the NCBI database (http://www.ncbi.nlm.nih.gov/blast). Based on sequence similarity, the *Apiospora* strains were identified as nine *Apiospora* species (*Ap. arundinis* (181 strains), *Ap. camelliae-sinensis* (17 strains), *Ap. hysterina* (two strains), *Ap. rasikravindrae* (31 strains), *Ap. saccharicola* (two strains), *Ap. sargassi* (one strain), *Ap. paraphaeosperma* (two strains), *Ap. lageniformis* sp. nov. (four strains), and *Ap. pseudohyphopodii* sp. nov. (two strains)). Figure 2S shows that the diversity of *Apiospora* was the highest in the culm, followed by the branch, and the most abundant species was *Ap. arundinis,* which accounted for >74% of the total isolates, followed by *Ap. rasikravindrae* (13%) and *Ap. camelliae-sinensis* (7%), respectively. The portion of *Ap. camelliae-sinensis* and *Ap. rasikravindrae* was higher in the bamboo branch but lower in the culm (Figure 2S). A few *Apiospora* species have been isolated from leaves, roots, and shoots. *Apiospora arundinis* was isolated from the highest proportion of bamboo tissues. *Apiospora sargassi* has only been isolated from the shoot tissues.

Apiospora hysterina strains were isolated from bamboo branches and *Ap. paraphaeosperma* strains were isolated from culms. The strains of *Ap. pseudohyphopodii* sp. nov. was isolated from bamboo culms, and *Ap. lageniformis* sp. nov. was isolated from the branches and culms. According to the present study, two species (*Ap. hysterina* and *Ap. paraphaeosperma*) and two novel species (*Ap. lageniformis* sp. nov. and *Ap. pseudohyphopodii* sp. nov.) have been recognized as new candidate species in Korea. Thus, phylogenetic and morphological analyses were performed for accurate taxonomic evaluation.

3.2. Phylogenetic analysis

The multigene alignments (ITS, LSU, TEF, and TUB combined datasets) contained 151 reference strains, and 10 new isolated strains in this study with 3717 characters, including gaps, were analyzed using ML and MB methods. The multigene alignments (ITS, LSU, TEF, and TUB combined datasets) contained 151 reference strains, and 10 new isolated strains in this study with 3717 characters, including gaps, were analyzed using ML and MB methods (Table 1). In MB analysis, ITS and LSU sequence alignments were assigned as GTR + I+G to the best-fit model, and TEF and TUB were assigned as GTR + G and HKY + I+G, respectively. Both ML and MB trees showed similar tree topologies, and the ML tree is represented. Two new *Apiospora* species (*Ap. lageniformis* sp. nov. and *Ap. pseudohyphopodii* sp. nov.) were distinct from other *Apiospora* clades and were clustered as monophyletic groups, respectively with high support (1/100, PP/bootstrap value (BS)) (Figure 1). Although *Ap. hysterina* KUC21437 and KUC21438 formed a monophyletic group with *Ap. hysterina* ICPM 6889 and *Ap. hysterina* AP29717, they were not distinguished from *Apiospora sasae* CPC 38165 and *Ap. yunnana* MFLUCC 18-1102. Furthermore, *Ap. paraphaeosperma* KUC21488 and KUC21688 were grouped together with *Ap. paraphaeosperma* GUCC 10126 and MFLUCC 13-06044, but the resolution was low in the concatenated tree (Figure 1). A morphoanatomical analysis is needed to interpret the low resolution of the two unrecorded *Apiospora* species.

3.3. Taxonomy

Apiospora lageniformis S.L. Kwon & J.J. Kim, sp. nov. (Figure 2)

MycoBank: MB845439

Type: KOREA, Jeollabuk-do, Damyang-gun, 32°34′27.4″N, 124°52′17.8″E, isolated from the culm of *Phyllostachys nigra* var. *henonis*, Apr. 2021, *S.L. Kwon* (NIBRFGC000509393= KUC21686).

Etymology: "*lageniformis*" refer to the lageniform shape of the conidiogenous cell.

Culture characteristics: PDA, colonies irregular form, flat, mycelium moderate, concentrically spreading, margin filiform; mycelia white;

Table 1. Strain informations included in the phylogenetic analyses.

				GenBank accession no.[b]			
Species	Strain no.[a]	Isolation source	Country	ITS	LSU	TEF	TUB
Apiospora acutiapica	KUMCC 20-0209	Clump of *Bambusa bambos*	China	MT946342	MT946338	MT947359	MT947365
	KUMCC 20-0210[c]	Clump of *Bambusa bambos*	China	MT946343	MT946339	MT947360	MT947366
Ap. agari	KUC21333[c]	*Agarum cribrosum*	Korea	MH498520	MH498440	MH544663	MH498478
	KUC21361	*Agarum cribrosum*	Korea	MH498519	MH498439	MN868914	MH498477
Ap. aquatica	S-642[c]	Submerged wood	China	MK828608	MK835806	–	–
Ap. arctoscopi	KUC21331[c]	Egg masses of *Arctoscopus japonicus*	Korea	MH498529	MH498449	MN868918	MH498487
	KUC21344	Egg masses of *Arctoscopus japonicus*	Korea	MH498528	MH498448	MN868919	MH498486
Ap. arundinis	CBS 124788	Living leaves of *Fagus sylvatica*	Switzerland	KF144885	KF144929	KF145017	KF144975
	CBS 133509	*Sclerotium* buried in sandy field	USA	KF144886	KF144930	KF145018	KF144976
Ap. aurea	CBS 244.83[c]	Air	Spain	AB220251	KF144935	KF145023	KF144981
Ap. balearica	AP24118[c]	Undetermined Poaceae	Spain	MK014869	MK014836	MK017975	MK017946
Ap. bambusicola	MFLUCC 20-0144[c]	Dead culms of bamboo	Thailand	MW173030	MW173087	MW183262	–
Ap. biseriale	CGMCC3.20135[c]	Dead culms of bamboo	China	MW481708	MW478885	MW522938	MW522955
	GZCC20-0099	Dead culms of bamboo	China	MW481709	MW478886	MW522939	MW522956
Ap. camelliae-sinsensis	LC5007[c]	*Camellia sinensis*	China	KY494704	KY494780	KY705103	KY705173
	LC8181	*Brassica rapa* subsp. *oleifera*	China	KY494761	KY494837	KY705157	KY705229
Ap. chiangraiense	MFLU:21-0046	Dead culms of bamboo	Thailand	MZ542520	MZ542524	–	MZ546409
Ap. chromolaenae	MFLUCC 17-1505[c]	Dead aerial culms of *Chromolaena odorata*	Thailand	MT214342	MT214436	MT235802	–
Ap. cordylines	GUCC 10026	*Cordyline fruticosa*	China	MT040105	–	MT040126	MT040147
	GUCC 10027	*Cordyline fruticosa*	China	MT040106	–	MT040127	MT040148
Ap. cyclobalanopsidis	CGMCC3.20136[c]	Leaf of *Cyclobalanopsi glauca* (Thunb.) Oerst	China	MW481713	MW478892	MW522945	MW522962
	GZCC20-0103	Leaf of *Cyclobalanopsi glauca* (Thunb.) Oerst	China	MW481714	MW478893	MW522946	MW522963
Ap. dendrobii	MFLUCC 14-0152[c]	Root of *Dendrobium harveyanum*	Thailand	MZ463151	MZ463192	–	–
Ap. descalsii	AP31118A[c]	*Ampelodesmos mauritanicus*	Spain	MK014870	MK014837	MK017947	MK017976
Ap. dichotomanthi	LC4950[c]	*Dichotomanthes tristaniicarpa*	China	KY494697	KY494773	KY705096	KY705167
	LC8175	*Dichotomanthes tristaniicarpa*	China	KY494755	KY494831	KY705151	KY705223
Ap. esporlensis	AP16717[c]	*Phyllostachys aurea*	Spain	MK014878	MK014845	MK017954	MK017983
Ap. euphorbiae	IMI 285638 b	*Bambusa* sp.	Bangladesh	AB220241	AB220335	–	AB220288
Ap. fermenti	KUC21288	Seaweed	Korea	MF615230	MF615217	MH544668	MF615235
	KUC21289[c]	Seaweed	Korea	MF615226	MF615213	MH544667	MF615231
Ap. gaoyouensis	CFCC 52301	*Phragmites australis*	China	MH197124	–	MH236793	MH236789
	CFCC 52302	*Phragmites australis*	China	MH197125	–	MH236794	MH236790
Ap. garethjonesii	JHB004[c]	Bamboo	China	KY356086	KY356091	–	–
Ap. gelatinosa	CS19-29[c]	Dead branch of bamboo	China	MW481706	MW478888	MW522941	MW522958
Ap. guiyangensis	HKAS 102403[c]	Dead culm of unidentified grass	China	MW240647	MW240577	MW759535	MW775604
Ap. guizhouensis	LC5318	Air	China	KY494708	KY494784	KY705107	KY705177
	LC5322[c]	Air	China	KY494709	KY494785	KY705108	KY705178
Ap. hispanica	IMI 326877[c]	Maritime sand	Spain	AB220242	AB220336	–	AB220289
Ap. hydei	CBS 114990[c]	Culms of *Bambusa tuldoides*	Hong Kong	KF144890	KF144936	KF145024	KF144982
	LC7103	Leaf of bamboo	China	KY494715	KY494791	KY705114	KY705183
Ap. hyphopodii	JHB003	Bamboo	China	KY356088	KY356093	–	–
	MFLUCC 15-003[c]	Bamboo	Thailand	KR069110	–	–	–
Ap. hysterina	AP29717	*Phyllostachys aurea*	Spain	MK014875	MK014842	MK017952	MK017981
	ICPM 6889[c]	Bamboo	New Zealand	MK014874	MK014841	MK017951	MK017980
	KUC21437	**Branch of *Phyllostachys bambusoides***	**Korea**	**ON764018**	**ON787757**	**ON806622**	**ON806632**
	KUC21438	**Branch of *Phyllostachys bambusoides***	**Korea**	**ON764019**	**ON787758**	**ON806623**	**ON806633**
Ap. iberica	AP10118[c]	*Arundo donax*	Portugal	MK014879	MK014846	MK017955	MK017984
Ap. intestini	CBS 135835	Gut of grasshopper	India	KR011352	KR149063	KR011351	KR011350
Ap. italica	AP29118	*Phragmites australis*	Spain	MK014881	MK014848	MK017957	MK017986
	AP221017[c]	*Arundo donax*	Italy	MK014880	MK014847	MK017956	MK017985
Ap. jatrophae	AMH-9557[c]	*Jatropha podagrica*	India	JQ246355	–	–	–
Ap. jiangxiensis	LC4494	*Phyllostachys* sp.	China	KY494690	KY494766	KY705089	KY705160
	LC4577[c]	*Maesa* sp.	China	KY494693	KY494769	KY705092	KY705163
Ap. kogelbergensis	CBS 113333	Dead culms of Restionaceae	South Africa	NR_120272	KF144938	KF145026	KF144984
	CBS 113332	Dead culms of *Cannomois virgata*	South Africa	KF144891	KF144937	KF145025	KF144983
Ap. koreana	KUC21332[c]	Egg masses of *Arctoscopus japonicus*	Korea	MH498524	MH498444	MH544664	MH498482
	KUC21348	Egg masses of *Arctoscopus japonicus*	Korea	MH498523	MH498443	MN868927	MH498481

(*continued*)

Table 1. Continued.

Species	Strain no.[a]	Isolation source	Country	GenBank accession no.[b]			
				ITS	LSU	TEF	TUB
Ap. lageniformis sp. nov	**KUC21681**	**Branch of *Phyllostachys pubescens***	**Korea**	**ON764020**	**ON787759**	**ON806624**	**ON806634**
	KUC21685	**Branch of *Phyllostachys pubescens***	**Korea**	**ON764021**	**ON787760**	**ON806625**	**ON806635**
	KUC21686[c]	**Top of culm of *Phyllostachys nigra* var. *henonis***	**Korea**	**ON764022**	**ON787761**	**ON806626**	**ON806636**
	KUC21687	**Top of culm of *Phyllostachys nigra* var. *henonis***	**Korea**	**ON764023**	**ON787762**	**ON806627**	**ON806637**
Ap. locuta-pollinis	LC11683[c]	Bee bread	China	MF939595	–	MF939616	MF939622
Ap. longistroma	MFLUCC 11-0481[c]	Bamboo	Thailand	KU940141	KU863129	–	–
Ap. malaysiana	CBS 251.29	Culm base of *Cinnamomum camphora*	Malaysia	KF144897	KF144943	KF145031	KF144989
	CBS 102053[c]	*Macaranga hullettii*	Malaysia	KF144896	KF144942	KF145030	KF144988
Ap. marii	CBS 497.90[c]	Beach sand	Spain	AB220252	KF144947	KF145035	KF144993
	CBS 114803	*Pseudosasa hindsii*	Hong Kong	KF144899	KF144945	KF145033	KF144991
Ap. marina	KUC21328[c]	Seaweed	Korea	MH498538	MH498458	MH544669	MH498496
	KUC21353	Seaweed	Korea	MH498537	MH498457	MN868923	MH498495
Ap. mediterranea	IMI 326875[c]	Air	Spain	AB220243	AB220337	–	AB220290
Ap. minutispora	1.70E-41[c]	Soil	Korea	LC517882	–	LC518889	LC518888
Ap. mytilomorpha	DAOM 214595	*Andropogon* sp.	India	KY494685	–	–	–
Ap. neobambusae	LC7106[c]	Leaf of bamboo	China	KY494718	KY494794	KY806204	KY705186
	LC7107	Leaf of bamboo	China	KY494719	–	KY705117	KY705187
Ap. neochinense	CFCC 53036[c]	*Fargesia qinlingensis*	China	MK819291	–	MK818545	MK818547
Ap. neogarethjonesii	HKAS 102408[c]	Bamboo	China	NR_171943	MK070898	–	–
Ap. neosubglobosa	JHB007[c]	Bamboo	China	KY356090	KY356095	–	–
Ap. obovata	LC4940[c]	*Lithocarpus* sp.	China	KY494696	KY494772	KY705095	KY705166
	LC8177	*Lithocarpus* sp.	China	KY494757	–	KY705153	KY705225
Ap. ovata	CBS 115042	*Pseudosasa hindsii*	Hong Kong	KF144903	KF144950	KF145037	KF144995
Ap. paraphaeosperma	GUCC 10126	–	–	MT040110	–	MT040131	MT040152
	MFLUCC 13-0644[c]	Dead culms of bamboo	Thailand	KX822128	KX822124	–	–
	KUC21488	**Culm of bamboo**	**Korea**	**ON764024**	**ON787763**	**ON806628**	**ON806638**
	KUC21688	**Culm of bamboo**	**Korea**	**ON764025**	**ON787764**	**ON806629**	**ON806639**
Ap. phragmitis	CPC 18900	Culms of *Phragmites australis*	Italy	KF144909	KF144956	KF145043	KF145001
Ap. phyllostachydis	MFLUCC 18-1101	Dead culms of *Phyllostachys heteroclada*	China	MK351842	–	MK340918	MK291949
Ap. piptatheri	AP4817A[c]	*Piptatheri miliaceum*	Spain	MK014893	MK014860	MK017969	–
	KUC21220	*Sargassum* sp.	Korea	KT207736	KT207686	MF615223	KT207636
	KUC21279	*Sargassum* sp.	Korea	MF615229	MF615216	MF615221	MF615234
Ap. psedospegazzinii	CBS 102052[c]	Culm colonized by ants	Malaysia	KF144911	KF144958	KF145045	KF145002
Ap. pseudoparenchymatica	LC7234[c]	Leaf of bamboo	China	KY494743	KY494819	KY705139	KY705211
Ap. pseudorasikravindrae	KUMCC 20-0208[c]	Sheath of *Bambusa dolichoclada*	China	MT946344	–	MT947361	MT947367
	KUMCC 20-0211	Sheath of *Bambusa dolichoclada*	China	MT946345	–	MT947362	MT947368
Ap. pseudosinensis	CPC 21546[c]	Leaf of bamboo	Netherlands	KF144910	KF144957	KF145044	MN868936
Ap. pterosperma	CPC 20193[c]	Leaf of *Lepidosperma gladiatum*	Australia	KF144913	KF144960	KF145046	KF145004
Ap. pusillisperma	KUC21321[c]	Seaweed	Korea	MH498533	MH498453	MN868930	MH498491
	KUC21357	Seaweed	Korea	MH498532	MH498452	MN868931	MH498490
Ap. qinlingensis	CFCC 52303[c]	*Fargesia qinlingensis*	China	MH197120	–	MH236795	MH236791
	CFCC 52304	*Fargesia qinlingensis*	China	MH197121	–	MH236796	MH236792
Ap. rasikravindrae	LC5449	Soil	China	KY494713	–	KY705112	KY705182
	LC7115	Leaf of bamboo	China	KY494721	KY494797	KY705118	KY705189
	NFCCI 2144[c]	Soil	Norway	JF326454	–	–	–
Ap. sacchari	CBS 301.49	Bamboo	Indonesia	KF144917	–	KF145048	KF145006
	CBS 372.67	Air		KF144918	KF144964	KF145049	KF145007
Ap. saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145051	KF145009
	CBS 463.83	Dead culms of *Phragmites australis*	Netherlands	KF144921	KF144968	KF145053	KF145011
Ap. sargassi	KUC21228[c]	*Sargassum* sp.	Korea	KT207746	KT207696	MH544677	KT207644
	KUC21232	*Sargassum* sp.	Korea	KT207750	KT207700	MH544676	KT207648
Ap. sasae	CPC 38165[c]	Dead culms of *Sasa veitchii*	Netherlands	MW883402	MW883797	MW890104	MW890120
Ap. septata	CGMCC3.20134[c]	Dead branch of bamboo	China	MW481711	MW478890	MW522943	MW522960
	GZCC20-0109	Dead branch of bamboo	China	MW481712	MW478891	MW522944	MW522961
Ap. serenensis	IMI 326869[c]	Excipients, atmosphere and home dust	Spain	AB220250	AB220344	–	AB220297
Ap. setariae	Beilin 024	*Setaria viridis*	China	MT492005	–	MW118457	MT497467
Ap. setostroma	KUMCC 19-0217	Dead branches of bamboo	China	MN528012	MN528011	MN527357	–
Ap. sichuanensis	HKAS 107008	Dead culm of Poaceae	China	MW240648	MW240578	MW759536	MW775605
Ap. stipae	CPC 38101[c]	Dead culm of *Celtica gigantea*	Spain	MW883403	MW883798	MW890105	MW890121
Ap. subglobosa	MFLUCC 11-0397[c]	Bamboo	Thailand	KR069112	KR069113	–	–
Ap. subrosea	LC7292[c]	Leaf of bamboo	China	KY494752	KY494828	KY705148	KY705220

(*continued*)

Table 1. Continued.

Species	Strain no.[a]	Isolation source	Country	GenBank accession no.[b]			
				ITS	LSU	TEF	TUB
Ap. taeanensis	KUC21322[c]	Seaweed	Korea	MH498515	MH498435	MH544662	MH498473
	KUC21359	Seaweed	Korea	MH498513	MH498433	MN868935	MH498471
Ap. thailandica	LC5630	Rotten wood	China	KY494714	KF144970	KY705113	KY806200
	MFLUCC 15-0202[c]	Dead culms of bamboo	Thailand	KU940145	KU863133	–	–
Ap. vietnamensis	IMI 99670	*Citrus sinensis*	Vietnam	KX986096	KX986111	–	KY019466
Ap. xenocordella	CBS 478.86	Soil from roadway	Zimbabwe	KF144925	KF144970	KF145055	KF145013
	CBS 595.66	Soil	Austria	KF144926	–	–	–
Ap. yunnana	MFLUCC 18-1102	Dead or nearly dead culms of *Phyllostachys heteroclada*	China	MK351843	KU863135	MK340919	MK291950
Ap. pseudohyphopodii sp. nov	**KUC21680[c]**	**Culm of *Phyllostachys pubescens***	**Korea**	**ON764026**	**ON787765**	**ON806630**	**ON806640**
	KUC21684	**Culm of *Phyllostachys pubescens***	**Korea**	**ON764027**	**ON787766**	**ON806631**	**ON806641**
Arthrinium austriacum	GZU 345006	*Carex pendula*	Austria	MW208929	MW208860	–	–
Ar. caricicola	AP23518	*Carex ericetorum*	Germany	MK014871	MK014838	MK017948	MK017977
Ar. crenatum	AG19066[c]	Probably *Festuca burgundiana*	France	MW208931	MW208861	–	–
Ar. curvatum	AP25418	Leaves of *Carex* sp.	Germany	MK014872	MK014839	MK017949	MK017978
Ar. japonicum	IFO 30500	–	Japan	AB220262	AB220356	–	AB220309
Ar. luzulae	AP7619-3	Dead leaves of *Luzula sylvatica*	Spain	MW208937	MW208863	–	–
Ar. morthieri	GZU 345043	*Carex digitata*	Austria	MW208938	MW208864	–	–
Ar. phaeospermum	CBS 114317	Leaf of *Hordeum vulgare*	Iran	KF144906	KF144953	KF145040	KF144998
	CBS 114318	Leaf of *Hordeum vulgare*	Iran	KF144907	KF144954	KF145041	KF144999
Ar. puccinioides	AP26418	*Carex arenaria*	Germany	MK014894	MK014861	MK017970	MK017998
	CBS 549.86	*Lepidosperma gladiatum*	Germany	AB220253	AB220347	–	AB220300
Ar. sorghi	URM 9300	*Sorghum bicolor*	Brazil	MK371706	–	–	MK348526
Ar. sphaerospermum	AP25619	Probably on Poaceae	Norway	MW208943	MW208865	–	–
Ar. sporophleum	AP21118	*Juncus* sp.	Spain	MK014898	MK014865	MK017973	MK018001
Ar. trachycarpum	CFCC 53038[c]	*Trachycarpus fortune*	China	MK301098	–	MK303396	MK303394
Ar. urticae	IMI 326344	–	–	AB220245	AB220339	–	–
Nigrospora aurantiaca	CGMCC3.18130[c]	*Nelumbo* sp.	China	KX986064	KX986098	KY019295	KY019465
N. camelliae-sinensis	CGMCC3.18125[c]	*Camellia sinensis*	China	KX985986	KX986103	KY019293	KY019460
N. chinensis	CGMCC3.18127[c]	*Machilus breviflora*	China	KX986023	KX986107	KY019422	KY019462
N. gorlenkoana	CBS 480.73[c]	*Vitis vinifera*	Kazakhstan	KX986048	KX986109	KY019420	KY019456
N. guilinensis	CGMCC3.18124[c]	*Camellia sinensis*	China	KX985983	KX986113	KY019292	KY019459
N. hainanensis	CGMCC3.18129[c]	*Musa paradisiaca*	China	KX986091	KX986112	KY019415	KY019464
N. lacticolonia	CGMCC3.18123[c]	*Camellia sinensis*	China	KX985978	KX986105	KY019291	KY019458
N. musae	CBS 319.34[c]	*Musa* sp.	Australia	MH855545	KX986110	KY019419	KY019455
N. oryzae	LC2693	*Neolitsea* sp.	China	KX985944	KX986101	KY019299	KY019471
N. osmanthi	CGMCC3.18126[c]	*Hedera nepalensis*	China	KX986010	KX986106	KY019421	KY019461
N. pyriformis	CGMCC3.18122[c]	*Citrus sinensis*	China	KX985940	KX986100	KY019290	KY019457
N. rubi	LC2698[c]	*Rubus* sp.	China	KX985948	KX986102	KY019302	KY019475
N. sphaerica	LC7298	*Nelumbo* sp.	China	KX985937	KX986097	KY019401	KY019606
N. vesicularis	CGMCC3.18128[c]	*Musa paradisiaca*	China	KX986088	KX986099	KY019294	KY019463
N. zimmermanii	CBS 290.62[c]	*Saccharum officinarum*	Ecuador	KY385309	–	KY385311	KY385317
Allelochaeta acuta	CBS 144168[c]	*Eucalyptus viminalis*	Australia	MH822973	MH823023	MH823113	MH823160
Sporocadus trimorphus	CBS 114203[c]	*Rosa canina*	Sweden	MH553977	MH554196	MH554395	MH554636

[a] AG, Alain Gardiennet; AP, Ángel Pintos; CBS, Westerdijk Fungal Biodiverity Institute (WI), Utrecht, The Netherlands; CFCC, China Forestry Culture Collection Center, Beijing, China; CGMCC, China General Microbiological Culture Collection Center, Beijing, China; CPC, Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; DAOM, Canadian Collection of Fungal Cultures, Ottawa, Canada; GUCC, Guizhou culture collection, Guizhou, China; GZU, arl-Franzens-Universität Graz, Austria; HKAS, Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China; IFO, Institute for Fermentation in Osaka, Japan; IMI, CABI Bioscience, Eggham, UK; JHB, H.B. Jiang; KUC, the Korea University Fungus Collection, Seoul, Korea; KUMCC, Kunming Institute of Botany Culture Collection, Kunming, China; LC, Personal culture collection of Lei Cai, housed at CAS, China; MFLUCC, Mae Fah Luang University Culture Collection, Thailand; NFCCI, National Fungal Culture Collection of India; and URM, URM culture collection in Brazil.

[b] The sequences generated in this study are shown in bold.

[c] Indicate the type materials.

sporulation observed after 7 days at 15 °C on hyphae; pigment not observed. MEA, colonies circular form, flat, mycelium low, concentrically spreading with sparse aerial mycelium, margin entire; mycelia hyaline to white colored; sporulation observed after 7 days at all temperatures on hyphae; pigment absent. OA, colonies circular form, mycelium abundant, fluffy, downy, crateriform, thick, concentrically spreading with abundant aerial mycelium, margin entire; mycelia white; sporulation not observed; pigment absent.

Colony diameters – 15 °C PDA 6.4–7 cm/14 days, MEA 6.5–6.6 cm/14 days, OA 5.1–5.5 cm/14 days; 20 °C PDA 7 cm/13 days, MEA 7 cm/12 days, OA 7 cm/13–14 days; 25 °C PDA 7 cm/13 days, MEA 7 cm/9 days, OA 7 cm/12–13 days.

Asexual morphology: Conidiophores are reduced to conidiogenous cells. Conidiogenous cells aggregated in

Figure 1. ML tree based on ITS, LSU, TEF, and TUB concatenated datasets. The node numbers indicate the Bayesian posterior probabilities (PP) > 0.70 and ML bootstrap support (BS) > 70% as PP/BS. The novel *Apiospora* cultures examined in this study are shown in bold-face orange color. The unrecorded species are denoted by a green color. Type materials indicated by "T".

a cluster on hyphae, basauxic, polyblastic, hyaline, lageniform, 8.0–10.5(–12) × 4.0–5.0 µm, apical neck 3.5–5.5 µm long, basal part 2.8–7.2 µm long. Conidia green to dark brown, surface smooth, globose to ellipsoid in surface view, (7.8–)8.1–9.0(–9.5) × (6.8–)7.5–8.5(–9.0) µm ($\bar{x}$ = 8.6 × 8.0 µm, $n = 30$); lenticular in side view, with equatorial slit, (7.0–)8.0–9.5(–9.5) × (5.3–)6.0–7.0(–7.5) µm ($\bar{x}$ = 8.6 × 6.4 µm, $n = 30$). Mycelium smooth, hyaline, branched, septate, 2.0–4.0 µm diam.

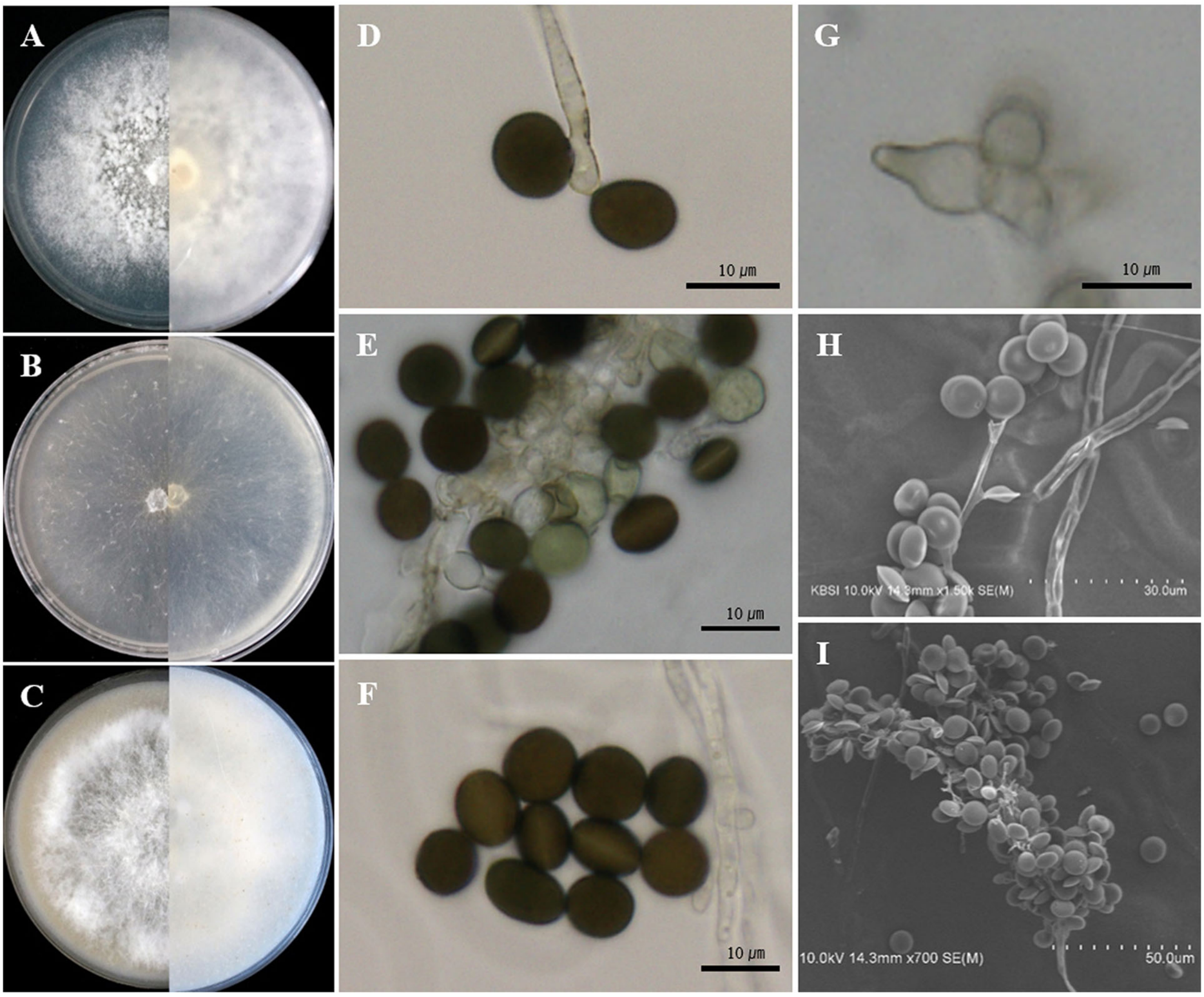

Figure 2. *Apiospora lageniformis* (KUC21686). (A) PDA; (B) MEA; (C) OA; (D, E) conidiogenous cell with conidia; (F) conidia; (G) lageniform conidiogenous cell; (H, I.) clustered conidia under UHR-SEM.

Additional materials examined: KOREA, Jeollabuk-do, Damyang-gun, 32°34′27.4″N, 124°52′17.8″E, isolated from the culm of *Phyllostachys nigra* var. *henonis*, Apr. 2021, *S.L. Kwon* (NIBRFGC000509394 = KUC21687); KOREA, Jeollabuk-do, Gochang-gun, 35°25′50.9″N, 126°42′16.9″E, isolated from a branch of *Phyllostachys pubescens*, Mar. 2021, *S.L. Kwon* (NIBRFGC000509391 = KUC21681 and NIBRFGC000509392 = KUC21685).

Remarks: The *Ap. lageniformis* sp. nov. is characterized by a lageniform conidiogenous cell. This species is closely related to *Apiospora jiangxiensis* LC4577 (M. Wang & L. Cai) Pintos & P. Alvarado (over 100% similarity in the ITS region, 100% in the LSU region, 99.77% in the TEF region, and 97.92% in the TUB region). However, they can be distinguished by phylogenetic analysis with high bootstrap values (1/100, PP/BS). In the original description, *Ap. jiangxiensis* LC4577 had luteous to sienna pigments on colonies and media [7]. However, no pigments were observed in the *Ap. lageniformis* sp. nov. Furthermore, the growth rate of *Ap. jiangxiensis* LC4577 (9 cm/10 days, at 25 °C on PDA) was faster than *Ap. lageniformis* sp. nov. KUC21686 at 25 °C on PDA (7 cm/13 days) [7]. *Apiospora lageniformis* sp. nov. also is closely related to *Apiospora obovata* (M. Wang & L. Cai) Pintos & P. Alvarado, and *Ap. arctoscopi* (S.L. Kwon, S. Jang & J.J. Kim) S.L. Kwon & J.J. Kim in concatenate phylogeny (Figure 1). However, *Ap. obovata* has obovoid, elongated to ellipsoidal conidia (size 16–31 × 9–16 μm), and *A. arctoscopi* has globose to elongated ellipsoid (in surface view, 9.5–13 × 7.5–12 μm) conidia (in lenticular side view, 5.5–7.5 μm) [5,7], which are different conidia characteristics of *Ap. lageniformis. Apiospora arctoscopi* also has different conidiogenous cell shapes (cylindrical, sometimes ampulliform) [5].

Apiospora pseudohyphopodii S.L. Kwon & J.J. Kim, sp. nov. (Figure 3)

MycoBank: MB845440

Type: KOREA, Jeollabuk-do, Gochang-gun, 35°25′50.9″N, 126°42′16.9″E, isolated from a branch of *Phyllostachys pubescens*, Mar. 2021, *S.L. Kwon* (NIBRFGC000509202 = KUC21680)

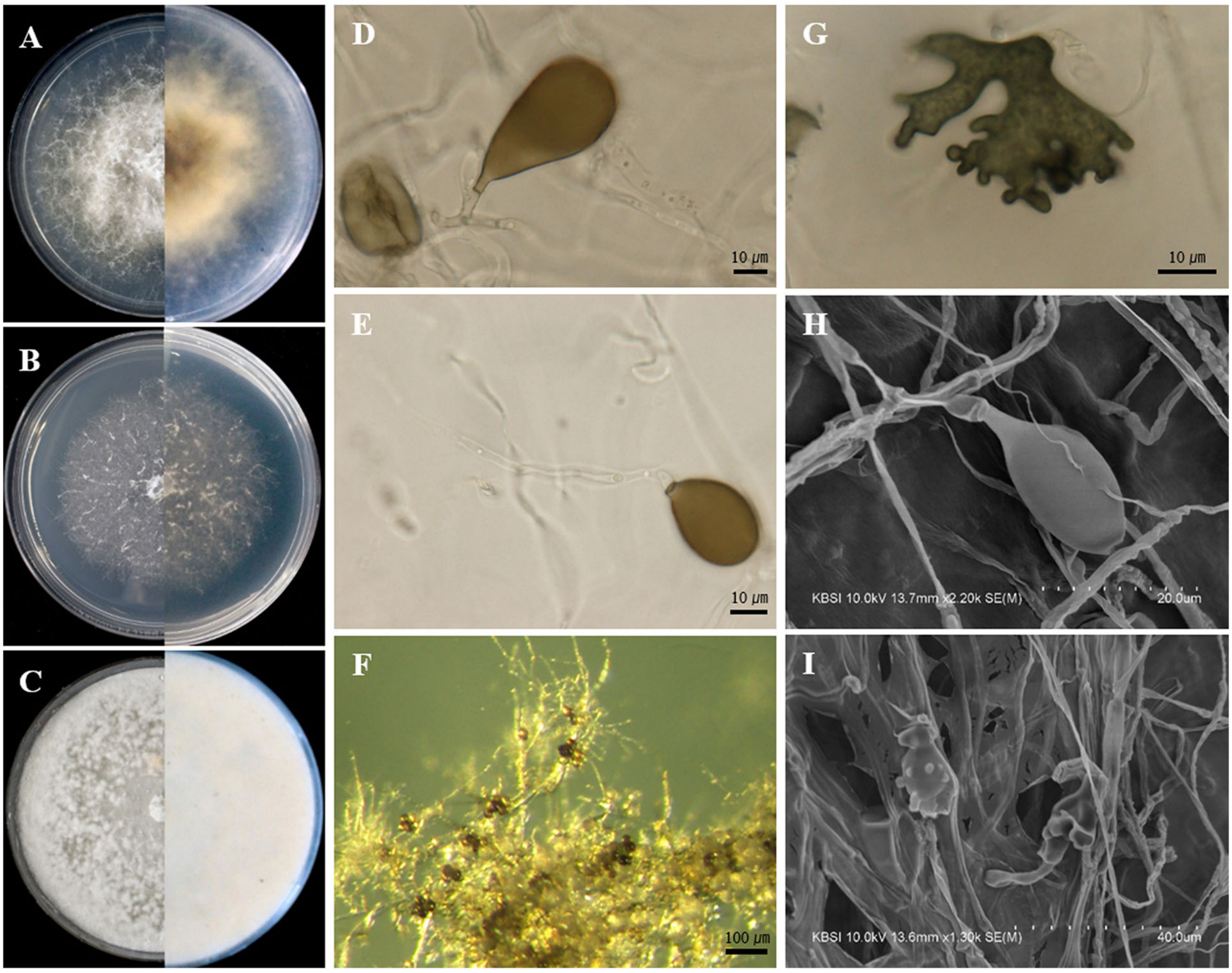

Figure 3. *Apiospora pseudohyphopodii* (KUC21680). (A) PDA; (B) MEA; (C) OA; (D, E) conidiogenous cell with conidia; (F) conidia generated on WA medium under light microscope; (G) lobed hyphopodia; (H) conidiogenous cell with ellipsoidal conidia under UHR-SEM; (I) lobed hyphopodia under UHR-SEM.

Etymology: Named after its morphological similarity to *Apiospora hyphopodii.*

Culture characteristics: PDA, colonies circular form, flat, mycelium dense around the center and become sparse at the margin, concentrically spreading with abundant aerial mycelium, margin filiform; mycelia white around the center, fading to hyaline at the margin; sporulation observed after 7 days at 15 °C on hyphae; yellow (2.5Y, 7/8) pigment diffused after 5 days, and becoming converted to dark olive gray (5Y, 3/2) pigment from the center in reverse. MEA, colonies circular form, flat, mycelium low, concentrically spreading with sparse aerial mycelium, margin filiform; mycelia white colored; sporulation observed around plug after 7–8 days at 15 °C; pigment absent. OA, colonies circular form, flat, mycelium abundant, dense, concentrically spreading with sparse aerial mycelium, margin entire; mycelia white; sporulation not observed; pigment absent.

Colony diameters – 15 °C PDA 3.2–3.5 cm/14 days, MEA 1.9–2.2 cm/14 days, OA 7 cm/12–13 days; 20 °C PDA 5.2–6.2 cm/14 days, MEA 4–4.3 cm/14 days, OA 7 cm/5–6 days; 25 °C PDA 7 cm/9 days, MEA 7 cm/11–12 days, OA 7 cm/5 days.

Asexual morphology: Conidiophores are reduced to conidiogenous cells. Conidiogenous cells solitary on hyphae, hyaline, cylindrical, 9.5–13(–24) × 4.5–5.5 μm. Conidia were brown, smooth, globose to ellipsoid, sometimes polygonal or irregular, 20–25(–26) × 18–23 μm ($\bar{x}$ = 22.4 × 21.1 μm, $n = 37$). Elongated conidia brown, smooth, obovoid, clavate, (25–)27–40(–44) × 12–20(–22) μm in size. Hyphopodia blackish, lobed, irregular in shape, resembling coral and sea squirt, 20–35(–42) × 5–35 μm. Mycelium smooth, hyaline, branched, and septate.

Additional material examined: KOREA, Jeollabuk-do, Gochang-gun, 35°25′50.9″N, 126°42′16.9″E, isolated from a branch of *Phyllostachys pubescens*, Mar. 2021, *S.L. Kwon* (NIBRFGC000509389 = KUC21684).

Remarks: The *Apiospora pseudohyphopodii* sp. nov. is closely related to *Apiospora pseudoparenchymatica* LC7234 (over 96.2% similarity in the ITS region, 99.52% in the LSU region, 92.92% in the TEF region, and 93.62% in the TUB region) and

Ap. hyphopodii MFLUCC 15-003 (over 98.68% similarity in the ITS region) in the phylogenetic analysis (Figure 1). This species is characterized by blackish-lobed hyphopodia and large and elongated conidia. Hyphopodia have also been observed in *Ap. hyphopodii* MFLU 15-0383 [31]. However, *Ap. pseudohyphopodii* sp. nov. KUC21680 has larger conidia (20–25(–26) × 18–22.5 µm ($x = 22.5 \times 21.2$ µm, $n = 37$)) than *Ap. hyphopodii* MFLU 15-0383 (5–10 × 4–8 µm ($x = 6.5 \times 5.6$ µm, $n = 20$)) [31]. The conidia of *Ap. pseudoparenchymatica* are similar in size to those of *Ap. pseudohyphopodii* sp. nov. KUC21680. However, they were clearly distinguished based on their phylogenies. Also, the growth rate of *Ap. pseudohyphopodii* sp. nov. KUC21680 (7 cm/9 days at 25 °C on PDA) is slower than *Ap. pseudoparenchymatica* (9 cm/8 days at 25 °C on PDA) [7].

Apiospora hysterina (Sacc.) Pintos & P. Alvarado, Fungal Systematics and Evolution 7:206 (2021) [MB837743] (Figure 4).

Culture characteristics: PDA, colonies circular form, flat, mycelium moderate, concentrically spreading with abundant aerial mycelium, margin entire; mycelia white; sporulation observed after 7–10 days at 15 °C and 20 °C on hyphae; reddish yellow (5YR, 7/8) pigment partially observed after 11 days. MEA, colonies circular form, flat, mycelium low, concentrically spreading with aerial mycelium, margin entire; mycelia hyaline to white colored; sporulation observed after 7–10 days at all temperatures on hyphae; pigment absent. OA, colonies circular form, flat, mycelium concentrically spreading with abundant aerial mycelium, margin entire; mycelia white; sporulation observed after 7–10 days at 20–25 °C on hyphae; pigment absent.

Colony diameters – 15 °C PDA 5.4–5.8 cm/14 days, MEA 4.8–4.9 cm/14 days, OA 5.5–6.8 cm/14 days; 20 °C PDA 7 cm/9–10 days, MEA 7 cm/11–12 days, OA 7 cm/9–10 days; 25 °C PDA 7 cm/7 days, MEA 7 cm/8 days, OA 7 cm/7 days.

Asexual morphology: Conidiophores basauxic, polyblastic, hyaline to pale brown, septate or not, smooth or finely roughened with granular pigments, cylindrical, straight or flexuous, 10–25 × 2–3.5 µm, sometimes exceeding 98 µm long. Conidia brown to dark brown, surface smooth, finely roughened,

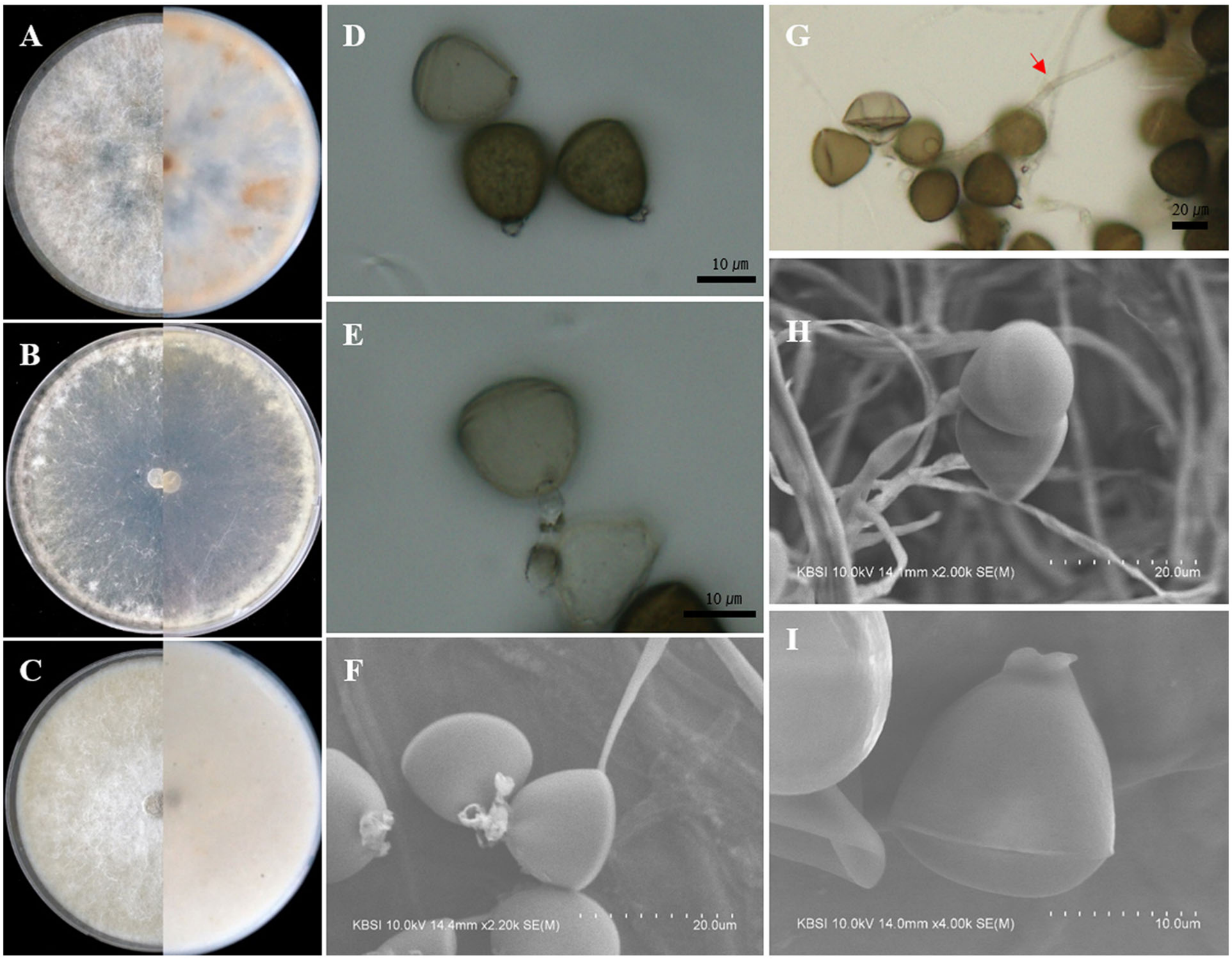

Figure 4. *Apiospora hysterina* (KUC21437). (A) PDA; (B) MEA; (C) OA; (D) conidia; (E, G) conidiogenous cell with conidia; (F, H, I) conidia under UHR-SEM.

globose to subglobose in surface view, 15.0–18.0 × (13.2–)14.0–16.5(–17.5) µm ($\bar{x}$ = 16.3 × 15.7 µm, $n = 30$); obovoid with a horizontal scar at the edge in side view, 15.0–18.0 × (11.5–)13.0–16(–17.5) µm ($\bar{x}$ = 16.7 × 14.9 µm, $n = 50$).

Specimen examined: KOREA, Chungcheongnam-do, Taean-gun, 36°29′51.0″N, 126°21′41.5″E, isolated from the branch of *Phyllostachys bambusoides*, Feb. 2020, *S.L. Kwon* (NIBRFGC000506558 = KUC21437 and NIBRFGC000509388 = KUC21438).

Remarks: The microscopic morphologies of *Ap. hysterina* KUC21437 and KUC21438 are well-matched with the original description. The former has longer conidiophores exceeding 98 µm and obovoid conidia with a horizontal scar resembling *Ap. hysterina* ICMP 6889 [32]. The diffused pigment of *Ap. hysterina* ICMP 6889 was observed on MEA [32]. However, the pigment of *Ap. hysterina* KUC21437 was not observed on the MEA medium but was observed on the PDA medium. The obovoid shape of conidia of *Ap. hysterina* are similar to those of *Apiospora yunnana* (D. Q. Dai & K.D. Hyde) Pintos & P. Alvarado, and *Ap. sasae* Crous & R.K. Schumach, and they are closely related in the concatenated phylogenetic tree (Figure 1). However, the long conidiophores and small conidia of *Ap. hysterina* KUC21437 differs from *Ap. yunnana* [33]. In the case of *Apiospora sasae*, it is morphologically similar to *Ap. hysterina* by producing subglobose, polygonal to urceolate (uniform) conidia ((16–)17–18(–20) × (15–)16–17(–19) µm) [34]. However, this species can be distinguished by the septate and long conidiophores of *Ap. hysterina* KUC21437.

Apiospora paraphaeosperma (Senan. & K.D. Hyde) Pintos & P. Alvarado, Fungal Systematics and Evolution 7:206 (2021) [MB837705] (Figure 5)

Culture characteristics: PDA, colonies circular form, mycelium thick, fluffy, concentrically spreading, margin entire; mycelia white, partially yellow; sporulation not observed; pigment absent. MEA, colonies circular form, flat, mycelium low, margin entire; mycelia hyaline to white colored; sporulation observed after 8–10 days at 20–25 °C on hyphae; pigment absent. OA, colonies circular form, flat, mycelium thick, fluffy, concentrically spreading with abundant aerial mycelium, margin entire; mycelia white, partially yellow; sporulation not observed; Yellow (2.5Y, 8/8) pigment partially diffused in media.

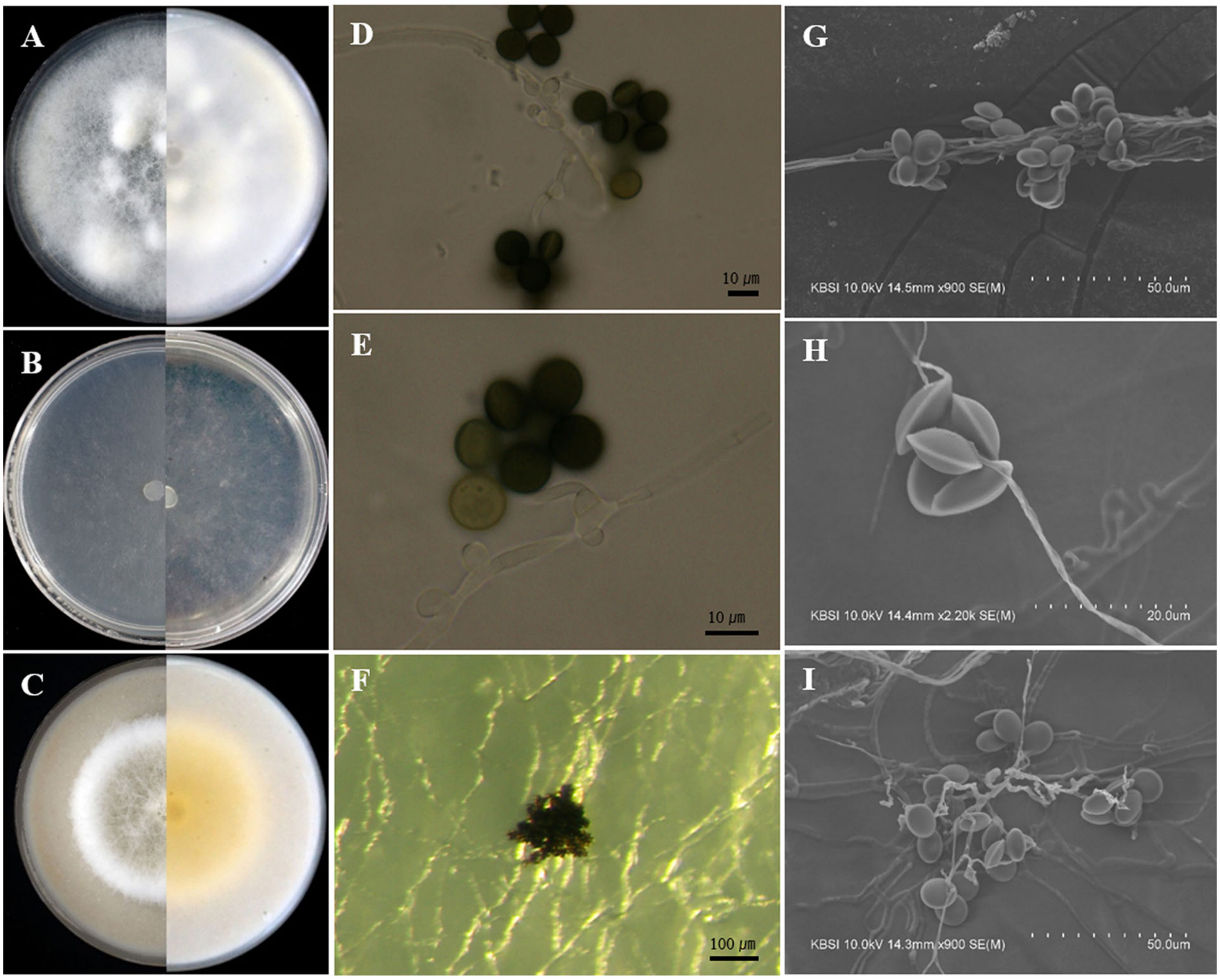

Figure 5. *Apiospora paraphaeosperma* (KUC21488). (A) PDA; (B) MEA; (C) OA; (D, E) conidiogenous cell with conidia; (F) conidia generated on WA medium under light microscope; (G–I) conidiogenous cell with conidia under UHR-SEM.

Colony diameters – 15 °C PDA 5.2–5.3 cm/14 days, MEA 4.3–4.5 cm/14 days, OA 4.0–4.2 cm/14 days; 20 °C PDA 7.0 cm/13 days, MEA 5.3–5.8 cm/14 days, OA 5.5–6.0 cm/14 days; 25 °C PDA 7.0 cm/11–12 days, MEA 7.0 cm/12–13 days, OA 6.5-7.0 cm/14 days.

Asexual morphology: Conidiophores are reduced to conidiogenous cells. Conidiogenous cells aggregated in clusters on hyphae, basauxic, polyblastic, hyaline, cylindrical, and ampulliform, 3.0–5.1(–8.7) × 1.5–3.0 µm, elongated conidiogenous cells length (11–)15–25(–34) µm. Conidia green to brown, surface smooth, globose to subglobose, 9.5–12.0 × 8.0–11.0 µm ($\overline{x}$ = 10.9 × 9.8 µm, $n = 47$) in surface view; lenticular in side view, with equatorial slit, 7.5–9.0 µm wide ($\overline{x}$ = 8.12 µm, $n = 37$) in side view, a slightly elongated cell was observed. Mycelium smooth, hyaline, branched, septate, 1.5–2.5 µm diam.

Specimen examined: KOREA, Jeju-do, Seogwipo-si, 33°15′26.4″N, 126°21′11.2″E, isolated from a culm of bamboo, 2018, J.J. Kim, (NIBRFGC000509203 = KUC21488 and NIBRFGC000509390 = KUC21688).

Remarks: In the original description, *Ap. paraphaeosperma* MFLUCC 13-0644 had a long conidiogenous cell (25–30 × 4–6 µm) [35]. Although the conidiogenous cells of *Ap. paraphaeosperma* KUC21488 usually were observed at an average of 3.0–5.1(–8.7) µm long, sometimes the elongated conidiogenous cells are also observed ((11–)15–25(–34) µm long). This species is closely related to *Apiospora rasikravindrae* (Shiv M. Singh et al.) Pintos & P. Alvarado, and *Apiospora marina* (S.L. Kwon, S. Jang & J.J. Kim) S.L. Kwon & J.J. Kim in the concatenated phylogenetic analysis. However, they could be distinguished by the presence or absence of elongated conidiogenous cells in *Ap. paraphaeosperma*.

4. Discussion

In this study, 242 bambusicolous *Apiospora* strains were isolated from various bamboo organs and identified based on their DNA similarity against the NCBI database. As a result, in the bamboo organs, the highest *Apiospora* diversity was detected on the culms (seven species), followed by branches (six species), leaves (two species), shoots epidermis (two species), and roots (one species) (Figure 2S). The finding that the most diverse *Apiospora* were found in bamboo culms is consistent with the previously reported result that most bambusicolous *Apiospora* species have been isolated from bamboo culms (23 species/34 species of total bambusicolous *Apiospora*) (Table 2) [4,10,31–33,35–45].

So far, only *Ap. rasikravindrae* species have been reported in bamboo shoots by Majeedano et al. [46]. In addition, no studies have reported on the isolation of *Apiospora* species from bamboo roots (Table 2). However, in this study, *Ap. arundinis* was isolated from all organs, including shoots and roots. In addition, this species had the highest abundance (74% of the total isolates) among the bambusicolous *Apiospora* species (Table 1S).

New records were identified based on morphological and phylogenetic analyses. The DNA barcode set (ITS, LSU, TEF, and TUB regions) was used in the phylogenetic analysis to distinguish them from cryptic species. In the case of *Ap. pseudohyphopodii* sp. nov., it is difficult to distinguish between them using only morphology. However, they were clearly distinguished in the phylogenetic analysis, with high bootstrap values (Figure 1). The *Ap. pseudohyphopodii* sp. nov. is morphologically noted to have hyphopodia and large conidia (Figure 3). Hyphopodia structures were also observed in the species *Ap. hyphopodii* within the genus *Apiospora* [31]. However, *Ap. hyphopodii* could be distinguished by having smaller conidia than *Ap. pseudohyphopodii* sp. nov. The conidia size of *Ap. pseudohyphopodii* sp. nov. (globose to ellipsoid, sometimes polygonal or irregular, 20–25(–26) × 18–22.5 µm ($x = 22.5$ × 21.2 µm, $n = 37$)) is similar to *Ap. neogarethjonesii* (globose to subglobose, 20–35 × 15–30 µm), *Ap. pseudoparenchymatica* (globose to subglobose, 13.5–27 × 12–23.5 µm), and *Ap. yunnana* (globose to obovoid, 17.5–26.5 × 15.5–25 µm) [7,33,42]. However, they could be distinguished by the shape of the conidia, the presence or absence of hyphopodia, and phylogeny. The *Ap. lageniformis* sp. nov. is closely related to *Ap. jiangxiense* (M. Wang & L. Cai) Pintos & P. Alvarado, *Ap. obvata* (M. Wang & L. Cai) Pintos & P. Alvarado, and *Ap. arctoscopi* (S.L. Kwon, S. Jang & J.J. Kim) S.L. Kwon & J.J. Kim in concatenate phylogeny (Figure 1). However, they could be distinguished by culture characteristics, growth rates, conidia size, and conidiogenous cell shape. The *Ap. lageniformis* sp. nov. is characterized by basauxic, polyblastic, and lageniform conidiogenous cells. The other two unrecorded species, *Ap. hysterina* and *Ap. paraphaeosperma*, could also be distinguished from cryptic species and identified as a new record species in this study, but both morphological and phylogenetic analyses are needed.

To date, 34 *Apiospora* species have been reported in bamboo materials worldwide (Table 2). In contrast, only two bambusicolous *Apiospora* species have been reported in Korea (*Ap. arundinis* and *Ap. camelliae-sinensis*) [15,17]. In the present study, nine *Apiospora* species contained two unrecorded species (*Ap.*

Table 2. List of bambusicolous *Apiospora* in worldwide.

Species	Bamboo species[a]	Organs	Country	Reference
Apiospora acutiapica	*Ba. bambos*	Clump	China	Senanayake et al. [36]
Ap. arundinis	*Sasa* sp., unidentified	Culm, leaf	Canada, China, Korea	Crous and Groenewald. [4], Wang et al. [7], Kim et al. [15]
Ap. bambusicola	Unidentified	Dead culm	Thailand	Tang et al. [37]
Ap. biseriale	Unidentified	Dead branch and culm	China	Feng et al. [38]
Ap. camelliae-sinensis	*Ph. bambusoides*	Leaf	Korea	Park et al. [17]
Ap. chiangraiense	Unidentified	Dead culm	Thailand	Tian et al. [10]
Ap. esporlensis	*Ph. aurea*	Dead culm	Spain	Pintos et al. [32]
Ap. euphorbiae	Unidentified	Dead culm	China	Jayasiri et al. [39]
Ap. garethjonesii	Unidentified	Dead culm and branch	China	Dai et al. [40], Feng et al. [38]
Ap. gelatinosa	Unidentified	Dead culm and branch	China	Feng et al. [38]
Ap. guizhouensis	*Ba. multiplex*	Branch	China	Senanayake et al. [36]
Ap. hydei	*Ba. tuldoides*, unidentified	Culm, leaf	Hong Kong, China	Crous and Groenewald. [4]
Ap. hyphopodii	*Ba. tuldoides*	Culm	Thailand	Senanayake et al. [31]
Ap. hysterina	*Bambusa* sp., *Ph. aurea*	Dead culm	New Zealand, Spain	Pintos et al. [32]
Ap. jiangxiensis	*Phyllostachys* sp., unidentified	Leaf	China	Wang et al. [7]
Ap. longistroma	Unidentified	Decaying culm	Thailand	Dai et al. [33]
Ap. neobambusae	Unidentified	Leaf	China	Wang et al. [7]
Ap. neochinensis	*Fa. qinlingensis*	Culm	China	Jiang et al. [41]
Ap. neogarethjonesii	Unidentified	Dead culm	China	Hyde et al. [42]
Ap. neosubglobosa	Unidentified	Dead culm	China	Dai et al. [40]
Ap. multiloculata	Unidentified	Dead culm	Thailand	Bhunjun et al. [43]
Ap. paraphaeosperma	*Bambusa* sp.	Dead clumps	Thailand	Hyde et al. [35]
Ap. phyllostachydis	*Ph. heteroclada*	Dead culm	China	Yang et al. [44]
Ap. pseudoparenchymatica	Unidentified	Leaf	China	Wang et al. [7]
Ap. pseudorasikravindrae	*Ba. dolichoclada*	Sheath	China	Senanayake et al. [36]
Ap. pseudosinensis	Unidentified	Leaf	Netherlands	Crous and Groenewald. [4]
Ap. qinlingensis	*Fa. qinlingensis*	Culm	China	Jiang et al. [45]
Ap. rasikravindrae	Unidentified, *L. intermedia*	Dead culm, Leaf, Shoot	China, Thailand	Wang et al. [7], Tian et al. [10], Majeedano et al. [46]
Ap. sacchari	Unidentified		Indonesia	Crous and Groenewald. [4]
Ap. septata	Unidentified	Dead culm	China	Feng et al. [38]
Ap. subglobosa	Unidentified	Culm	Thailand	Senanayake et al. [31]
Ap. subroseum	Unidentified	Leaf	China	Wang et al. [7]
Ap. thailandica	Unidentified	Culm	Thailand	Dai et al. [33]
Ap. yunnana	Unidentified	Culm	China	Dai et al. [33]

[a]The genus names of bamboo were abbreviated as: *Ba.*, *Bambusa*; *Ph.*, *Phyllostachys*; *Fa.*, *Fargesia*; and *L.*, *Lignania*.

hysterina and *Ap. paraphaeosperma*), five recorded species (*Ap. arundinis*, *Ap. camelliae-sinensis*, *Ap. rasikravindrae*, *Ap. sargassi*, and *Ap. saccharicola*), and two novel species (*Ap. pseudohyphopodii* sp. nov. and *Ap. lageniformis* sp. nov.) were found in bamboo forests. Two previously unrecorded species have been reported from bamboo materials in New Zealand (*Ap. hysterina*), Spain (*Ap. hysterina*), and Thailand (*Ap. paraphaeosperma*) [32,35]. Moreover, one recorded species, *Ap. rasikravindrae* has been reported in bamboo in China [7]. However, the other two recorded species (*Ap. sargassi* and *Ap. saccharicola*) have not been reported in bamboo until now; thus, this is the first report of these species isolated from bamboo materials.

Research on bambusicolous fungi may provide opportunities to control bamboo pathogens and promote bamboo cultivation [47]. However, the ecological roles of most of the *Apiospora* remain unknown. Therefore, *Apiospora* diversity and their ecological roles need to be explored further. This study will serve as a basis for the taxonomic study of *Apiospora* and is expected to be the groundwork for potentially determining the diversity of *Apiospora* in the bamboo forests of Korea.

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