

Sexual Morph of *Furcasterigmium furcatum* (Plectosphaerellaceae) from *Magnolia liliifera* Collected in Northern Thailand

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Abstract: We isolated an interesting fungus from dead leaves of *Magnolia liliifera* collected from Chiang Mai, Thailand. The novel strain is related to Plectosphaerellaceae based on the morphology of its asexual morph and the analysis of sequence data. Phylogenetic analyses using a combined gene analysis of LSU and ITS sequence data showed that this strain is clustered in the same clade with *Furcasterigmium furcatum* with high statistical support. The new strains produced the asexual morph in culture which is morphologically similar to *F. furcatum*. Thus, we identified this strain as the sexual morph of *F. furcatum*. This is the first record of sexual morph for the monotypic genus *Furcasterigmium* and the first record of this genus on *Magnolia*.

Keywords: *Acremonium*; phylogeny; sexual morph; sordariomycetes; taxonomy

1 Introduction

The family Plectosphaerellaceae was introduced by Zare et al. [1], with the generic type *Plectosphaerella* [1,2]. Members in this family are found in diverse habitats, and most of them are soil-borne saprobes and plant pathogens [3–5]. Some species are opportunistic animal pathogens: *Gibellulopsis serrae* and *Plectosphaerella oratosquillae* [6–8]. The family Plectosphaerellaceae was placed in the order Glomerellales by Maharachchikumbura et al. [9]. At present, 24 genera are accepted in the family, with two holomorphic genera; *Plectosphaerella* and *Sodiomyces* [10]. However, the asexual morphs are more similar among genera, with simple or verticillate conidiophores, mono- or polyphialidic conidiogenous cells and mostly cylindrical or ellipsoidal one or two celled conidia arranged in slimy heads [1,11]. The sexual morphs are characterised by perithecial or cleistothecial ascomata, superficial, brown to dark brown, with clavate or saccate asci and hyaline to pale brown ascospores [11].

Acremonium represents a polyphyletic genus, with *A. alternatum* as the type species. Most of the species are related to Bionectriaceae (Hypocreales) [12], whereas, other *Acremonium* species belong to either Glomerellales or other Hypocreales [1,4,9,13–17]. *Acremonium furcatum* was established by Moreau et al. [18] with the invalid name; *Cephalosporium furcatum*. Gams et al. [19] validated and transferred the species to *Acremonium*, which grouped in the section Nectrioidea [20]. Glenn et al. [21] showed that



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A. furcatum is likely related to *Microascus* and *Ceratocystis* of the order Microascales. However, the taxonomic placement of the species was uncertain [21], until, Zare et al. [1] established the family Plectosphaerellaceae and accommodated *Acremonium furcatum*. Giraldo et al. [11] proposed a new monotypic genus, *Furcasterigmium* with *F. furcatum* as the type species in Plectosphaerellaceae based on their phylogenetic analyses of combined LSU, ITS, TEF1- α and RPB2 sequence data.

During an investigation on diversity of leaf litter fungi in northern Thailand, we isolated an interesting fungus resembling a species of *Plectosphaerellaceae* collected from *Magnolia liliifera* in Chiang Mai Province. Based on morphological studies and phylogenetic analyses of combined LSU and ITS sequence data, this fungus is referred to *Furcasterigmium furcatum*. The sexual morph of *F. furcatum* is reported for the first time in this study with description and illustrations.

2 Materials and Methods

2.1 Fungal Collection, Isolation and Morphology

Dead leaves of *Magnolia liliifera* was collected from Chiang Mai, Thailand, following the methods outlined by Monkai et al. [22] and taken back to the laboratory using plastic Ziploc bags. Samples were placed in a plastic box with moistened tissue paper and maintained at room temperature (28–32°C) for 1–2 weeks. Morphological characters were observed using a Motic SMZ 168 Series stereomicroscope (Motic Incorporation Ltd., Hong Kong). Microscopy photographs were taken using Nikon ECLIPSE 80i compound microscope equipped with a Canon EOS 600D digital camera. Measurements of fungal structures were calculated using Tarosoft (R) Image Frame Work program. Figures were arranged using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). A pure culture was acquired using the single spore isolation method [23] and sub-cultured onto potatoes dextrose agar (PDA) and sabouraud dextrose agar (SDA) The characteristics of asexual morph were observed on cultures. Culture was deposited in Mae Fah Luang University Culture Collection (MFLUCC) and the type specimen was deposited in the herbarium of Mae Fah Luang University (MFLU). The Faces of Fungi number was issued as described in Jayasiri et al. [24].

2.2 DNA Extraction, PCR Amplification and Sequencing

Fresh mycelium was scraped from the margin of a colony cultivated on PDA for 2 weeks at 28°C. Genomic DNA was extracted from the fresh mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, China) according to the manufacturer protocol. DNA sequence data was processed by the sequences of the internal transcribed spacers region of ribosomal DNA (ITS) using primer pairs ITS5 and ITS4 [25], and partial large subunit nuclear ribosomal DNA (LSU) using primer pairs LR0R and LR5 [26]. The polymerase chain reaction (PCR) amplification included the mixtures of 50 ng template DNA, PCR Master Mix 1.3, 0.5 mM of each primer in a 25 mL volume, 50 U *Taq* DNA polymerase 400 mM of each dNTP, and 3 mM MgCl₂. The thermal cycling programme consisted of 95°C for 2 min, followed by 35 cycles of denaturation at 94°C 1 min, annealing at 50°C 1 min, extension at 72°C 1 min and a final extension step of at 72°C for 10 min. PCR products were examined for the quality by staining with red gel on 1% agarose gel electrophoresis. Sequencing were conducted by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The obtained nucleotide sequence data was deposited in GenBank (Tab. 1).

2.3 Phylogenetic Analysis

The sequences generated in this study were analysed with the reference sequence data of representative genera in Plectosphaerellaceae which were derived from GenBank, and relevant publications [11,12] (Tab. 1). The alignments of consensus sequences were performed using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>; [27]), and improved manually using Bioedit v. 7.0.9.1 [28]. The final

Table 1: Details of the taxa used in the analyses and their GenBank accession numbers

Taxa	Culture Accession No.	GenBank Accession No.	
		LSU	ITS
<i>Acrostalagmus annulatus</i>	CBS 121.84	LR025802	LR026673
<i>Acrostalagmus luteoalbus</i>	CBS 112.16	LR025797	LR026668
<i>Acrostalagmus luteoalbus</i>	JW 1001	LR590267	LR590089
<i>Brunneochlamydosporium cibotii</i>	CBS 109240 ^{IT}	LR025807	LR026678
<i>Brunneochlamydosporium nepalense</i>	CBS 971.72 ^{IT}	HQ231970	DQ825971
<i>Brunneochlamydosporium terrestre</i>	CBS 112777 ^T	LR025819	LR026690
<i>Brunneomyces brunnescens</i>	CBS 559.73 ^T	HQ231966	LN810520
<i>Brunneomyces europaeus</i>	CBS 652.96 ^T	LN810512	LN810519
<i>Brunneomyces hominis</i>	FMR 10429 ^T	LN810509	KP131517
<i>Chlamydosporiella restricta</i>	CBS 178.40 ^T	LR025822	LR026693
<i>Chlamydosporiella restricta</i>	CBS 443.66	LR025824	LR026695
<i>Chordomyces albus</i>	CBS 987.87 ^T	JX158444	DQ825970
<i>Chordomyces albus</i>	CBS 299.70E	LR025830	LR026701
<i>Chordomyces antarcticus</i>	CBS 120045 ^T	KJ443109	KJ443241
<i>Colletotrichum fructicola</i>	LC0032	JN940418	JN943079
<i>Colletotrichum gloeosporioides</i>	LC0555	JN940412	JN943090
<i>Furcaterigmium furcatum</i>	CBS 299.70C	MH871416	MH859660
<i>Furcaterigmium furcatum</i>	CBS 122.42 ^T	LR025838	LR026709
<i>Furcaterigmium furcatum</i>	CBS 299.70A	LR025839	LR026710
<i>Furcaterigmium furcatum</i>	CBS 116548	LR025842	LR026712
<i>Furcaterigmium furcatum</i>	CBS 116550	LR025843	LR026713
<i>Furcaterigmium furcatum</i>	JW 12002	LR590307	LR590130
<i>Furcaterigmium furcatum</i>	JW 125004	LR590308	LR590131
<i>Furcaterigmium furcatum</i>	JW 170003	LR590309	LR590132
<i>Furcaterigmium furcatum</i>	MFLUCC 10-0282	KC775775	KC775774
<i>Fuscohypha expansa</i>	CBS 418.89 ^T	LR025845	LR026715
<i>Fuscohypha expansa</i>	CBS 103.95	LR025844	LR026714
<i>Gibellulopsis fusca</i>	CBS 560.65 ^T	LR025854	LR026724
<i>Gibellulopsis nigrescens</i>	CBS 120949 ^{NT}	LR025857	LR026727
<i>Gibellulopsis serrae</i>	CBS 290.30 ^T	LR025872	LR026742
<i>Lectera colletotrichoides</i>	IMI 303685	LR025894	JQ647450
<i>Lectera humicola</i>	IMI 265740 ^T	LR025896	JQ647449
<i>Lectera phaseoli</i>	IMI 366179 ^T	LR025898	JQ693168
<i>Musicillium elettariae</i>	CBS 252.80 ^T	LR025899	LR026765

(Continued)

Table 1 (continued).

Taxa	Culture Accession No.	GenBank Accession No.	
		LSU	ITS
<i>Musicillium theobromae</i>	CBS 968.72 ^{NT}	LR025907	LR026773
<i>Musicillium tropicale</i>	CBS 120009 ^T	LR025917	LR026783
<i>Musidium stromaticum</i>	CBS 863.73 ^T	MH872546	MH860814
<i>Musidium stromaticum</i>	CBS 132.74	LR025919	LR026785
<i>Nigrocephalum collariferum</i>	CBS 124586 ^T	MH874911	MH863392
<i>Nigrocephalum collariferum</i>	CBS 124585	LR025928	FJ765365
<i>Paragibbellulopsis chrysanthemi</i>	MAFF 242621 ^T	KC287230	KC287235
<i>Paragibbellulopsis chrysanthemi</i>	MAFF 243429	KC287229	KC287234
<i>Paramusicillium asperulatum</i>	CBS 120158 ^T	LR025930	LR026792
<i>Phialoparvum bifurcatum</i>	CBS 299.70B ^T	LR025931	LR026793
<i>Phialoparvum maaspleinense</i>	JW 266001 ^T	LR590368	LR590190
<i>Plectosphaerella cucumerina</i>	CBS 137.37 ^T	MH867359	MH855856
<i>Plectosphaerella plurivora</i>	CBS 131742 ^T	LR025967	LR026829
<i>Sayamraella subulata</i>	BCC 78964 ^T	LR025971	LR026833
<i>Sodiomyces alcalophilus</i>	CBS 114.92 ^{IT}	JX158443	JX158421
<i>Sodiomyces alkalinus</i>	CBS 110278 ^T	JX158427	NR_145378
<i>Sodiomyces tronii</i>	CBS 137618 ^T	KJ443147	KJ443277
<i>Stachylidium bicolor</i>	CBS 121802 ^{ET}	LR025972	LR026834
<i>Stachylidium pallidum</i>	BCC 79031	LR025973	LR026835
<i>Summerbellia oligotrophica</i>	CBS 657.94 ^T	LR025849	LR026719
<i>Summerbellia oligotrophica</i>	CBS 299.70G	LR025846	LR026716
<i>Theobromium fuscum</i>	CBS 112271 ^T	LR025976	LR026839
<i>Verticillium dahliae</i>	CBS 130341 ^{ET}	LR026028	LR026889
<i>Verticillium longisporum</i>	CBS 124.64 ^T	LR026040	LR026901
<i>Verticillium tricorpus</i>	CBS 447.54 ^T	LR026083	LR026944

The newly generated sequence is indicated in bold. ^TEx-type, ^{ET}Ex-pitype, ^{IT}Ex-sotype, ^{NT}Ex-eotype. BCC: BIOTEC Culture Collection, Pathumthani, Thailand; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; FMR: Faculty of Medicine of Reus, Reus, Spain; IMI: International Mycological Institute, CABI-Bioscience, Egham, BAKEHAM Lane, UK; JW: Johanna Westerdijk Collection, Utrecht, The Netherlands; LC: Working collection of Lei Cai, housed at the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; MAFF: Ministry of Agriculture, Forestry and Fisheries, Ibaraki, Japan; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand.

alignment and tree were deposited in TreeBASE, submission ID 25582 (Study Accession URL: <http://purl.org/phylo/treebase/phyloids/study/S25582>). Phylogenetic analyses of both the individual and combined dataset were carried out using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses.

A ML analysis was generated by raxmlGUI v.1.3 [29] using ML + rapid bootstrap setting with 1000 replicates. A MP analysis was conducted in PAUP v.4.0b 10 [30] using the heuristic search option with 1,000 random replicates and maxtrees set as 1000. Alignment gaps were treated as missing characters.

The branches of zero length were collapsed and all equally parsimonious trees were saved. Clade stability was evaluated using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa [31]. Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were analyzed for all trees. The Kishino-Hasegawa tests [32] were used to check whether the trees inferred under different optimality criteria were significantly different. The BI analysis was generated by MrBayes v3.1.2 using Markov chain Monte Carlo sampling (BMCMC) to assess posterior probabilities [33,34]. Six simultaneous Markov chains were run for 2000000 generations and trees were sampled every 200th generation. The first 10% generated trees were discarded after checking the effective sampling size (ESS) using the Tracer v. 1.6 [35]. While the remaining 90% of trees were applied for calculating the posterior probability (PP) of the majority rule consensus tree. Phylograms were observed in FigTree v1.4.0 program [36] and reorganized in Adobe Photoshop CS6 software (Adobe Systems, USA).

3 Results

3.1 Phylogenetic Analyses

The combined LSU and ITS sequence dataset including our strain and other related taxa in Plectosphaerellaceae comprised 58 taxa (Fig. 1). Phylogenetic analyses obtained from ML, MP and BI analyses show similar topologies and were not significantly different. The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -8388.191939 . The matrix had 453 distinct alignment patterns, with 4.37% of undetermined characters or gaps. Parameters for the GTR + I + G model of the combined LSU and ITS were as follows: Estimated base frequencies; A = 0.231405, C = 0.264478, G = 0.296765, T = 0.207353; substitution rates AC = 1.048443, AG = 1.746751, AT = 2.332002, CG = 0.591076, CT = 5.613633, GT = 1.000000; gamma distribution shape parameter $\alpha = 0.198778$. The maximum parsimony dataset consists of 1353 characters, including 918 constant characters, 40 variable parsimony-uninformative characters and 395 parsimony-informative characters. The most parsimonious tree showed, TL = 1287, CI = 0.501, RI = 0.777, RC = 0.389, HI = 0.499. The new strain clustered with the type strains (CBS 122.42) and other isolates of *F. furcatum* (100% ML, 100 % MP and 1.00 PP) (Fig. 1).

3.2 Taxonomy

Furcasterigmium furcatum (W. Gams) A. Giraldo & P.W. Crous, Studies in Mycology 92: 227–286 (2019). (Figs. 2 and 3)

Index Fungorum Number: IF 828042; *Facesoffungi Number*: FoF 07081

Holotype: MFLU 12–2222

Saprobic on dead leaves of *Magnolia liliifera*. **Sexual morph**: *Ascomata* solitary, immersed to semi-immersed, becoming erumpent, perithecial, black, papillate. *Papilla* central, black, conical. *Paraphyses* widest at the base up to 8. μm and narrowest at the end up to 3 μm , septate, unbranched. *Asci* 47–61 \times 5–10 μm ($\bar{x} = 53 \times 8 \mu\text{m}$), 8-spored, unitunicate, cylindrical, clavate, thin-walled, swollen in the centre in squash mount, with a short pedicel, no apical ring observed in Melzer's reagent. *Ascospores* 8–11 \times 2–3 μm ($\bar{x} = 3 \times 9 \mu\text{m}$), uniseriate at the base, overlapping uniseriate to biseriate in the main part, cylindrical with slightly narrowing ends, hyaline, one-celled, with several guttules, rough-walled, surrounded by a gelatinous sheath, 1.5–2.4 thick ($\bar{x} = 2 \mu\text{m}$). **Asexual morph**: Hyphomycetous. *Conidiophore* hyaline, unbranched, commonly proliferating sympodially. *Conidiogenous cell* 5–42 μm long, 1.5–3 μm wide at the base, with cylindrical collarette, and a conspicuous periclinal thickening at the conidiogenous locus, commonly with a percurrent proliferation, polyphialidic with up to three conidiogenous loci. *Conidia* broadly ellipsoidal, concave in the middle and thickening toward the end, one cell, hyaline, smooth- and thin-walled, 1.8–3.3 \times 1.3–2.3 μm ($\bar{x} = 2.2 \times 1.7 \mu\text{m}$), arranged in slimy heads. *Chlamydospore* terminal or intercalary, mostly in chains, ellipsoidal to pyriform, smooth- and thick-walled, 12–23 \times 3–5 μm ($\bar{x} = 16 \times 4 \mu\text{m}$).

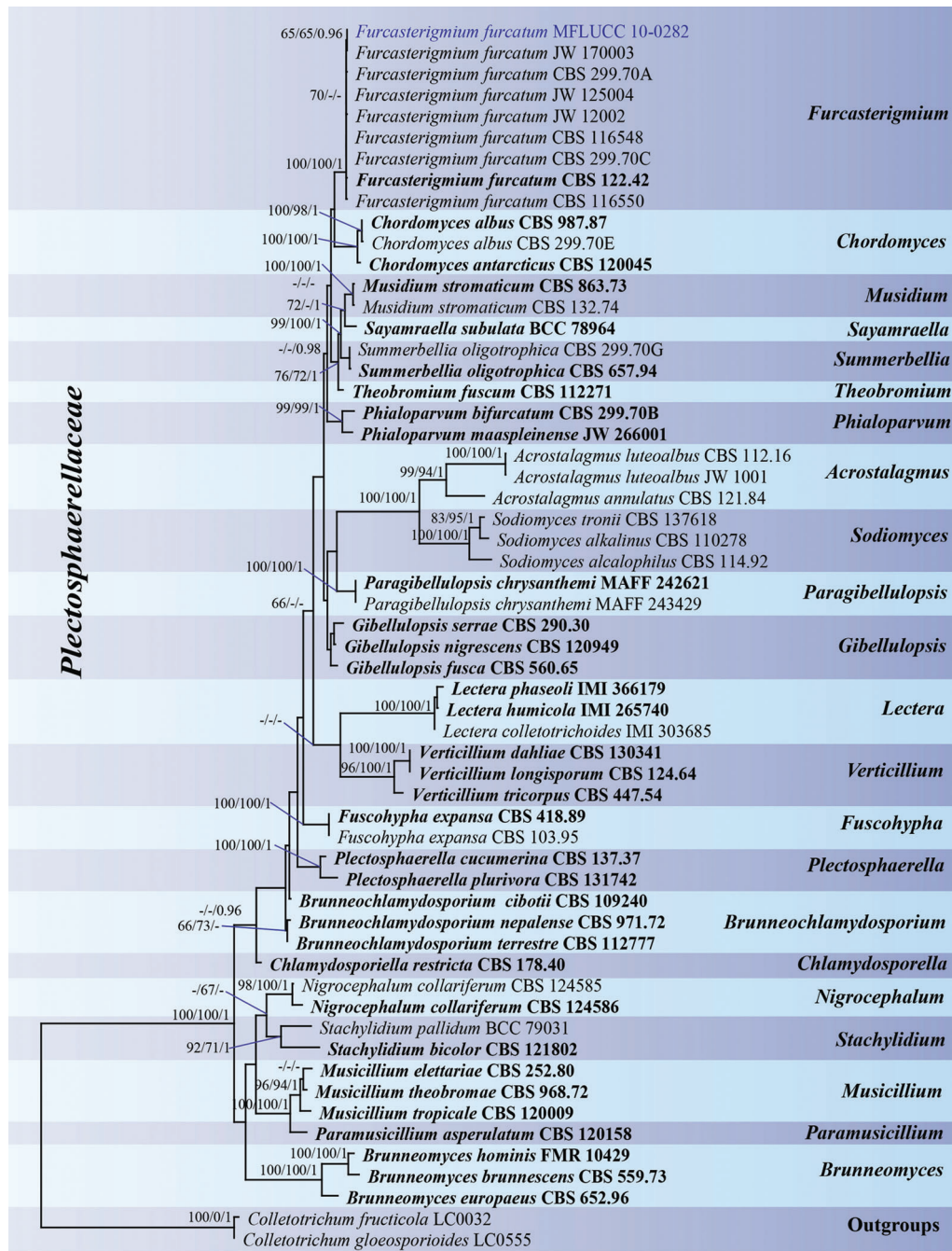


Figure 1: Phylogenetic tree obtained from RAxML analyses of combined LSU and ITS sequence data. Bootstrap support values for ML, MP equal or greater than 65% and PP greater than 0.95 are indicated at the nodes. Ex-epitype, Ex-isotype, Ex-neotype and Ex-type strains are in bold and the new isolate of this study is in dark blue. The tree is rooted to *Colletotrichum fructicola* (LC0032) and *C. gloeosporioides* (LC0555)

Culture characteristics: Colonies on sabouraud dextrose agar (SDA) effuse, mostly superficial, white, 10 mm diam in 15 days at 28°C. Mycelium mucoid, irregular, crenated edge, no pigment diffusing into agar. Asexual morph sporulated after two months.

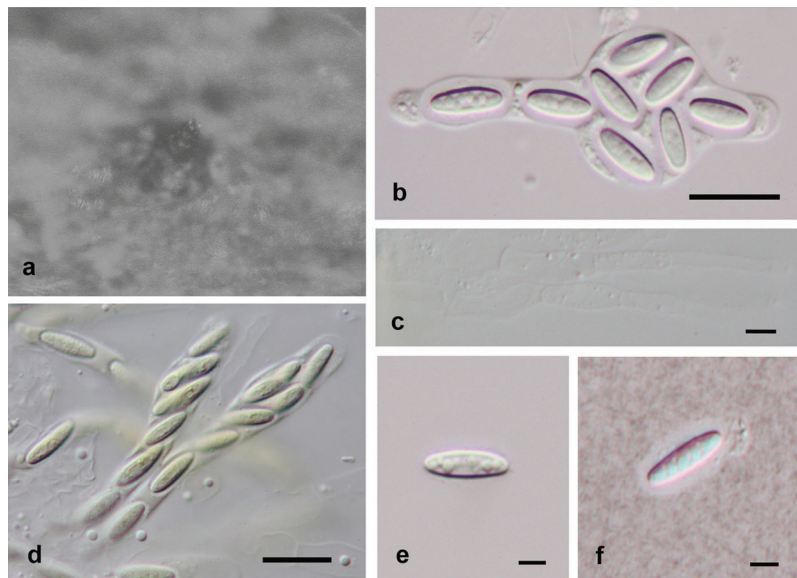


Figure 2: Sexual morph of *Furcasterigmium furcatum* (MFLUCC 12–2222). (a) Ascoma on the host surface. Note the papilla. (b) Ascus in wet mount slide by squashing. (c) Paraphyses. (d) Asci in Melzer's reagent with no visible apical ring. (e) Ascospore. (f) Ascospore in India ink. Scale bars: (b–d) = 10 μm , c = 5 μm , (e–f) = 3 μm

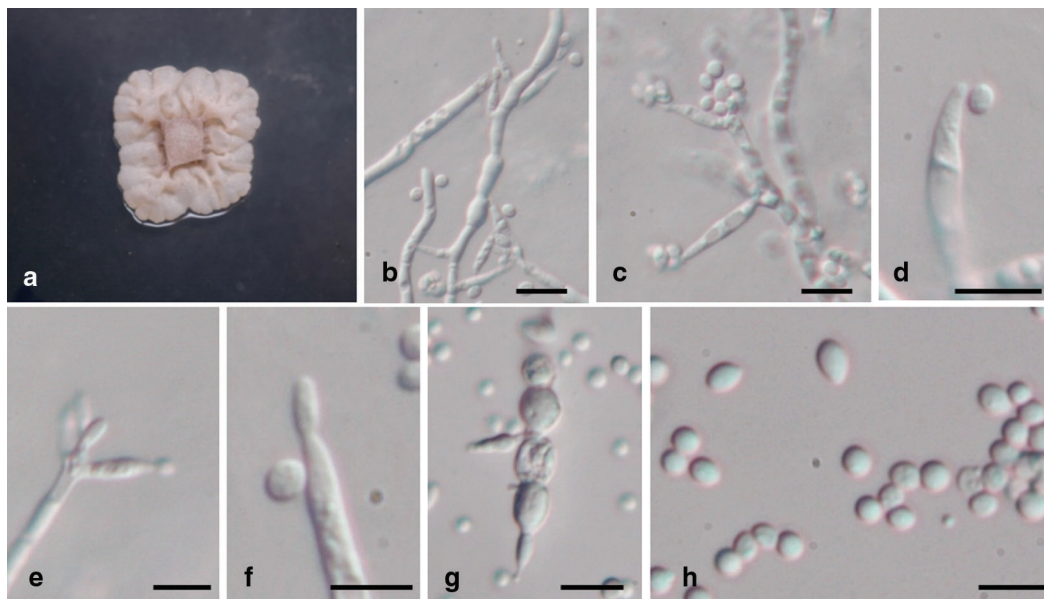


Figure 3: Asexual morph of *Furcasterigmium furcatum* (MFLUCC 10–0282). (a) Colony on SDA after 15 days at 28°C. (b–c) Conidiophores. (d–f) Conidiogenous cells. (g) Chlamydospores. (h) Conidia. Scale bars: (a–h) = 5 μm

Material examined: THAILAND: Chiang Mai, Doi Suthep-Pui forest, on dead leaves of *Magnolia liliifera* (L.) Baill. (Magnoliaceae), 11 January 2010, J. Monkai (MFLU 12–2222), living culture, MFLUCC 10–0282.

Notes: our strain belongs to *F. furcatum*. The sexual morph of *F. furcatum* is similar to *Plectosphaerella cucumeris* and *P. plurivora*, but differs in having paraphyses and ascospores with a gelatinous sheath [4,10]. The sexual morph of *F. furcatum* is compared to other related species in Plectosphaerellaceae (Tab. 2). The asexual morph of our strain is similar to the type strains of *F. furcatum* (CBS 122.42) [11]. However, our strain has broadly ellipsoidal conidia with a slightly different size [*F. furcatum* MFLUCC 10-0282, $1.8\text{--}3.3 \times 1.3\text{--}2.3 \mu\text{m}$ vs. $2.7\text{--}3.8 \times 1.5\text{--}2.1 \mu\text{m}$, *F. furcatum* CBS 122.42] and the production of chlamydospores in cultures in our strain [11]. However, the asexual characters of the type strain were observed on malt extract agar (MEA). *Chordomyces albus*, *Phialoparvum bifurcatum* and *Theobromium fuscum* were previously identified as *A. furcatum* [5,11]. However, they are phylogenetic distant and these species differ from *F. furcatum* in having polyphialides with up to two conidiogenous loci [11]. The asexual morph of *F. furcatum* is compared to other related species in Plectosphaerellaceae (Tab. 3).

Table 2: Synopsis of the sexual morph species in Plectosphaerellaceae discussed in this study

Species	Ascomata	Paraphyses	Asci	Ascospores	References
<i>Furcasterigmium furcatum</i>	Perithecial solitary, immersed to semi-immersed, becoming erumpent	Septate, unbranched, $8 \mu\text{m}$ widest at the base and $3 \mu\text{m}$ narrowest at the end	Cylindrical, clavate, $47\text{--}61 \times 5\text{--}10 \mu\text{m}$, swollen in the centre in squash mount, no apical apparatus	Cylindrical with slightly narrowing ends, 1-celled, $8\text{--}11 \times 2\text{--}3 \mu\text{m}$, gelatinous sheath present	This study
<i>Plectosphaerella cucumerina</i>	Globose to pyriform, $90\text{--}130 \mu\text{m}$ wide	Absent	Cylindrical $50\text{--}80 \times 6\text{--}9 \mu\text{m}$, no apical apparatus	Ellipsoid, both ends rounded, 2-celled, $(9\text{--}) 10.5\text{--}14(\text{--}15) \times 2.5\text{--}3(\text{--}4) \mu\text{m}$	[4]
<i>P. plurivora</i>	Perithecial solitary or gregarious, superficial, subglobose to pyriform, $100.3\text{--}209 \times 86\text{--}156 \mu\text{m}$	Absent	Clavate, $31.4\text{--}43 \times 6.2\text{--}8.2 \mu\text{m}$, no apical apparatus	Ellipsoidal, 1- or 2-celled, $6.1\text{--}13.2 \times 2.4\text{--}3.7 \mu\text{m}$	[11]
<i>Sodiomyces alkalinus</i>	Cleistothecial, $120\text{--}150 \mu\text{m}$ wide	Absent	Saccate, scattered irregularly in the ascocarp, no apical apparatus	Ellipsoidal or ovoid, 2-celled, $12\text{--}15 \times 5\text{--}7 \mu\text{m}$	[15]

4 Discussion

This study is the first report of the sexual morph of *Furcasterigmium furcatum* in the family Plectosphaerellaceae and is supported by the molecular evidence. Strains of *F. furcatum* are commonly isolated from soil [11,20]. However, this strain was isolated from moist house and also as an endophyte of *Vitis vinifera* [11]. The distribution of *F. furcatum* was restricted to Europe with reports of this species

Table 3: Synopsis of the asexual morph species in Plectosphaerellaceae discussed in this study

Species	Basionym (previous identification)	Conidiophores	Conidiogenous cell	Conidia	Chlamydospore	References
<i>Furcasterigmium furcatum</i>	<i>A. furcatum</i>	Unbranched, proliferating sympodially	Phialides subulate, 18–36 × 2–2.5 µm, cylindrical collarete, polyphialides with up to three conidiogenous loci	Ellipsoidal, 1-celled, 2.7–3.8 × 1.5–2.1 µm, arranged in slimy heads	–	[11]
<i>Brunneochlamydosporium nepalense</i>	<i>Acremonium nepalense</i>	Simple or poorly branched	Phialides cylindrical or subulate, 25–55 × 2–2.5 µm, minute cylindrical collarete, polyphialides with up to two conidiogenous loci	Cylindrical with rounded ends to ellipsoidal, 1- celled, 3.2–4.7 × 1.9–2.4 µm, arranged in slimy heads	Lateral, terminal, solitary, in pairs, subglobose or irregularly shaped 4.4–5 × 3.5–3.6 µm	[11]
<i>Chlamydosporiella restricta</i>	<i>Verticillium dahliae f. restrictum</i>	Unbranched or basitonously branched	Phialides cylindrical, slightly wavy at the apex, 22.7–45 × 1.5–2 µm, short collarete	Obovoid, widely ellipsoidal with apiculate base, 1-celled, 2.2–4.7 × 1.5–2.3 µm, arranged in slimy heads	Terminal or intercalary, single or branched chains, subglobose, 3–5.6 × 2.3– 5.6 µm	[11]
<i>Chordomyces albus</i>	<i>A. furcatum</i>	Unbranched, bearing 3–4 phialides	Phialides cylindrical or subulate, 12–22 × 2–2.5 µm, cylindrical collarettes	Ellipsoidal to near cylindrical, 1-celled, 3–4 × 2–2.5 µm, arranged in slimy heads	–	[11]
<i>Musidium stromaticum</i>	<i>A. stromaticum</i>	Unbranched or basitonously branched	Phialides subulate, 23–55 × 2–2.5 µm, cylindrical collarete, percurrent proliferation	Cylindrical with rounded ends or ellipsoidal, 1- celled, 4.2–6.2 × 1.4–2.3 µm, arranged in slimy heads	Stromatic hyphae branched	[11]
<i>Nigrocephalum collariferum</i>	<i>A. collariferum</i>	Simple or basitonously branched	Phialides sub- cylindrical to subulate, slightly wavy at the apex, 17–51.5 × 1.5–2.5 µm, conspicuous funnel-shaped collarete, percurrent proliferation	Widely ellipsoidal, concave in lateral view, 1-celled, 2.9–5 × 2.1–2.7 µm, pale brown to brown, arranged in slimy heads	–	[11]

(Continued)

Table 3 (continued).

Species	Basionym (previous identification)	Conidiophores	Conidiogenous cell	Conidia	Chlamyospore	References
<i>Phialoparvum bifurcatum</i>	<i>A. furcatum</i>	Unbranched or poorly branched	Phialides subulate to ampulliform, 8–15 × 1.5–2.7 μm, cylindrical collarette, polyphialides with up to two conidiogenous loci	Cylindrical, 1-celled, 2.8–4.4 × 1.2–1.8 μm, arranged in slimy heads	–	[11]
<i>Plectosphaerella cucumerina</i>	<i>Venturia cucumerina</i>	Unbranched or rarely irregularly branched	Phialides cylindrical, 6–10 × 35–69 μm, cylindrical collarette	Ellipsoidal, tapering gradually to rounded apex and base, widest in the middle, septate or aseptate, aseptate conidia 6.7–7 × 2.7–2.8 μm, septate conidia 8.6–8.9 × 2.8–2.9 μm, arranged in slimy heads	–	[4]
<i>Sayamraella subulata</i>	–	Unbranched or poorly branched, proliferating sympodially	Phialides subulate, 0.3–73.7 × 2.1–3 μm, polyphialides with up to two conidiogenous loci	Ellipsoidal, 1-celled, 3.6–4.7 × 1.7–2.4 μm, arranged in slimy heads	–	[11]
<i>Summerbellia oligotrophica</i>	<i>Gliocladium cibotii</i>	Long	Phialides subcylindrical, 13–50 × 1.5–2 μm, minute cylindrical collarette	Ellipsoidal or cylindrical, 1-celled, 2.3–4.3 × 1.2–2 μm, arranged in slimy heads	Terminal or intercalary, mostly in chains, subglobose, 3–4 × 3–4 μm	[11]
<i>Theobromium fuscum</i>	<i>A. furcatum</i>	Unbranched or basitonously branched, bearing up to two phialides, proliferating sympodially	Phialides subulate, 23–38 × 2–3 μm, minute cylindrical collarette, polyphialides with up to two conidiogenous loci	Cylindrical or ellipsoidal, 1-celled, 2.7–4.1 × 1.3–2 μm, arranged in slimy heads	–	[11]

from France, Germany Italy, and the Netherlands [8,11,20]. Our study reports the first record of *Furcasterigmium* from dead leaves of *Magnolia liliifera* and the first geographic distribution for Thailand.

The identification of species in Plectosphaerellaceae based on only the asexual morph is difficult as morphological characters in culture can differ in different culture media [11]. Combination of sexual and asexual morph characters together with DNA sequence data can help to improve the taxonomic concept of the species in the family Plectosphaerellaceae. This study provides a good example for the connection of sexual and asexual morphs with supporting phylogenetic analyses.

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References

1. Zare, R., Gams, W., Starink-Willemse, M., Summerbell, R. C. (2007). *Gibellulopsis*, a suitable genus for *Verticillium nigrescens*, and *Musicillium*, a new genus for *V. theobromae*. *Nova Hedwigia*, 85(3), 463–489. DOI 10.1127/0029-5035/2007/0085-0463.
2. Kirk, P., Cannon, P. F., Minter, D. W., Stalpers, J. A. (2008). *Ainsworth & bisby's dictionary of the fungi*. 10th edn. Wallingford: CAB International.
3. Cannon, P., Buddie, A., Bridge, P., de Neergaard, E., Lübeck, M. et al. (2012). *Lectera*, a new genus of the *Plectosphaerellaceae* for the legume pathogen *Volutella colletotrichoides*. *MycKeys*, 3(5), 23–36. DOI 10.3897/mycokeys.3.3065.
4. Carlucci, A., Raimondo, M. L., Santos, J., Phillips, A. J. L. (2012). *Plectosphaerella* species associated with root and collar rots of horticultural crops in southern Italy. *Persoonia*, 28(1), 34–48. DOI 10.3767/003158512X638251.
5. Giraldo, A., Gené, J., Sutton, D. A., Wiederhold, N., Guarro, J. (2017). New acremonium-like species in the *Bionectriaceae* and *Plectosphaerellaceae*. *Mycological Progress*, 16(4), 349–368. DOI 10.1007/s11557-017-1271-7.
6. Batista, A. C., Maia, H. da S. (1959). Uma nova doença fúngica de peixe ornamental. *Anais da Sociedade de Biologia de Pernambuco*, 16, 153–159.
7. Duc, P. M., Hatai, K., Kurata, O., Tensha, K., Yoshitaka, U. et al. (2009). Fungal infection of mantis shrimp (*Oratosquilla oratoria*) caused by two anamorphic fungi found in Japan. *Mycopathologia*, 167(5), 229–247. DOI 10.1007/s11046-008-9174-4.
8. Giraldo, A., Hernández-Restrepo, M., Crous, P. W. (2019). New plectosphaerellaceous species from Dutch garden soil. *Mycological Progress*, 18(9), 1135–1154. DOI 10.1007/s11557-019-01511-4.
9. Maharachchikumbura, S. S. N., Hyde, K. D., Jones, E. G., McKenzie, E. H. C., Bhat, J. D. et al. (2016). Families of Sordariomycetes. *Fungal Diversity*, 79(1), 1–317. DOI 10.1007/s13225-016-0369-6.
10. Wijayawardene, N. N., Hyde, K. D., Al-Ani, L. K. T., Tedersoo, L., Haelewaters, D. (2020). Outline of *Fungi* and fungi-like taxa. *Mycosphere*, 11(1), 1060–1456. DOI 10.5943/mycosphere/11/1/8.
11. Giraldo, A., Crous, P. W. (2019). Inside *Plectosphaerellaceae*. *Studies in Mycology*, 92, 227–286. DOI 10.1016/j.simyco.2018.10.005.
12. Summerbell, R. C., Gueidan, C., Schroers, H. J., De Hoog, G. S., Starink, M. et al. (2011). *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. *Studies in Mycology*, 68, 139–162. DOI 10.3114/sim.2011.68.06.

13. Giraldo, A., Gené, J., Cano, J., de Hoog, S., Guarro, J. (2012). Two new species of *Acremonium* from Spanish soils. *Mycologia*, 104(6), 1456–1465. DOI 10.3852/11-402.
14. Gräfenhan, T., Schroers, H. J., Nirenberg, H. I., Seifert, K. A. (2011). An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Studies in Mycology*, 68, 79–113. DOI 10.3114/sim.2011.68.04.
15. Grum-Grzhimaylo, A. A., Debets, A. V., Van Diepeningen, A. D., Georgieva, M. L., Bilanenko, E. N. (2013). *Sodiomyces alkalinus*, a new holomorphic alkaliphilic ascomycete within the *Plectosphaerellaceae*. *Persoonia*, 31(1), 147–158. DOI 10.3767/003158513X673080.
16. Lombard, L., Van der Merwe, N. A., Groenewald, J. Z., Crous, P. W. (2015). Generic concepts in *Nectriaceae*. *Studies in Mycology*, 80, 189–245. DOI 10.1016/j.simyco.2014.12.002.
17. Maharachchikumbura, S. S. N., Hyde, K. D., Jones, E. G., McKenzie, E. H., Huang, S. K. et al. (2015). Towards a natural classification and backbone tree for Sordariomycetes. *Fungal Diversity*, 72(1), 199–301. DOI 10.1007/s13225-015-0331-z.
18. Moreau, F., Moreau, V. (1941). Première contribution à l'étude de la microflore des dunes. *Revue de Mycologie*, 6, 49–94.
19. Gams, W., Domsch, K. H. (1969). Bemerkungen zu einigen schwer bestimmbareren Bodenpilzen. *Nova Hedwigia*, 18, 1–29.
20. Gams, W. (1971). *Cephalosporium-artige Schimmelpilze (Hyphomycetes)*. Stuttgart: Gustav Fischer Verlag.
21. Glenn, A. E., Bacon, C. W., Price, R., Hanlin, R. T. (1996). Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia*, 88(3), 369–383. DOI 10.1080/00275514.1996.12026664.
22. Monkai, J., Promputtha, I., Kodsueb, R., Chukeatirote, E., McKenzie, E. H. C. et al. (2013). Fungi on decaying leaves of *Magnolia liliifera* and *Cinnamomum iners* show litter fungi to be hyperdiverse. *Mycosphere*, 42, 92–301.
23. Chomnunti, P., Hongsanan, S., Aguirre-Hudson, B., Tian, Q., Peršoh, D. et al. (2014). The sooty moulds. *Fungal Diversity*, 66(1), 1–36. DOI 10.1007/s13225-014-0278-5.
24. Jayasiri, S. C., Hyde, K. D., Ariyawansa, H. A., Bhat, J., Buyck, B. et al. (2015). The faces of fungi database: fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity*, 74(1), 3–18. DOI 10.1007/s13225-015-0351-8.
25. White, T. J., Bruns, T., Lee, S., Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J. (eds.) *PCR protocols: a guide to methods and applications*. pp. 315–322. New York: Academic.
26. Vilgalys, R., Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172(8), 4238–4246. DOI 10.1128/JB.172.8.4238-4246.1990.
27. Katoh, K., Standley, D. M. (2017). MAFFT Multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780. DOI 10.1093/molbev/mst010.
28. Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids. Symposium Series*, 41, 95–98.
29. Silvestro, D., Michalak, I. (2012). raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution*, 12(4), 335–337. DOI 10.1007/s13127-011-0056-0.
30. Swofford, D. L. (2002). *PAUP: phylogenetic analysis using parsimony, version 4.0 b10*. Sunderland: Sinauer Associates.
31. Hillis, D. M., Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology*, 42(2), 182–192. DOI 10.1093/sysbio/42.2.182.
32. Kishino, H., Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data. *Journal of Molecular Evolution*, 29(2), 170–179. DOI 10.1007/BF02100115.
33. Huelsenbeck, J. P., Ronquist, F. (2001). MRBAYES: bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754–755. DOI 10.1093/bioinformatics/17.8.754.

34. Zhaxybayeva, O., Gogarten, J. P. (2002). Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *Genomics*, 3(6), 1–15. DOI 10.2174/1389202023350246.
35. Rambaut, A., Drummond, A. J. (2007). *Tracer v1.4*. Available from: <http://beast.bio.ed.ac.uk/Tracer>.
36. Rambaut, A. (2012). *FigTree, version 1.4. 2*. Edinburgh: University of Edinburgh.