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Acrocalymmaceae (Pleosporales) from freshwater habitats in Thailand with the introduction of *Acrocalymma bilobatum* sp. nov.

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Abstract

An observation of lignicolous freshwater fungi in Thailand resulted in the discovery of a novel taxon and recollection of *Acrocalymma aquaticum*. Morphology coupled with phylogenetic analysis support the placement of the new species in *Acrocalymma*. *Acrocalymma bilobatum* sp. nov. resembles *A. ampeli, A. bipolare,* and *A. medicaginis* in conidial shape and the mucoid appendages at both ends but differs in the sizes of conidia and appearance of appendages. Phylogenetic analysis of SSU, LSU, ITS, and TEF1-α sequence data shows that *A. bilobatum* is closely related to *A. bipolare*, *A. chuxiongense*, *A. medicaginis,* and *A. pterocarpi*. *Acrocalymma aquatica* was recollected in Thailand and a detailed description and photographic documentation of its morphological characteristics is provided. The herbarium specimen of *Acrocalymma fici,* collected from freshwater habitats in Thailand, was reexamined to complete a review of Acrocalymmaceae species in the country.

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Introduction

Studies on the taxonomy and phylogeny of freshwater fungi in Thailand, mostly focused on the northern region, and resulted in the discovery of new taxa[1–11]. A checklist of freshwater fungi of Thailand published up to the end of 2010 was provided by Zhang et al.[12] and reported 173 identified species (in 112 genera), of which 26 were new species. The number of novel fungi from freshwater habitats in Thailand increased significantly over the past years wherein Calabon et al.[8] listed an additional 129 new species, dominated by Dothideomycetes (86 species) and Sordariomycetes (40 species), between the years 2015–2020.

In freshwater habitats, Dothideomycetes is the second largest class in Ascomycota (677 species, 229 genera), next to Sordariomycetes with 823 species (in 298 genera)[10,13]. Dong et al.[7] provided a monograph of freshwater dothideomycetous taxa with a comprehensive taxonomic and phylogenetic account. Among these diverse taxa are members of Pleosporales with 391 species dominated by Dictyosporiaceae (55 species), Aigialaceae (27), Lindgomycetaceae (25), and Astrosphaeriellaceae (24). For the monotypic family Acrocalymmaceae, three species were reported from freshwater habitats: *A. aquaticum*[14], *A. bipolare*[7] and *A. fici*[11]. *Acrocalymma aquatica*, characterized by conidia having a single polar appendage, was introduced by Zhang et al.[12] collected from a freshwater stream in Chiang Mai, Thailand. This is the first species in the genus known to thrive in freshwater habitats. *Acrocalymma fici*, a species with flaring mucoid polar appendage, was reported by Boonmee et al.[11] from a freshwater stream in western Thailand. The species was introduced by Trakunyingcharoen et al.[15] which was collected from a terrestrial habitat on *Ficus* sp. The latest addition is *Acrocalymma bipolare* introduced by Dong et al.[7] characterized by conidia filled with oil droplets and bipolar appendages that elongate in contact with water forming filaments

The present study aims to broaden our knowledge of the taxonomy of lignicolous freshwater fungi using morpho-phylogenetic approach. Freshwater habitats in Thailand were explored to document extant and novel aquatic fungi. A new lignicolous freshwater species belonging to *Acrocalymma* from submerged wood in Thailand was discovered and *Acrocalymma aquatica* was recollected from Tak Province. A detailed description with morphological illustrations and multi-gene phylogenetic analyses to confirm the placement of new findings are provided in this paper.

Materials and methods

Sample collection, morphological observation, and fungal isolation

Samples of submerged decayed wood were collected from a stream in Ban Mae Ja Wang, Tha Song Yang District, Tak Province, Thailand on 17 October 2019. Samples were

incubated for five days and observed using a stereomicroscope for the presence of fruiting bodies[16]. The specimens were examined using a Motic SMZ 168 Series stereomicroscope with built-in camera for fungal fruiting bodies on the woody substrate. Micromorphological characters (e.g., ascoma, asci, ascospores) were photographed using a Nikon Eclipse 80i compound light microscope equipped with a Canon EOS 600D digital camera. Single spore isolation was used to obtain pure culture as described by Senanayake et al.[16]. Briefly, hand-sectioned ascomata were aseptically transferred from the wood to sterile glass slides containing distilled water. Asci were teased out to release spores and transferred to malt extract agar (MEA) medium. After 24 h, germinated spores were aseptically transferred into fresh MEA medium. Culture plates were incubated at 25 °C for one month and checked weekly for growth. Herbarium specimens were deposited in Mae Fah Luang University (MFLU). Living cultures were deposited at Mae Fah Luang University Culture Collection (MFLUCC). The new species was registered in Faces of Fungi (www.facesoffungi.org)[17] and Index Fungorum databases[18]. Herbarium material of *Acrocalymma fici* MFLU 21–0124 was loaned from Mae Fah Luang University Fungarium, for comparison with the new taxon.

DNA extraction, PCR amplification, and sequencing

Fungal mycelia from pure cultures grown in MEA for 30 d were scraped using a sterilized scalpel and kept in a 1.5 mL microcentrifuge tube. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction kit (BioFlux®, China) following the manufacturer's protocol. Polymerase chain reaction (PCR) was used to amplify four markers: the large subunit (LSU), small subunit (SSU), internal transcribed spacers (ITS) of rDNA, and the translation elongation factor 1-alpha gene (TEF1-α). The LSU was amplified using the primers LR0R and LR5[19]. For SSU and ITS, primers NS1/NS4 and ITS5/ITS4 were used[20]. TEF1-α was amplified using primers EF1-983F and EF1-2218R[21]. Polymerase chain reaction was performed in a volume of 25 µl, which contained 12.5 µl of 2× Power Taq PCR Master Mix (Bioteke Co., China), 1 µl of each primer (10 µM), 1 µl genomic DNA, and 9.5 µl deionized water. The amplification conditions used were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 90 s, annealing at 55 °C (28S) and 48 °C (18S) for 1 min, and final extension at 72 °C for 10 min. Agarose gel electrophoresis was carried out to confirm the presence of amplicons at the expected molecular weight. PCR products were purified and sequenced with the primers mentioned above at a commercial sequencing provider (BGI, Ltd., Shenzhen, China). A BLAST search of the newly generated sequences was carried out to exclude contamination and to search for related taxa in the GenBank database (www.ncbi.nlm.nih.gov/blast).

Phylogenetic analysis

The taxa table was assembled based on the closest matches from the BLAST search results and from recently published data by Mortimer et al.[22] and Konta et al.[23]. Sequences generated from the four loci were analyzed along with other sequences retrieved from GenBank (Table 1). Four datasets, one for each locus, were aligned with MAFFT v. 7 using the web server (http://mafft.cbrc.jp/alignment/server[24]). Alignment was further refined manually, where necessary, using BioEdit v.7.0.9.0[25]. Aligned sequences were automatically trimmed using TrimAl v. 1.3 on the web server (http://phylemon.bioinfo.cipf.es/utilities.html). The online tool 'ALTER'[26] was used to convert the alignment file to phylip format. Phylogenetic analysis of both individual and combined gene data was performed using maximum likelihood (ML) and Bayesian inference (BI).

Maximum likelihood analysis was performed using RAxML v. 8 software on the CIPRES web portal[27–29] (www.phylo.org/portal2[30]). The GTR+GAMMA model of nucleotide evolution was used. RAxML rapid bootstrapping of 1000 replicates was performed. Bayesian inference analysis was performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES webportal[31], using the parameter setting of 2 parallel runs, 4 chains, run for 1,000,000 generations at which point the standard deviation of split frequencies was below 0.01. Trees were sampled every 200th generations and all other parameters were left as default. In the phylogenetic tree, a bootstrap value of 95%–100% ML and 0.95–1.00 BYPP was considered high support. Newly generated sequences have been deposited in GenBank (Table 1).

Results

Phylogenetic analysis

The combined SSU, LSU, ITS and TEF1-α gene dataset comprised of 32 taxa from Acrocalymmaceae and closely related taxa, with *Boeremia exigua* (CBS 431.74) and *B. foveata* (CBS 341.67) as the outgroup taxa (Table 1).

The analyzed dataset, after trimming, comprised a total of 4103 characters including gaps (SSU = 987, LSU = 1,336 bp, ITS = 869, TEF1-α = 911) with 701 distinct alignment patterns and 49.47% proportion of gaps and completely undetermined-characters. The ML analysis for the combined dataset provided the best scoring tree (Fig. 1) with a final ML optimization likelihood value of –11459.976 (ln). Parameters for the GTR model of the combined SSU, LSU, and ITS dataset are as follows: estimated base frequencies; A = 0.250, C = 0.250, G = 0.250, T = 0.250; substitution rates AC = 1.47340, AG = 1.83232, AT = 1.47340, CG = 1.079563, CT = 4.16678, GT = 1.000000; gamma distribution shape parameter α = 0.227521. Bayesian analysis resulted in 5001 samples of which 3751 samples were included after 1000000 generations. Phylogenetic analyses of the combined data matrix resulted in considerably high bootstrap support and well-resolved clades (Fig. 1). The tree topologies resulted from ML and BI analyses are similar. Support values for maximum likelihood (ML) above 75%, and Bayesian posterior probabilities (BYPP) greater than 0.95 are given at the nodes.

In the phylogenetic analysis (Fig. 1), *Acrocalymma* formed a well-supported monophyletic clade separate from the closely related family Ascocylindricaceae (100% ML, 1.00 BYPP). The novel species *A. bilobatum* (MFLUCC 20–0125) clustered with *Acrocalymma* sp. (K.L. Chen L119), which was named as *A. bilobatum* (K.L. Chen L119) in this study, see notes of *A. bilobatum*. Six distinct clades were formed: Clade I [*A. bilobatum* (MFLUCC 20–0125; K.L. Chen L119), *A. pterocarpi* (MFLUCC 17–0926; MFLUCC 18–0718; NC13-171), *A. medicaginis* (MFLUCC 17-1423; MFLUCC 17-1439), *A. bipolare* (MD1321), *Acrocalymma chuxiongense* (IFRDCC3104)]; Clade II [*A. hongheense* (HKAS 111907; HKAS 111908; HKAS 111909), *Acrocalymma arengae* (MFLUCC 15–0327A; MFLUCC 15–0327B), *A. vagum* (CPC 24225; CPC 24226), *A. walkeri* (UTHSC DI16-195); *A. aquaticum* (MFLUCC 11–0208; MFLUCC 20–0124)]; Clade III [*A. ampeli* (MFLUCC 20-0159; NCYUCC 19-0288)]; Clade IV [*A. guizhouense* (CGMCC

Table 1. Taxa used in this study for the analysis of combined SSU, LSU, and ITS rDNA sequence data and their GenBank accession numbers. The newly generated sequences are indicated with * and the ex-type strains are indicated in bold.

Taxon	Strain / voucher number	SSU	LSU	ITS	TEF1-α
Acrocalymma ampeli	**MFLUCC 20-0159**	**MW079341**	**MW063211**	**MW063150**	–
Acrocalymma ampeli	NCYUCC 19-0288	MW079342	MW063212	MW063151	–
Acrocalymma aquaticum	**MFLUCC 11-0208**	**JX276953**	**NG_042698**	**NR_121544**	–
Acrocalymma aquaticum	MFLUCC 20-0124	–	MT875393	MT875395	MT897894
Acrocalymma arengae	**MFLUCC 15–0327A**	**ON650177**	**ON650673**	**ON650154**	**–**
Acrocalymma arengae	**MFLUCC 15–0327B**	**ON650178**	**ON650674**	**ON650155**	**–**
Acrocalymma bilobatum	K.L. Chen L119	–	–	KX034339	–
Acrocalymma bilobatum*	**MFLUCC 20-0125**	–	**MT875394**	**MT875396**	**MT897895**
Acrocalymma bipolare	**MD1321**	–	**NG_075326**	–	–
Acrocalymma chuxiongense	**IFRDCC3104**	–	**ON596248**	**ON595715**	–
Acrocalymma cycadis	**CBS 137972**	–	**NG_057046**	**NR_137884**	–
Acrocalymma fici	**CBS 317.76**	–	**NG_057056**	**NR_137953**	**KP170663**
Acrocalymma fici	MFLUCC 21-0103	–	MT860429	MT864351	–
Acrocalymma guizhouense	**CGMCC 3.20853**	**OM838471**	**OM838474**	**OM838410**	–
Acrocalymma guizhouense	GZUIFR H22.028	OM838472	OM838475	OM838411	–
Acrocalymma guizhouense	GZUIFR H22.029	OM838473	OM838476	OM838412	–
Acrocalymma hongheense	HKAS 111907	MW424792	MW424777	MW424763	–
Acrocalymma hongheense	HKAS 111908	MW424791	MW424776	MW424762	–
Acrocalymma hongheense	**HKAS 111909**	**MW424790**	**MW424775**	**MW424761**	–
Acrocalymma magnoliae	MFLUCC 18–0545	OL331094	OK655819	OL413439	–
Acrocalymma magnoliae	MFLUCC 21–0204	OL331095	OK655820	OL413440	–
Acrocalymma medicaginis	**CPC 24340**	–	**KP170713**	**KP170620**	–
Acrocalymma medicaginis	MFLUCC 17-1423	MT214387	MT214432	MT214338	–
Acrocalymma medicaginis	MFLUCC 17-1439	MT214388	MT214433	MT214339	–
Acrocalymma pterocarpi	**MFLUCC 17-0926**	**MK347840**	**NG_066306**	**MK347732**	**MK360040**
Acrocalymma pterocarpi	MFLUCC 18–0718	OL331093	OK655818	OL413438	–
Acrocalymma pterocarpi	NC13-171	–	LC517881	LC517880	–
Acrocalymma vagum	CPC 24225	–	–	KP170635	–
Acrocalymma vagum	CPC 24226	–	–	KP170636	–
Acrocalymma walkeri	UTHSC DI16-195	–	LN907338	LT796832	LT797072
Acrocalymma yuxiense	**HKAS 111910**	**MW424793**	**MW424778**	–	–
Ascocylindrica marina	MD6011	KT252907	KT252905	–	–
Ascocylindrica marina	MF416	MK007124	MK007123	–	–
Boeremia exigua	CBS 431.74	EU754084	EU754183	FJ427001	KY484687
Boeremia foveata	CBS 341.67	GU238203	GU237947	GU237834	KY484716

3.20853; GZUIFR H22.028; GZUIFR H22.029)]; Clade V [*A. magnoliae* (MFLUCC 18–0545; MFLUCC 21–0204)]; Clade VI [*A. fici* (CBS 317.76; MFLUCC 21–0103); *A. yuxiense* (HKAS 111910)].

Acrocalymma aquaticum Huang Zhang & K.D. Hyde, Cryptogr. Mycol. 33(3): 337 (2012)

Mycobank number: 835894; Facesoffungi number: FoF 07098, Fig. 2

Saprobic on submerged wood in a freshwater stream. **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 240–270 μm diam., 100–140 μm high, dark brown or black, solitary, pycnidial, immersed to semi-immersed, unilocular, globose to subglobose, glabrous, papillate, ostiolate. *Ostiole* single, centrally located. *Peridium* 12–18 μm thick, composed of thick-walled, dark brown to black cells of *textura angularis* in the outer layers, becoming hyaline cells towards conidial hymenium. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 6–12 × 3.5–6 μm, hyaline, enteroblastic, phialidic, ampulliform to lageniform, with a narrow channel, determinate, formed from the inner cells of the pycnidial wall. *Conidia* 11.5–16.5 × 2–4 μm ($\bar{x}$ = 13.9 × 2.7 μm, n = 20), hyaline, cylindrical with an obtuse apex and a narrow truncate base, straight, 0–1-septate, not constricted at the septa, thin and smooth-walled, guttulate, bearing a mucilaginous appendage (2–3 μm diam.) at the apex.

Culture characters: Conidia germinated on MEA within 24 h. Colonies on MEA reaching 4.5–5 cm diam., after 4 weeks at room temperature, colonies circular, medium dense, flat with smooth and entire margins; smoke-gray to gray in top view, reverse gray.

Material examined: THAILAND, Tak Province, Tha Song Yang, Ban Mae Ja Wang stream, on submerged wood, 17 October 2019, OD Padaruth, CC36 (MFLU 22–0114); living culture MFLUCC 20–0124.

Known distribution: THAILAND: Chiang Mai Province (Zhang et al.[14]), Tak Province (present study).

Notes: *Acrocalymma aquaticum* was introduced by Zhang et al.[14], which was collected from a submerged wood in a freshwater stream in Chiang Mai, Thailand. Based on the phylogenetic analysis of combined SSU, LSU, ITS, and TEF1-α sequence data, *A. aquaticum* (MFLUCC 20–0124) clustered with the ex-type strain of *A. aquaticum* (MFLUCC 11–0208) with high bootstrap support (100% ML, 1.00 BYPP). Our isolate resembles *A. aquaticum* in morphology and measurements of the conidiomata, conidiogenous cells, and conidia but most of the conidiomata were immersed. A comparison of the ITS nucleotides of *A. aquaticum* (MFLUCC 11–0208) and the new strain (MFLUCC 20–0124) revealed 1 bp (0.19%; 540 bp) nucleotide difference which indicates that the new strain is *A. aquaticum*[32].

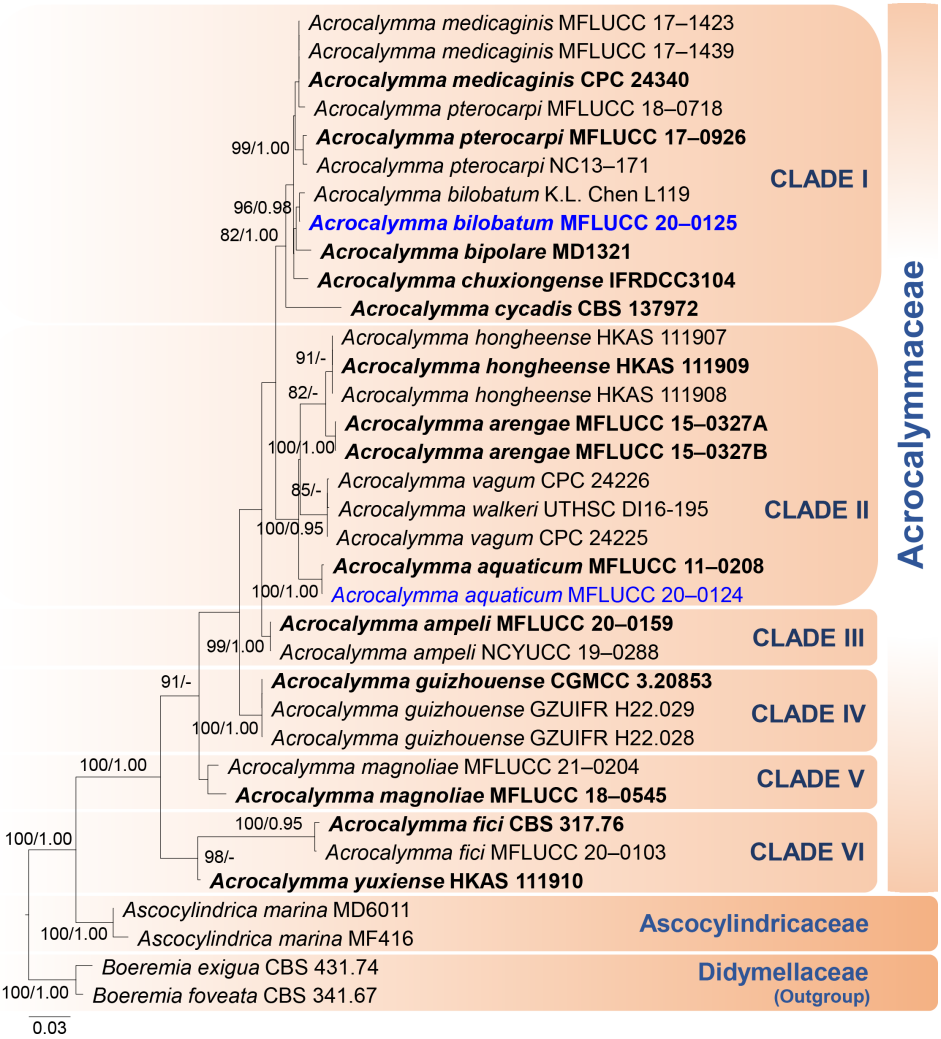

Fig. 1 Phylogenetic tree based on RAxML analyses of combined SSU, LSU, ITS, and TEF1-α sequence data. Bootstrap support values for maximum likelihood (ML) higher than 75% and Bayesian posterior probabilities (BYPP) greater than 0.95 are indicated above the nodes as ML/BYPP. The new species are represented in blue bold and type species are in bold. The tree is rooted to *Boeremia foveata* (CBS 341.67) and *B. exigua* (CBS 431.74) (Didymellaceae). Bar = 0.03 estimated number of nucleotide substitutions per site per branch

Acrocalymma bilobatum M.S. Calabon, E.B.G. Jones & K.D. Hyde ***sp. nov.***

Mycobank number: 848523; *Facesoffungi number*: FoF 13985; Fig. 3

Etymology: derived from the bilobed polar appendages in the conidia

Holotype: MFLU 22–0115

Saprobic on submerged wood in freshwater habitat. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 170–275 µm diam., 135–205 µm thick/high, dark brown, pycnidial, solitary to gregarious, immersed with only the white neck visible in surface view, globose or subglobose, unilocular, glabrous and ostiolate. *Ostiole* cylindrical, centrally located. *Peridium* 12–27 µm thick, composed of thick-walled, dark brown cells of *textura angularis* in the outer layer, become hyaline cells of *textura globulosa* in the inner layer. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 4.5–11 × 3–5 µm, hyaline, heteroblastic, doliiform to ampulliform, determinate, smooth-walled, formed from the inner cells of the pycnidial wall. *Conidia* 7–12 × 2.5–4 µm ($\bar{x}$ = 9.2 × 3.5, n = 30), hyaline, cylindric-clavate to fusiform, rounded at apex, truncate at base, straight, unicellular, thin and smooth-walled, guttulate, with rounded mucoid bilobed polar appendage (0.4–0.9 × 2–4 µm), and basal globose to hemispherical flaring mucoid appendage (0.8–1.1 × 0.1–1.4 µm).

Culture characters: Conidia germinated on MEA within 24 h. Colonies on MEA reaching 4–5 cm diam., after 4 weeks at room temperature, colonies circular, medium dense, flat with smooth and entire margins; gray to pale brown in top view, reverse dark brown to black in center and reddish in the outer region.

Material examined: THAILAND, Tak Province, Tha Song Yang, Ban Mae Ja Wang stream, on submerged wood, 17 October 2019, OD Padaruth, CC40 (MFLU 22–0115, holotype); ex-type culture MFLUCC 20–0125.

Notes: In the phylogenetic analysis, *Acrocalymma bilobatum* (MFLUCC 20–0125) grouped with *Acrocalymma* sp. (K.L. Chen L119), with high bootstrap support (96% ML, 0.98 BYPP). Comparison of morphological features of *Acrocalymma* sp. (K.L. Chen L119) and *Acrocalymma bilobatum* (MFLUCC 20–0125) was not possible because the morphology of the former is not available but comparison of the ITS nucleotides of *Acrocalymma* sp. (K.-L. Chen L119) and *A. bilobatum* (MFLUCC

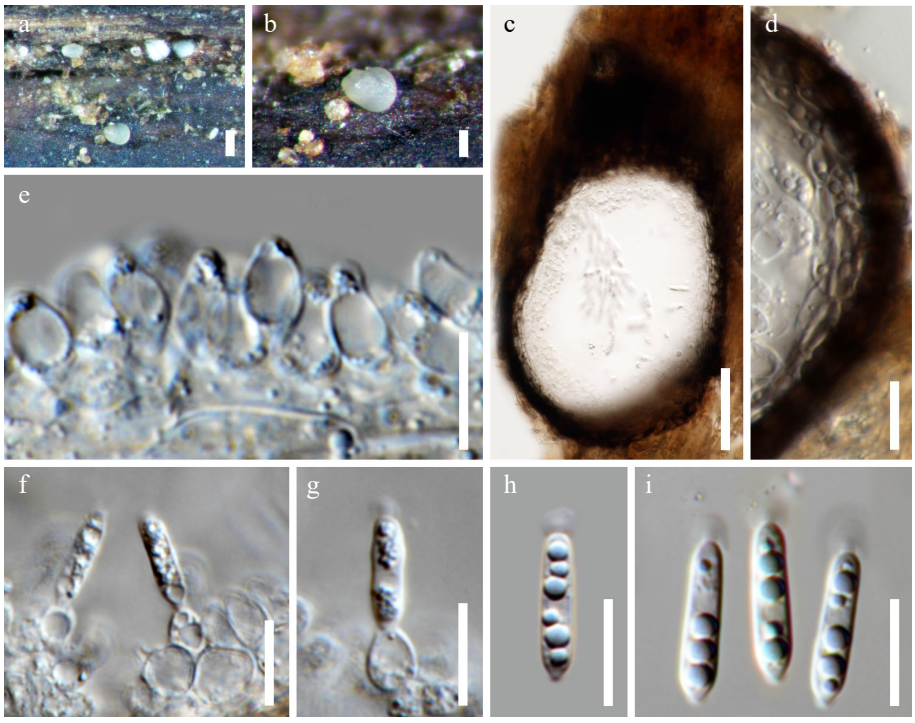

Fig. 2 *Acrocalymma aquatica* (MFLU 22–0114). (a), (b) Erumpent conidiomata on wood surface. (c) Vertical sections of a conidioma. (d) Section through the peridium. (e)–(g) Conidiogenous cells. (h), (i) Conidia with apical appendages. Scale bars: (a) = 200 μm, (b) = 100 μm, (c) = 50 μm, (d)–(i) = 10 μm.

20–0125) revealed two (0.37%) base pair differences, which indicates that the former is *A. bilobatum*. The two strains of *A. bilobatum* shared the same subclade with *Acrocalymma chuxiongense* (IFRDCC3104) and *Acrocalymma bipolare* (MD1321). *Acrocalymma bilobatum* resembles *A. ampeli, A. medicaginis*, and *A. bipolare* in the shape of conidia and appendages at both ends but differs in the sizes of conidia and appearances of the appendages. *Acrocalymma bipolare* has a mucoid polar appendage filled with oil droplets, which elongates in water to form filaments[7], while *A. medicaginis* has globose to hemispherical or helmet-shaped apical appendage and tapered, short, cylindrical to hemispherical basal appendage[33]. *Acrocalymma ampeli* has a flaring mucoid basal appendage with a hemispherical to bilobed apical appendages[34].

Acrocalymma fici P.W. Crous & T. Trakunyingcharoen, IMA Fungus 5 (2): 405 (2014)

Mycobank number: 810838; *Facesoffungi number*: FoF 09155, Fig. 4

Saprobic on submerged decaying wood. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* 80–150 × 40–100 μm, white, separate to gregarious, immersed to semi-immersed, pycnidial, globose to subglobose, unilocular, glabrous, ostiolate. *Peridium* 40–50 μm thick, composed of thick-walled, dark brown to hyaline cells of *textura angularis*, become darker cells at the ostiolar region. *Ostiole* 40–55 μm diam., centrally located. *Conidiophores* reduced to conidiogenous cells or a supporting cell. *Conidiogenous cells* 4–10 × 2–5 μm, hyaline, enteroblastic, ampulliform to doliiform, smooth-walled. *Conidia* 12–15 × 2–3 ($\bar{x}$ = 13.4 × 2.8 μm, n = 10), hyaline, cylindrical with sub-obtuse apex, acutely tapered at base to a small flattened central scar, 0–1-septate, not constricted at septum, smooth-walled, guttulate, with flaring mucoid apical appendage (2–5 μm diam.), visible in water mounts.

Material examined: THAILAND, Kanchanaburi, Sangkhla Buri, Liwo, on decaying wood submerged in a stream, 27 June 2019, N. Chaiwan, TFW5 (MFLU 21–0124); ex-type culture, MFLUCC 21–0103.

Notes: *Acrocalymma fici* (CBS 317.76) was collected from a terrestrial habitat on *Ficus* sp.[15] and on *A. fici* (MFLUCC 21–0103) on submerged decaying wood in Thailand[11]. *Acrocalymma fici* (MFLUCC 21–0103) resembles *A. fici* (CBS 317.76), but almost all the conidia are aseptate, while Trakunyingcharoen et al.[15] observed septate conidia. The differences in conidial septation may be explained by conidiomatal differences. A comparison of the ITS and TEF nucleotides of *A. fici* (CBS 317.76) and *A. fici* (MFLUCC 21–0103) revealed no nucleotide difference, which indicates that the new strain is *A. fici*[11,32].

Discussion

Acrocalymmaceae, whose establishment was supported using divergence time estimates, comprises a monotypic genus *Acrocalymma* typified by *A. medicaginis*[15,35,36]. Presently, 16 species are included in this genus: *A. aquatica*[14], *A. ampeli*[34], *A. arengae*[23], *A. cycadis*[37], *A. bilobatum* (this study), *A. bipolare*[7], *A. fici*, *A. vagum*, *A. walkeri*[15], *A. chuxiongense*[38], *A. guizhouense*[39], *A. magnoliae*[40], *A. medicaginis*[33], *A. pterocarpi*[41], *A. hongheense, A. yuxiense*[22]. *Acrocalymma* species are known to thrive as pathogens (i.e., *A. medicaginis*, *A. vagum*) and saprobes (e.g., *A. ampeli, A. pterocarpi*) of various plant hosts (e.g., *A. ampeli* on *Ficus ampelas*, *A. arengae* on *Arenga pinnata*, *A. fici* on *Ficus* sp., *A. cycadis* on *Cycas calcicola*, *A. magnoliae* on *Magnolia* sp. and *Anomianthus dulcis*, *A. yixienxe* on *Quercus glauca*) in terrestrial habitats. Five species, *A. arengae*, *A. chuxiongense*, *A. hongheense, A. pterocarpi*, and *A. walkeri* are sexual morphs and the rest are asexual

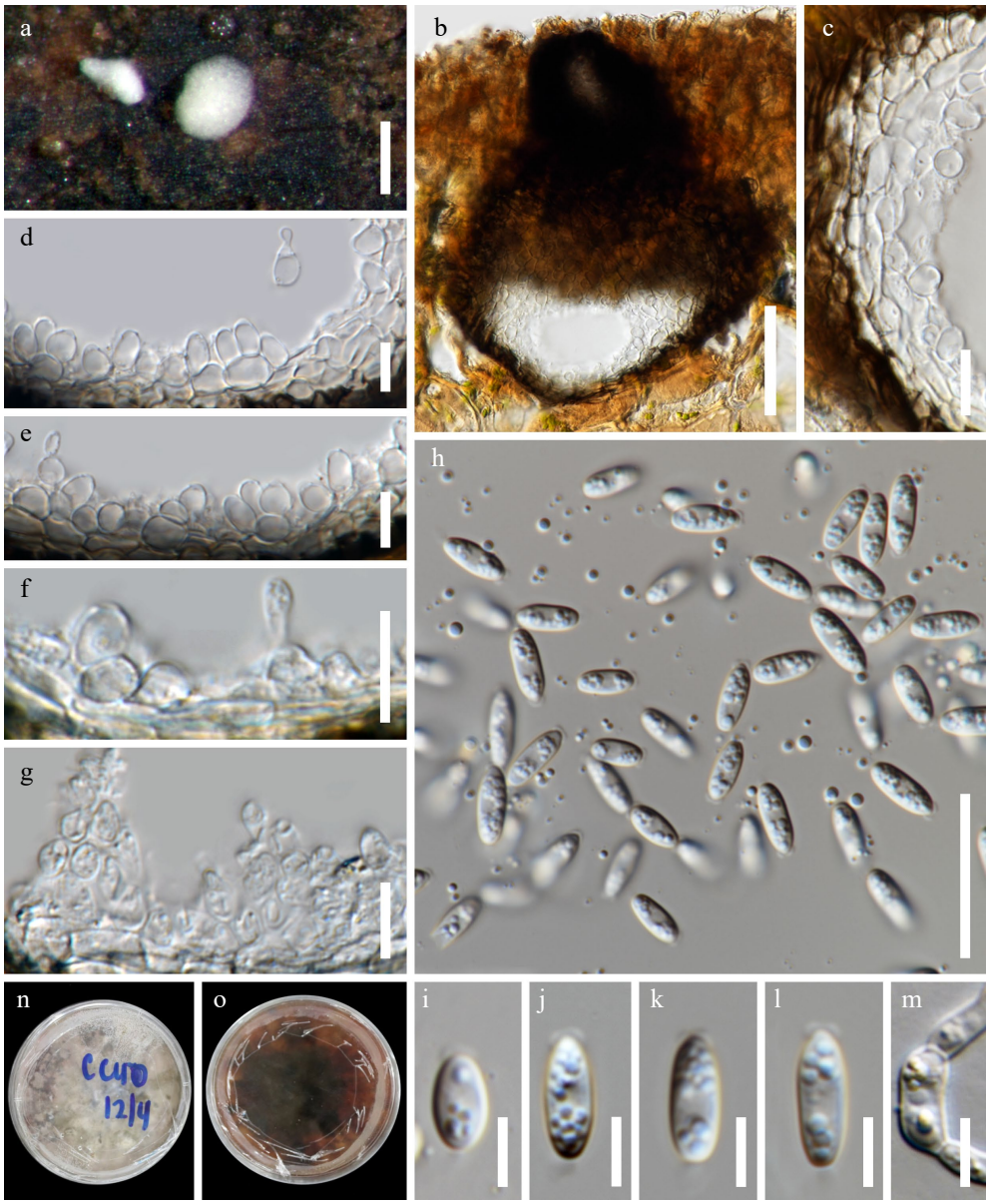

Fig. 3 *Acrocalymma bilobatum* (MFLU 22–0115, holotype). (a) Appearance of erumpent dark brown conidiomata on wood surface. (b) Vertical section of conidioma. (c) Section through the peridium. (d)–(g) Conidiogenous cells. (h)–(l) Conidia with appendages. (m) Germinated conidium. Colony on MEA: (n) obverse. Scale bars: (a) = 200 µm, (b) = 50 µm, (c)–(g) = 10 µm, (h) = 20 µm, (i)–(l) = 5 µm.

coelomycetous species. Mortimer et al.[22] discovered the sexual (HKAS 111909) and asexual morph (HKAS 111907; HKAS 111908) of *A. hongheense* collected on woody litter in China. The morphological differences of sexual and asexual morphs and comparison of habitats and localities of *Acrocalymma* spp. are provided by Liu & Zeng[38] and de Silva et al.[40], respectively. A key to species is provided as Table 2.

In freshwater habitats, four species are reported as saprobes: *A. aquaticum, A. bilobatum, A. bipolare, A. fici*. Three of these, except for *A. bipolare*, are recorded in the freshwater environments of Thailand. The amazing biodiversity of freshwater fungi in Thailand is exceptional with many novel taxa identified[42–44]. Also, it is likely that there are more species of *Acrocalymma* awaiting discovery as we continuously explore both terrestrial and aquatic habitats and study the different plant hosts for their fungal associates.

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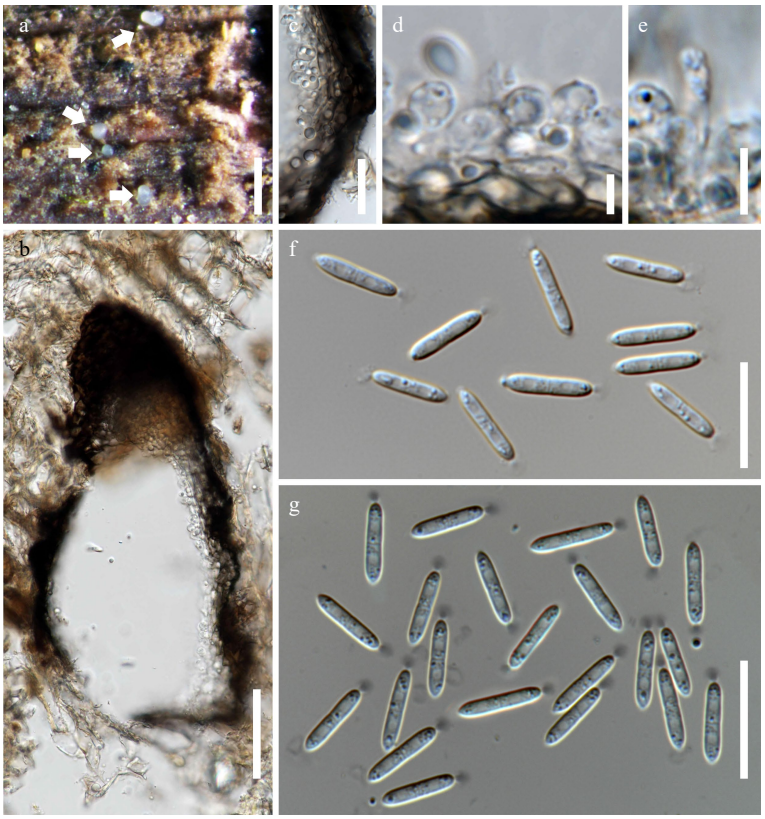

Fig. 4 *Acrocalymma fici* (MFLU 21–0124). (a) Appearance of conidiomata on wood surface. (b) Vertical section of conidioma. (c) Section through the peridium. (d), (e) Conidiogenous cells. (f), (g) Conidia with apical appendages. (g) Conidia stained with nigrosin. Scale bars: (a) = 500 μm, (b) = 50 μm, (c), (f), (g) = 20 μm, (d), (e) = 5 μm.

Table 2. Key to species of *Acrocalymma*

Step	Features	Species
1a	Sexual morph	2
1b	Asexual morph	6
2a	Asci, <100 μm	3
2a	Asci, >100 μm	4
3a	Ascospores, 17–21 × 3–5 μm	*A. pterocarpi*
3b	Ascospores, 19–22 × 4.5–5.5 μm	*A. walkeri*
4a	Ascospores, 1-septate	*5*
4b	Ascospores, 1–3-septate	*A. arengae*
5a	Ascospores, 35–45 × 18–20 μm	*A. chuxiongense*
5b	Ascospores, 20–35 × 7–9 μm	*A. hongheense*
6a	Conidia lacks mucoid cap	7
6b	Conidia with mucoid caps	8
7a	Conidia, (16–)18–25(– 28) × (4.0–)4.5–6.0(–6.9) μm	*A. vagum*
7b	Conidia, 15–21 × 4–5 μm	*A. yuxiense*
8a	Conidia, mucoid caps in apex	9
8b	Conidia, mucoid caps in both ends	13
9a	Conidia, <20 μm	10
9b	Conidia, >20 μm	12
10a	Conidia <10	*A. guizhouense*
10b	Conidia >10	11
11a	Conidia, 12–17 × 3–4 μm	*A. cycadis*
11b	Conidia, 12–16 × 2.5–3 μm	*A. magnoliae*
12a	Conidia, 22–30 × 5–7 μm	*A. aquatica*
12b	Conidia, (25–)28–32(–35) × (4–)5 μm	*A. fici*
13a	Conidia <15	14
13b	Conidia >15	15
14a	Conidia, 7–12 × 2.5–4 μm	*A. bilobatum*
14b	Conidia, 9–12 × 3–5 μm	*A. bipolare*
15a	Conidia, 17–19 × 5.5–6.5 μm	*A. ampeli*
15b	Conidia, 11–21 × 3.5–5.0 μm	*A. medicaginis*

Conflict of interest

The authors declare that they have no conflict of interest. Kevin D. Hyde is the Editorial Board member of *Studies in Fungi*. He was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and his research groups.

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