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Ceramothyrium chiangraiense, a novel species of Chaetothyriales (Eurotiomycetes) from *Ficus* sp. in Thailand

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Abstract

In our investigation of epifoliar fungi, a novel species of *Ceramothyrium*; *C. chiangraiense* is isolated from the living leaves of *Ficus* sp. in Thailand. Genus *Ceramothyrium* is characterized by the ascomata covered with pellicle mycelium and the circumferential space around the maturing ascomata, bitunicate asci and phragmospores which lack setae. The new species resembles genus *Ceramothyrium* by its ascomata with superficial mycelial pellicle over the fruiting structures and scattered ascomata that cupulate when dry and 8-spored, bitunicate asci which contain hyaline, pluriseptate ascospores. We have processed the phylogeny based on Maximum Likelihood (ML) and Bayesian (BI) analyses using combined ITS and LSU sequence data. Based on phylogeny, *C. chiangraiense* is confirmed its placement within the genus *Ceramothyrium* and closely related to *C. aquaticum*, *C. carniolicum*, *C. exiguum*, *C. linnaeae* and *C. phuquocense*. Morphologically, *C. chiangraiense* is distinguished from phylogenetically related species (*Ceramothyrium carniolicum*, *C. ficus*, *C. linnaeae*, *C. longivolcaniforme*, *C. menglunense*, *C. paiveae and C. thailandicum*) by having obpyriform asci and 3–4 seriate, oblong to ellipsoid ascospores with 4–7 longitudinal and angled septa. An updated phylogenetic tree for the family Chaetothyriaceae (Eurotiomycetes) is constructed. The relationships of *C. chiangraiense* to other *Ceramothyrium* species are discussed based on comparative morphology and phylogenetic analyses.

Key words – new species – epifoliar fungi – morphology – phylogeny

Introduction

Order Chaetothyriales was introduced by Barr (1976) in Loculoascomycetes which are plant parasitic ascomycetes. Currently, the order is placed in the Eurotiomycetes based on the nucleotide data of 18s rDNA gene (Winka et al. 1998, Schoch et al. 2006). Members of Chaetothyriales are consisted of lichenized fungi, plant pathogens and opportunistic human and animal pathogens mostly in tropical and temperate ecosystems (Teixeira et al. 2017, Maharachchikumbura et al. 2018).

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The family Chaetothyriaceae was introduced by Hansford (1946) and it was considered to be the type family of the order Chaetothyriales by Batista & Ciferri (1962). Members of the Chaetothyriaceae are superficially colonizing on leaf surfaces and form a dark, loose mycelium net on leaf surface (Chomnunti et al. 2012a). The mycelia are not penetrating in to the host tissues but appressed to the leaf cuticle (Batista & Ciferri 1962, von Arx & Müller 1975, Chomnunti et al. 2012a). They are also characterized by having ascomata with or without setae, and the ascomata are surrounded by a thin mycelial pellicle comprises a loose hyphal net looks like sooty molds which is covering the host surfaces between maturing ascomata (Hansford 1946, Batista & Ciferri 1962, von Arx & Müller 1975, Hughes 1976, Pereira et al. 2009, Chomnunti et al. 2012a, Yang et al. 2014, Zeng et al. 2016). Therefore, family Chaetothyriaceae was earlier classified as a capnodiaceous Dothideomycetes (Batista & Ciferri 1962). Later, Eriksson (1982) placed Chaetothyriaceae in the order Dothideales. Finally, the family is accepted in Chaetothyriales by Barr (1987). Most of the Chaetothyriaceae species are reported as saprotrophic or biotrophic (Barr 1987, Chomnunti et al. 2012b, 2014, Hongsanan et al. 2016, Maharachchikumbura et al. 2018).

Wijayawardene et al. (2018) included 16 genera within Chaetothyriaceae and later, 17 genera are accepted by Crous et al. (2018). *Chaetothyrium* Speg. is accounted as the type genus of the family Chaetothyriaceae (Hughes 1976). The genus *Ceramothyrium* was introduced by Batista & Maia (1956) in family Phaeosaccardinulaceae with *C. paiveae* as the type species. Hughes (1976) accepted the genus in family Chaetothyriaceae based on morphological evidences. *Ceramothyrium* is characterized by ascomata cover with pellicle mycelium and the circumferential space around the maturing ascomata, lack of setae, olivaceous to fuscous and hyaline to subhyaline or straw-coloured hyphae, bitunicate, 8-spored asci and hyaline, transversely pluriseptate ascospores (Batista & Maia 1956, Hughes 1976, Chomnunti et al. 2012a, Zeng et al. 2016). Constantinescu et al. (1989) stated that the genus *Ceramothyrium* has hyphomycetous *Stanhughesia* asexual morphs. The asexual morphs of *Ceramothyrium carniolicum* (= *Stanhughesia carniolica*), *C. linnaeae* (= *S. linnaeae*) and *C. lycopodii* (= *S. lycopodii*) are obtained from pure cultures (Constantinescu et al. 1989). Crous et al. (2012) introduced the novel asexual morphs of *Ceramothyrium* as *C. melastoma* and *C. podocarpi*. There are 38 species epithets listed under the genus *Ceramothyrium* (Index Fungorum 2019). However, sequence data are available only for 11 species in GenBank (Yang et al. 2018, Yen et al. 2018).

In this study, a novel foliar epiphyte, *Ceramothyrium chiangraiense* is introduced from fresh and healthy leaves of *Ficus* sp. in Northern Thailand. Illustrations, comprehensive morphological descriptions and DNA analyses based on ITS and LSU sequence data are provided for the novel species.

Materials & Methods

Specimen collection, isolation and identification

The fresh leaves with black spots on the surface were collected from *Ficus* sp. in Mae Fah Luang University premises at the end of the dry season (May 2018). Samples were taken to the laboratory inside the paper envelopes. Photographs of enlarged host leaves and ascomata were taken under a Motic SMZ 168 compound stereomicroscope. Morphological characters were examined by hand sectioning of fruiting bodies on the upper leaf surface. The micro-morphological structures of ascomata were photographed using a Nikon ECLIPSE 80i compound stereo microscope fitted with a Canon 600D digital camera. All structures (ascomata, asci and ascospores) were processed for photographs by immersing sections in water mounted clean glass slides. In the observation of asci and apical rings, 10 % KOH and Congo red were used when necessary. Following morphological characters were observed: ascomata-diameter, height, colour and shape; peridium-width; asci and ascospores-length and width (at the longest and widest point) and pseudoparaphyses-width. The measurements of photomicrograph structures were measured using Tarosoft (R) Image Frame Work version 0.9.7. program. Images used for figures were processed with Adobe Photoshop CS6 Extended version 13.0.1 software (Adobe Systems, USA).

Single spore isolation technique was carried out to isolate fungal species present on the leaf surface. An ascoma was picked out on the host surface and it was immersed in 300 µl of sterilized distilled water on a center curved glass slide and crushed using a sterilized needle until an ascospore suspension is obtained. Small drops of the spore suspension were placed on potato dextrose agar (PDA) media plates. Petri-plates were kept at 25 °C for 12–24 h for the ascospores germination. Single germinated ascospores aseptically transferred onto potato dextrose agar (PDA) plates. The plates were incubated at 25 °C for 15 to 20 days for pure cultures. Colony characters were observed and measured weekly. After a month, well-grown cultures were used to extract DNA. The holotype and isotype materials were deposited at Mae Fah Luang University herbarium (MFLU) in Chiang Rai, Thailand and Herbarium of Mycology, Chinese Academy of Sciences (HMAS) in China respectively. The ex-type cultures were deposited at Mae Fah Luang culture collection (MFLUCC). Facesoffungi number was obtained and Index Fungorum number was registered (Jayasiri et al. 2015, Index Fungorum 2019).

DNA isolation, PCR amplification and Sequencing

Genomic DNA was extracted from the scraped fresh fungal mycelium grown on PDA media for 6 weeks at 25 °C by using Biospin Fungus Genomic DNA extraction Kit - BSC14S1 (BioFlux, P.R. China) following the instructions of the manufacturer. Template DNA was stored at 4 °C for use in regular work and duplicated at -20 °C for long-term storage. DNA sequence data were obtained from the sequences of two regions, the internal transcribed spacers (ITS) and large subunit (LSU). ITS region was amplified using the primers ITS5 and ITS4 (White et al. 1990) and LSU region was amplified using the primers LR0R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994).

Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl which contained 12.5 µl of 2× Power Taq PCR MasterMix (Bioteke Co., China), 20 mM Tris-HCL pH 8.3, 100 mM KCl, 3 mM $MgCl_2$ (for stabilizing and enhancing), 1 µl of each forward and reverse primers (10 µM), 1 µl template genomic DNA, and 9.5 µl deionized water. In PCR profile, the reactions for ITS and LSU regions were initialized with the denaturation at 95 °C for 5 mins, followed by 35 thermal cycles of denaturation at 95 °C for 90 s, annealing at 55 °C for 90 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min. Sequencing of the PCR amplicons was conducted using the same primers used for the amplification reactions. The PCR products were verified by staining with ethidium bromide on 1 % agarose electrophoresis gels. The amplified PCR fragments were sent to a commercial sequencing provider (BGI, Ltd., Shenzhen, China).

Molecular data analyzing

Lasergene SeqMan Pro v.7 was used to obtain consensus sequences from sequences generated from forward and reverse primers. Contig sequences were analyzed with other sequences retrieved from GenBank. Sequences with high similarity indices were determined by BLAST search and the related literature (Liu et al. 2015, Zeng et al. 2016, Crous et al. 2018, Maharachchikumbura et al. 2018, Yang et al. 2018, Yen et al. 2018). Final alignment consists of the new taxa proposed in this study and sequences of Chaetothyriaceae downloaded from the GenBank representing genera *Aphanophora, Camptophora, Ceramothyrium, Chaetothyrium, Cyphellophoriella, Nullicamyces, Phaeosaccardinula* and *Vonarxia* (Table 1). The single and multiple (ITS and LSU) alignments of all reference sequences, were automatically generated with MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh & Standley 2013, Katoh et al. 2017). BioEdit v. 7.0.5.2 software was used when manual improvement is needed (Hall 1999). The terminal ends and ambiguous regions of the alignment were deleted.

Phylogenetic analyses of both individual and combined data-sets were based on Maximum Likelihood (ML) and Bayesian (BI) analyses. In BI analysis, the sequence alignments were converted from FASTA into NEXUS file format (.nex) using ClustalX2 v.1.83 (Thompson et al. 1997). The NEXUS file was prepared for MrModeltest v. 2.2 after (Nylander 2004) performing in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) to estimate the best

evolutionary model. For RAxML analysis (Randomized Accelerated Maximum Likelihood), the sequence alignments were converted from FASTA into PHYLIP (.phy) file format using ALTER (alignment transformation environment, http://www.sing-group.org/ALTER/) bioinformatics web tool (Glez-Peña et al. 2010). The evolutionary models for both BI and ML analysis were evaluated independently for each locus. MrModeltest v. 2.3 (Nylander 2004) was run under the AIC (Akaike Information Criterion) implemented in PAUP v. 4.0b10. The evaluated best-fit model was GTR + I + G for each locus in both BI and ML analyses.

ML trees were generated with RAxML-HPC2 on XSEDE (v. 8.2.10) tool (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010). The optimal ML tree search was conducted with 1,000 separate runs. The nonparametric bootstrap iterations (Stamatakis et al. 2008) were run in 1,000 replicates with the using GTR + I + G model of evolution. ML trees were generated with RAxML-HPC2 on XSEDE (v. 8.2.10) tool (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010).

Table 1 Taxa used for molecular study and their GenBank numbers.

Species	Strain/Voucher	GenBank accessions	
		ITS	LSU
Aphanophora eugeniae	**CBS 124105**	**FJ839617**	**FJ839652**
Brycekendrickomyces acaciae	**CBS 124104**	**FJ839606**	**FJ839641**
Camptophora hylomeconis	**CBS 113311**	**EU035415**	**EU035415**
C. schimae	IFRDCC 2664	MF285231	MF285233
Ceramothyrium aquaticum	VTCC:F-1210	LC360299	LC360296
C. carniolicum	**CBS 175.95**	**KC455237**	**KC455251**
C. chiangraiense	**MFLUCC 18-1354**	**MN481190**	**MN449441**
C. linnaeae	CBS 742.94	MH862502	MH874144
C. exiguum	**VTCC:F-1209**	**LC360297**	**LC360295**
C. ficus	MFLUCC 15-0229	KT588602	KT588600
C. ficus	**MFLUCC 15-0228**	**KT588601**	**KT588599**
C. longivolcaniforme	**MFLU 16-1306**	**KP324929**	**KP324931**
C. melastoma	**CPC 19837**	**KC005771**	**KC005793**
C. menglunense	**MFLU 16-1874**	**KX524148**	**KX524146**
C. podocarpi	**CPC 19826**	**KC005773**	**KC005795**
C. phuquocense	VTCC:F-1206	LC360298	LC360294
C. thailandicum	**MFLUCC 10-0008**	**HQ895838**	**HQ895835**
Chaetothyrium agathis	**MFLUCC 12-0113**	**KP744437**	**KP744480**
C. brischoficola	MFLU(CC)10-0012	HQ895839	HQ895836
Cyphellophoriella pruni	CPC 25120	KR611878	_
Nullicamyces eucalypti	**CPC 32942**	**MH327807**	**MH327843**
Phaeosaccardinula dendrocalami	IFRDCC 2663	KF667242	KF667246
P. coffeicola	**COF25**	**MH345730**	**MH345729**
P. ficus	**MFLU(CC)10-0009**	**HQ895840**	**HQ895837**
P. multiseptata	**IFRDCC 2639**	**KF667241**	**KF667244**
Vonarxia vagans	**CBS 123533**	**FJ839636**	**FJ839672**

* Newly generated sequence is indicated in blue-bold and type materials are in bold

MrBayes v.3.0b4 (Ronquist & Huelsenbeck 2003) was used to conduct the BI analyses to evaluate Bayesian posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002). Markov chain Monte Carlo sampling (BMCMC) with GTR + I + G was used as the best fit model of evolution for PP. Six simultaneous Markov chains were run for 1,000,000 generations, and trees were sampled every 1000th generation. The distribution of log-likelihood scores was examined to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence, using the program Tracer 1.5 (Rambaut & Drummond 2007). Phylograms were envisioned with FigTree v1.4.0 program (Rambaut 2012) and modified in Microsoft PowerPoint (2010). Sequences generated in this study were deposited in GenBank (Table 1).

Results

Phylogenetic analyses

Final combined LSU and ITS alignment was processed to resolve the species relationship in the Chaetothyriaceae (Fig. 1). The alignment comprised 26 strains including the outgroup taxon *Brycekendrickomyces acaciae* (CBS 124104) and the manually adjusted dataset is comprised 1467 characters including gaps (LSU: 823; ITS: 641). The tree topology of the ML analysis is similar to the BI analysis and not shown significant difference. Also, the tree topology is similar to previous analysis performed by Yang et al. (2018). Novel strain *Ceramothyrium chiangraiense* (MFLUCC 18-1354) is grouped within the genus *Ceramothyrium* and constitutes a moderately-supported clade (77% ML/0.98 PP, Clade A, Fig. 1) that comprises *C. aquaticum*, *C. carniolicum*, *C. exiguum*, *C. linnaeae* and *C. phuquocense*.

The best sorting RAxML tree with the final ML optimization likelihood value of -9007.254229 is represented in Fig. 1. The matrix had 587 distinct alignment patterns, with 15.53% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.242155, C = 0.241240, G = 0.281076, T = 0.235529; substitution rates AC = 1.718900, AG = 2.557961, AT = 2.201386, CG = 0.990328, CT 6.413590, GT = 1.000000; gamma distribution shape parameter α = 0.209737. In BI analysis, first 10 % of generated trees were discarded and remaining 90 % of trees were used to calculate posterior probabilities of the majority rule consensus tree. There are twenty seven credible sets of trees are sampled and final average standard deviation of split frequencies = 0.002.

Taxonomy

Ceramothyrium chiangraiense Wijesinghe & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF556793; Facesoffungi number: FoF01680

Etymology – In reference to the locality, Chiang Rai province, Thailand where the fungus was collected

Holotype – MFLU 19-1351

Epifoliar growing on the leaf surfaces of *Ficus* spp. Upper surface covers with dark mycelium. *Mycelial pellicle* 3.2–5.5 µm wide ($\bar{x}$ = 4.5 µm, n = 15), elongated, subiculum-like, pale brown to hyaline, mostly narrow, septate, slightly constricted at septa, circumferential space between matured ascomata spreading to outwards from center, anastomose at ostiole with the hyphal network. Sexual morph: *Ascomata* 185–210 × 165–205 µm ($\bar{x}$ = 195 × 180 µm, n = 10), superficial, solitary or scattered, coriaceous, covered by subiculum, blackish to greenish brown, setae absent, globose to subglobose, uniloculate, flattened or cupulate when dry, ostiolate. *Ostiole* central, minutely papillate, single. *Peridium* 18–60 µm ($\bar{x}$ = 35 µm, n = 20) widest at the sides, comprising several thick-walled cell layers, outer layer comprising dark brown to black, pseudoparenchymatous, cells of *textura prismatica*, innermost layer comprising light brown cells of *textura angularis*. *Hamathecium* comprising numerous, 2–5 µm wide ($\bar{x}$ = 3 µm, n = 15), filamentous, septate, hyaline pseudoparaphyses. *Asci* 35–60 × 25–35 µm ($\bar{x}$ = 48 × 29 µm, n = 15),

8-spored, bitunicate, obpyriform sessile or short pedicellate, mostly evanescent, minute ocular chamber, with an apical ring. *Ascospores* 20–30 × 5–9 µm ($\bar{x}$ = 25 × 7 µm, n = 40), crowded, 3–4 seriate, hyaline, oblong-ellipsoid, 4–7 longitudinal septa that are sometimes angled, no transverse septa, slightly constricted at the septa, without a mucilaginous sheath, smooth-walled, germ tubes developing from numerous cells. Asexual morph: Undetermined.

Culture characteristics – Ascospores germinating on PDA within 24 hours, from single spore isolation. Colonies on PDA reaching 6–8 mm diam. after 14 days at 24 °C, slow growing, circular, undulate, convex with papillate surface, black to dark grey in upper surface and completely blackish grey in lower surface, greenish pale grey at the outer edge in entire circle.

Known distribution – Northern Thailand

Material examined – Thailand, Chiang Rai Province, Mae Fah Luang University, Opposite side of the swimming pool, on living leaves of *Ficus* sp. (Moraceae), 21 May 2018, Wijesinghe SN, NN022 (MFLU 19-1351, holotype), (isotype, HMAS 290496). Ex-type living culture MFLUCC 18-1354.

GenBank numbers – ITS: MN481190 and LSU: MN449441

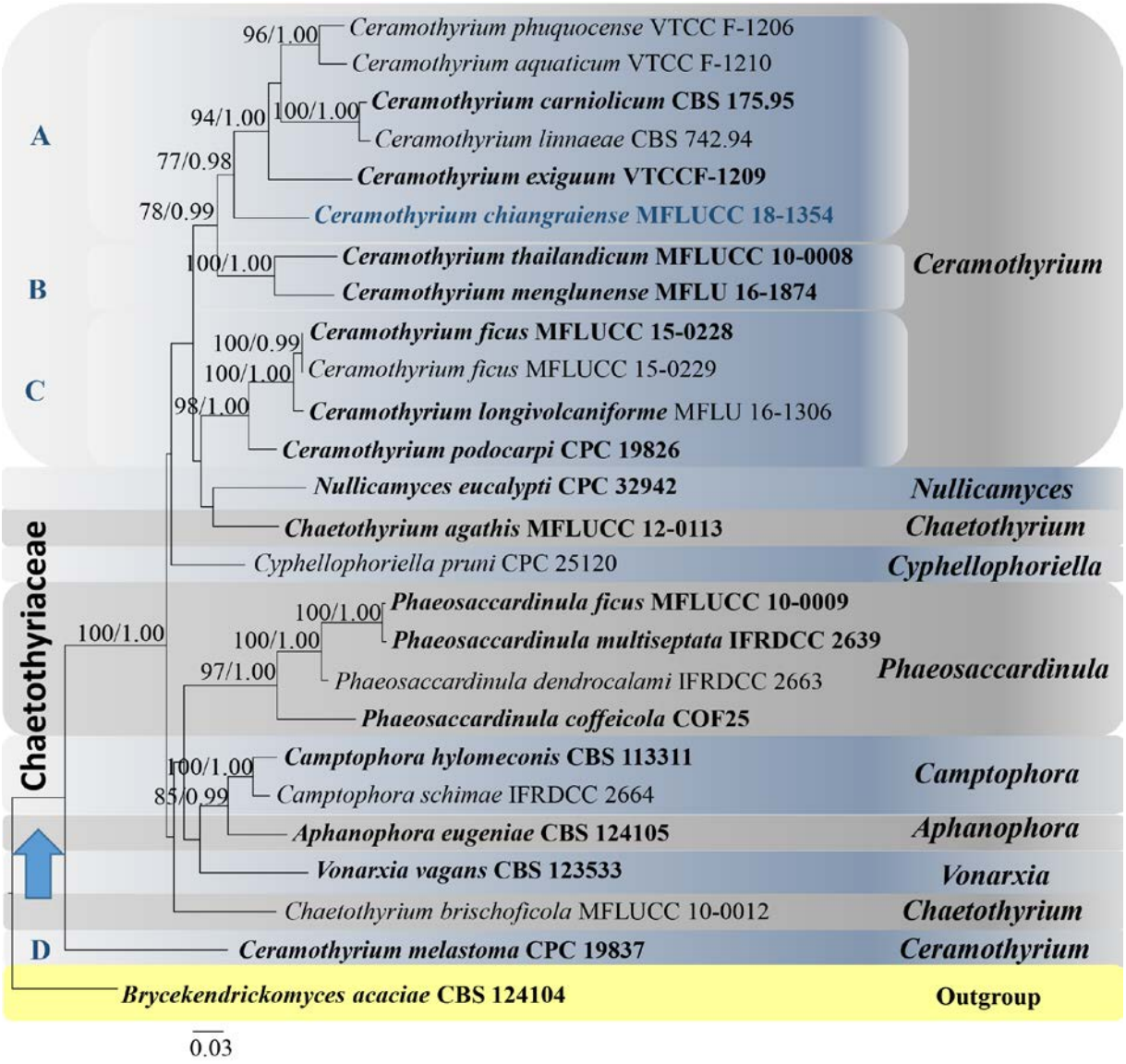

Fig. 1 – Phylogram generated from maximum likelihood analysis based on combined LSU and ITS sequence data for Chaetothyriaceae. ML ≥ 70% and PP ≥ 0.95 are given above each node. The tree is rooted to *Brycekendrickomyces acaciae* (CBS 124104). Novel species is visualized in blue-bold and type strains are symbolized in bold.

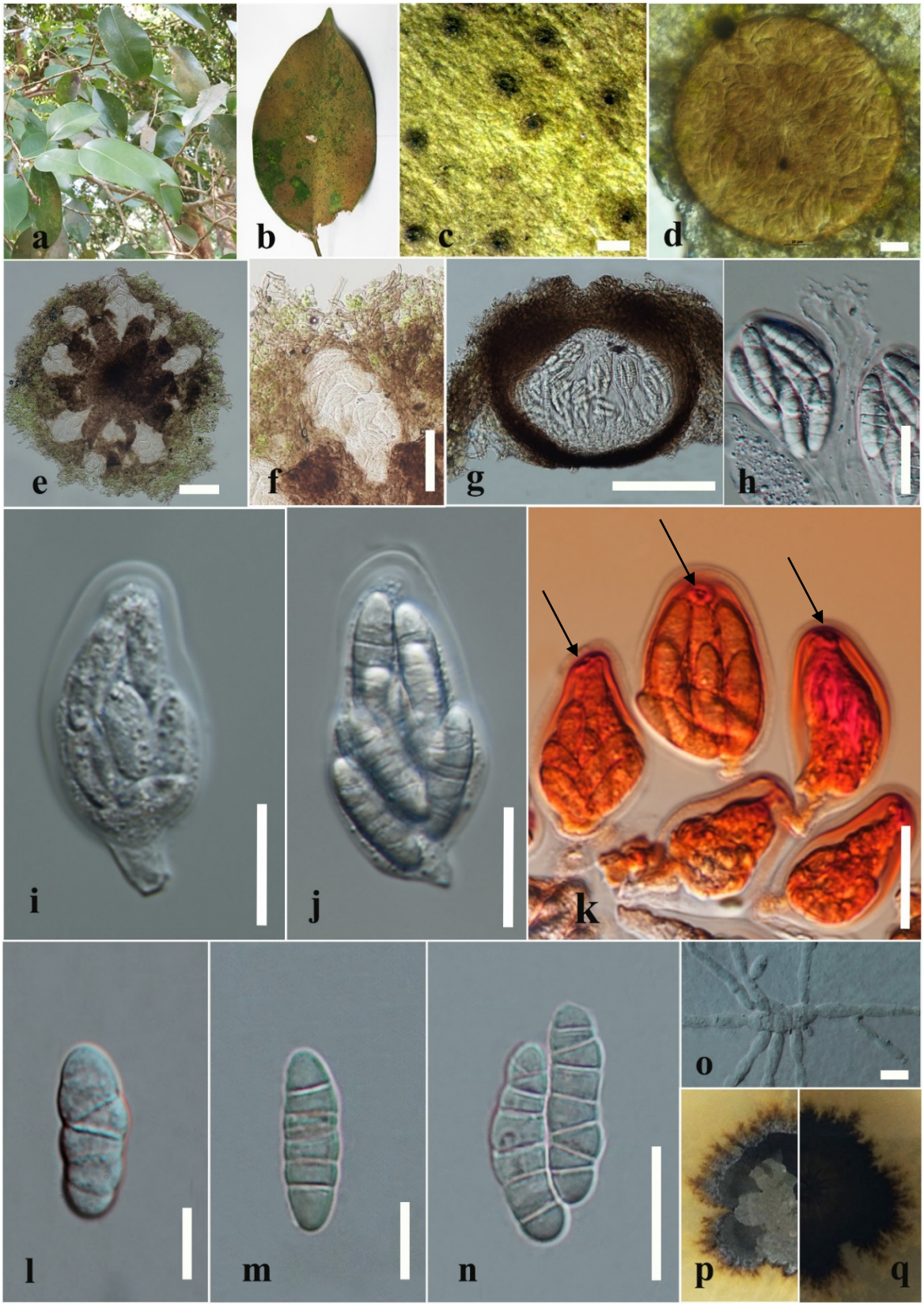

Fig. 2 – *Ceramothyrium chiangraiense* (MFLU 19-1351, holotype). a Host plant of *Ficus* sp. b, c Ascomata on living leaf surface. d, e Ascomata covered by a subiculum or layer. f Hyphae attaching to the margin of ascomata. g Longitudinal section of an ascoma with 10 % KOH. h Pseudoaraphyses. i, j Asci. k Asci stained with Congo red (Arrows indicate apical rings). l–n Ascospores. o Germinated ascospore. p, q Colonies on PDA (p upper, q lower). Scale bars: e = 200 μm, f, g, h = 100 μm, d, i–k n, o = 20 μm, l, m = 10 μm.

Discussion

Our multi-gene phylogenetic analysis (Fig. 1), indicates the new taxon is phylogenetically distinct to other taxa of *Ceramothyrium* (Clade A). The base pair similarities were analyzed for the taxa which show the close phylogenetic affinities to *C. chiangraiense*. Based on ITS sequence alignment *C. chiangraiense* has closest similarity to *C. phuquocense* (LC360298, Identities = 405/484 (83.67%), 2 gaps (0.41%)), to *C. exiguum* (LC360297, Identities = 411/496 (82.86%), 14 gaps (2.82%)), to *C. aquaticum* (LC360299, Identities = 405/489 (82.82%), 7 gaps (1.43%)), to *C. linnaeae* (MH862502, Identities = 426/545 (78.16%), 15 gaps (2.75%)) and to *C. carniolicum* (KC455237, Identities = 393/508 (77.36%), 13 gaps (2.55%)). Based on LSU sequence alignment the similarity of *C. chiangraiense* is closest to *C. carniolicum* (KC455251, Identities = 784/817 (95.96%), 3 gaps (0.36%)), to *C. linnaeae* (MH874144, Identities = 779/816 (95.46%), 3 gaps (0.36%)), to *C. phuquocense* (LC360294, Identities = 501/527 (95.06%), 6 gaps (1.13%)), to *C. aquaticum* (LC360296, 495/525 (94.28%), 7 gaps (1.33%)) and to *C. exiguum* (LC360295, 493/527 (93.54%), 6 gaps (1.13%)).

Species of the genus *Ceramothyrium* are separated into four major clades viz. A, B, C and D (Fig. 1). In clade A, new species *Ceramothyrium chiangraiense* forms a separate lineage sister to *C. aquaticum*, *C. carniolicum*, *C. exiguum*, *C. linnaeae* and *C. phuquocense*. *Ceramothyrium chiangraiense* is morphologically similar to taxa in genus *Ceramothyrium*, in having ascomata covered with a subiculum, circumferential space around matured ascomata, lack of setae, hyaline and transversely pluriseptate ascospores which are characteristic to the genus *Ceramothyrium* (Chomnunti et al. 2012a, Yen et al. 2018). The synopsis of morphological characters for the reported sexual morphs of *Ceramothyrium* species are presented in Table 2. *Ceramothyrium chiangraiense* is distinct from other *Ceramothyrium* species in having obpyriform asci, 3–4 seriate and oblong-ellipsoid ascospores with 4–7 transverse septa (without distinct pattern). *Ceramothyrium carniolicum* and *C. linnaeae* are morphologically and phylogenetically close to *C. chiangraiense* (Fig. 1). *Ceramothyrium carniolicum*, *C. linnaeae* and *C. chiangraiense* comprise epiphyllous, scattered, superficial and brownish black ascomata without setae which can be easily wiped when dried. The mycelial pellicle of them are formed in surrounding area of ascomata on upper host surface. Also, they have 8-spored, bitunicate asci with a deeply staining apical ring (k, Fig. 2) in Congo red (von Arx & Müller 1975, Ainsworth et al. 2015) and multi-septate ascospores which are strongly or slightly constricted at the septa and lack gelatinous sheath. However, *C. chiangraiense* and *C. carniolicum* have moderately large ascomata, asci and ascospores than those of *C. linnaeae* (Table 2). Occasionally *C. linnaeae* has amphigenous ascomata (Sutton et al. 1990). Both *C. carniolicum* and *C. linnaeae* have mucronate (small pointed appendages at both apices) ascospores (Ainsworth et al. 2015), while pointed appendages are absent in ascospores of *C. chiangraiense*.

Clade B which consisted of *C. menglunense* and *C. thailandicum* is sister to clade A. Both species in clade B, have clavate to pyriform asci (Chomnunti et al. 2012a, Hyde et al. 2016) while *C. chiangraiense* has obpyriform asci. Also, ascospores of *C. menglunense* are muriform (Hyde et al. 2016) while ascospores of *C. chiangraiense* are phragmosporic. Also, *C. menglunense* differs from *C. chiangraiense* and other *Ceramothyrium* species by the presence of brown setae (Hyde et al. 2016). However, *C. menglunense* and *C. chiangraiense* both have an inner peridium of *textura angularis* but differ to each other from the outer layer of *textura globulosa* in *C. menglunense* and *textura prismatica* in *C. chiangraiense*. *Ceramothyrium thailandicum* can be distinguished from *C. chiangraiense* by the presence of mucilaginous sheath of ascospores (Chomnunti et al. 2012a) while *C. chiangraiense* do not have a sheath. However, the presence of the sheath may be changed according to maturity.

Clade C is represented *Ceramothyrium ficus*, *C. longivolcaniforme* and *C. podocarpi*. *Ceramothyrium chiangraiense* has a similar character to *C. longivolcaniforme* by having oblong-ellipsoid ascospores (Zeng et al. 2016). However, *C. chiangraiense* differs from *C. ficus* and *C. longivolcaniforme* through shape of asci, *C. ficus* has clavate asci and *C. longivolcaniforme* has clavate to pyriform or obovoid asci, while *C. chiangraiense* has obpyriform asci. Also, *C.*

longivolcaniforme is similar to *C. chiangraiense* by having short pedicellate asci while *C. ficus* comprises long pedicellate asci (Hongsanan et al. 2015, Zeng et al. 2016).

Clade D is consisted of *C. melastoma* (CPC 19837). Only asexual morph is known for *C. melastoma* (Crous et al. 2012). The recent analysis performed by Yen et al. (2018) and Yang et al. (2018) excluded *C. melastoma* (CPC 19837) in their analysis. In present study, *Chaetothyrium brischoficola* (MFLUCC 10-0012) and *C. agathis* (MFLUCC 12-0113) are not form a stable clade within family Chaetothyriaceae (Fig. 1). This is probably, due to short LSU and ITS sequences of *C. brischoficola*. In the recent study done by Yang et al. (2018), these two species were shown this unstable placement.

Table 2 Synopsis of morphological characters of related sexual morphs of *Ceramothyrium* species to *C. chiangraiense*

Taxa	Ascomata (µm)	Asci (µm)	Ascospores				Reference
			Size(µm)	Shape	Septation	Arrangement	
Ceramothyrium chiangraiense	185–210 × 165–205	35–60 × 25–35	20–31 × 5–9	Oblong-ellipsoid	4–7 longitudinal septa	3–4 seriate	This study
C. carniolicum	150–200	40 × 20	18–26 × 4–6	Oblong-fusiform, mucronate	3 septate	-	Petrak (1961), Sutton et al. (1990), Ainsworth et al. (2015), Zeng et al. (2016)
C. ficus	475–550 × 120–130	95–110 × 30–39	36–39 × 7–8	Sub-cylindrical	7–8 transversal, 1 longitudinal septum	3–5 seriate	Hongsanan et al. (2015)
C. linnaeae	90–120	25–30 × 12–15	14–19 × 3–5	Fusoid, mucronate	3 septate -	4–5 seriate	Batista & Ciferri (1963), Hughes (1976), Sutton et al. (1990), Ainsworth et al. (2015), Zeng et al. (2016)
C. longivolcaniforme	650–900	67–90 × 27–45	28–37 × 7–13	Oblong to ellipsoid	7 transversals, 6 longitudinal septa	2 seriate	Zeng et al. (2016)
C. menglunense	245–255 × 140–165	50–75 × 24–30	25–35 × 10–12	Ellipsoid to obovoid	4–7 transversal, 1–5 longitudinal septa	2–3 seriate	Hyde et al. (2016)
C. paiveae	-	-	12.5–22 × 3.7–6	-	1–4 septate	-	Batista & Maia (1956), Zeng et al. (2016)
C. thailandicum	200–255 × 100–160	70–96 × 39–53	24.7–35.5 × 5.7–8.7	Cylindro-clavate	7–9 transversal septa	3–5-seriate	Chomnunti et al. (2012a)

"-": no information

In most recent studies above gene regions have been used for the phylogenetic analysis of family Chaetothyriaceae (Hongsanan et al. 2015, Zeng et al. 2016, Yang et al. 2018, Yen et al. 2018). Thus, we used combined LSU and ITS regions for the identification of species boundaries in genus *Ceramothyrium*. It is estimated that the genus *Ceramothyrium* includes 38 species (Yen et al. 2018). Considering the asexual morphs of this genus, stanhughesia-like asexual morphs are reported for *Ceramothyrium aquaticum*, *C. exiguum* and *C. phuquocense* from submerged decaying leaves in Vietnam (Yen et al. 2018). However, we could not observe the asexual morph of our new species. However, in the future if we can obtain the asexual morph of *C. chiangraiense* we will introduce more samples to stabilize its taxonomic placement and the reference sequences to GenBank.

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