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***Discopycnothyrium palmae* gen. & sp. nov. (Asterinaceae)**

SINANG HONGSANAN^{1,2}, ALI H. BAHKALI³, PUTARAK CHOMNUNTI^{1,2},
JIAN-KUI LIU^{1,2}, JUN-BO YANG⁴ & KEVIN D. HYDE^{1,2,3}

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

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

³ *Department of Botany and Microbiology, College of Sciences, King Saud University,
Riyadh, KSA*

⁴ *Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany,
Chinese Academy of Science, Kunming 650201, Yunnan, China*

* CORRESPONDENCE TO: putarak.cho@mfu.ac.th

ABSTRACT—A new genus and species, *Discopycnothyrium palmae*, is described from palms (*Arecaceae*) in Narathiwat Province, southern Thailand, and is known only from its asexual morph. The genus is characterized by circular pycnothyria with darkened cells around the central ostiole, variably long hyphae at the margin, and pycnothyriospores that are 1-septate with the septum between a larger brown upper cell and hyaline lower cell. LSU gene sequence analysis clustered *D. palmae* in the *Asterinaceae* clade and supported (59% ML and 0.88 PP support) the taxon as phylogenetically distinct from other *Asterinaceae*. Morphological and phylogenetic differences confirm the new genus, here introduced and illustrated.

KEY WORDS—*Asterinales*, foliar epiphytes, taxonomy

Introduction

The family *Asterinaceae* was introduced by Hansford (1946), with the generic type *Asterina* Lév. (Müller & von Arx 1962, Luttrell 1973, Eriksson 1981, Hosagoudar 2004, Kirk et al. 2008, Hofmann 2010, Hyde et al. 2013, Hongsanan et al. 2014). The family comprises biotrophs found on living plant leaves and is distributed worldwide (Kirk et al. 2001, Barr & Huhndorf 2001, Taylor et al., Hofmann et al. 2010, Hyde et al. 2013, Hongsanan et al. 2014). While most previous studies focused on the morphology, Hofmann (2010) provided the first molecular data of *Asterinaceae* that supported its phylogenetic

placement in *Dothideomycetes* and which were included in the phylogenetic tree representing *Asterinales* sensu stricto (Hyde et al. 2013, 2016, Wijayawardene et al. 2014). The order comprises a single family *Asterinaceae*, which contains 17 genera that are differentiated using both morphological and phylogenetic characters (Hongsanan et al. 2014). The asexual morph in the family can be either coelomycetous (*Asterostomella*) or hyphomycetous (*Asterina*), although classification of the asexual morphs needs further clarification (Hyde et al. 2013, Hongsanan et al. 2014). Redescription of the asexual morphs of *Asterinaceae* (Hofmann 2010, Hosagoudar 2010) and key to genera by Hosagoudar (2010) were based mainly on morphology and host association

In this study, we introduce a new genus *Discopycnothyrium* and its type species *D. palmae* based on phylogeny, morphology, and host association. *Discopycnothyrium* is an asexual morph genus that somewhat resembles *Asterostomula* and *Prillieuxina* in its circular pycnothyria with radially arranged cells. However, *Asterostomula* and *Prillieuxina* differ in having pycnothyria with star-like or irregular openings and brown pycnothyriospores, while pycnothyria in *Discopycnothyrium* have a central ostiole and 1-septate pycnothyriospores comprising brown upper cells and hyaline lower cells. The placement of *Discopycnothyrium* in *Asterinaceae* is also supported by rDNA sequence analysis.

Materials & methods

An *Asterinaceae*-like specimen was collected from Narathiwat Province in the southern part of Thailand. Morphological characters were observed under Nikon 80i stereo and compound microscopes. Measurements were determined using Tarosoft (R) Image Frame Work (v. 0.9.7). We attempted to isolate the specimen by using a single spore technique (Chomnunti et al. 2011, 2014), but the spores did not grow in culture. The fresh specimen was therefore dried in silica gel, and DNA was extracted directly from pycnothyria. Type material was conserved in the Herbarium, Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand (MFLU), and the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China (KUN).

Genomic DNA was extracted from dried pycnothyria using the E.Z.N.A® Forensic Genomic DNA Extraction Kit (OMEGA Bio-tek Norcross GA 2013). Individual pycnothyria were placed into 1.5 ml sterilized tubes and kept in -20 °C overnight, after which DNA was extracted following the manufacturer's instructions.

Universal primers LR5 and LROR were successfully used to amplify the 28S rDNA (LSU) region. The PCR thermal cycle program was: an initial cycle at 94°C for 3 min; 40 cycles of denaturation (94°C for 30 s), annealing (54°C for 40 s), elongation (72°C for 1 min); final extension (72°C for 10 min). After visualization on 1% agarose electrophoresis gels stained with ethidium bromide, the PCR products were sent to

TABLE 1. Taxa used in the phylogenetic analysis: specimen codes and GenBank accession numbers (LSU)

SPECIES	VOUCHER/CULTURE	GENBANK NO.
<i>Apiosporina collinsii</i>	CBS 118973	GU301798
<i>Asterina cesticola</i>	TH 591	GU586215
<i>Asterina fuchsiae</i>	TH 590	GU586216
<i>Asterina phenacis</i>	TH589	GU586217
<i>Asterina siphocampyli</i>	ppMP 1324	HQ701140
<i>Asterina</i> sp.	MFLU13-0619	KM386978
<i>Asterina weinmanniae</i>	TH 592	GU586218
<i>Asterina zanthoxyli</i>	TH 561	GU586219
<i>Discopycnothyrium palmae</i>	MFU13-0485	KM386979
<i>Asterotexis cucurbitacearum</i>	PMA M-0141224	HQ610510
<i>Chaetothyriotheceum elegans</i>	CPC 21375	KF268420
<i>Coleroa robertiani</i>	CBS 458.64	JQ036231
<i>Fusicladium africanum</i>	CPC 12828	EU035423
<i>Fusicladium pini</i>	CBS 463.82	EU035436
<i>Gibbera conferta</i>	CBS 191.53	GU301814
<i>Gloniopsis praelonga</i>	CBS 112415	FJ161173
<i>Gloniopsis subrugosa</i>	CBS:123346	FJ161210
<i>Hysterium angustatum</i>	CBS 236.34	FJ161180
<i>Hysterobrevium smilacis</i>	CBS 114601	FJ161174
<i>Lembosia albersii</i>	MFLU13-0377	KM386982
<i>Lichenothelia convexa</i>	L1608	KC015085
<i>Microthyrium microscopicum</i>	CBS 115976	GU301846
<i>Natipusilla limonensis</i>	L_AF286_1A	HM196370
	PE3_2b	JX474862
<i>Natipusilla naponensis</i>	L_AF217_1A	HM196371
<i>Oedohysterium insidens</i>	CBS:238.34	FJ161142
<i>Oedohysterium sinense</i>	CBS:123345	FJ161209
<i>Protoventuria barriae</i>	CBS 300.93	JQ036232
<i>Psiloglonium araucanum</i>	CBS:112412	FJ161172
<i>Psiloglonium clavisorum</i>	CBS:123338	FJ161197
<i>Psiloglonium simulans</i>	CBS:206.34	FJ161178
<i>Rhytidhysterium rufulum</i>	AFTOL-ID 2109	FJ469672
<i>Symptoventuria capensis</i>	CPC 12840	DQ885904
	CBS 120136	KF156104
<i>Tyrannosorus pinicola</i>	AFTOL-ID 1235	DQ470974
<i>Venturia inaequalis</i>	CBS 815.69	GU301878
<i>Veronaepsis simplex</i>	CBS 588.66	EU041877
<i>Zeloasporium siamense</i>	IFRDCC 2194	JQ036228

¹LSU: 28s rDNA (Newly generated sequence in bold)

Majorbio Co., Ltd. (China) for purification and sequencing. The sequence generated for *Discopycnothyrium palmae* was deposited in GenBank (KM386979).

Other LSU sequence data in this study were obtained from GenBank to compile the dataset (TABLE 1), which represents *Asterinaceae*, *Hysteriaceae* Chevall., *Microthyriaceae* Sacc., *Mytiliniidiaceae* Kirschst., *Natipusillaceae* Raja et al., *Sympoventuriaceae* Y. Zhang et al., and *Venturiaceae* E. Müll. & Arx ex M.E. Barr following the publications by Schoch et al. (2009), Hyde et al. (2013), Boonmee et al. (2014), Hongsanan et al. (2014), and Wijayawardene et al. (2014). *Lichenothelia convexa* Henssen was selected as outgroup. Alignments were performed automatically using BioEdit v 7.1.9 and Clustal X 2.0.11 (Thompson et al. 1997) and improved manually when necessary (Hall 2004).

Maximum likelihood analysis was performed in RAxML with raxmlGUIv.0.9b2 (Silvestro & Michalak 2012); the search strategy was set to rapid bootstrapping and carried out using GTRGAMMAI model of nucleotide substitution for a small dataset to account for rate heterogeneity. The nucleotide substitution model was selected with MrModel test 2.2 (Nylander 2008); the number of replicates was automatically inferred using the stopping criterion (Pattengale et al. 2009). The evolution model was performed with MrModeltest 2.2 (Nylander 2008). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes (v. 3.0b4; Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains ran for 1,000,000 generations, with trees sampled every 100th generation. 10,000 trees were obtained; the first 2000 trees, representing the burn-in phase were discarded, and the remaining 8000 trees were used to calculate posterior probabilities; the default convergence criterion was set at 0.001 (Cai et al. 2006, 2008). Phylogenetic trees were viewed in TreeView (v. 1.6.6; Page 2001). Maximum likelihood bootstrap values higher than 50% are given as the first set of numbers above the nodes (FIG. 2). Bayesian posterior probabilities (BYPP) equal or higher than 0.9 are given as the second set of numbers above the nodes (FIG. 2).

Taxonomy

Discopycnothyrium Hongsanan & K.D. Hyde, *gen. nov.*

INDEX FUNGORUM IF551024

Differs from other genera in *Asterinaceae* by its pycnothyria with darker cells surrounding a rounded central ostiole, and 1-septate pycnothyriospores with a hyaline lower cell.

TYPE SPECIES: *Discopycnothyrium palmae* Hongsanan & K.D. Hyde

ETYMOLOGY: from Latin *disco* meaning “circular” or “plate-like”, and *pycnothyrium* referring to the technical term for the type of fructification.

FACES OF FUNGI FoF00569

Foliar epiphytes. SUPERFICIAL MYCELIUM aseptate, pale brown to brown. SEXUAL MORPH: undetermined. ASEXUAL MORPH: PYCNOTHYRIUM superficial on the host surface, circular, walls comprising radially arranged cells, dark brown, with central ostiole surrounded by dark cells. PERIDIUM poorly

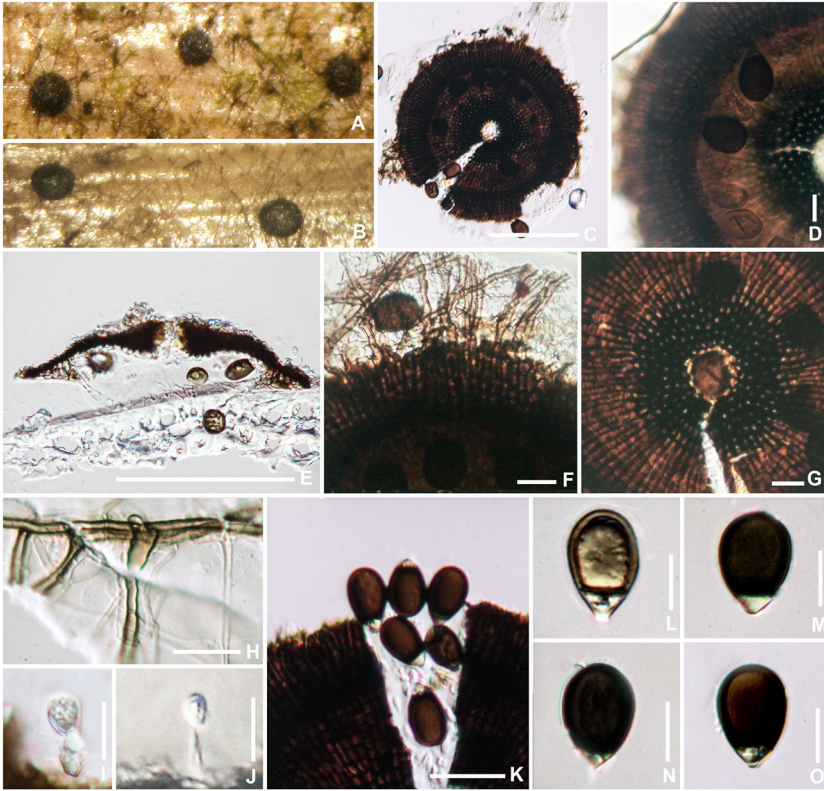


FIG 1. *Discopycnothyrium palmae* (MFLU 13-0485, holotype). A, B. Pycnothyria on surface of host. C. Pycnothyrium when viewed in squash mounts. D. Pycnothyriospore arrangement in pycnothyrium. E. Vertical section through pycnothyrium. F. Upper wall of pycnothyrium when viewed in squash mounts. G. Darkened cells around ostiole. H. Aseptate hyphae without appressoria. I, J. Conidiogenous cells giving rise to pycnothyriospores. K, M–O. Mature pycnothyriospore. L. Immature pycnothyriospore. Bars: C–E = 100 μ m; K = 20 μ m; D, F–J, L–O = 10 μ m.

developed at the base. CONIDIogenous CELLS holoblastic, cylindrical, hyaline. PYCNOTHYRIOSPORES ovoid or broadly clavate, widest and rounded near the apex and tapering towards lower end, 1-septate near the tapering base, upper cell pale brown to brown, lower cell hyaline.

Discopycnothyrium palmae Hongsanan & K.D. Hyde, sp. nov.

FIG. 1

INDEX FUNGORUM IF551023

Differs from other species in *Asterinaceae* by its darker brown cells surrounding a rounded central ostiole, and its 1-septate pycnothyriospores with a hyaline lower cell.

TYPE: Thailand, Narathiwat Province, on the branches of palm (*Arecaceae*), September 2013, K.D. Hyde (**holotype** MFLU13-0485; **isotype** KUN; GenBank KM386979).

ETYMOLOGY: Referring to the host palm on which the fungus was found.

FACES OF FUNGI FoF00570

Foliar epiphytes on surface of palm leaves (*Arecaceae*). SUPERFICIAL MYCELIUM 1–2 μm diam ($x = 2 \mu\text{m}$, $n = 10$), aseptate, pale brown to brown, dark brown at the margin, lacking appressoria and setae.

SEXUAL MORPH: Undetermined.

ASEXUAL MORPH: PYCNOTHYRIUM 162–195 μm diam ($x = 187 \mu\text{m}$, $n = 10$), superficial on host surface, easily removed, mostly solitary, circular, hyphae of different lengths at the margin, wall comprising radial cells, dark brown, with central ostiole, and with darker cells around the central ostiole. PERIDIUM poorly developed at the base. PSEUDOPARAPHYSES not observed. CONIDIOPHORES not observed. CONIDIOGENOUS CELLS 6–8 \times 2–3 μm ($x = 8 \times 3 \mu\text{m}$, $n = 5$), holoblastic in cavity of pycnothyria, cylindrical, hyaline, smooth. PYCNOTHYRIOSPORES 18–22 \times 12–15 μm ($x = 20 \times 13 \mu\text{m}$, $n = 10$), ovoid or broadly clavate, widest and rounded near the apex and tapering towards the lower end, 1-septate near the tapering base, not constricted at the septum, upper cell with 2 layers when immature, outer layer disappearing at maturity, pale brown in upper cell when immature and dark brown at maturity with smooth-walls, lower cell always hyaline, smooth-walled or sometimes rough.

NOTES: *Discopycnothyrium palmae* is most similar to *Asterostomula loranthei* Theiss. by having a superficial mycelium without appressoria and setae and pycnothyrium walls composed of radial cells, but *A. loranthei* differs in the stellate dehiscence of the pycnothyrium centers and its unicellular pycnothyriospores (Hosagoudar 2010).

The new species also somewhat resembles the asexual morph of *Prillieuxina*, which also has circular pycnothyria and dark pycnothyriospores with a single septum, and a similar host association (Hofmann 2010), with *P. calami* (Syd. & P. Syd.) R.W. Ryan and *P. saginata* (Syd. & P. Syd.) R.W. Ryan also described from arecaceous hosts. *Prillieuxina* differs from *Discopycnothyrium* by its circular pycnothyria with X- or Y-shaped fissures at maturity and its 1-septate pycnothyriospores with a brown (not hyaline) lower cell (Hongsanan et al. 2014).

Allothyrium marcgraviae Syd. also has circular pycnothyria but differs from *D. palmae* by the star-like fissures of its pycnothyrial opening and its multi-septate ascospores (Hongsanan et al. 2014). The LSU sequence analyses cluster *D. palmae* in the clade of *Asterinaceae* but in its own lineage, with 59% ML and 0.88 PP support (FIG. 2), confirming it as a new genus.

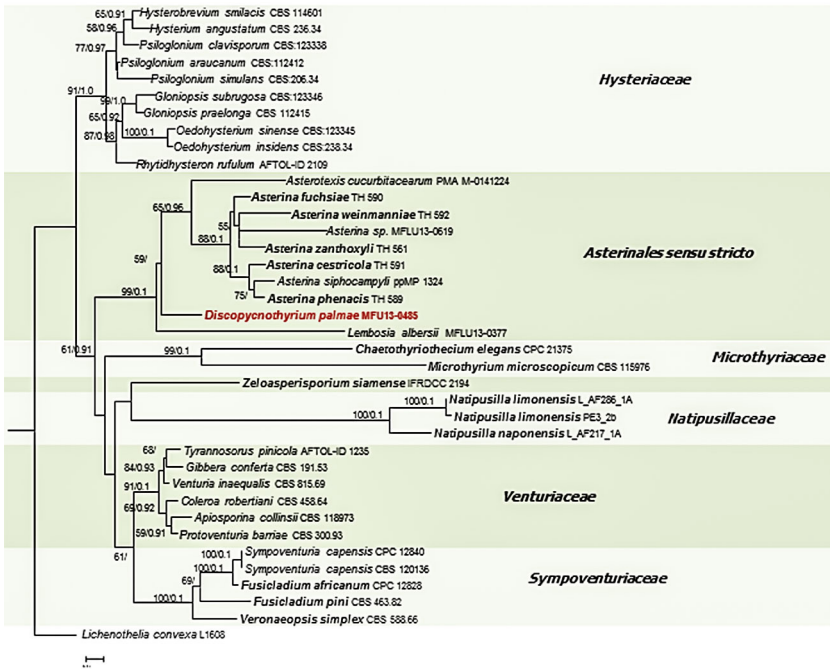


FIG 2. RAXML maximum likelihood phylogenetic tree based on sequence analysis of LSU dataset. The first set of numbers above the nodes are RAXML values >50%; the second set are Bayesian posterior probabilities ≥0.9. Strain numbers are indicated after species names. The new species introduced is in red bold, other types are in black bold.

Molecular phylogeny

LSU sequence data from six families (*Hysteriaceae*, *Microthyriaceae*, *Mytiliniaceae*, *Natipusillaceae*, *Sympoventuriaceae*, *Venturiaceae*) were included in the phylogenetic analysis (FIG. 2). The *Asterinaceae* clade includes six strains of *Asterina*: *A. cesticola* (R.W. Ryan) Hosag. & T.K. Abraham, *A. fuchsiae* Syd., *A. phenacis* Syd., *A. siphocampyli* Syd., *A. weinmanniae* Syd., and *A. zanthoxyli* W. Yamam. The new taxon, *Discopycnothyrium palmae*, forms a moderately supported (59% ML, 0.88 PP) clade sister to the other *Asterina* species, supporting *D. palmae* as clearly separate from other species in *Asterinaceae*. Moreover, *Lembosia albersii* Henn. (MFLU13-0377) forms a well-supported clade basal to the other genera in *Asterinaceae* with high (99% ML, 1.0 PP) bootstrap support.

The *Microthyriaceae* clade comprises two strains: *Chaetothyriothecium elegans* Hongsanan & K.D. Hyde and *Microthyrium microscopicum* Desm.,

also with high (99% ML, 1.0 PP) bootstrap support. The *Natipusillaceae* clade includes three strains of freshwater fungi: *Natipusilla limonensis* A. Ferrer et al. (two strains) and *N. naponensis* A. Ferrer et al. with 100% ML and 1.0 PP support; although they are phylogenetically related to *Zeloasperisporium siamense* Hongsanan et al., there is no morphological similarity between *Natipusilla* and *Zeloasperisporium*, so these should be regarded as tentatively placed lineages.

The *Venturiaceae* clade) with high bootstrap support of 91% ML, 1.0 PP) includes six representative strains—*Apiosporina collinsii*(Schwein.) Höhn., *Coleroa robertiani* (Fr.) E. Müll., *Gibbera conferta* (Fr.) Petr., *Protoventuria barriae* Carris & A.P. Poole, *Tyrannosorus pinicola* (Petrini & P.J. Fisher) Unter. & Malloch, and *Venturia inaequalis* (Cooke) G. Winter. The clade of *Venturiaceae*—and is closely (66% ML support) related to the *Symptoventuriaceae* clade with five strains: *Symptoventuria capensis* Crous & Seifert (two strains), *Fusicladium africanum* Crous, *F. pini* Crous & de Hoog, and *Veronaeopsis simplex*(Papendorf) Arzanlou & Crous.

Discussion

Discopycnothyrium (type species *D. palmae*) is introduced as a new genus in the family *Asterinaceae* (characterised by a superficial pycnothyrium comprising radiating cells) that differs from other genera in having circular pycnothyria with variably long marginal hyphae, darkened cells around a central ostiole, and pycnothyriospores that are widest near the apex, taper towards lower end, and have one septum near the tapering base that separates the larger pale brown to brown upper cell from the smaller hyaline basal cell.

There are limited sequence data for taxa in *Asterinaceae* because members of this family are mostly obligate parasites/biotrophs, and pure cultures are difficult to obtain; therefore direct DNA extraction and sequencing must be obtained from specimen. However, sequence data from *Asterina* species do support the *Asterinaceae* within *Dothideomycetes* (Hofmann 2010, Wu et al. 2011, Hyde et al. 2013, Boonmee et al. 2014, Hongsanan et al. 2014, Wijayawardene et al. 2014). Although phylogenetic LSU analyses of *Discopycnothyrium* cluster *D. palmae* within the *Asterinaceae* clade, it is distinctly separate from other genera in that clade. Bootstrap values are not high because of low number of populations representing this group, and further study is needed to clarify the divergence of the species. Based on

Nonetheless, both morphology and phylogeny supports the new taxon as a species in a new genus within *Asterinaceae*, which we introduce here as *Discopycnothyrium*.

Acknowledgments

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