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Palawaniaceae fam. nov., a new family (Dothideomycetes, Ascomycota) to accommodate *Palawania* species and their evolutionary time estimates

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Abstract

Palawania species are common on palms, occurring on dried fronds and spines, rarely on leaves, at first appearing as solitary, circular, black spots and then being confluent and lacking superficial hyphae. The taxonomy of the genus has been problematic because of lack of some important morphological characters as well as molecular data. Two collections made in Thailand are characterized based on analyses of combined LSU, SSU and RPB2 sequence datasets. Phylogenetic analyses indicate that *Palawania* species form a sister clade with Pleurotremataceae (= Dyfrolomycetaceae) and have a close relationship with Muyocopronales and Acrospermales. Thus, we introduce a new family Palawaniaceae (Dothideomycetes family, *incertae sedis*). A new *Palawania* species is also introduced from northern Thailand based on its distinct phylogeny and comparison of morphological characteristics. The present study clarifies the phylogenetic placement of *Palawania* and divergence time estimates are provided for the new family.

Key words – Ascomycota – Dothideomycetes – evolution – new species – phylogeny – taxonomy

Introduction

The genus *Palawania* was introduced by Sydow & Sydow (1914) and is typified by *Palawania grandis* (Niessl) Syd. & P. Syd. The genus was previously place in the family Microthyriaceae (Müller & von Arx 1962, Lumbsch & Huhndorf 2010, Wu et al. 2011, Hyde et al. 2013, Wijayawardene et al. 2014) based on its morphological characteristics being similar to *Microthyrium*, while sharing some characters with Asterinaceae (Wu et al. 2011, Hongsanan et al. 2014). Index Fungorum (2016) list seven species epithets for the genus. However, five species (i.e. *P. brosimi* Bat. & J.L. Bezerra, *P. dovyalidis* (Doidge) G.C. Nel, *P. eucleae* (Doidge) G.C. Nel, *P. halleriae* (Dippen.) Doidge, *P. orbiculata* (Syd. & P. Syd.) Doidge, have been transferred to other genera in the family Parmulariaceae. Two species (*P. cocois* Syd. & P. Syd and *P. grandis* (Niessl) Syd. & P. Syd) from palms were accepted in the family Microthyriaceae. Material of *P. grandis* (Niessl) Syd. & P. Syd. was re-examined by Wu et al. (2011) who provided descriptions, together

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with illustrations. However, fresh collections and sequence data are needed to confirm the relationship and phylogenetic placement of *Palawania* (Wu et al. 2011).

The aim of the present study is to determine the phylogenetic placement of the genus *Palawania*. Divergence time estimates for Palawaniaceae are provided. A new species is also introduced, based on molecular and morphological comparison with descriptions, together with illustrations.

Materials & Methods

Sample collection and specimen examination

Fresh materials were collected from Chiang Rai Horticultural Research Center during 2013–2014. Fungal micromorphology was studied following the methods of Mapook et al. (2016a). Single spore isolations were obtained using the methods of Chomnunti et al. (2014). Ascospores germinating within 12–24 h were transferred to new malt extract agar (MEA) plates and incubated at 25–30 °C in the dark, one of these cultures was used for molecular study. The specimens and living cultures are deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Culture collection Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand and Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), China. Facesoffungi numbers and Index Fungorum numbers were registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

Isolates were grown on MEA at 25–30 °C for two weeks. The fungal mycelium was scraped off and transferred to microcentrifuge tubes (1.5 ml.). The fungal genomic DNA from mycelium was extracted by the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, China) following the manufacturer's instructions (Hangzhou, P.R. China). The fungal genomic DNA was extracted directly from the ascomata using The E.Z.N.A.® Forensic DNA Kit (Omega Bio-Tek, Inc., United States) following the manufacturer's protocol. DNA amplifications were performed by polymerase chain reaction (PCR). The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5 (Vilgalys & Hester 1990). The partial small subunit nuclear rDNA (SSU) was amplified with primer pairs NS1 and NS4 (White et al. 1990). The partial RNA polymerase second largest subunit (RPB2) was amplified by using primers fRPB2-5F and fRPB2-7cR (Liu et al. 1999). Methods for PCR amplification and sequencing were performed as in Mapook et al. (2016a).

Phylogenetic analysis

The most closely related taxa were determined using nucleotide BLAST searches online in GenBank (http://www.ncbi.nlm.nih.gov/). Combined LSU, SSU and RPB2 sequence data from representative closest relatives to Palawaniaceae were selected following Hongsanan et al. (2016) and Mapook et al. (2016b), to confirm the phylogenetic placement of the *Palawania* strains. Representative stains from Lecanoromycetes were selected as outgroup taxa based on their placement close to the ingroup, following Hongsanan et al. (2016). The closest matched taxa were determined through nucleotide blast searches in GenBank. The sequences used for analyses with accession numbers are given in Table 1. All sequence data were aligned using MAFFT (v7.110) online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). The alignments were checked and uninformative gaps minimized manually where necessary in BioEdit 7.0.1 (Hall 1999). Maximum likelihood (ML) and Bayesian inference (BI) analyses were used in the following the methodology as described in Mapook et al. (2016a). The GTR+I+G model with inverse gamma rate were selected for each partition based on the results from MrModeltest v. 2.2 (Nylander et al. 2004). Phylogenetic trees were drawn using Treeview v. 1.6.6 (Page 1996). The new nucleotide sequence data are deposited in GenBank.

Fossil calibrations

The fossil calibrations were used in the analyses following the methodology as described in Beimforde et al. (2014), Hongsanan et al. (2016) and Pérez-Ortega et al. (2016) for dating molecular clock analyses. Estimating divergences time of the common ancestor of Palawaniaceae in Dothideomycetes was performed using BEAST analysis.

A molecular clock tree was generated using the calibration points from Beimforde et al. (2014), Hongsanan et al. (2016) and Pérez-Ortega et al. (2016). Metacapnodiaceae was used as a minimum age of Capnodiales (normal distribution, mean = 100, SD = 150, CI = 400) with *Calicium* (Rikkinen 2003) (gamma distribution shape = 1.0, scale = 70, offset = 35) and *Microthyrium* (gamma distribution scale = 70, offset = 35) for calibrations in this study.

Molecular clock analysis

Molecular clock analyses were performed using BEAST 1.8.0 (Drummond et al. 2012) following the methodology as described in Hongsanan et al. (2016). LSU, SSU and RPB2 aligned sequences data partitions were prepared separately. Each data partitions were loaded to BEAUti 1.8.0 (Drummond et al. 2012). GTR+I+G model were selected for LSU, and TrN+I+G model for SSU and RPB2 based on the results from jModelTest 2.1.10 (Darriba et al. 2012). Taxa sets were prepared for the most recent common ancestor (TMRCA) and other interesting groups. A lognormal relaxed clock (uncorrelated) model was selected, and a birth/death incomplete sampling tree prior was used to model the speciation of nodes in the topology.

BEAST analyses were run for 50 million generations, and sampling parameters every 1000 generations. Convergence, mixing and effective sample sizes (ESS > 200) of parameters were checked using Tracer v1.6 (Rambaut et al. 2014). The first 50,000 trees representing the burn-in phase were discarded. The remaining trees were combined using LogCombiner 1.8.0 and generate a maximum clade credibility tree (MCC) with TreeAnnotator 1.8.0. Molecular clock trees were drawn using FigTree v. 1.4.0 (Rambaut 2009).

The ages for geological time periods have taken from the most recent International Chronostratigraphic Chart (v2016/04) by International Commission on Stratigraphy (IUGS) and available from www.stratgraphy.org, are indicated at the base of the evolution tree (Fig. 2)

Results

Phylogenetic analysis

The combined of LSU, SSU and RPB2 sequence data (93 taxa) including our new strains were analyzed by maximum likelihood (ML) and Bayesian analyses. Representative stains from Lecanoromycetes were selected as outgroup taxa. A best scoring RAxML analysis is shown in Fig. 1. The trees generated from both methods are similar in topology with no significant difference. In the phylogenetic tree (Fig. 1) our new strains clustered within Dothideomycetes, and as a sister clade with Pleurotremataceae with moderate support (62% ML) and related to the order Muyocopronales with 93% ML, 1.0 PP support.

Molecular clock and Divergence time estimates

The maximum clade credibility (MCC) tree indicates the divergence estimates at 319 Mya (247−400), in the Carboniferous for Dothideomycetes and Lecanomycetes. Divergence time estimates in this study for the target groups and the splits between them are shown in Table 2. A simplified of diagrammatic scheme representation for the target groups is show in Fig. 3.

Acrospermales has an estimated crown date in the Cretaceous of 124 Mya (90−160). The family shares the most common ancestor with Pleurotremataceae, Palawaniaceae and Muyocopronales at 215 Mya (164−272) in the late Triassic. Pleurotremataceae has an estimated crown date in the early Cenozoic of 55 Mya (38−74) and shares the most common ancestor with Palawaniaceae and Muyocopronales in the early Jurassic at 192 Mya (145−243). The split between Muyocopronales which was introduced as a new order in Mapook et al. (2016b) and Palawaniaceae (in this study) is estimated at 172 Mya (130−218), during the Jurassic with estimated crown dates of Muyocopronales during the Cenozoic at 52 Mya (38−66).

Table 1 Taxa used in this study and their GenBank accession numbers. New sequences are in bold.

Taxon	Culture accession no.	GenBank accession no.		
		LSU	SSU	RPB2
Acrospermum adeanum	M133	EU940104	EU940031	EU940320
Acrospermum compressum	M151	EU940084	EU940012	EU940301
Acrospermum gramineum	M152	EU940085	EU940013	EU940302
Alternaria alternata	KFRD-18	KX609781	KX609769	-
Alternariaster bidentis	CBS 134021	KC609341	-	KC609347
Antennariella placitae	CBS:124785	GQ303299	-	-
Asterina fuchsiae	TH590	GU586216	GU586210	-
Asterina phenacis	TH589	GU586217	GU586211	-
Bambusicola massarinia	MFLUCC 11-0389	JX442037	JX442041	KU940169
Bambusicola splendida	MFLUCC 11 439	JX442038	JX442042	-
Botryosphaeria agaves	MFLUCC 11-0125	JX646808	JX646825	-
Botryosphaeria tsugae	AFTOL-ID 1586	DQ767655	-	DQ767644
Calicium viride	10-VII-1997 (DUKE)	AF356670	AF356669	AY641031
Calicium salicinum	CBS 100898	KF157982	KF157970	KF157998
Camarosporium quaternatum	CBS 483.95	GU301806	GU296141	GU357761
Capnodium salicinum	AFTOL-ID 937	DQ678050	DQ677997	-
Caryospora minima	-	EU196550	EU196551	-
Chaetothyriothecium elegans	CPC 21375	KF268420	-	-
Corynespora cassiicola	CBS 100822	GU301808	GU296144	GU371742
Corynespora smithii	CABI 5649b	GU323201	-	GU371783
Cucurbitaria berberidis	MFLUCC 11-0387	KC506796	KC506800	-
Cyphelium tigillare	Tibell 22343 (UPS)	AY453641	AF241545	-
Cyphelium inquinans	Tibell 22283 (UPS)	AY453639	U86695	-
Cystocoleus ebeneus	L161	EU048578	EU048571	-
Didymella exigua	CBS 183.55	JX681089	EU754056	GU371764
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436588	-
Dothiora cannabinae	AFTOL ID 1359	DQ470984	DQ479933	DQ470936
Elsinoe fawcettii	CPC 18535	JN940382	JN940559	-
Elsinoe verbenae	CPC 18561	JN940391	JN940562	-
Extremus antarcticus	CCFEE 5312	KF310020	-	KF310086
Helicascus nypae	BCC 36751	GU479788	GU479754	GU479826
Julella avicenniae	BCC 20173	GU371822	GU371830	GU371786
Karschia cezannei	Cezanne-Eichler B26	KP456152	-	-
Katumotoa bambusicola	KT 1517a	AB524595	AB524454	AB539095
Labrocarpon canariense	Ertz 16907 (BR)	KP456157	-	-
Lentithecium fluviatile	CBS 123090	FJ795450	FJ795492	FJ795467
Leptosphaeria doliolum	MFLUCC 15-1875	KT454719	KT454734	-
Leptosphaerulina australis	CBS 317.83	EU754166	GU296160	GU371790
Leptoxyphium cacuminum	MFLUCC10-0049	JN832602	JN832587	-
Lophiotrema nucula	CBS 627 86	GU301837	GU296167	GU371792
Lophium mytilinum	AFTOL-ID 1609	DQ678081	DQ678030	DQ677979
Massarina bambusina	H 4321	AB807536	AB797246	-
Massarina eburnea	CBS 473.64	GU301840	GU296170	GU371732
Melanomma pulvis-pyrius	CBS 371 75	GU301845	FJ201989	GU371798
Melaspileopsis cf. diplasiospora	Ertz 16247 (BR)	KP456164	-	-

Taxon	Culture accession no.	GenBank accession no.		
		LSU	SSU	RPB2
Microsphaeropsis olivacea	CBS 233 77	GU237988	-	KT389643
Microthyrium microscopicum	CBS 115976	GU301846	GU296175	GU371734
Microthyrium buxicola	MFLUCC 15-0213	KT306552	KT306550	-
Murispora rubicunda	IFRD 2017	FJ795507	GU456308	-
Muyocopron castanopsis	MFLUCC 10-0042	-	JQ036225	-
Muyocopron castanopsis	MFLUCC 14-1108	KU726965	KU726968	KY225778
Muyocopron dipterocarpi	MFLUCC 14-1103	KU726966	KU726969	KY225779
Muyocopron lithocarpi	MFLUCC 10-0041	JQ036230	JQ036226	-
Muyocopron lithocarpi	MFLUCC 14-1106	KU726967	KU726970	KY225780
Myriangium duriaei	CBS 260.36	NG_027579	AF242266	KT216528
Myriangium hispanicum	CBS 247.33	GU301854	GU296180	GU371744
Mytilinidion rhenanum	CBS 135.34	FJ161175	FJ161136	FJ161115
Natipusilla decorospora	AF236 1a	HM196369	HM196376	-
Natipusilla naponensis	AF217 1a	HM196371	HM196378	-
Neocylindroseptoria pistaciae	CBS 471.69	KF251656	-	KF252161
Multiseptospora thailandica	MFLUCC 11-0183	KP744490	KP753955	-
Phaeodimeriella cissampeli	MFLU 16-0558	KU746806	KU746808	KU746810
Phaeodimeriella dilleniae	MFLU 14-0013	KU746805	KU746807	KU746809
Phaeotrichum benjaminii	CBS 541.72	AY004340	AY016348	GU357788
Physcia aipolia	AFTOL-ID 84	DQ782904.1	DQ782876	DQ782862
Piedraia hortae	CBS 480.64	GU214466	-	-
Palawania thailandense	**MFLUCC 14-1121**	**KY086493**	**KY086495**	**KY086496**
Palawania thailandense	**MFLU 16-1871**	**KY086494**	**-**	**-**
Platystomum crataegi	MFLUCC 14-0925	KT026109	KT026113	-
Pleomassaria siparia	AFTOL-ID 1600	DQ678078	DQ678027	DQ677976
Pleospora herbarum	IT 956	KP334709	KP334729	KP334733
Pleurotrema rhizophorae	JK 5456A	GU479799	-	-
Pleurotrema rhizophorae	BCC15481	-	KF160009	
Pleurotrema tiomanensis	NTOU3636	KC692156	KC692155	-
Preussia funiculata	CBS 659.74	GU301864	GU296187	GU371799
Pseudomassariosphaeria bromicola	IT-1333	KT305994	KT305996	-
Pseudostrickeria muriformis	MFLUCC 13-0764	KT934254	KT934258	-
Ramularia endophylla	CBS 113265	KF251833	-	KP894673
Rasutoria pseudotsugae	rapssd	EF114704	EF114729	-
Rasutoria tsugae	ratstk	EF114705	EF114730	-
Salsuginea ramicola	KT 2597.1	GU479800	GU479768	GU479833
Schizothyrium pomi	CBS 406.61	EF134949	-	KF902384
Stictographa lentiginosa	Ertz 17570 (BR)	KP456170	-	-
Sympoventuria capensis	CBS 120136	KF156104	KF156094	-
Teratosphaeria fibrillosa	CBS 121707	GU323213	GU296199	GU357767
Trichodelitschia munkii	Kruys 201 (UPS)	DQ384096	DQ384070	-
Uwebraunia commune	NC132C1d	-	-	KT216546
Venturia inaequalis	CBS 594.70	GU301879	GU296205	GU357757
Xenolophium applanatum	CBS 123127	GU456330	GU456313	GU456355
Zeloasperisporium wrightiae	MFLUCC 15-0225	KT387737	KT387738	-
Zeloasperisporium hyphopodioides	CBS 218.95	EU035442	-	-
Zeloasperisporium siamense	IFRDCC 2194	JQ036228	JQ036223	-

Table 2 Divergence time estimates of the target groups in this study

	Geological period	Time (Mya)
Acrospermales crown group	Cretaceous	124 (90–160)
Muyocopronales crown group	Cenozoic Era, Tertiary, Paleogene	52 (38–66)
Pleurotremataceae crown group	Cenozoic Era, Tertiary, Paleogene	55 (38–74)
Palawaniaceae + Muyocopronales	Jurassic	172 (130–218)

Taxonomy

Palawaniaceae Mapook & K.D. Hyde, **fam. nov.**

Index Fungorum number: IF552528, *Facesoffungi number*: FoF 02653

Type genus: ***Palawania*** Syd. & P. Syd., Philipp. J. Sci., C, Bot. 9(2): 171 (1914).

Saprobic on surface of dead rachides and spines of Arecaceae, rarely on leaves, at first appearing as solitary, circular, black spots, then being confluent, superficial hyphae absent. **Sexual morph**: *Ascomata* superficial, scattered, or some solitary, flattened, sub-carbonaceous, dark brown to black, basal layer poorly developed, with a central ostiole. *Peridium* wide, upper wall comprising of dark brown or black to light brown cell of *textura epidermoidea*, with light brown cells of *textura angularis* at the sides. *Hamathecium* comprising asci with cylindrical to filiform, septate, pseudoparaphyses which are longer than the asci and inclined toward the central ostiole. *Asci* 8-spored, bitunicate, inequilateral to ovoid, pedicellate, straight or slightly curved, short pedicellate, without ocular chamber. *Ascospores* overlapping, irregularly arranged, oblong to broadly fusiform, hyaline, becoming brown at maturity, 1-septate, constricted at the septum, guttulate, surrounded by hyaline, gelatinous sheath, observed clearly when mounted in Indian ink. **Asexual morph**: Undetermined.

Notes – Based on phylogenetic analysis, Palawaniaceae forms a distinct family in the clade comprising Acrospermales, Pleurotremataceae and Muyocopronales (Fig. 1). Palawaniaceae differs from Acrospermales and Pleurotremataceae (= Dyfrolomycetaceae) in morphology (Riddle 1920, Minter et al. 2007, Hyde et al. 2013, Pang et al. 2013, Maharachchikumbura et al. 2016, Mapook et al. 2016b). Although, the new family has similarity with Muyocopronales in its superficial, flattened ascomata, and pseudoparaphyses that are longer than the asci, they differ in peridium wall patterns.

Palawania Syd. & P. Syd., Philipp. J. Sci., C, Bot. 9(2): 171 (1914).

Saprobic on dead palm rachides. **Sexual morph**: *Ascomata* superficial, solitary or scattered, coriaceous, appearing as circular, scattered, flattened, dark brown to black spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central. *Peridium* 10–50 µm wide, comprising of dark brown or black to light brown cell of *textura epidermoidea* at the top, with light brown cells of *textura angularis* at the sides. *Hamathecium* comprising 1.5–2.5 µm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* 8-spored, bitunicate, inequilateral to ovoid, pedicellate, straight or slightly curved, short pedicel, without ocular chamber. *Ascospores* overlapping, irregularly arranged, oblong to broadly fusiform, hyaline, becoming brown at maturity, 1-septate, constricted at the septum, guttulate, surrounded by hyaline gelatinous sheath, observed clearly when mounted in Indian ink. **Asexual morph**: Undetermined.

Type species – *Palawania grandis* (Niessl) Syd. & P. Syd., Philipp. J. Sci., C, Bot. 9(2): 171 (1914)

Notes – The type specimen of *Palawania grandis* was first reported from *Calamus* sp.

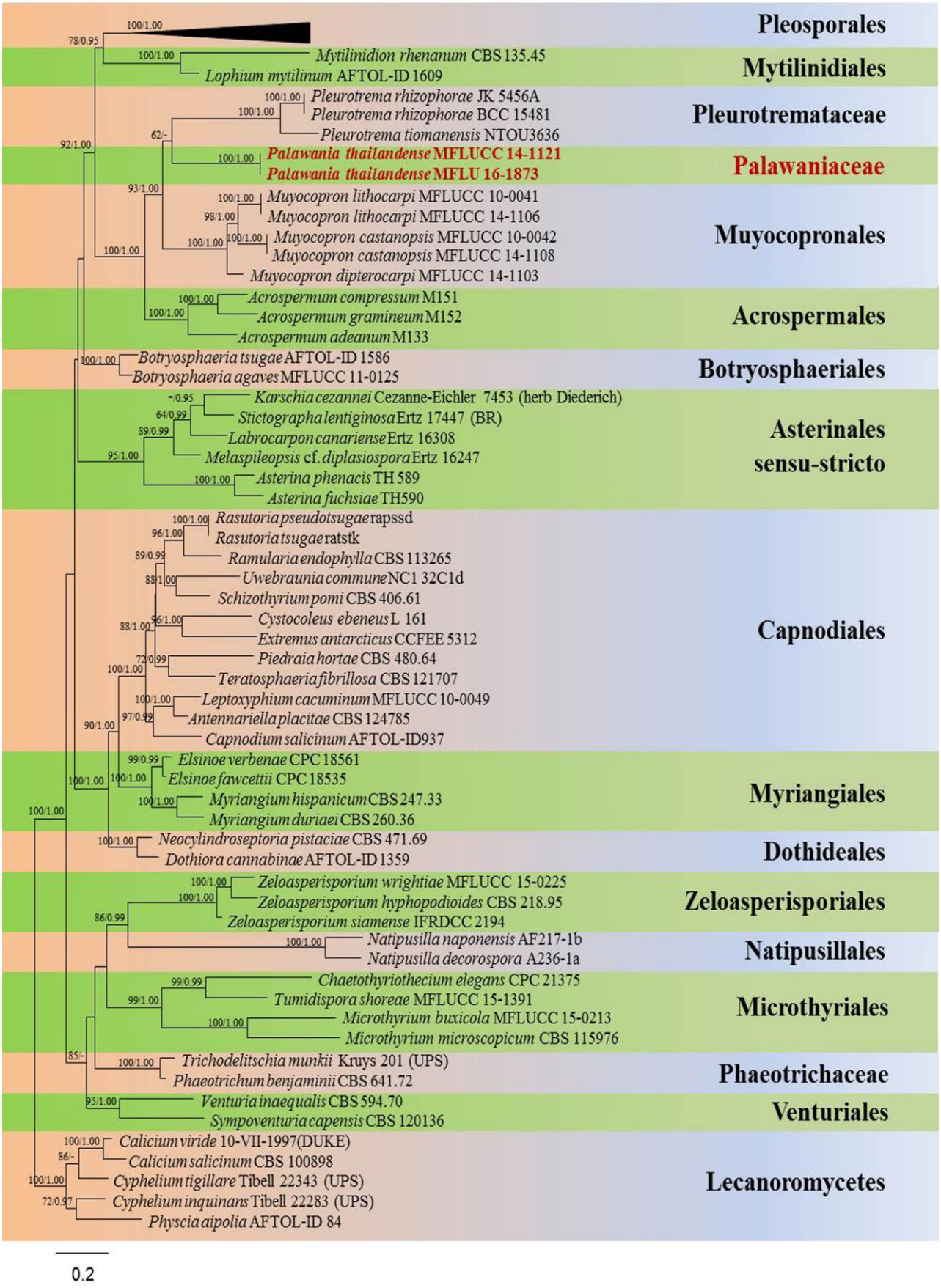

Fig 1 – Phylogram generated from RAxML based on combined LSU, SSU and RPB2 sequence data to establish the new family Palawaniaceae. Bootstrap support values for maximum likelihood (ML) greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are given above the nodes. The new isolates are in red. The tree is rooted with Lecanomycetes.

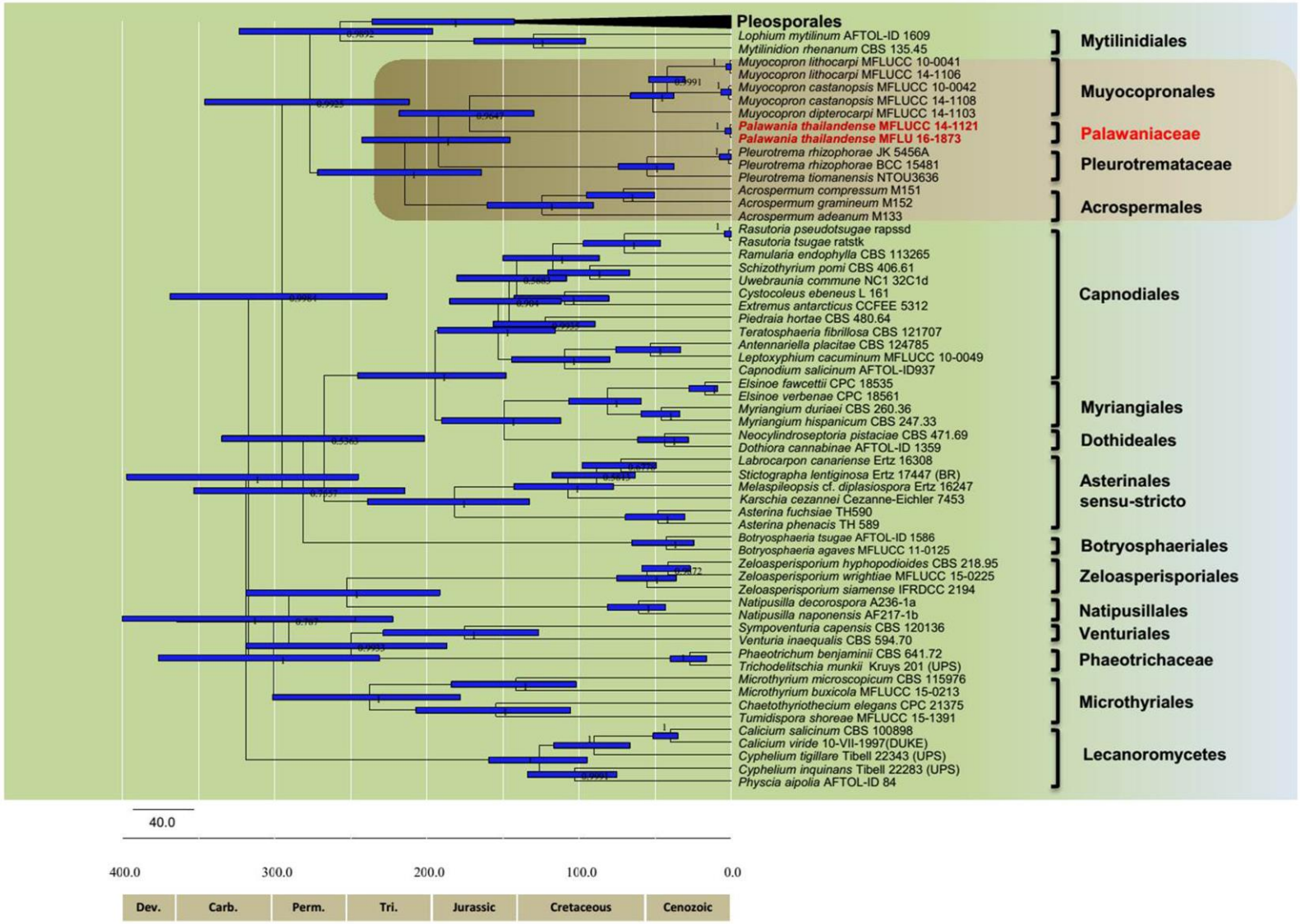

Fig 2 – Divergence time estimates from Dothideomycetes obtained from a Bayesian approach (BEAST). Bars correspond to the 95% highest posterior density (HPD) intervals. The fossil minimum age constraints and second calibrations used in this study are marked with red dots. Geological periods are indicated at the base of the tree and abbreviated as: Dev. = Devonian, Carb. = Carboniferous, Perm. = Permian, Tri. = Triassic. The *Palawania* species discussed in this study are highlighted in red.

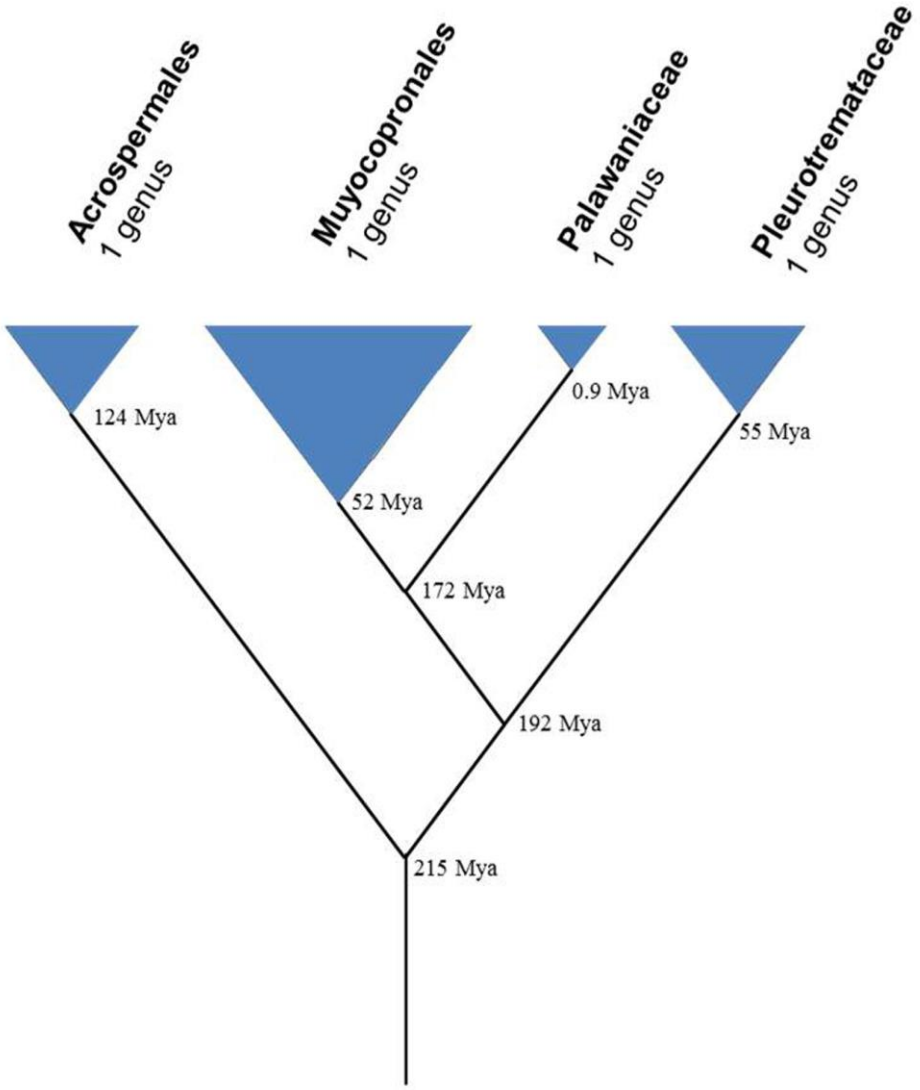

Fig 3 – A simplified of diagrammatic scheme of the evolution representation for the target groups. Divergence time estimates are given at the branches as millions of years ago (Mya).

Palawania thailandense Mapook & K.D. Hyde, **sp. nov.**

Index Fungorum number: IF552527, *Facesoffungi number*: FoF 02654 Fig 4

Etymology – Name reflects the country from where this species was collected, Thailand

Holotype – MFLU 16-1872

Saprobic on dead rachis of *Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf. **Sexual morph**: *Ascomata* 75–115(–125) µm high × (400–)475–495(–575) µm diam. ($\bar{x}$ = 105 × 485 µm, n = 5), superficial, solitary or scattered, coriaceous, appearing as circular, scattered, flattened, dark brown to black spots, covering the host, without a subiculum, with a poorly developed basal layer and an irregular margin. *Ostiole* central. *Peridium* (15–)20–40 µm wide, comprising dark brown or black to light brown cells of *textura epidermoidea* from above, with light brown cells of *textura angularis* at the sides. *Hamathecium* comprising 1.5–2.5 µm wide, cylindrical to filiform, septate, pseudoparaphyses. *Asci* (60–)80–110 × 20–30 µm ($\bar{x}$ = 85 × 25 µm, n = 20), 8-spored, bitunicate, inequilateral to ovoid, pedicellate, straight or slightly curved, without ocular chamber. *Ascospores* 20–27 × (6–)7–10 µm ($\bar{x}$ = 25 × 8 µm, n = 40), overlapping, irregularly arranged, oblong to

broadly fusiform, hyaline, 1-septate, constricted at the septum, guttulate, surrounded by hyaline gelatinous sheath, observed clearly when mounted in Indian ink. **Asexual morph**: Undetermined.

Material examined – THAILAND, Chiang Rai, Chiang Rai Horticultural Research Center, on dried twigs of *Dypsis lutescens* (Arecaceae), 9 September 2014, A. Mapook, (MFLU 16-1872, **holotype**), ex-type living culture MFLUCC 14–1121, (**isotype** in HKAS, under the code of HKAS 95075), on dried twigs of *Phoenix roebelenii* (Arecaceae), 9 September 2014, A. Mapook, (MFLU 16–1873, HKAS 95076, **paratype**).

Notes – *Palawania thailandense* (MFLU 16-1872) is a saprobe on dead rachis of *Dypsis lutescens*. Our collection is most similar to *Palawania grandis* in size and shape of ascomata and ascospores (Table 3), but it differs in having light brown to brown thyriothecia, the peridium radiating cell pattern and longer asci. Thus, we consider our taxon is a new species based on morphology.

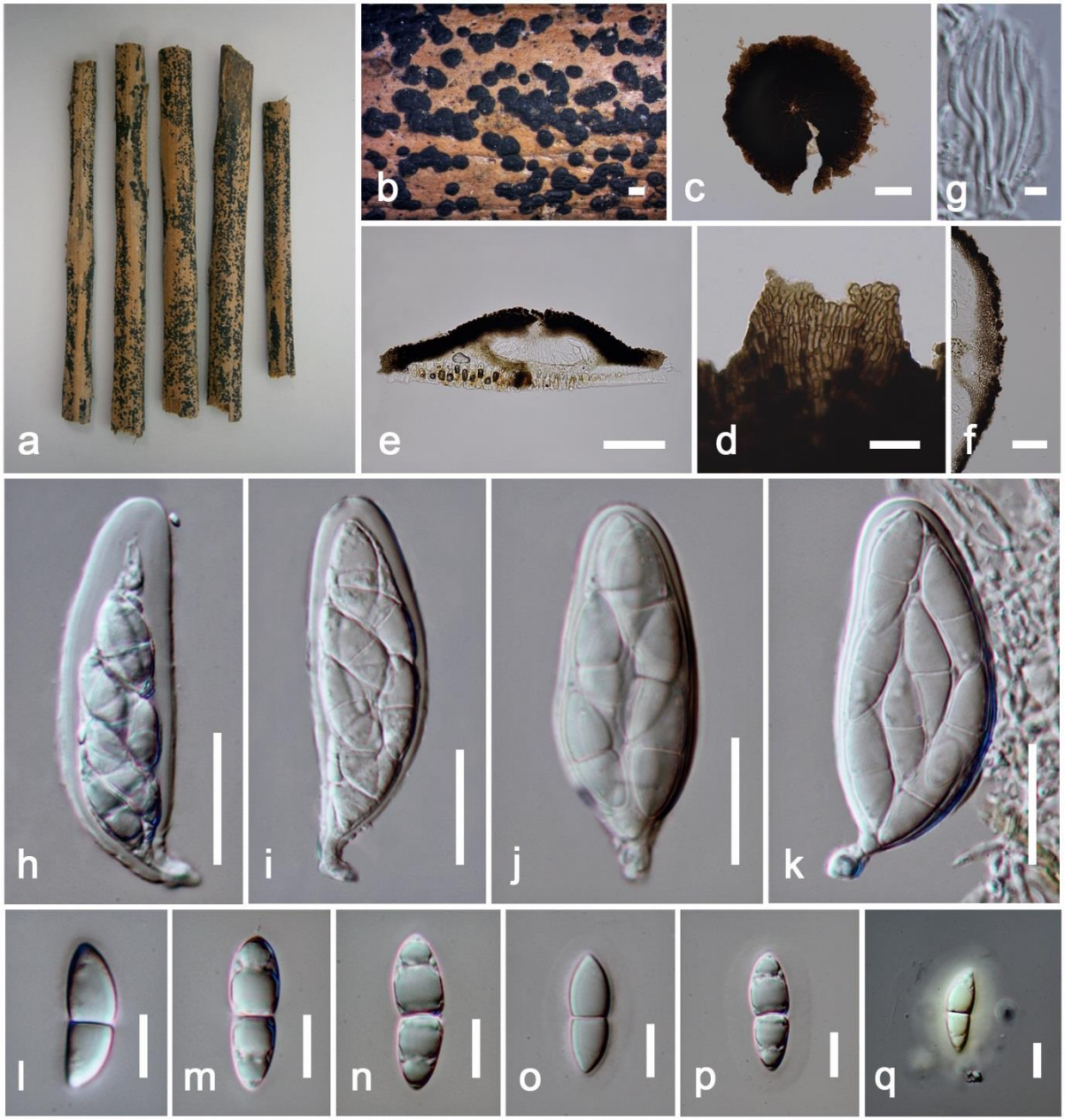

Fig 4 – *Palawania thailandense* (holotype). **a, b** Superficial ascomata on substrate. **c**, **d** Squash mounts showing ascomata walls. **e** Section of ascoma. **f** Peridium. **g** Pseudoparaphyses. **h–k** Asci. **l–p** Ascospores. **q** Ascospores surrounded by hyaline gelatinous sheath in Indian ink. – Bars: b = 500 µm, c, e = 100 µm, f, h–k = 20 µm, d, l–q = 10 µm, g = 5 µm

Table 3 Synopsis of *Palawania* species with some similar morphological features.

Species	Ascomata	Asci size (µm)	Ascospores size (µm)	Septation	Host association family	Source references
***Palawania grandis* (Niessl) Syd. & P. Syd. 1914**	86–110 µm high × 310–480 µm diam.	80–86 × 24–30	26–31 × 9–15, fusiform to ellipsoid-fusiform, brown when mature	1-septate, surrounded by a hyaline sheath.	*Calamus* sp. (Arecaceae)	Wu et al. 2011. (M-0155393, F8214, F90149, F8873)
***Palawania grandis* (Niessl) Syd. & P. Syd. 1914**	65–93 µm high × 330–560 µm diam.	65–85 × 20–22.5	22.5–27 × 8–10	1-septate	*Calamus* sp. (Arecaceae)	Batista & Vital 1960
***Palawania cocois* Syd. & P. Syd. 1914**	0.3–1 (8) mm	50–75 × 20–26	22–24 × 8–10	1-septate	*Cocos nucifera* L. (Arecaceae)	Syd. & P. Syd. 1914
***Palawania thailandense* (MFLUCC 14-1121)**	75–115(–125) µm high × (430–)475–495 µm diam.	(60–)80–110 × 22–25	22–27 × 7–10	1-septate, surrounded by a hyaline sheath.	*Dypsis lutescens* (Arecaceae)	This study

Discussion

The genus *Palawania* has been placed in the family Microthyriaceae (Müller & von Arx 1962, Lumbsch & Huhndorf 2010, Wu et al. 2011, Hyde et al. 2013, Wijayawardene et al. 2014), based on morphology. Wu et al. (2011) re-examined the type material (*P. grandis* (Niessl) Syd. & P. Syd) and provided descriptions and illustrations. However, fresh collections and sequence data are required to confirm the phylogenetic placement of *Palawania* (Wu et al. 2011). In this study, two fresh collections were made from the same location associated with palms (Arecaceae). The collections are similar to *Palawania* and are morphologically and phylogenetically the same genus (Fig. 1). However, morphological comparisons within other *Palawania* species show that our collections differ and we introduce our collection as a new species. *Palawania cocois* and *P. grandis* were listed under Microthyriales (Index Fungorum 2016), both being common on Arecaceae. *Palawania grandis* is morphologically most similar with our new species in the size and shape of its ascomata and ascospores (Wu et al. 2011). However, it differs in having light brown to brown thyriothecia, in the peridium radiating cell pattern and longer asci.

Based on phylogenetic analyses, we conclude that the genus *Palawania* cannot be placed in Microthyriales, as the genus formed a sister clade with Pleurotremataceae and has a close phylogenetic relationship with Muyocopronales and Acrospermales (Fig. 1). Species of Acrospermales and Pleurotremataceae are morphologically very different from *Palawania* species. Therefore, we introduce a new family Palawaniaceae to accommodate this group of unique fungi. The new family is ecologically similar to Muyocopronales and shares some morphological characters, but differs in its peridium wall pattern, shape of asci and ascospores that are surrounded by hyaline gelatinous sheath.

Several of divergence times estimates in Fungi have been published with available fossil evidence from ascomycetes (Vijaykrishna et al. 2006, Gueidan et al. 2008, Schoch et al. 2009, Gazis et al. 2012, Prieto et al. 2013, Beimforde et al. 2014, Hongsanan et al. 2016 and Pérez-Ortega et al. 2016), and events in the geological record and their correlations with fungal fossils have been reported in Berbee and Taylor (2010). The fossil fungal spore record was associated with the Permian–Triassic extinction event (252 Mya) (Visscher et al. 1996, Shen et al. 2011). During this period, the numbers of marine and terrestrial fungi are through to have increased (Visscher et al. 1996). More than 95% of land plants however, disappeared (Labandiera and

Sepkoski 1993, Retallack 1995, Eshet et al. 1995). Furthermore, 34% of marine genera disappeared, land vertebrates and many of amphibians also became extinct after the Triassic–Jurassic extinction (201.3 Mya) according to Benton (1988) and Ryder et al. (1996).

Sequence data from *Palawania thailandense* cluster in the new family Palawaniaceae. The maximum clade credibility (MCC) tree with divergence estimates using three calibration points (Fig. 2) is topologically quite similar to the Bayesian and ML analyses in most of the major lineages (Fig. 1). A difference occurred in the clade comprising our target group, with the families, Acrospermaceae (Acrospermales), Muyocopronaceae (Muyocopronales), Palawaniaceae (Dothideomycetes family, *incertae sedis*), and Pleurotremataceae (Dothideomycetes family, *incertae sedis*), which are generally poorly-studied and lack molecular data. In the maximum likelihood analysis (Fig. 1), Palawaniaceae shared the most common ancestor with Pleurotremataceae, while the MCC tree provided by BEAST suggests that Palawaniaceae is closely related and shared the most common ancestor with Muyocopronales having diverged in the Jurassic period (approximately 172 Mya), which was around 29 Mya after the Triassic–Jurassic extinction (201.3 Mya). However, the relationship between Palawaniaceae and Pleurotremataceae in the ML tree is moderately supported. The relationship between Palawaniaceae and Muyocopronaceae is well-supported in the MCC tree, and these families share many characters. Thus, we believe that the new family is most closely related to Muyocopronaceae and should probably be placed in the order Muyocopronales.

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