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ORIGINAL ARTICLE

Acuminatispora palmarum gen. et sp. nov. from mangrove habitats

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Abstract
Fungi play a vital role as decomposers in mangrove ecosystems. A new ascomycete species, *Acuminatispora palmarum*, inhabiting decayed petioles and rachides of palms in mangrove habitats, is introduced in this paper based on morphological and phylogenetic evidence. Phylogenetic relationships of related taxa were inferred from combined LSU, SSU, *TEF1α*, and *RPB2* sequence data, and the analyses indicate that *A. palmarum* could be recognized as a distinct group in Pleosporales, but its familial placement needs to be further resolved. The morphological characters of this new taxon are also different from other members in Pleosporales by its deeply immersed ascomata, long pedicellate asci, and biseriate to triseriate, 1-(rarely 3) septate, brown, fusiform ascospores with acute or narrowly pointed ending cells. *Acuminatispora* gen. nov. (Pleosporales, *incertae sedis*) is therefore established to accommodate the new taxon *A. palmarum*. Furthermore, phylogenetic relationships of *Acrocordiopsis* and *Caryospora* are discussed with a consideration of morphological observations.

Keywords 2 new taxa · Dothideomycetes · Phylogeny · Sexual morph · Taxonomy

Introduction

Mangroves are distinctive coastal ecosystems comprising a diverse group of predominantly tropical trees and shrubs that are adapted to life in coastal intertidal marine locations (Tomlinson 1986). Breathing roots, salt-excreting leaves, and viviparous water-dispersed propagules are the three most important morphological and physiological traits (Duke 1992; Shi et al. 2005). Most studies on fungi colonizing mangroves are regarding to taxonomy (Cribb and Cribb 1955; Kohlmeyer and Kohlmeyer 1971, 1977; Kohlmeyer 1981, 1985; Kohlmeyer and Schatz 1985; Kohlmeyer and Volkmann-Kohlmeyer 1991), ecology and inventory (Kohlmeyer 1966, 1969; Hyde 1988; Hyde and Jones 1988; Hyde 1989a, b, c; Jones and Kuthubutheen 1989; Hyde et al. 1992; Hyde and Lee 1995; Alias et al. 2010; Pang et al. 2011), as well as a series of reviews (Jones 2000; Sarma and Hyde 2001; Jones 2011a, b; Jones et al. 2015; Sivakumar 2016), while other studies have considered the molecular phylogeny of mangrove fungi (Jones et al. 2009; Suetrong et al. 2009; Jones and Pang 2012). There are 74 mangrove species in 53 genera and 35 families that occurred along the protected shorelines of Thailand (Plathong and Plathong 2011), and 184 fungal species have been documented from Thai mangroves, of which approximately 85% are Ascomycota (Suetrong et al. 2017).

Pleosporales Luttr. ex M.E. Barr is the largest and most diverse group in Dothideomycetes (Ascomycota), including 75 families, 400 genera, and 52 genera *incertae sedis* (Schoch et al. 2006b, 2009; Hyde et al. 2013; Ariyawansa et al. 2015; Liu et al. 2017; Wijayawardene et al. 2018), and four new families were recently proposed in this order using multi-locus phylogenetic evidence (Valenzuela-Lopez et al. 2018). Since molecular phylogeny has been used to rank marine fungi, most of the species found from intertidal mangrove wood, twigs, and leaves were identified as members of Pleosporales and are distributed in 12 accepted families: Aigialaceae,

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Biatriosporaceae, Caryosporaceae, Halojulellaceae, Halotthiaceae, Lophiostomataceae, Morosphaeriaceae, Pseudoastrosphaeriellaceae, Salsugineaceae, Testudinaceae, Trematosphaeriaceae, and Zopfiaceae (Suetrong et al. 2009; Zhang et al. 2012b; Hyde et al. 2013; Jones et al. 2015; Devadatha et al. 2017; Hyde et al. 2017; Suetrong et al. 2017; Hashimoto et al. 2018; Wijayawardene et al. 2018). Four families: Didymellaceae, Leptosphaeriaceae, Lindgomycetaceae, and Melanommataceae also include manglicolous taxa but some genera lack molecular data to confirm their phylogenetic positions (Suetrong et al. 2009; Jones and Pang 2012; Jones et al. 2015).

During examination of collections of intertidal fungi from *Nypa fruticans* (brackish water palm) and *Phoenix paludosa* (mangrove date palm) in Thailand, a novel ascomycete species, *Acuminatispora palmarum*, was discovered with total four isolates obtained from both hosts. Morphological comparison and multi-gene phylogenetic analysis were carried out to reveal their taxonomical classification and delineate the phylogenetic relationships with related groups. A monotypic genus *Acuminatispora* gen. nov. (Pleosporales, *incertae sedis*) is introduced to accommodate the new taxon *Acuminatispora palmarum*. In addition, the phylogenetic relationships of the mangrove species *Acrocordiopsis patilii* and the genus *Caryospora* are discussed.

Materials and methods

Specimen collection, examination, and single spore isolation

Decayed petioles and rachides of palms were collected from mangrove habitats in Ranong, Trat, and Chanthaburi provinces, Thailand. The specimens were packaged in plastic bags in the field and washed under running water, and then examined via laboratory procedures following those of Jones and Hyde (1988). Morphological characters were observed using a Carl Zeiss stereo microscope fitted with an AxioCam ERC 5S camera and photographed by a Nikon ECLIPSE 80i compound microscope fitted with a Canon EOS 600D digital camera. Free hand sections of fruiting bodies were made into slides within water mounts and observed under Motic SMZ 168 stereo microscope. Measurements were taken by Tarosoft Image Frame Work program v. 0.9.7 and images used for figures were processed with Adobe Photoshop CS6 Extended v. 13.0 software. Isolations were obtained from single spores as described in Choi et al. (1999). The strains isolated in this study were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Herbarium specimens were deposited at the herbaria of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and Kunming Institute of Botany Academia Sinica (HKAS), Kunming, China. MycoBank numbers and Facesoffungi numbers (Jayasiri et al. 2015) of the new taxa were provided, and the new taxa were established following recommendations outlined by Jeewon and Hyde (2016).

DNA extraction, PCR amplification, and sequencing

Fungal genomic DNA was extracted from fresh mycelia scraped from the margin of a colony on PDA that was incubated at 25–28 °C for 30 days, followed by the Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech (Shanghai) Co., Ltd., China) manufacturer instructions. Two partial gene portions and two protein coding genes were used in this study: the large subunits of the nuclear ribosomal RNA genes (LSU), the small subunits of the nuclear ribosomal RNA (SSU), the translation elongation factor-1 alpha (*TEF1*α), and the second largest subunit of RNA polymerase II (*RPB2*). The primers used were LROR and LR5 for LSU (Vilgalys and Hester 1990), NS1/NS4 for SSU (White et al. 1990), EF1-983F/EF1-2218R for *TEF1*α (Rehner and Buckley 2005), and fRPB2-5F/fRPB2-7cR for *RPB2* (Liu et al. 1999). The amplification reactions were performed in 25 μL of PCR mixtures containing 9.5 μL ddH$_2$O, 12.5 μL 2× PCR MasterMix (TIANGEN Co., China), 1 μL DNA temple, and 1 μL of each primer. The PCR thermal cycle programs for LSU, SSU, and *TEF1*α amplification were as follows: initially denaturing step of 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 50 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR thermal cycle program for the partial RNA polymerase second largest subunit (*RPB2*) was followed as initially 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 2 min, elongation at 72 °C for 90 s, and final extension at 72 °C for 10 min. PCR products were observed on 1% Agarose gel electrophoresis strained with ethidium bromide. Purification and sequencing of PCR products were carried out at Sangon Biotech (Shanghai) Co., Ltd., China.

Sequence alignment and phylogeny analyses

The closely related strains of the new taxa were retrieved using nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and other strains included in this study are mainly referred to Tanaka et al. (2009), Ariyawansa et al. (2015), Hashimoto et al. (2017), and Liu et al. (2017). The final dataset of LSU, SSU, *TEF1*α, and *RPB2* sequence data used for the phylogenetic analyses along with original references and GenBank accession numbers is listed in Table 1.

Sequences were aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013) and then checked visually and manually optimized using BioEdit v.7.0.9 (Hall 1999). A maximum likelihood (ML) analysis

Table 1 Taxa used in this study and their GenBank accession numbers. The type species of each genus are marked with superscript ^{T} and ex-type strains are in bold

Taxa	Strain / Culture	GenBank Accession number				References
		LSU	SSU	*TEF1α*	*RPB2*	
Acrocordiopsis patilii^{T}	BCC28167	GU479772	GU479736	–	GU479811	Suetrong et al. (2009)
Acrocordiopsis patilii	BCC28167	GU479773	GU479737	–	GU479812	Suetrong et al. (2009)
Acuminatispora palmarum^{T}	**MFLUCC 18-0264**	MH390437	MH390401	MH399248	–	This study
Acuminatispora palmarum	MFLUCC 18-0460	MH390438	MH390402	MH399249	MH399252	This study
Acuminatispora palmarum	MFLUCC 18-0461	MH390439	MH390403	MH399250	MH399253	This study
Acuminatispora palmarum	MFLUCC 18-0462	MH390440	MH390404	MH399251	–	This study
Aigialus grandis^{T}	BCC18419	GU479774	GU479738	GU479838	GU479813	Suetrong et al. (2009)
Aigialus grandis	BCC20000	GU479775	GU479739	GU479839	GU479814	Suetrong et al. (2009)
Alternaria alternata	CBS 916.96	DQ678082	DQ678031	DQ677927	DQ677980	Suetrong et al. (2009)
Amniculicola lignicola^{T}	**Ying01**	EF493861	EF493863	–	EF493862	Zhang et al. (2008b)
Angustospora nilensis	**MFLU 15-1511**	KT944072	–	–	–	Li et al. (2016)
Anteaglonium abbreviatum^{T}	ANM 925a	GQ221877	–	GQ221924	–	Mugambi and Huhndorf (2009b)
Anteaglonium globosum	**ANM 925.2**	GQ221879	–	GQ221925	–	Mugambi and Huhndorf (2009b)
Anteaglonium parvulum	GKM 1218	GQ221880	–	GQ221922	–	Mugambi and Huhndorf (2009b)
Antealophiotrema brunneosporum^{T}	CBS 123095	LC194340	–	LC194382	LC194419	Hashimoto et al. (2017)
Aquasubmersa japonica	KT 2862	LC061587	LC061582	–	LC194421	Ariyawansa et al. (2015)
Aquasubmersa mircensis^{T}	**MFLUCC 11-0401**	JX276955	JX276956	–	–	Zhang et al. (2012a)
Ascocratera manglicola^{T}	BCC 09270	GU479782	GU479747	GU479846	GU479821	Suetrong et al. (2009)
Astrosphaeriella fusispora^{T}	MFLUCC 10-0555	KT955462	–	–	KT955413	Phookamsak et al. (2015)
Astrosphaeriella neofusispora	MFLUCC 11-0161	KT955463	KT955444	–	KT955418	Phookamsak et al. (2015)
Astrosphaeriella stellata	KT998	AB524592	AB524451	–	–	Tanaka et al. (2009)
Astrosphaeriellopsis bakeriana^{T}	CBS 115556	GU301801	–	GU349015	–	Schoch et al. (2009)
Astrosphaeriellopsis bakeriana	MFLUCC 11-0027	JN846730	–	–	–	Liu et al. (2011)
Bimuria novae-zelandiae^{T}	**CBS 107.79**	AY016356	AY016338	DQ471087	DQ470917	Lumbsch and Lindemuth (2001)
Byssosphaeria villosa	GKM 204N	GU385151	–	GU327751	–	Mugambi and Huhndorf (2009a)
Byssothecium circinans^{T}	CBS 675.92	AY016357	–	GU349061	DQ767646	Lumbsch and Lindemuth (2001)
Camarographium koreanum	CBS 117159	JQ044451	–	–	–	Crous et al. (2011)
Caryospora minima	–	EU196550	EU196551	–	–	Cai and Hyde (2007)
Caryospora aquatica	**MFLUCC 11-0008**	MH057847	MH057850	–	–	Ariyawansa et al. (2015)
Cochliobolus sativus	DAOM 226212	DQ678045	DQ677995	–	DQ677939	Schoch et al. (2006b)
Cryptocoryneum akitaense	**KT 3019**	LC194348	–	LC096136	LC194430	Hashimoto et al. (2017)
Cryptocoryneum condensatum	CBS 113959	LC194350	LC194308	LC096138	LC194432	Hashimoto et al. (2017)
Cryptocoryneum condensatum	CBS 122629	LC194351	LC194309	LC096139	LC194433	Hashimoto et al. (2017)
Cryptocoryneum pseudorilstonei	**CBS 113641**	LC194364	LC194322	LC096152	LC194446	Hashimoto et al. (2017)

Table 1 (continued)

Taxa	Strain / Culture	GenBank Accession number				References
		LSU	SSU	*TEF1α*	*RPB2*	
Delitschia chaetomioides	SMH 3253.2	GU390656	–	–	–	Mugambi and Huhndorf (2009a)
Delitschia winteri	CBS 225.62	DQ678077	DQ678026	DQ677922	DQ677975	Schoch et al. (2006b)
Didymella bryoniae	CBS 133.96	GU301863	–	–	GU371767	Schoch et al. (2009)
Didymella exigua^{T}	CBS 183.55	EU754155	EU754056	–	–	de Gruyter et al. (2010)
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023	KJ436586	KJ436588	–	–	Ariyawansa et al. (2014)
Dothidotthia aspera	CPC 12933	EU673276	EU673228	–	–	Phillips et al. (2008)
Dothidotthia symphoricarpi^{T}	CPC 12929	EU673273	EU673224	–	–	Phillips et al. (2008)
Falciformispora lignatilis^{T}	BCC 21117	GU371826	–	–	–	Schoch et al. (2009)
Falciformispora lignatilis	BCC 21118	GU371827	–	–	–	Schoch et al. (2009)
Fissuroma bambusae	MFLUCC 11-0160	KT955468	KT955448	KT955430	KT955417	Phookamsak et al. (2015)
Flammeascoma lignicola	MFLUCC 10-0128	KT324583	–	KT324585	KT324586	Ariyawansa et al. (2015)
Halotthia posidoniae^{T}	BBH 22481	GU479786	–	–	–	Suetrong et al. (2009)
Helminthosporium velutinum^{T}	MAFF 243854	AB807530	AB797240	–	AB808505	Tanaka et al. (2015)
Hermatomyces iriomotensis	**MAFF 245730**	LC194367	–	LC194394	LC194449	Hashimoto et al. (2017)
Hermatomyces tectonae	**MFLUCC 14-1140**	KU764695	KU712465	KU872757	KU712486	Doilom et al. (2017)
Hermatomyces thailandica	**MFLUCC 14-1143**	KU764692	KU712468	KU872754	KU712488	Doilom et al. (2017)
Herpotrichia diffusa	CBS 250.62	DQ678071	DQ678019	DQ677915	DQ677968	Schoch et al. (2006b)
Hypsostroma caimitalense	GKM 1165	GU385180	–	–	–	Mugambi and Huhndorf (2009a)
Hypsostroma saxicola^{T}	SMH 5005	GU385181	–	–	–	Mugambi and Huhndorf (2009a)
Latorua caligans^{T}	CBS 576.65	KR873266	–	–	–	Crous et al. (2015)
Latorua grootfonteinensis	CBS 369.72	KR873267	–	–	–	Crous et al. (2015)
Lepidosphaeria nicotiae	CBS 101341	DQ678067	–	–	DQ677963	Schoch et al. (2006b)
Leptosphaeria biglobosa	CBS 303.51	GU301826	–	GU349010	–	Schoch et al. (2009)
Leptosphaeria doliolum^{T}	CBS 505.75	GU301827	GU296159	GU349069	–	Schoch et al. (2009)
Ligninsphaeria jonesii	GZCC 15-0080	KU221038	–	–	–	Zhang et al. (2016)
Ligninsphaeria jonesii^{T}	**MFLUCC 15-0641**	KU221037	–	–	–	Zhang et al. (2016)
Lindgomyces cinctosporae	R56-1	AB522431	AB522430	–	–	Hirayama et al. (2010)
Lindgomyces ingoldianus^{T}	ATCC 200398	AB521736	AB521719	–	–	Hirayama et al. (2010)
Lindgomyces rotundatus	KT1096	AB521740	AB521723	–	–	Hirayama et al. (2010)
'Lophiotrema' boreale	CBS 114422	LC194375	–	LC194402	LC194457	Hashimoto et al. (2017)
Lophiotrema lignicola	CBS 122364	GU301836	GU296166	GU349072	–	Schoch et al. (2009)
Lophiotrema nucula^{T}	CBS 627.86	GU301837	GU296167	GU349073	GU371792	Schoch et al. (2009)
Lophiotrema vagabundum	JCM 17674	AB619022	–	–	–	Hirayama and Tanaka (2011)
Loratospora aestuarii^{T}	JK 5535B	GU301838	GU296168	–	GU371760	Schoch et al. (2009)

Table 1 (continued)

Taxa	Strain / Culture	GenBank Accession number				References
		LSU	SSU	*TEF1α*	*RPB2*	
Macrodiplodiopsis desmazieri^{T}	CBS 140062	KR873272	–	–	–	Crous et al. (2015)
Massaria anomia	CBS 591.78	GU301839	GU296169	–	GU371769	Schoch et al. (2009)
Massaria gigantispora	M26	HQ599397	HQ599447	HQ599337	–	Voglmayr and Jaklitsch (2011)
Massaria inquinans^{T}	M19	HQ599402	HQ599444	HQ599342	HQ599460	Voglmayr and Jaklitsch (2011)
Massarina eburnea^{T}	CBS 473.64	GU301840	GU296170	GU349040	GU371732	Schoch et al. (2009)
Mauritiana rhizophorae^{T}	BCC 28866	GU371824	–	GU371817	GU371796	Schoch et al. (2009)
Melanomma pulvis-pyrius^{T}	CBS 124080	GU456323	GU456302	GU456265	GU456350	Zhang et al. (2009a)
Montagnula opulenta	CBS 168.34	DQ678086	AF164370	–	DQ677984	Schoch et al. (2006b)
Murispora rubicunda^{T}	IFRD 2017	FJ795507	GU456308	–	–	Zhang et al. (2009b)
Mytilinidion andinense	CBS 123562	FJ161199	FJ161159	FJ161107	FJ161125	Boehm et al. (2009)
Mytilinidion mytilinellum	CBS 303.34	FJ161184	FJ161144	FJ161100	FJ161119	Boehm et al. (2009)
Neoastrosphaeriella krabiensis^{T}	**MFLUCC 11-0025**	JN846729	JN846739	–	–	Liu et al. (2011)
Neoroussoella bambusae^{T}	**MFLUCC 11-0124**	KJ474839	–	KJ474848	KJ474856	Liu et al. (2014)
Neotestudina rosatii	CBS 690.82	DQ384107	DQ384069	–	–	Kruys et al. (2006)
Ophiosphaerella herpotricha	CBS 620.86	DQ678062	–	DQ677905	DQ677958	Schoch et al. (2006b)
Phaeosphaeria elongata	CBS 120250	GU456327	–	GU456261	GU456345	Zhang et al. (2009a)
Phaeosphaeria oryzae^{T}	CBS 110110	GQ387591	GQ387530	–	KF252193	de Gruyter et al. (2010)
Phoma exigua	CBS 431.74	EU754183	–	GU349080	GU371780	de Gruyter et al. (2010)
Phoma herbarum^{T}	CBS 276.37	DQ678066	DQ678014	DQ677909	DQ677962	Schoch et al. (2006b)
Pleomassaria siparia^{T}	CBS 279.74	DQ678078	DQ678027	DQ677923	DQ677976	Schoch et al. (2006b)
Pleospora herbarum^{T}	**CBS 191.86**	DQ247804	DQ247812	DQ471090	DQ247794	Schoch et al. (2006a)
Polyplosphaeria fusca^{T}	**KT 1616**	AB524604	AB524463	–	–	Tanaka et al. (2009)
Polyschema larviformis	CBS 463.88	EF204503	–	–	–	Shenoy (2007)
Polyschema terricola^{T}	CBS 301.65	EF204504	EF204519	–	–	Shenoy (2007)
Preussia funiculata^{T}	CBS 659.74	GU301864	GU296187	GU349032	GU371799	Schoch et al. (2009)
Prosthemium orientale	KT1669	AB553748	AB553641	–	–	Tanaka et al. (2010)
Pseudoastrosphaeriella bambusae	**MFLUCC 11-0205**	KT955475	–	KT955437	KT955414	Phookamsak et al. (2015)
Pseudoastrosphaeriella longicolla	**MFLUCC 11-0171**	KT955476	–	KT955438	KT955420	Phookamsak et al. (2015)
Pseudoastrosphaeriella thailandensis^{T}	**MFLUCC 11-0144**	KT955478	–	KT955440	KT955416	Phookamsak et al. (2015)
Pseudochaetosphaeronema larense^{T}	CBS 640.73	KF015611	KF015652	KF015684	–	Ahmed et al. (2014)
Pseudomassariosphaeria bromicola^{T}	**MFLUCC 15-0031**	KT305994	–	–	–	Ariyawansa et al. (2015)
Pseudotetraploa curviappendiculata^{T}	**HC 4930**	AB524608	AB524467	–	–	Tanaka et al. (2009)
Quadricrura septentrionalis^{T}	**HC 4984**	AB524616	AB524475	–	–	Tanaka et al. (2009)
Repetophragma ontariense	HKUCC 10830	DQ408575	–	–	DQ435077	Shenoy (2006)

Table 1 (continued)

Taxa	Strain / Culture	GenBank Accession number				References
		LSU	SSU	*TEF1α*	*RPB2*	
Roussoella nitidula^{T}	**MFLUCC 11-0182**	KJ474843	–	KJ474852	KJ474859	Liu et al. (2014)
Roussoellopsis macrospora	MFLUCC 12-0005	KJ474847	–	KJ474855	KJ474862	Liu et al. (2014)
Salsuginea ramicola^{T}	KT 2597.1	GU479800	GU479767	GU479861	GU479833	Suetrong et al. (2009)
Salsuginea ramicola	KT 2597.2	GU479801	GU479768	GU479862	GU479834	Suetrong et al. (2009)
Setomelanomma holmii^{T}	CBS 110217	GU301871	GU296196	GU349028	GU371800	Schoch et al. (2009)
Spegazzinia deightonii	MAFF 243876	AB807581	AB797291	AB808557	–	Tanaka et al. (2015)
Sporormiella minima	CBS 524.5	DQ678056	DQ678003	DQ677897	DQ677950	Aveskamp et al. (2010)
Stagonospora duoseptata	CBS 135093	KF251758	–	–	–	Quaedvlieg et al. (2013)
Stagonospora paludosa	CBS 135088	KF251760	–	–	–	Quaedvlieg et al. (2013)
Tetraplosphaeria sasicola^{T}	**KT563**	AB524631	AB524490	–	–	Tanaka et al. (2009)
Thyridaria acaciae	CBS 138873	KP004497	–	–	–	Crous et al. (2014)
Thyridaria broussonetiae	CBS 141481	KX650568	–	KX650539	KX650586	Jaklitsch and Voglmayr (2016)
Thyridaria broussonetiae	CBS 141482	KX650570	–	KX650540	KX650587	Jaklitsch and Voglmayr (2016)
Torula herbarum	CBS 111855	KF443386	–	KF443403	KF443396	Ahmed et al. (2014)
Torula hollandica	CBS 220.69	KF443384	–	–	–	Ahmed et al. (2014)
Trematosphaeria pertusa	CBS 122368	FJ201990	–	–	FJ795476	Zhang et al. (2008a)
Trematosphaeria pertusa^{T}	**CBS 122371**	FJ201992	–	–	GU371801	Zhang et al. (2008a)
Triplosphaeria maxima^{T}	**KT 870**	AB524637	AB524496	–	–	Tanaka et al. (2009)
Ulospora bilgramii^{T}	CBS 101364	DQ678076	DQ678025	DQ677921	DQ677974	Schoch et al. (2006b)
Verruculina enalia^{T}	BCC 18401	GU479802	–	GU479863	GU479835	Suetrong et al. (2009)
Westerdykella angulata	CBS 610.74	DQ384105	DQ384067	–	–	Kruys et al. (2006)
Wicklowia aquatica	AF289-1	GU045446	–	–	–	Raja et al. (2010)
Wicklowia aquatica^{T}	**F76-2**	GU045445	GU266232	–	–	Raja et al. (2010)
Zopfia rhizophila^{T}	CBS 207.26	DQ384104	–	–	–	Kruys et al. (2006)

Abbreviations: *ATCC* American Type Culture Collection, Virginia, USA, *BBH* Biotec Bangkok Herbarium, Thailand, *BCC* BIOTEC Culture Collection, Bangkok, Thailand, *CBS* Centraal bureau voor Schimmel cultures, Utrecht, The Netherlands, *CPC* Collection of Pedro Crous housed at CBS, *DAOM* Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada *GZCC* Guizhou Culture Collection, *HKUCC* University of Hong Kong, Centre for Research in Fungal Diversity, Culture collection, *IFRD* Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China, *JCM* the Japan Collection of Microorganisms, Japan, *MAFF* Ministry of Agriculture, Forestry and Fisheries, Japan, *MFLU* Mae Fah Luang University Herbarium Collection, *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, *ANM* A.N. Miller, *GKM* G.K. Mugambi, *JK* J. Kohlmeyer, *KT* K. Tanaka, *SMH* S.M. Huhndorf was performed at the CIPRES web portal (Miller et al. 2010) using RAxML v.7.2.8 as part of the "RAxML-HPC Blackbox (8.2.10)" tool (Stamatakis 2006; Stamatakis et al. 2008). A general time-reversible (GTR) model was applied with a discrete gamma distribution and four rate classes. Fifty thorough ML tree searches were carried out in RAxML v.7.2.7 under the same model. One thousand non-parametric bootstrap iterations were run with the GTR model and a discrete gamma distribution. The resulting replicates were plotted onto the best scoring tree obtained previously.

Maximum parsimony (MP) analyses were performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equally weight; gaps were treated as missing data. Maxtrees setting was 1000, and zero-length branches were collapsed, and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis and Bull 1993). Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated.

The Bayesian analysis was performed using PAUP v.4.0b10 (Swofford 2002) and MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). The best model for different gene partition in the concatenated dataset was determined by MrModeltest 2.3 (Nylander 2004). Posterior probabilities (Rannala and Yang 1996) were determined by Markov Chain Monte Carlo (MCMC) sampling (Larget and Simon 1999) in MrBayes v.3.1.2. Four simultaneous Markov chains were run for 10 million generations and trees were sampled every 1000th generation; thus, 10,000 trees were obtained. The suitable burn-in phases were determined by inspecting likelihoods and parameters in Tracer version 1.6 (Rambaut et al. 2013). Based on the tracer analysis, the first 1000 trees representing 10% were discarded as the burn-in phase in the analysis. The remaining trees were used to calculate posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic set to 0.01). Phylogenetic tree was visualized by FigTree v.1.4.0 (Rambaut 2012), and the alignment was deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S22887).

Results

Phylogeny

Four fungal isolates from palms were obtained from mangrove-inhabiting decayed substrates and identified as members of the order Pleosporales. LSU, SSU, *TEF1α*, and *RPB2* sequence data and morphological characters were used to determine their placement and to describe the novel taxon. The combined dataset comprised 126 taxa with *Mytilinidion andinense* (CBS 123562) and *Mytilinidion mytilinellum* (CBS 303.34) as the outgroup taxa. The dataset comprises 3980 characters (LSU 1–902; SSU 903–2129; *TEF1α* 2130–3032; *RPB2* 3033–3980) after alignment, including gaps. The maximum parsimonious dataset consists of 3980 characters, of which 2462 characters were constant and 279 variable characters parsimony uninformative. Maximum parsimony analysis of the remaining 1239 parsimony-informative characters resulted in 1000 trees with TL = 9053, CI = 0.277, RI = 0.614, RC = 0.170, and HI = 0.723. RAxML, maximum-parsimony (MP), and Bayesian analysis of the combined dataset resulted in phylogenetic reconstructions with similar topologies, and the best sorting RAxML tree is shown in Fig. 1.

Representatives of the sequenced families (with molecular data) of Pleosporales were included in our phylogenetic analysis (Fig. 1). The phylogenetic analysis based on the combined LSU, SSU, *TEF1α*, and *RPB2* sequence data showed that the two suborders Massarineae and Pleosporineae are well-supported, and the familial assignment of Pleosporales is similar to previous studies (Schoch et al. 2009; Hyde et al. 2013; Ariyawansa et al. 2015; Liu et al. 2017). The four newly obtained fungal isolates formed a well-supported monotypic clade and can be identified as a new genus (namely *Acuminatispora*) in Pleosporales, which clustered together with *Acrocordiopsis* Borse & K.D. Hyde, *Astrosphaeriella* Syd. & P. Syd., *Astrosphaeriellopsis* Phook., Jian K. Liu & K.D. Hyde, and *Caryospora* De Not., and placed as basal lineages in the order. Additionally, *Acuminatispora palmarum* formed a sister clade to *Caryospora*, while *Acrocordiopsis patilii* clustered together with *Astrosphaeriella* and *Astrosphaeriellopsis* in an unsupported clade. However, the phylogenetic relationships of *Acrocordiopsis*, *Astrosphaeriella*, *Astrosphaeriellopsis*, *Acuminatispora*, and *Caryospora* do not form a stable clade and further taxon sampling is required to resolve their phylogenetic relationships.

Taxonomy

Acuminatispora S.N. Zhang., K.D. Hyde & J.K. Liu, gen. nov.

MycoBank: MB 825525; *Facesoffungi number*: FoF 04671

Etymology: Name refers to the ascospores with acute or narrowly pointed ending cells.

Saprobic in mangrove habitats. **Sexual morph:** *Ascomata* black, subglobose, solitary, scattered, immersed, with an erumpent short neck. *Ostiole* central, periphysate, cylinder-like opening. *Peridium* composed several layers with cells of *textura angularis*. *Hamathecium* comprising numerous, filamentous, hyaline, branched, trabeculate pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, cylindrical, slightly curved, long pedicellate, apically rounded with an ocular chamber.

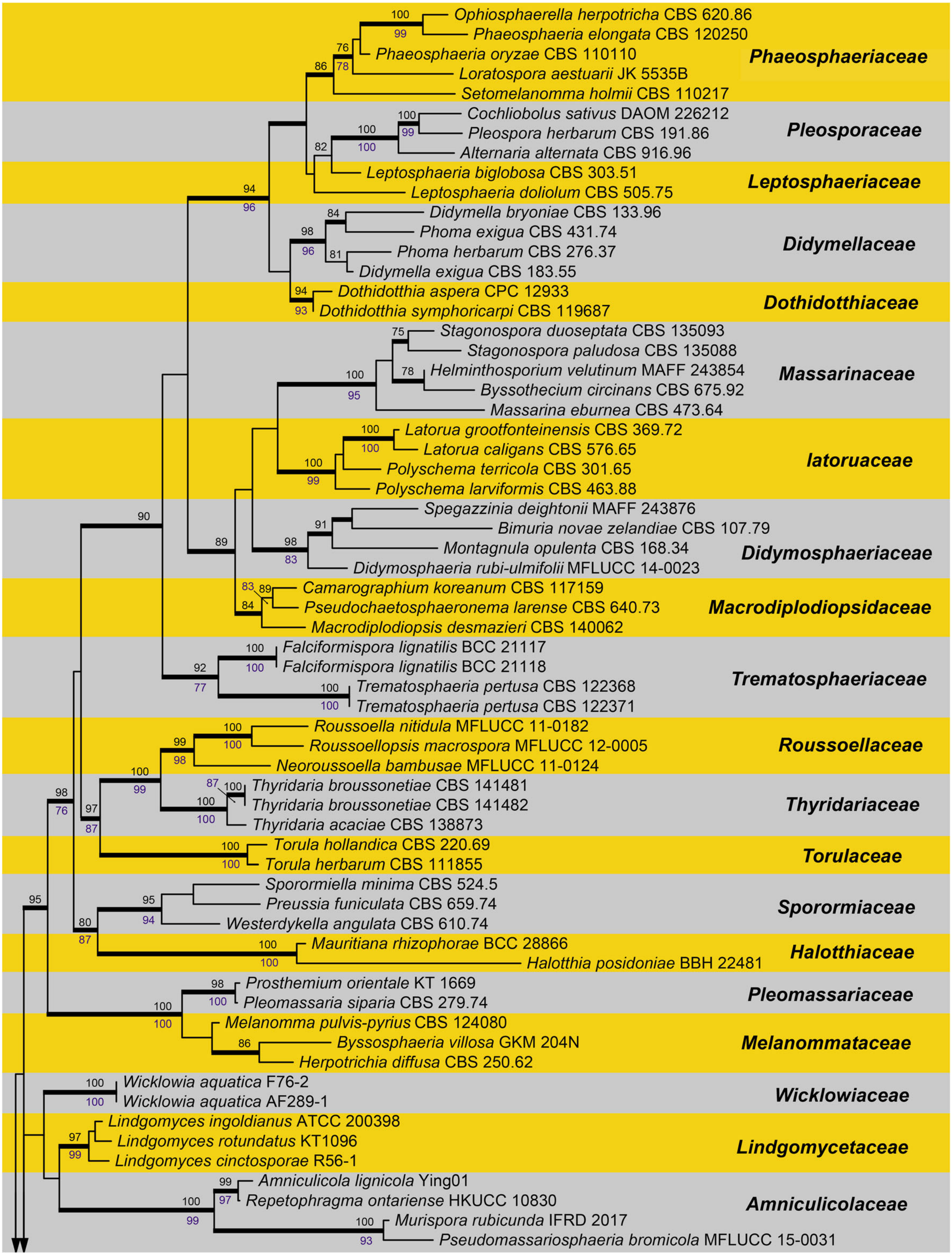

Fig. 1 RAxML tree of Pleosporales based on analysis of combined LSU, SSU, *TEF1α*, and *RPB2* sequence data. Bootstrap values for ML and MP equal to or greater than 75 are placed above and below the branches respectively. Branches with Bayesian posterior probabilities (PP) from MCMC analysis equal or greater than 0.95 are in bold. Newly generated sequences are indicated in blue. The tree is rooted with *Mytilinidion mytilinellum* (CBS 303.34) and *Mytilinidion andinense* (CBS 123562)

Ascospores overlapping biseriate to triseriate, hyaline to brown, fusiform with acute or narrowly pointed ending cells, 1-(rarely 3) septate, constricted at the central septa, guttulate, smooth-walled. **Asexual morph:** Undetermined.

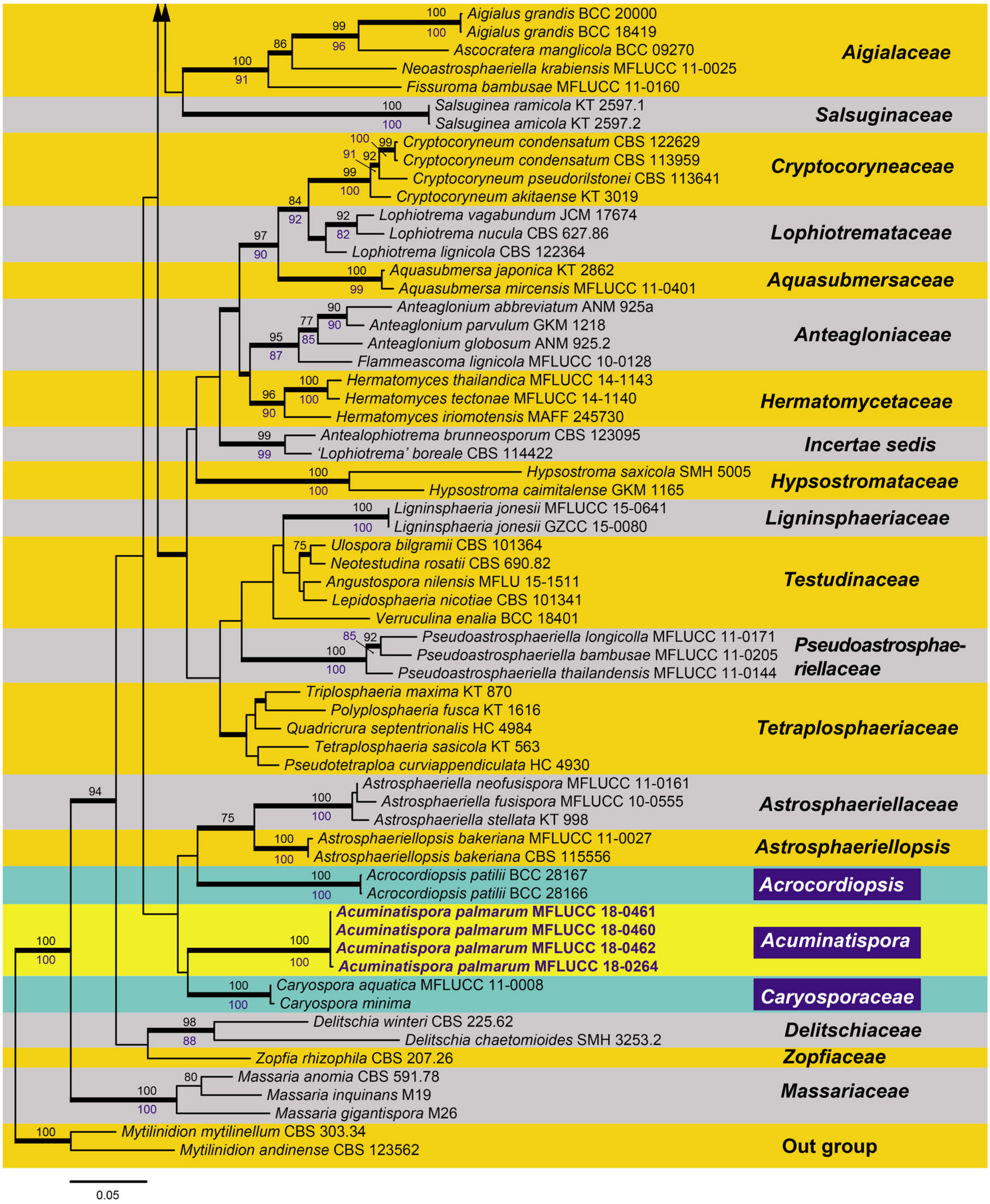

Fig. 1 continued.

Type species: ***Acuminatispora palmarum*** S.N. Zhang, K.D. Hyde & J.K. Liu

Acuminatispora palmarum S.N. Zhang, K.D. Hyde & J.K. Liu, sp. nov. Fig. 2.

MycoBank: MB 825526; *Facesoffungi number*: FoF 04672

Etymology: The epithet referring to the host on which the fungus was collected.

Holotype: MFLU 18-1068

Saprobic on petioles and rachides of palms in mangrove habitats. **Sexual morph:** *Ascomata* 380–610 μm high (including neck), 150–395 μm diam. ($\overline{x}$ = 531.2 × 275.8 μm, n = 10), black, subglobose, solitary, scattered, immersed, with an erumpent short neck. *Ostiole* 72–85 μm diam., central, periphysate, cylinder-like opening. *Peridium* 10–20 μm wide, composed several brown outside layers and inter layers with hyaline cells of *textura angularis*. *Hamathecium* up to 2.5 μm

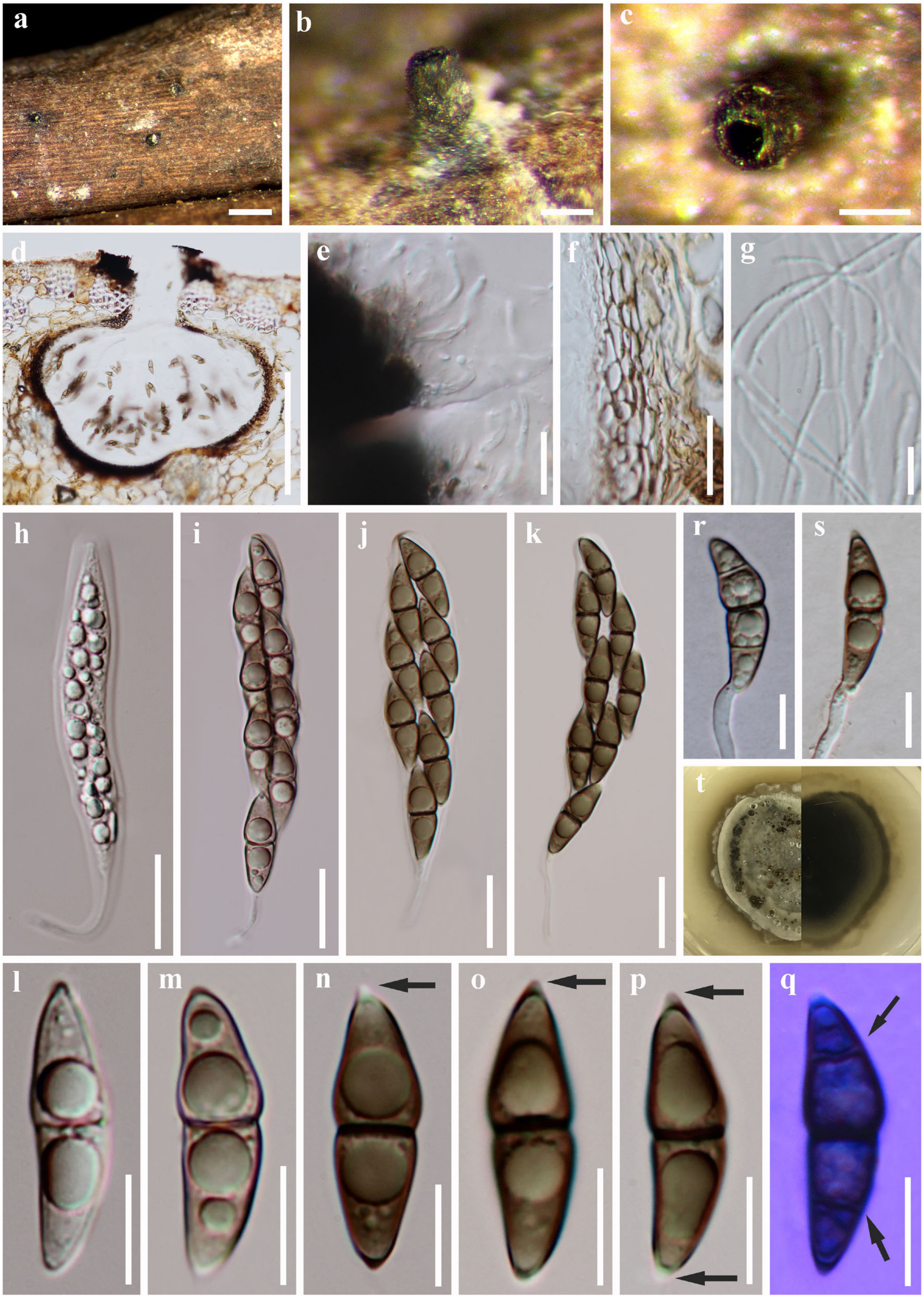

Fig. 2 *Acuminatispora palmarum* (MFLU 18-1068, holotype; MFLU 18-1071, paratype). **a–c** Appearance of ascomata on host surface. **d** Vertical section of ascoma. **e** Ostiole with periphyses. **f** Structure of peridium. **g** Trabeculate pseudoparaphyses. **h–k** Asci. **l–q** Ascospores. q, 3-septate ascospore in lactophenol cotton blue reagent with clearly acute ends. **s–r** Germinating ascospores. **t** Colony on PDA. Scale bars: **a** = 500 μm, **b**, **c** = 100 μm, **d** = 200 μm, **f**, **h–k** = 20 μm, **e**, **g**, **l–s** = 10 μm

wide, comprising numerous, filamentous, hyaline, branched, trabeculate pseudoparaphyses, embedded in a gelatinous matrix. *Asci* 93–125 × 13–22 μm ($\bar{x}$ = 109.5 × 16 μm, $n = 15$), 8-spored, bitunicate, cylindrical, slightly curved, long pedicellate, apically rounded with an ocular chamber. *Ascospores* 24–30 × 7–10 μm ($\bar{x}$ = 27.2 × 8.1 μm, $n = 30$), overlapping biseriate to triseriate, hyaline to brown, fusiform with small, hyaline, acute, or narrowly pointed ending cells, 1-(rarely 3) septate, strongly constricted at the central septa, the upper cell broader, each cell with one large guttule and sometimes several small ones, smooth-walled, lacking a sheath or appendages. **Asexual morph:** Undetermined.

Culture characteristics: Ascospores germinating on PDA within 24 h at 25–28 °C under natural light. Germ tubes produced from each end. Colonies growing well on both PDA and MEA media and attaining a diameter about 1.5 cm on PDA after 21 days at 25 °C, obverse olive to gray-green or light gray-green, tufted colony center elevated, reverse dark green. Mycelium 2.5–3.5 μm wide, hyaline to pale brown, aerial, septate, branched, and anastomosing, producing chlamydospores.

Material examined: Thailand, Ranong, Ngao Mangrove Forest Research Center, intermittently submerged on decayed rachis of *Phoenix paludosa* (Roxb. 1832), 6 December 2016, S.N. Zhang, SNT53, holotype (MFLU 18-1068), isotype (HKAS 97478), ex-type living culture MFLUCC 18-0264 = GZCC 18-0001; Thailand, Trat, Ko Chang District, 12° 1′ 14″ N, 102° 23′ 19″ E, intermittently submerged on decayed rachis of *Phoenix paludosa*, 27 April 2017, S.N. Zhang, SNT107, paratype (MFLU 18-1069), living culture MFLUCC 18-0460 = GZCC 18-0002; Thailand, Trat, Ko Chang District, 12° 1′ 14″ N, 102° 23′ 19″ E, submerged on decayed petiole of *Nypa fruticans* (Wurmb 1779), 27 April 2017, S.N. Zhang, SNT111, paratype (MFLU 18-1070), living culture MFLUCC 18-0461 = GZCC 18-0003; Thailand, Chanthaburi, 12° 26′ 43″ N, 102° 15′ 47″ E, intermittently submerged on decayed petiole of *Phoenix paludosa*, 25 April 2017, S.N. Zhang, SNT133, paratype (MFLU 18-1071), living culture MFLUCC 18-0462 = GZCC 18-0004.

Habitat and distribution: Inhabiting mangrove forests. The Gulf of Thailand (east) and Andaman sea (west) coastline, Thailand.

Notes: *Acuminatispora palmarum* is phylogenetically distinct from other members in Pleosporales (Fig. 1) and its unique morphological features of deeply immersed ascomata with an erumpent short neck, 8-spored, long pedicellate asci, and biseriate to triseriate, brown, fusiform ascospores, 1-(rarely 3) septate, with a broader upper cell and small hyaline acute or conical ending cells, also distinguish it from its phylogenetically closely related genera *Acrocordiopsis*, *Astrosphaeriella*, *Astrosphaeriellopsis*, and *Caryospora* (Barr 1979; Borse and Hyde 1989; Alias et al. 1999; Phookamsak et al. 2015), as well as two other genera *Caryosporella* Kohlm. and *Zopfia* Rabenh (Arnaud 1913; Kohlmeyer 1985), which are distinct from *A. palmarum* in phylogeny but having similarity in ascospore morphology. *Acuminatispora palmarum* shares similar immersed ascomatal morphology and 3-septate brown ascospores with tapering ends to *Coronopapilla mangrovei* (K.D. Hyde) Kohlm. & Volkm.-Kohlm. (≡ *Caryospora mangrovei* K.D. Hyde), and another mangrove species *Passeriniella savoryellopsis* Hyde & Mouzouras (Hyde and Mouzouras 1988; Hyde 1989c; Kohlmeyer and Volkmann-Kohlmeyer 1990, 1991). However, *Acuminatispora palmarum* differs from *Co. mangrovei* in having distinctly smaller (24–30 × 7–10 μm) biseriate to triseriate, acute or narrowly pointed ending cells of fusiform ascospores, while the latter has large (36–60 × 16–24 μm) uniseriate, ellipsoidal, thick-walled ascospores with tapering rounded ends. In addition, the permutation and the number of ascospores in asci, and the position of secondary septa are also reliable to distinguish *Ac. palmarum* and *P. savoryellopsis* (Table 2), especially the obviously different size of ascospores (24–30 × 7–10 μm vs. 64–88 × 24–28 μm). Phylogenetically, *Ac. palmarum* is distinct from *P. savoryellopsis* based on multi-gene phylogeny (data not shown), and the phylogenetic relationship of *Ac. palmarum* and *Co. mangrovei* is unresolved as the molecular data of *Coronopapilla* spp. is not available. We hereby introduce *Acuminatispora palmarum* as a new species and establish the monotypic genus *Acuminatispora* to accommodate it.

Discussion

The phylogenetic placement of *Acuminatispora palmarum* is problematic and even equivocal in this study because of (i) the grouping pattern of the *Acuminatispora*, *Acrocordiopsis*, *Caryospora*, and Astrosphaeriellaceae, which is inconsistent with the phylogenetic analysis of single- and multi-gene studies (Suetrong et al. 2009; Ariyawansa et al. 2015; Phookamsak et al. 2015); (ii) the family Caryosporaceae was established mainly based on the phylogeny with one species representing each genus (*Acrocordiopsis* and *Caryospora*) (Ariyawansa et al. 2015), which was not justified enough to define the family boundary; (iii) the relatively low corresponding bootstrap support values of *Acuminatispora*, *Acrocordiopsis*, and *Caryospora* might due to the few discovered species and little available molecular data of these groups. Therefore, it is difficult to justify the phylogenetic placement of these groups, and further taxon sampling and re-isolation of species is required before the taxonomic position of the new genus can be resolved.

The phylogeny based on multi-gene analysis also indicated that *Acuminatispora palmarum* clustered together with *Caryospora*, and formed a monospecific clade distinct from *Acrocordiopsis* and *Caryospora.* It is reasonable that with more taxa included, the phylogenetic positions of some groups become unstable and their phylogenetic relationships with others even

Table 2 Morphological comparison of *Acuminatispora palmarum*, *Coronopapilla mangrovei* and *Passeriniella savoryellopsis*

	Acuminatispora palmarum (this study)	*Coronopapilla mangrovei* (Hyde 1989c, Kohlmeyer and Volkmann-Kohlmeyer 1990, 1991)	*Passeriniella savoryellopsis* (Hyde and Mouzouras 1988)
Ascomata	380–610 μm high, 150–395 μm diam., subglobose, immersed with an erumpent short neck. Ostiole 72–85 μm diam., central, cylinder-like opening, periphysate	440–640 μm high, 360–480 μm diam., globose-pyriform, immersed becoming erumpent; Ostiolate with short necks, papillate	500–700 μm high, 800–1300 μm diam., globose to subglobose, immersed, ostiolate, periphyses, papillate
Peridium	10–20 μm thick, cells *textura angularis*	14–40 μm thick, at the side elongate cells, at the base angular cells	80–100 μm thick, cells *textura angularis*
Hamathecium	Trabeculate pseudoparaphyses up to 2.5 μm wide	Pseudoparaphyses very thin, less than 1.0 μm wide	Pseudoparaphyses 3.9–5.9 μm
Asci	93–125 × 13–22 μm, long pedicellate	215–260 × 28–34 μm	280–440 × 24–32 μm, 4-spored
Ascospores	24–30 × 7–10 μm, overlapping biseriate to triseriate, fusiform, with acute or narrowly pointed ending cells, 1-(rarely 3) septate, constricted at the central septa, the upper cell broader	36–60 × 16–24 μm, uniseriate, ellipsoidal, with tapering rounded tips, primary septum median, constricted, developing pseudosepta, thick-walled	64–88 × 24–28 μm, uniseriate, ellipsoidal, 3-septate, constricted at central septum, end cells small, conical and hyaline

change in a phylogenetic tree. Furthermore, the corresponding taxonomical classification may need to be modified.

Acrocordiopsis

The initial phylogenetic study of *Acrocordiopsis patilii* was conducted by Suetrong et al. (2009) who noted it grouped as a residual paraphyletic assemblage and was not assigned to any family. Subsequently, the genus was assigned to Salsugineaceae K.D. Hyde & S. Tibpromma (Hyde et al. 2013) based on the same sequence data as Suetrong et al. (2009), while Ariyawansa et al. (2015) placed *Acrocordiopsis* in the new family, Caryosporaceae with *Caryospora* and both of the above two treatments were based on phylogenetic analyses with few taxa representing the genera and families.

Wijayawardene et al. (2018) referred *Ac. patilii* in Salsugineaceae following Hyde et al. (2013). Jones and Pang (2012) also consider the phylogenetic placement of *Ac. patilii* unresolved. In our study, the phylogenetic position of the new taxon is not stable, as well as the taxa *Acrocordiopsis*, *Astrosphaeriella*, *Astrosphaeriellopsis*, and *Caryospora*. Currently, *Ac. patilii* and *Ac. sphaerica* are referred to the Salsugineaceae, although morphologically they have little in common with *Salsuginea ramicola* (website: marinefungi.org). Similarly, *Acrocordiopsis* is distinct from *Astrosphaeriellopsis bakeriana* both morphologically and phylogenetically. Further collections of *Ac. patilii*, *Ac. sphaerica*, and *Salsuginea ramicola* and new sequence data are required for all these species before their taxonomic positions are resolved.

Caryospora

The genus *Caryospora* and *Acuminatispora* formed an unsupported sister clade (ML/31, MP/17, and BYPP/0.55). *Caryospora* is an old genus, which currently includes approximately 12 species (*Caryospora aquatic* Huang Zhang, K.D. Hyde & Ariyaw., *C. australiensis* Abdel-Wahab & E.B.G. Jones, *C. callicarpa* (Curr.) Nitschke ex Fuckel, *C. coffeae* Pat. & Gaillard, *C. daweiensis* G.C. Zhao & R.L. Zhao, *C. langloisii* Ellis & Everh., *C. masonii* D. Hawksw., *C. minima* Jeffers, *C. obclavata* Raja & Shearer, *C. olearum* (Castagne) Sacc., *C. phyllostachydis* (Hara) I. Hino & Katum., *C. putaminum* (Schwein.) De Not.), and all have superficial or erumpent to nearly superficial ascomata, a carbonaceous peridium, and trabeculate pseudoparaphyses (Jeffers 1940; Barr 1979; Hawksworth 1982; Abdel-Wahab and Jones 2000; Raja and Shearer 2008; Hawksworth et al. 2010; Hu 2010; Ariyawansa et al. 2015). Only two species have been sequenced and further studies are required.

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