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An advance in the endophyte story: *Oxydothidaceae* fam. nov. with six new species of *Oxydothis*

Konta S¹, Hongsanan S¹, Tibpromma S^{1,2}, Thongbai B¹, Maharachchikumbura SSN³, Bahkali AH⁴, Hyde KD^{1,2} & Boonmee S^{1*}

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

²Key Laboratory for Plant Biodiversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, China

³Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 8, 123, Al Khoud, Oman

⁴Department of Botany and Microbiology, King Saudi University, Riyadh, Saudi Arabia

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Abstract

Oxydothis species are associated with monocotyledons including *Arecaceae* (palms), *Pandanaceae*, *Poaceae* (Bamboo) and *Liliaceae* and have been recorded as endophytes, pathogens and saprobes. Species of *Oxydothis* form singly or in clusters, as darkened, raised regions or dots on the surface of host. This paper clarifies the placement of *Oxydothis* and related species based on morphological characteristics and phylogenetic analyses using recent collections from Thailand. *Oxydothis* species are characterized by cylindrical asci, with a J+ (rarely J-) subapical ring and filiform to fusiform, hyaline, 1-septate ascospores, tapering from the center to spine-like, pointed or rounded ends. Phylogenetic analyses generated from maximum likelihood and Bayesian analysis of combined ITS, LSU and SSU sequence data indicate that *Oxydothis* species form a distinct lineage related to *Vialaeaceae* and *Iodosphaeriaceae*. We introduce a new family *Oxydothidaceae* in *Xylariales* with six new species based on morphological characteristics and phylogenetic analyses. We provided notes on the lifestyle of this genus which may throw light on the nature of many saprobic microfungi.

Key words – ascomycetes – hyaline ascospores – palm fungi – Xylariales.

Introduction

Oxydothis (Sordariomycetes) species are usually found on monocotyledons, especially on palms (Hyde 1993a, b, 1994, Wong & Hyde 1999, 2001, Fröhlich & Hyde 2000, Taylor & Hyde 2003, Shenoy et al. 2005, Hidayat et al. 2006). The genus *Oxydothis* was introduced by Penzig & Saccardo (1897), and placed in *Amphisphaeriaceae* within *Xylariales* (Müller & Arx 1962, 1973, Wehmeyer 1975, Samuels & Rossman 1987, Eriksson & Hawksworth 1991). The genus is characterized by the often-horizontal orientation of its ascomata; unitunicate asci with a J+ (rarely

J-), wedge-shaped or discoid subapical ring, and fusiform or filiform, 1-septate ascospores with spine-like or rounded ends (Hyde 1994). *Oxydothis* was transferred to the family *Hyponectriaceae* Petr. (*Xylariales*) based on morphological data (Barr 1990, Hawksworth et al. 1995). Wang & Hyde (1999) excluded *Oxydothis* from *Hyponectriaceae* based on its morphological characteristics differing from the generic type of *Hyponectriaceae* (*Hyponectria buxi*). The asci of *Oxydothis* are most similar to the asci of *Diatrypaceae* Nitschke species (Wong & Hyde 1999). However, the genus was placed in *Clypeosphaeriaceae* G. Winter based on morphological and phylogenetic analyses, and treated as polyphyletic (Kang et al. 1998, 1999). Jeewon et al. (2003) reported a close phylogenetic relationship between *Oxydothis* and *Leiosphaerella* Höhn., (*Pseudomassariaceae* Senan. & K.D. Hyde), with uncertain phylogenetic placement within *Xylariales*. Many researchers treated *Oxydothis* as genus *incertae sedis* in *Xylariales* (Shenoy et al. 2005, Hidayat et al. 2006, Maharachchikumbura et al. 2016). Seventy-eight species of *Oxydothis* are listed in Index Fungorum (2016); with 81 species in MycoBank (2016). In this paper, we accept 75 species of *Oxydothis* as some species have been transferred to other genera based on their morphological characteristics (Theissen & Sydow 1914, Hyde 1994, Hyde & Canon 1999). Most *Oxydothis* species have been introduced using only morphological characteristics, while less than 10 species have sequence data. *Oxydothis* is poorly represented with sequence data in GenBank.

Oxydothis species have been recorded as endophytes, pathogens and saprobes (Fröhlich & Hyde 1994). Taylor (1988) isolated *Oxydothis* species (e.g., *O. obducens* K.D. Hyde) as endophytes in studies of endophytes on palm leaves and rachides. Numerous species have been recorded from dead fronds and leaves of mainly palms (e.g., *O. acutata* (Syd. & P. Syd.) K.D. Hyde, *O. alexandrarum* K.D. Hyde; Hyde 1994, Hyde et al. 1997), while *O. parasitica* J. Fröhl. & K.D. Hyde and *O. oraniopsis* J. Fröhl. & K.D. Hyde were associated as leaf spots of *Licuala ramsayi* F. Muell. and *Oraniopsis appendiculata* (Becc.) J. Dransf. et al., respectively (Fröhlich & Hyde 1994). In this study, we discuss the lifestyles of *Oxydothis* species.

In this study, we introduce *Oxydothidaceae* as a new family with six new species of *Oxydothis* from palms (*Arecaceae*). The new species are morphologically distinguished from other species in *Oxydothis*, and also supported by combined ITS, LSU and SSU sequence analyses. Full descriptions, photo plates of macro-and micro-morphological characteristics and a phylogenetic tree to show the phylogenetic placement of the new family and the new species are provided.

Materials and methods

Collection, isolation and identification

Dead parts of different palm species were collected from Thailand (Fig. 1). Materials were brought to the laboratory in Zip lock plastic bags and morphological characteristics were observed under a Motic SMZ 168 series dissecting stereo microscope. Ascospores were photographed using Axio camera. Hand sections of the fruiting structures were done with a razor blade and mounted in water for microscopic studies and photomicrography. Micro-structures were observed under a Nikon ECLIPSE 80i compound microscope and photographed by a Canon 600D digital camera fitted on the microscope. Measurements were made with an Image Frame Work program. Figures were processed by Adobe Photoshop CS5 Extended version 10.0 for making photo-plates.

Single ascospore isolates were obtained following the method of Chomnunti et al. (2014). Contents of the sectioned ascospores were transferred to a drop of sterile water on a flame-sterilized slide. Drops of the spore suspension were spread on a Petri-dish containing MEA and incubated at 25°C overnight. Germinating ascospores were transferred to a fresh MEA media (Alves et al. 2006, Liu et al. 2012).

Holotypes of *Oxydothis* species introduced in this study are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand, and duplicated in the Cryptogamic Herbarium, Kunming Institute of Botany, Academia Sinica (HAKS). Ex-type living cultures are deposited in Mae Fah Luang Culture Collection (MFLUCC). *Facesoffungi* and *Index Fungorum* numbers were obtained following Jayasiri et al. (2015) and Index Fungorum (2016) respectively.

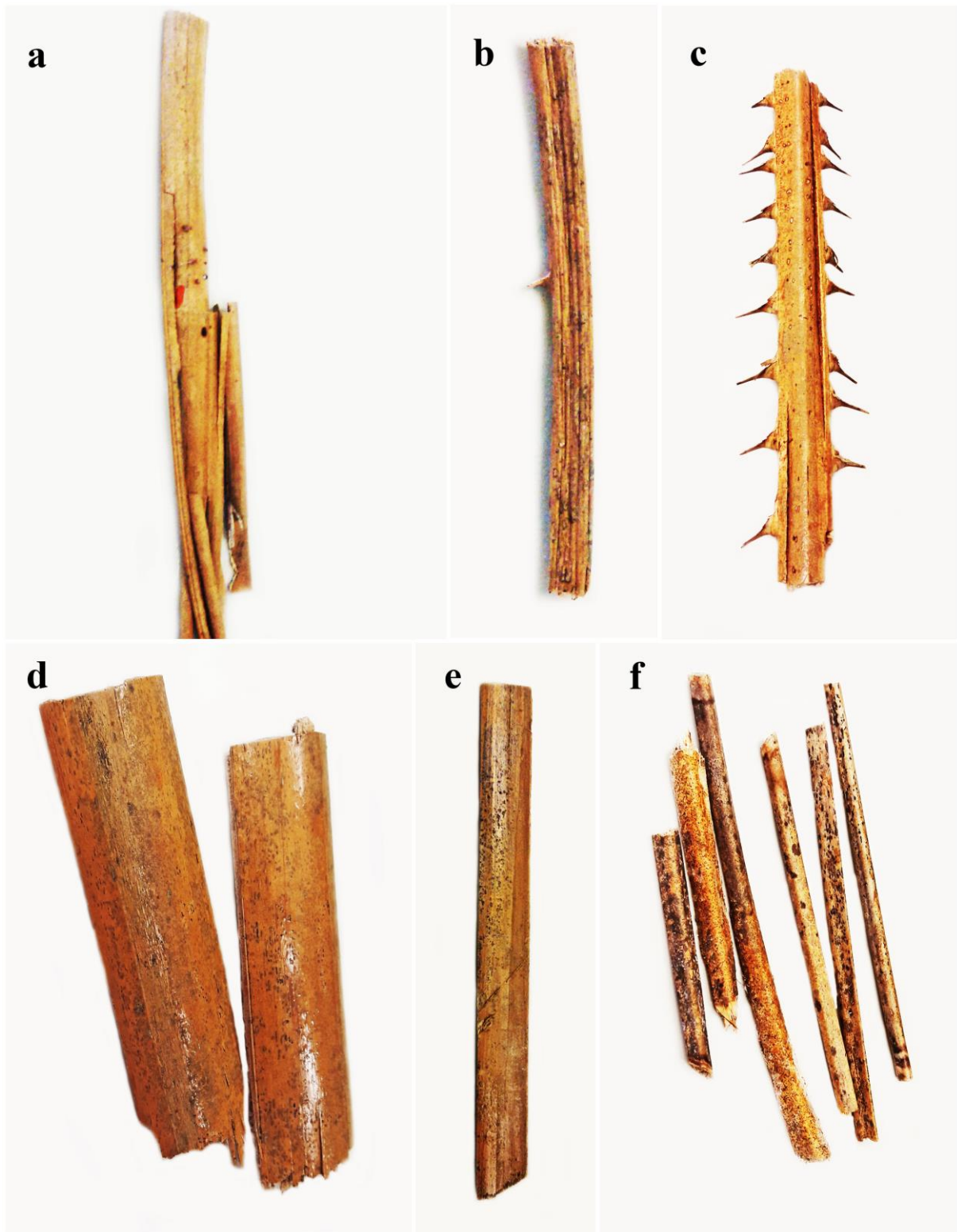


Fig. 1 Different parts of dead palms where *Oxydothis* species were found. a. Leaflets of *Eleais guineensis*. b–c. Rachides of *Calamus* spp. and *Eleais* sp. d–f. Petioles of *Metroxylon sagu*.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh mycelium grown on MEA for two weeks at 25–28°C using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer’s protocol (Hangzhou, P. R. China). ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S region of the rDNA molecule (White et al. 1990). LSU was amplified using LROR and LR5 primers pairs (Vilgalys & Hester 1990). NS1 and NS4 primer pairs were used to

amplify SSU. Amplification reactions were performed in 25 µl of total reaction which contained 9.5 µl of sterilized water, 12.5 µl of 2 × Easy Taq PCR Super Mix (mixture of Easy Taq™ DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, PR China), 1 µl of each primers, and 1 µl of DNA template. The amplification were performed with an initial denaturing step of 3 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 50 seconds annealing at 52°C, then 1 minute at 72°C for ITS, 30 seconds at 94°C, 50 seconds annealing at 55°C, then 1 minute at 72°C for LSU and SSU, a final extension of 10 minutes at 72°C and final hold at 4°C. PCR products were viewed on 1% agarose electrophoresis gels stained with ethidium bromide. PCR products were sequenced by Shangkai Majorbio Biopharm Technology Co, Ltd, China.

Sequence alignment and phylogenetic analyses

A BLAST search was used to reveal the closest matching taxa. Sequence alignment was performed using MAFFT (Kato et al. 2013) online software (<http://www.ebi.ac.uk/Tools/msa/mafft/>). Sequences were firstly analyzed and prepared in MEGA6 (Tamura et al. 2013). Data were converted from fasta to nexus format with Clustal X (Thompson et al. 1997). The models of evolution were determined with MrModeltest 2.2 (Nylander 2004) under the Akaike information criterion (AIC). The models selected were SYM+I+G for ITS and SSU, GTR+I+G for LSU and GTR-GAMMA for the combined dataset (Nylander 2004). Maximum likelihood analysis was performed by RAxML GUI v.0.9b2 with 1000 bootstrap replicates (Silvestro & Michalak 2010). The number of replications was inferred using the stopping criterion. Bootstrap values greater than 50% were accepted. Four chains were run for the individual and combined data sets. Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), with fifteen million generations and sampling frequency of every 100 generations. The first 15,000 trees were excluded as burn-in phase based on suggestion from Tracer. Bayesian posterior probabilities (BYPP) were calculated from the remaining 12,000 trees and values greater than 0.90 were accepted. The phylogenetic tree was visualized with Tree View32 (Page 1996).

Table 1 GenBank accession numbers of the isolates used in this study.

Species name	strain	GenBank accession number		
		ITS	LSU	SSU
<i>Acrocordiella occulta</i>	RS10	KT949894	-	-
<i>Acrocordiella occulta</i>	RS9	KT949893	-	-
<i>Albertiniella polyporicola</i>	CBS 457.88	-	AF096185	AF096170
<i>Amphibambusa bambusicola</i>	MFLUCC 11-0617	KP744433	KP744474	-
<i>Amphisphaeria sorbi</i>	MFLUCC 13-0721	-	KP744475	-
<i>Amphisphaeria umbrina</i>	AFTOL-ID 1229	-	FJ176863	FJ176809
<i>Arecophila bambusae</i>	HKUCC 4794	-	AF452038	AY083802
<i>Arthrimum bambusae</i>	ICMP 6889	-	DQ368630	DQ368662
<i>Arthrimum hydei</i>	CBS 114990	KF144890	KF144936	-
<i>Arthrimum montagnei</i>	AFTOL-ID 951	-	DQ471018	FJ190614
<i>Arthrimum phaeospermum</i>	HKUCC 3395	-	AY083832	AY083816
<i>Bartalinia robillardoides</i>	CBS 122705	KJ710460	KJ710438	-
<i>Beltrania pseudorhombica</i>	CPC 23656	KJ869158	KJ869215	-
<i>Beltraniella endiandrae</i>	CPC 22193	KJ869128	KJ869185	-
<i>Broomella vitalbae</i> 28S	MFLUCC 15-0023	KP757755	KP757751	KP757759
<i>Cainia graminis</i>	CBS 136.62	-	AF431949	AF431948
<i>Cephalotheca foveolata</i>	UAMH11631	KC408422	KC408398	-
<i>Clypeosphaeria uniseptata</i>	HKUCC6349	-	DQ810219	DQ810255
<i>Colletotrichum gloeosporioides</i>	LC0555	JN943090	JN940412	JN940356
<i>Coniocessia anandra</i>	Co108	GU553338	GU553349	-
<i>Coniocessia maxima</i>	Co117	GU553332	GU553344	-

Species name	strain	GenBank accession number		
		ITS	LSU	SSU
<i>Conioecessia nodulisporioides</i>	Co126	GU553333	GU553352	-
<i>Cordana abramovii</i>	PE 0053-24a	-	KF833358	-
<i>Cordana inaequalis</i>	CBS 508.83	HE672146	HE672157	-
<i>Cordana pauciseptata</i>	CBS 121804	HE672149	HE672160	-
<i>Creosphaeria sassafras</i>	CM-AT 018	-	DQ840056	-
<i>Cryptendoxyla hypophloia</i>	WM10.89	-	HQ014708	-
<i>Diatrype disciformis</i>	AFTOL-ID 927	-	DQ470964	DQ471012
<i>Diatrype palmicola</i>	MFLUCC 11-0020	KP744438	KP744482	KP753950
<i>Diatrype whitmanensis</i>	ATCC-MYA 4417	FJ746656	-	-
<i>Eutypa lata</i>	CBS 208.87	DQ006927	-	-
<i>Hyalotiella spartii</i>	MFLUCC 13-0397	KP757760	KP757752	KP757756
<i>Hyponectria buxi</i>	UME 31430	-	AY083834	AF130976
<i>Immersidiscosia eucalypti</i>	HHUF:29920	AB594793	AB593722	AB593724
<i>Iodosphaeria tongrenensis</i>	MFLU 15-0393	KR095282	KR095283	KR095284
<i>Lepteutypa cupressi</i>	IMI 052255	-	AF382379	AY083813
<i>Lopadostoma turgidum</i>	LT2	KC774618	-	-
<i>Melogramma campylosporom</i>	MBU	JF440978	-	-
<i>Microdochium phragmitis</i>	CBS 423.78	KP858948	KP858948	-
<i>Microdochium trichocladiopsis</i>	CBS 623.77	KP858998	KP858934	-
<i>Monosporascus cannonballus</i>	FMR6682	-	-	AF340016
<i>Oxydothis calamicola</i>	MFLUCC 14-1165	-	KY206761	KY206767
<i>Oxydothis cyrtostachicola</i>	FIH 151	DQ660334	DQ660337	-
<i>Oxydothis daemonoropsicola</i>	FIH 019	DQ660335	DQ660338	-
<i>Oxydothis frondicola</i>	HKUCC 1001	AF009803	AY083835	AY083818
<i>Oxydothis garethjonesii</i>	MFLUCC 15-0287	KY206773	KY206762	KY206768
<i>Oxydothis inaequalis</i>	FIH 018	DQ660336	DQ660339	-
<i>Oxydothis metroxylonicola</i>	MFLUCC 15-0281	KY206774	KY206763	KY206769
<i>Oxydothis metroxylonis</i>	MFLUCC 15-0283	KY206775	KY206764	KY206770
<i>Oxydothis palmicola</i>	MFLUCC 15-0806	KY206776	KY206765	KY206771
<i>Oxydothis rhapsodicola</i>	MFLUCC 14-0616	-	KY206766	KY206772
<i>Phialemonium atrogriseum</i>	CBS 604.67	HE599384	HQ231981	-
<i>Pseudomassaria chondrospora</i>	It 1200	KR092790	KR092779	-
<i>Pseudomassaria chondrospora</i>	PC1	JF440982	-	-
<i>Pseudoestalotiopsis theae</i>	SAJ-0021	JN943623	JN940838	JN940785
<i>Requienella seminuda</i>	RS12	KT949912	-	-
<i>Requienella seminuda</i>	RS13	KT949913	-	-
<i>Robillarda sessilis</i>	CBS 114312	KR873256	KR873284	-
<i>Robillarda terrae</i>	CBS 587.71	KJ710484	KJ710459	-
<i>Seiridium phylicae</i>	CPC 19962	KC005785	KC005807	-
<i>Seynesia erumpens</i>	SMH 1291	-	AF279410	AF279409
<i>Subramaniomyces fusisaprophyticus</i>	CBS 418.95	EU040241	-	-
<i>Vialaea minutella</i>	BRIP 56959	KC181926	KC181924	-
<i>Vialaea mangifia</i>	MFLUCC12-0808	KF724974	KF724975	-
<i>Xylaria hypoxylon</i>	CBS 122620	AM993141	-	-
<i>Xylaria polymorpha</i>	MUCL: 49904	FN689809	-	-

Results

Phylogenetic analyses

Sequences used in the analyses were selected based on recent publications on Xylariomycetidae (Daranagama et al. 2015, Senanayake et al. 2015, Maharachchikumbura et al. 2015, 2016). *Cordana inaequalis* and *C. pauciseptata* were used as outgroup taxa. The topology of the trees produced by (ML) and Bayesian analyses methods were similar and the best scoring ML tree is shown in Fig. 2. Species of *Oxydothis* clustered together and form a distinct clade within *Xylariales* with low bootstrap support in the ML analysis but high posterior probabilities in Bayesian analysis (51% ML, 0.95 PP), this is probably because of lack of molecular data for many missing or extinct genera. The *Oxydothidaceae* clade formed a sister clade to *Vialaeaceae* and

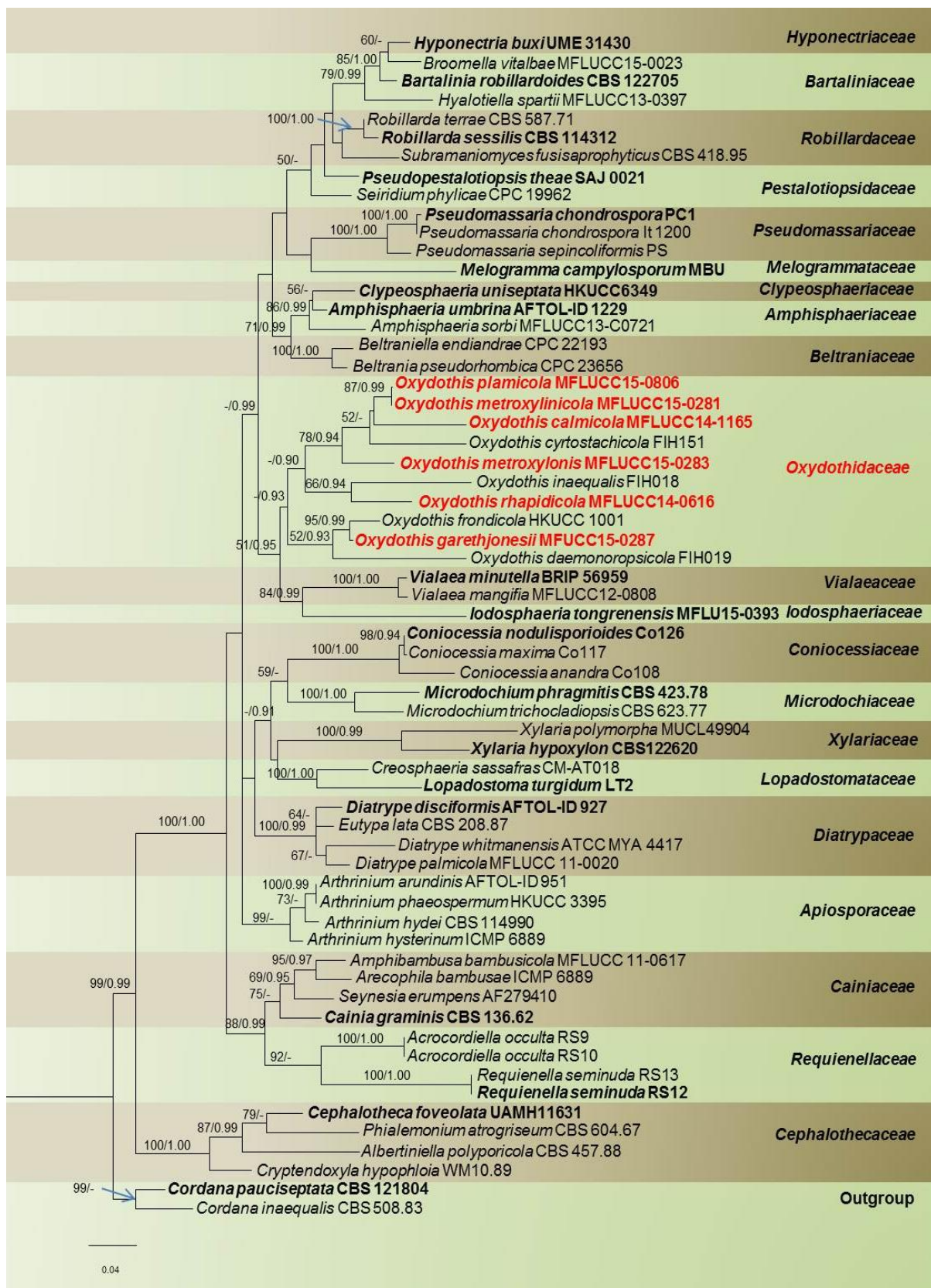


Fig. 2 – ML tree resulting from analyses of combined ITS, LSU and SSU sequence data of selected taxa in Xylariales. Maximum likelihood bootstrap values $\geq 50\%$ and Bayesian posterior probabilities (BYPP) greater than 0.90 are given above the nodes. The tree is rooted to *Cordana inaequalis* and *C. pauciseptata*. The new family *Oxydothidaceae* and new strains are in red bold and ex-type strains are in black bold.

Iodosphaeriaceae. *Oxydothis rhapsidicola* is closely related to *O. inaequalis* with moderate support (66% ML, 0.94 PP), while related to the sister clade which comprises *Oxydothis metroxylonis*, *O. cyrtostachicola*, *O. calamicola*, *O. metroxylonicola* and *O. palmicola* which group together with moderate support (78% ML, 0.94 PP), and are a sister group to *O. palmicola* (87% ML, 0.99 PP). *Oxydothis calamicola* and *O. cyrtostachicola* are phylogenetically poorly supported (52% ML), *O. cyrtostachicola* lacks SSU sequence in GenBank and *O. calamicola* lacks ITS sequence data. The basal clade in *Oxydothidaceae* comprises the strains of *Oxydothis daemonoropsicola*, *O. garethjonesii* and *O. frondicola* (52% ML, 0.93 PP). *Oxydothis garethjonesii* is a distinct species which is closely related to *O. frondicola* (95% ML, 0.99 PP).

Taxonomy

Oxydothidaceae Konta & K.D. Hyde, *fam. nov.*

Index Fungorum number: IF 552561; Facesoffungi number: FoF 02700

Saprobic, *endophytic* or *parasitic* on petioles or leaves of monocotyledons, especially palms. *Sexual morph*: *Ascomata* immersed, solitary or aggregated, clustered in large groups or single, ellipsoidal or subglobose, slightly raised from host surface with light or darkened discs, with most taxa lying horizontal to the host surface. *Peridium* 1–18 × 2–36 (\bar{x} = 8 × 16 μm , n = 15) thick-walled, brown to dark brown, and/or peridium cells merging with the host tissue. *Paraphyses* hypha-like, filamentous, irregular, septate, persisting between asci, but often fragmenting in dried material. *Asci* 8-spored, unitunicate, cylindrical, pedicellate, with a J+ (rarely J-), subapical ring. *Ascospores* fasciculate, fusiform or filiform, 1-septate, tapering from the center to spine-like, pointed or rounded ends. *Appressoria* (11–)22(–36) μm high × (5)–12(–22) μm diam., solitary, hyaline, light green, light brown, irregular in shape, thick-walled. *Asexual morph*: Hyphomycetous *Selenospora* sp. *Conidiophores*, mononematous, 30–45 × 4–6 μm diam. at the base, (1–)2–3-septate, unbranched or 1-branched, brown olivaceous, thick-walled at below, thin-walled and colourless at above. *Conidiogenous cells* 10–15 μm high, with a minute scar. *Conidia* (17–)23–27(–29) × 1–1.5(–2) μm , arcuate, unicellular, colorless, distinct obviously differentiated apex or base (described from Samuels & Rossman, 1987). *Appressoria* produced by germinating ascospores in some species, solitary, hyaline, light green, light brown, irregular in shape, thick-walled.

Notes – *Oxydothidaceae* species are mostly saprobic or endophytic on leaves, rachides or petioles of palms, although one species appears as a pathogen (Fröhlich & Hyde 1994, Hyde 1994, Pinnoi et al. 2006). Species of *Oxydothidaceae* are characterized by thin-walled ascomata, which usually develop within a darkened stroma or a raised blistering area on the host surface, unitunicate and cylindrical asci, often with a J+ apical ring, and 1-septate, hyaline, fusiform or filiform ascospores, which taper at the ends. Phylogenetic analysis indicates that *Oxydothidaceae* species cluster together as a distinct clade (51%, ML, 0.95, PP), and sister to *Vialaeaceae* and *Iodosphaeriaceae* in *Xylariales* (Fig. 2). The new family differs from others families in *Xylariales* in having immersed ascomata or slightly raised from the host tissues, and filiform or fusiform, 1-septate, hyaline ascospores. Thus, *Oxydothidaceae* is introduced as a new family in *Xylariales* (Sordariomycetes) based on morphology and phylogeny.

Oxydothidaceae, *Vialaeaceae*, and *Iodosphaeriaceae* share similar characters, as asci have J+, subapical rings and ascospores are hyaline (Cannon 1995, Senanayake et al. 2014, Li et al. 2015). They probably also share similar endophytic life modes and becoming saprobes when the host dies (Wong & Hyde 1999, Promputtha et al. 2007) and may be pathogenic when the host is stressed (Fröhlich & Hyde 1994, McTaggart et al. 2013). They differ as in *Vialaeaceae* ascomata form in pseudostroma in circular groups, and have central ostioles and elongate necks. In *Iodosphaeriaceae* species, ascomata are superficial, with brown flexuous hairs radiating from the peridium surface, with pore-like ostioles and lacking necks. In *Oxydothidaceae* species, ascomata form under slightly raised, blistering areas, and have their axis oblique or perpendicular to the host surface, each with an individual neck (Cannon 1995, McTaggart et al. 2013, Senanayake et al. 2014, Li et al. 2015, Maharachchikumbura et al. 2016). The ascospores also differ: in *Vialaeaceae*

they are 1–3-septate, elongate, strongly isthmoid, with fusiform to rhombic ends (Cannon 1995, McTaggart et al. 2013, Senanayake et al. 2014, Maharachchikumbura et al. 2016), in *Iodosphaeriaceae* they are ellipsoidal to fusiform, aseptate, and surrounded by thick mucilaginous sheath (Li et al. 2015), while in *Oxydothidaceae* they are fusiform or filiform, 1-septate and tapering from the center, to spine-like, pointed or rounded ends. *Oxydothidaceae* species are mostly endophytes and saprobes on palms and other monocotyledons, while *Vialaeaceae* and *Iodosphaeriaceae* species are only known from dicotyledonous trees, where they are also endophytes, weak parasites, and saprobes (Fröhlich & Hyde 1994, Cannon 1995, Li et al. 2015). Although the three families are sister taxon, they are ecologically, morphologically and phylogenetically well-resolved groups, therefore we are confident that they are distinct families. Samarakoon et al. (2016) date the divergence times for *Oxydothidaceae* and *Vialaeaceae* at 200 MYA.

Type genus – *Oxydothis* Penz. & Sacc., *Malpighia* 11(11-12): 505 (1898) [1897]

Oxydothis Penz. & Sacc., *Malpighia* 11(11-12): 505 (1898) [1897]

Saprobic, endophytic or parasitic on dead rachides, petioles or leaves of mostly palms and some other monocotyledons. *Sexual morph*: *Ascomata* immersed in host tissues, solitary or aggregated, ellipsoidal or globose to subglobose, lenticular, slightly raised, as light or darkened discs, or under raised light or darkened blistering areas, usually lying horizontal to the host surface. *Peridium* comprising thick-walled, flattened, brown to dark brown, cells of *textura prismatica* to *angularis*, sometimes occasionally integrated with host cell walls. *Hamathecium* comprising hypha-like, filamentous, irregular, septate, paraphyses persisting between asci, but often fragmenting in dried material. *Asci* 8-spored, unitunicate, cylindrical, thin-walled, with a J+ (occasionally J-), wedge-shaped or discoid, subapical ring. *Ascospores* 1–4 seriate or fasciculate, hyaline, fusiform or filiform, 1-septate at the center, gradually tapering from the center to the ends, ends sometimes spine-like or rounded, smooth-walled. *Asexual morph*: *Selenosporella* sp. (descriptions from Samuels & Rossman 1987). *Appressoria* produced by germinating ascospores in some species, solitary, hyaline, light green, light brown, irregular in shape, thick-walled.

Notes – Penzig and Saccardo (1897) introduced *Oxydothis* with *O. grisea* Penz. & Sacc. as the type species. Hyde (1994) reported that most of *Oxydothis* species were found on palms. Some species were later introduced from other monocotyledonous (Fröhlich & Hyde 2000, Shenoy et al. 2005). The placement of *Oxydothis* has been unclear. Hyde (1993) suggested that the genus should be transferred from *Amphisphaeriaceae* to *Hyponectriaceae* based on its morphology. Subsequently, Kang et al. (1999) moved the genus to *Clypeosphaeriaceae*. According to the phylogenetic analyses of Jeewon et al. (2003), *Oxydothis* is closely related to *Leiosphaerella*, but the placement was unclear. Our phylogenetic analyses indicate that *Oxydothis* is best placed in *Xylariales* in a monophyletic lineage (*Oxydothidaceae*) with the *Vialaeaceae* and *Iodosphaeriaceae* clades (Fig. 2). The other genera that may be related to *Oxydothidaceae* are *Ceriospora* Niessl, *Frondispora* K.D. Hyde, *Lasiobertia* Sivan. and *Leiosphaerella* (Barr 1990, Hyde 1993a, Shenoy et al. 2005).

Type species – *Oxydothis grisea* Penz. & Sacc., *Malpighia* 11(11–12): 505 (1898) [1897]

Oxydothis calamicola Konta & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF552542, Facesoffungi number: FoF 02701 Fig. 3

Etymology: The specific epithet refers to the host genus *Calamus*, and *cola* meaning loving.

Holotype: MFLU: 15-0016

Saprobic on rachis of *Calamus* L. (*Areaceae*). *Sexual morph*: *Ascomata* 280–835 μm diam. (\bar{x} = 673 μm diam., n = 40, up to 1,700 μm), solitary or aggregated, mostly solitary when young, becoming grouped at maturity, immersed, comprising non-blistering areas through the host tissue, axis oblique or perpendicular to the host surface, with central papilla, 30–46 μm high \times 186–230 μm diam. (\bar{x} = 35 \times 222 μm , n = 5), in transverse section, lenticular, globose to subglobose, darker at the central region, light towards the outer darker rim on the host surface. *Peridium* 8–15 μm (\bar{x} = 11

μm , $n = 10$), outer cells merging with the host epidermal cells, comprising dark brown to black, walled cells of *textura angularis*. Asci $142\text{--}150 \times 8\text{--}11 \mu\text{m}$ ($\bar{x} = 144 \times 10 \mu\text{m}$, $n = 10$), 8-spored, unitunicate, cylindrical, pedicellate, with a J+, wedge-shaped, subapical ring. Ascospores $48\text{--}50 \times 4\text{--}5 \mu\text{m}$ ($\bar{x} = 48 \times 5 \mu\text{m}$, $n = 20$), 1–2-seriate, fusiform, tapering gradually from the center to the ends, centrally 1-septate, not constricted at the septum, with pointed ends, hyaline, with one large guttule at the central when immature, becoming two large guttules when mature in each cells, smooth-walled. Asexual morph: Undetermined. Appressoria not formed.

Culture characters – Ascospores germinating on MEA within 24 hours. Colonies on MEA, grey to olivaceous, surface rough, margins smooth, produced rudimentary ascomata, hyphae, septate, branched, smooth-walled (Fig. 9 viz a).

Material examined – THAILAND, Chiang Mai, on dead rachis of *Calamus* L. (*Arecaceae*), 11 August 2014, S. Konta, P04c (MFLU 15-0016, holotype); *Ibid.* (HKAS95039, isotype); ex-type living culture, MFLUCC 14-1165.

Notes – *Oxydothis calamicola* is similar to *O. maculosa* Penz. & Sacc. in its ellipsoidal ascomata in transverse section, as well as the shape and size of ascospores. *Oxydothis calamicola* differs from *O. maculosa* in forming globose to subglobose rims on the host surface, with dark ostiolar dots at the center, while *O. maculosa* forms blackened regions on host surface. Phylogenetically, *Oxydothis calamicola* is placed in a basal clade to *O. palmicola* and *O. metroxylinicola* (Fig. 2). *Oxydothis calamicola* is distinct from these two species in having smaller ascomata, and ascospores with spine-like ends.

Oxydothis Garethjonesii Konta & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF552541, Facesoffungi number: FoF 02702, Fig. 4

Etymology: The specific epithet is named after Professor E.B.G. Jones in recognition of his contribution to mycology on his 80th birthday.

Holotype: MFLU: 15-0033

Saprobic on petiole of *Eleais* Jacq. (*Arecaceae*). Sexual morph: Ascomata $221\text{--}348 \mu\text{m}$ diam. ($\bar{x} = 293 \mu\text{m}$ diam., $n = 40$, up to $400 \mu\text{m}$), solitary or aggregated, mostly solitary when young, becoming grouped at maturity, immersed, comprising slightly raised blistering areas through the host tissue, axis oblique or perpendicular to the host surface, with central papilla, $118\text{--}133 \mu\text{m}$ high $\times 122\text{--}141 \mu\text{m}$ diam. ($\bar{x} = 123 \times 131 \mu\text{m}$, $n = 5$), in transverse section, globose to subglobose, darker at the central region, light towards the outer darker rim on the host surface. Peridium $15\text{--}34 \mu\text{m}$ ($\bar{x} = 25 \mu\text{m}$, $n = 10$) thick-walled, outer cells merging with the host epidermal cells, dark brown to black, walled cells of *textura angularis*. Asci $210\text{--}229 \times 14\text{--}21 \mu\text{m}$ ($\bar{x} = 216 \times 19 \mu\text{m}$, $n = 10$), 8-spored, fasciculate, unitunicate, cylindrical-clavate, pedicellate, with a J+, wedge-shaped, subapical ring. Ascospores $47\text{--}50 \times 3\text{--}5 \mu\text{m}$ ($\bar{x} = 49 \times 4 \mu\text{m}$, $n = 20$), 2–4-seriate, filiform to fusiform, tapering gradually from the center to the ends, centrally 1-septate, not constricted at the septum, with blunt to pointed ends, hyaline, with large guttules in each cell when immature, and lack in maturity, smooth-walled. Appressoria $15\text{--}36 \mu\text{m}$ high $\times 18\text{--}20 \mu\text{m}$ diam. ($\bar{x} = 23 \times 19 \mu\text{m}$, $n = 10$) solitary, hyaline, irregular in shape, thick-walled. Asexual morph: Undetermined.

Culture characters – Ascospores germinating on MEA within 24 hours and germ tube producing appressoria. Colonies on MEA, smoky-grey to dark green, margins smooth, dense at the center, with fairly fluffy, as double rings, hyphae, septate, branched, smooth-walled (Fig. 9 viz b).

Material examined – THAILAND, Krabi, on petiole and rachis of *Eleais* sp. (*Arecaceae*), 3 December 2014, S. Konta, KBM01c (MFLU 15-0033, holotype); *Ibid.* (HKAS95038); ex-type living culture, MFLUCC 15-0287.

Notes – *Oxydothis Garethjonesii* is similar to *O. megalospora* J. Fröhl. & K.D. Hyde and *O. daemonoropsicola* J. Fröhl. & K.D. Hyde in characteristics of ascospores. It however differs from these species in host, the size of ascomata and ascospores, and shape of ascal apical ring (Fröhlich & Hyde 2000). *Oxydothis Garethjonesii* is phylogenetically related to *O. frondicola* (95% ML, 0.99 PP) and *O. daemonoropsicola* as basal branches (52% ML, 0.93 PP) (Fig. 2).

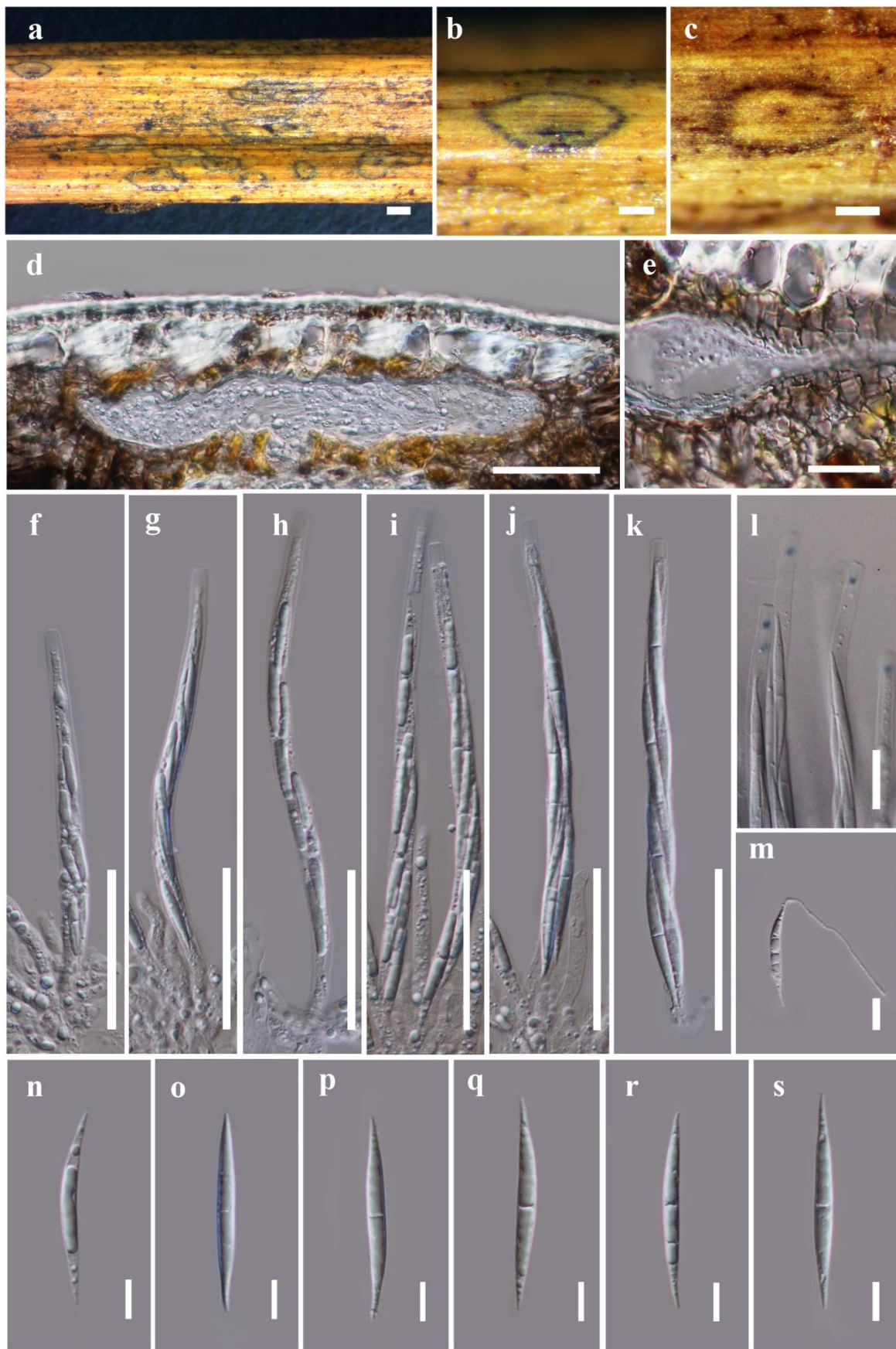


Fig. 3 – *Oxydothis calamicola* (holotype) a. Appearance of fruiting bodies on host substrate. b–c. Close up of fruiting bodies. d. Section of ascoma. e. Peridium. f–k. Asci. l. J+ reaction of apical ring in Melzer's reagent. m. Germinating ascospore. n–s. Ascospores. Scale bars: a = 500 μm , b–c = 200 μm d = 50 μm , e–l = 20 μm , m–s = 10 μm

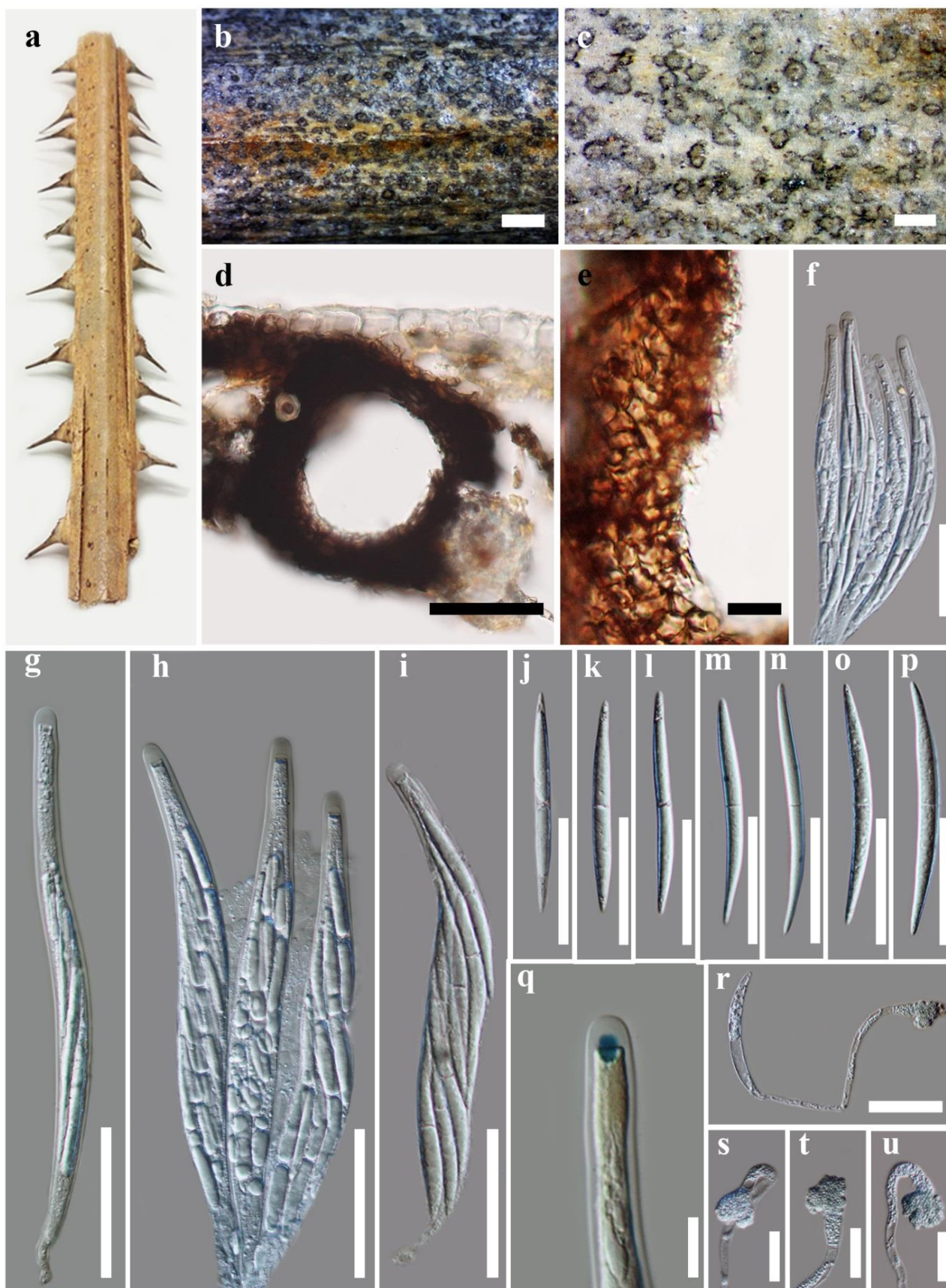


Fig. 4 – *Oxydothis Garethjonesii* (holotype) a. Host substrate. b–c. Close up of ascomata. d. Section of ascoma. e. Peridium. f. Asci. g–h. Immature asci. i. Mature Ascus. j–p. Ascospores. q. J+ reaction of apical ring in Melzer's reagent. r. Germinating ascospore. s–u. Appressoria. Scale bars: b = 1000 μ m, c = 500 μ m, d = 50 μ m, e = 10 μ m, f–p, r = 50 μ m, q = 5 μ m, s–u = 10 μ m.

Oxydothis metroxylo-nicola Konta & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552539, Facesoffungi number: FoF 02703, Fig. 5

Etymology: The specific epithet refers to the host genus *Metroxylon* and *cola* meaning loving.

Holotype: MFLU: 15-0027

Saprobic on petiole of *Metroxylon sagu* Rottb. (*Arecaceae*). *Sexual morph*: Ascomata 210–490 μm diam. (\bar{x} = 337 μm diam., n = 40, up to 700 μm), solitary or aggregated, mostly solitary when young, becoming grouped at maturity, immersed, comprising slightly raised blistering areas through the host tissue, axis oblique or perpendicular to the host surface, with central papilla, 62–110 μm high \times 193–310 μm diam. (\bar{x} = 89 \times 263 μm , n = 5), in transverse section, lenticular, globose to subglobose, darker at the central region, light towards the outer darker rim on the host surface. *Peridium* 15–36 μm (\bar{x} = 24 μm , n = 10) thick-walled, outer cells merging with the host epidermal cells, dark brown to black, walled cells of *textura prismatica*. *Asci* 137–150 \times 13–19 μm (\bar{x} = 143 \times 16 μm , n = 10), 8-spored, fasciculate, unitunicate, cylindrical-clavate, pedicellate, with a J+, wedge-shaped, subapical ring. *Ascospores* 52–58 \times 8–9 μm (\bar{x} = 57 \times 8 μm , n = 20), 2-seriate, fusiform, curved, tapering gradually from the center to the ends, centrally 1-septate, swollen at the central of each cell, not constricted at the septum, with pointed ends, hyaline, with large globose multi-guttules in each cell, smooth-walled. *Asexual morph*: Undetermined. *Appressoria* 12–23 μm high \times 7–12 μm diam. (\bar{x} = 17 \times 10 μm , n = 10), solitary, hyaline to light green, irregular in shape, thick-walled.

Culture characters – Ascospores germinating on MEA within 24 hours and germ tube was produced from end cell with developing appressoria structure. Colonies on MEA, olivaceous, rough on surface, produced ascomata-like, colony growing like double rings, hyphae, septate, branched, smooth (Fig. 9 *viz* c).

Material examined – THAILAND, Krabi, on dead petiole of *Metroxylon sagu* (*Arecaceae*). 8 December 2014, S. Konta, KBR04c (MFLU 15-0027, holotype); *ibid.* (HKAS 95036, isotype); ex-type living culture, MFLUCC 15-0281.

Notes – *Oxydothis metroxylo-nicola* resembles *O. elaeidis* (Beeli) Sivan., *O. parasitica* and *O. sabalensis* (Cooke) Petr. in the shape of its ascospores. However, *O. metroxylo-nicola* differs from these species in having small, immersed ascomata, while *O. elaeidis*, *O. parasitica* and *O. sabalensis* have raised subglobose ascomata (ellipsoidal ascomata in *O. sabalensis*), and differently shaped ascospores (Sivanesan 1970, Fröhlich & Hyde 1994, Hyde 1994). *Oxydothis parasitica* is a pathogen causing leaf spot disease of *Licuala ramsayi* (Fröhlich & Hyde 1994).

Phylogenetic analyses indicate that *Oxydothis metroxylo-nicola* is closely related to *O. palmicola* (87% ML, 0.99 PP), with *O. calamicola* as a basal clade without bootstrap support. *Oxydothis metroxylo-nicola* differs from *O. calamicola* and *O. palmicola* in having raised blistering ascomata on host surface, and differently shaped, J+, subapical rings.

Oxydothis metroxylo-nis Konta & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552543, Facesoffungi number: FoF 02704, Fig. 6

Etymology: The specific epithet refers to the host genus *Metroxylon*.

Holotype: MFLU: 15-0029

Saprobic on petiole of *Metroxylon sagu* Rottb. (*Arecaceae*). *Sexual morph*: Ascomata 716–1,580 μm diam. (\bar{x} = 1,091 μm diam., n = 40), solitary or aggregated, mostly solitary when young, becoming grouped at maturity, immersed, comprising non-blistering areas, axis oblique or perpendicular to the host surface, with central papilla. *Peridium* 10–33 μm (\bar{x} = 21 μm , n = 10), outer cells merging with the host epidermal cells, comprising dark brown to black, cells of *textura angularis*. *Asci* 165–181 \times 9–15 μm (\bar{x} = 175 \times 14 μm , n = 10), 8-spored, unitunicate, cylindrical-clavate, pedicellate, with a J+, wedge-shaped, subapical ring. *Ascospores* 47–57 \times 4–6 μm (\bar{x} = 52 \times 5 μm , n = 20), 2–3-seriate, fusiform, tapering gradually from the center to the ends, centrally 1-septate, not constricted at the septum, with pointed ends, hyaline, with large multi-guttules in each cell, smooth-walled. *Asexual morph*: Undetermined. *Appressoria* 14–31 μm high \times 6–17 μm diam.

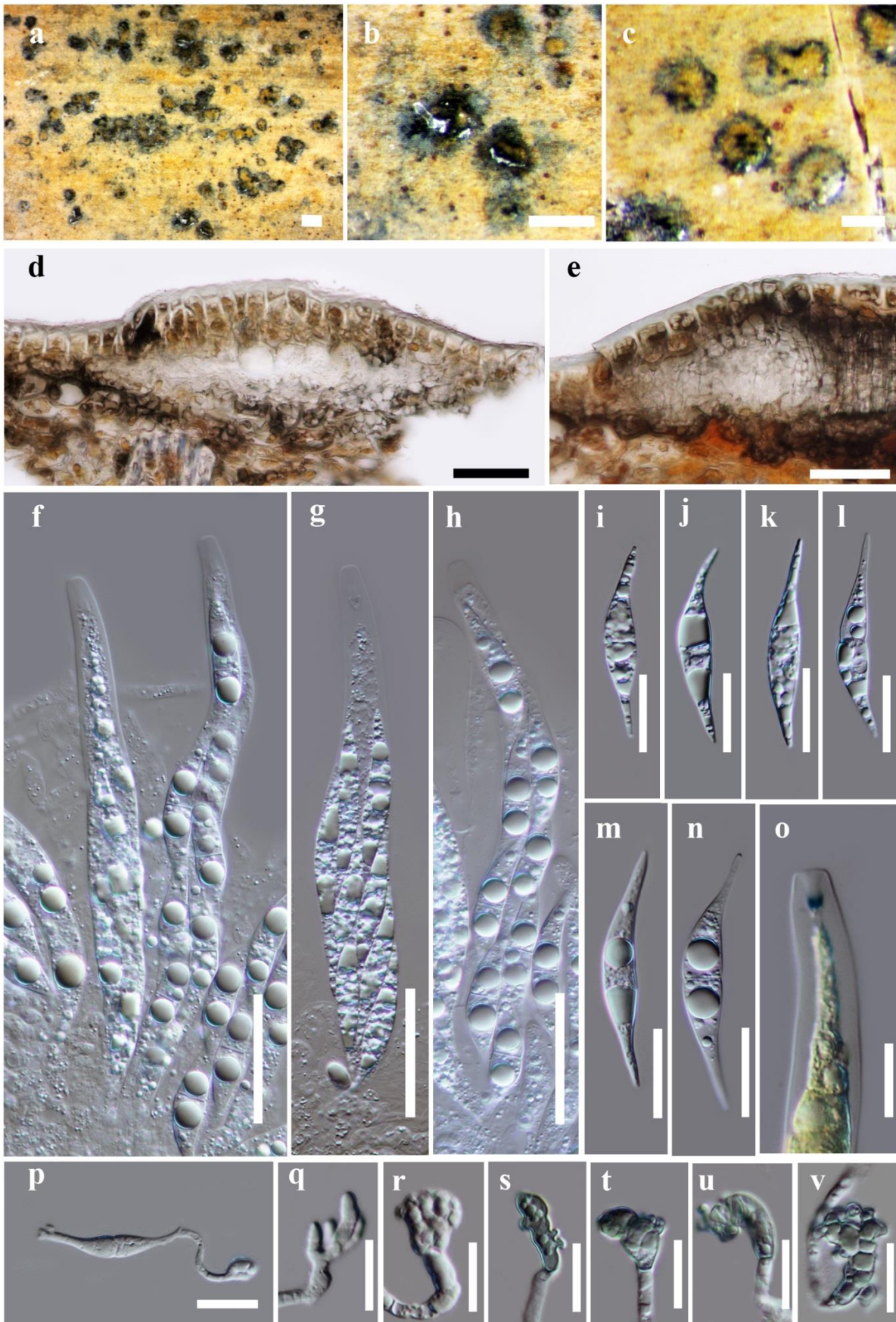


Fig. 5 – *Oxydothis metroxylonicola* (holotype) a. Ascomata on host substrate. b–c. Close up of ascomata. d. Section of ascoma. e. Peridium. f–h. Asci. i–n. Ascospores. o. J+ reaction of apical ring in Melzer's reagent. p. Germinating ascospore. q–v. Appressoria. Scale bars: a–b = 500 μ m, c = 200 μ m, d = 20 μ m, e = 20 μ m, f–n = 20 μ m, o = 10 μ m, p = 20 μ m, q–v = 10 μ m.

($\bar{x} = 25 \times 10 \mu\text{m}$, $n = 10$) solitary, single, hyaline to light green, irregular in shape, thick-walled.

Culture characters – Ascospores germinating on MEA within 24 hours and germ tubes developing appressoria. Colonies on MEA, olivaceous, rough, producing rudimentary ascomata at the center, slightly fluffy at the margins, hyphae, septate, branched, smooth-walled (Fig. 9 viz d).

Material examined – THAILAND, Krabi, on dead petiole palm, *Metroxylon sagu* Rottb. (Arecaceae), 8 December 2014, S. Konta, KBR04e (MFLU 15-0029, holotype; HKAS 95040, isotype); ex-type living culture, MFLUCC 15-0283.

Notes – *Oxydothis metroxylonis* is similar to *Oxydothis oraniopsis* in ascospore shape, however, it is distinct in having globose to subglobose, non-blistering ascomata, ellipsoidal in transvers-section, asci with a J+, wedge-shaped apical ring, and multi-guttulate ascospores. *Oxydothis oraniopsis* forms dome-shaped, subglobose to cylindrical ascomata, has J+, discoid ascus rings. Phylogenetic analysis place *Oxydothis metroxylonis* as a basal clade clustering with *O. calamicola*, *O. cyrtostachicola* Hidayat et al., *O. metroxylonicola* and *O. palmicola* with moderate support (78% ML, 0.94 PP) (Fig. 2), but the species have very different ascomata.

Oxydothis palmicola Konta, B. Thongbai & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552540, Facesoffungi number: FoF 02705, Fig. 7

Etymology: The specific epithet refers to the host substrate (palm).

Holotype: MFLU: 15-2339

Saprobic on dead leaves of *Eleais guineensis* Jacq. (Arecaceae). *Sexual morph*: Ascomata 111–195 μm diam. ($\bar{x} = 166 \mu\text{m}$ diam., $n = 40$, up to 220 μm), solitary or aggregated, mostly solitary when young, becoming grouped at maturity, immersed, appearing as non-blistering areas through the host tissue, axis oblique or perpendicular to the host surface, with a central, 59–79 μm high \times 182–263 μm diam. ($\bar{x} = 67 \times 215 \mu\text{m}$, $n = 5$) papilla curving upwards and piercing the host cuticle. *Peridium* 18–34 μm ($\bar{x} = 25 \mu\text{m}$, $n = 10$), thick-walled, outer cells merging with the host epidermal cells, comprising dark brown to black, cells of *textura angularis*. *Asci* 138–145 \times 15–20 μm ($\bar{x} = 141 \times 18 \mu\text{m}$, $n = 10$), 8-spored, unitunicate, cylindrical-clavate, pedicellate, with a J+, wedge-shaped, subapical ring. *Ascospores* 51–53 \times 6–8 μm ($\bar{x} = 52 \times 7 \mu\text{m}$, $n = 20$), 2–4-seriate, fusiform, tapering gradually from the center to the ends, centrally 1-septate, not constricted at the septum, with pointed ends, hyaline, one large guttule at the center when immature, with one large guttules when in each cell mature, sometimes smaller near the ends, smooth-walled. *Asexual morph*: Undetermined. *Appressoria* 18–36 μm high \times 12–16 μm diam. ($\bar{x} = 23 \times 14 \mu\text{m}$, $n = 10$) solitary, hyaline to light brown, irregular in shape, thick-walled.

Culture characters – Ascospores germinating on MEA within 24 hours and germ tube developing appressoria. Colonies on MEA, grey to olivaceous, white at the center, dense, with fairly fluffy surface, hyphae, septate, branched, smooth-walled (Fig. 9 viz e).

Material examined – THAILAND, SongKhla, Hatyai, on dead leaf *Eleais guineensis* (Arecaceae), 16 June 2015, B. Thongbai, SK01c (MFLU 15-2339, holotype, HKAS 95037, isotype); ex-type living culture, MFLUCC 15-0806.

Notes – *Oxydothis palmicola* resembles *O. linospadicis* J. Fröhl. & K.D. Hyde in ascospore shape, but differs in its lenticular ascomata, and shape of ascospores. In our phylogenetic analysis, *O. palmicola* clusters with *O. metroxylonicola*. *Oxydothis palmicola*, however, it differs from *O. metroxylonicola*; in its host, in having non-blistering ascomata, the shape of the ascospores, the J+, subapical ring (Figs. 5, 7) and in culture characteristics (Fig. 9 viz c, e). Both *O. palmicola* and *O. metroxylonicola* produce appressoria from germinating ascospores.

Oxydothis rhapsidicola Konta & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552538, Facesoffungi number: FoF 02706, Fig. 8

Etymology: The specific epithet refers to the host genus *Rhapis*

Holotype: MFLU: 15-0002

Saprobic on petiole of *Rhapis excelsa* (Thunb.) Rehder (Arecaceae). *Sexual morph*: Ascomata 303–480 μm diam. ($\bar{x} = 361 \mu\text{m}$ diam., $n = 40$, up to 600 μm), solitary or aggregated,

mostly solitary when young, becoming grouped at maturity, immersed, comprising slightly raised blistering areas through the host tissue, axis oblique or perpendicular to the host surface, with central papilla, 25–44 μm high \times 82–124 μm diam. (\bar{x} = 40 \times 117 μm , n = 5), in transverse section, lenticular, globose to subglobose, darker at the central region, light towards the outer darker rim on

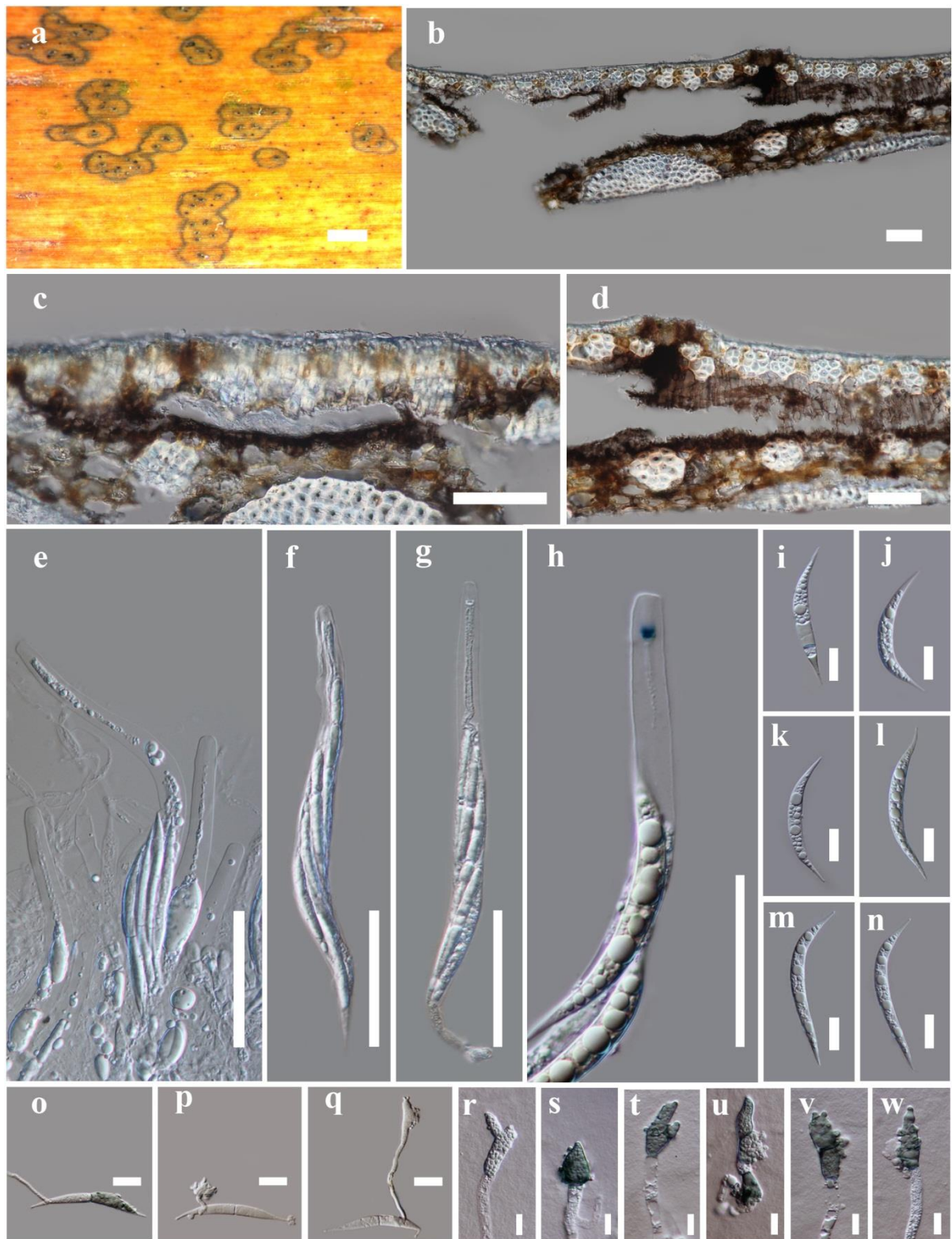


Fig. 6 – *Oxydothis metroxylonis* (holotype) a. Ascomata on host substrate. b–c. Section of ascomata. d. Peridium. e–g. Asci. h. J+ reaction of apical ring in Melzer's reagent. i–n. Ascospores. o–q. Germinating spores. r–w. Appressoria. Scale bars: a = 1 mm, b–g = 50 μm , h = 20 μm , o–q = 10 μm , r–w = 5 μm .

the host surface. *Peridium* 10–14 ($\bar{x} = 12 \mu\text{m}$, $n = 10$), outer cells merging with the host epidermal cells, comprising dark brown to black, cells of *textura angularis*. *Asci* 103–150 \times 8–12 μm ($\bar{x} = 123 \times 10 \mu\text{m}$, $n = 10$), 8-spored, unitunicate, cylindrical, pedicellate, with a J+, wedge-shaped, subapical ring. *Ascospores* 47–50 \times 3–5 μm ($\bar{x} = 49 \times 4 \mu\text{m}$, $n = 20$), 2–4-seriate, fusiform, tapering gradually from the center to the ends, centrally 1-septate, not constricted at the septum, with pointed ends, hyaline, with large multi-guttules in each cell, sometimes smaller near the ends, smooth-walled. *Asexual morph*: Undetermined. *Appressoria* not formed.

Culture characteristics – Ascospores germinating on MEA within 24 hours and germ tubes produced from end cells. Colonies on MEA, white, grey and olivaceous, rough on the surface, producing ascomata-like structures, hyphae, septate, branched, smooth-walled (Fig. 9 *viz* f).

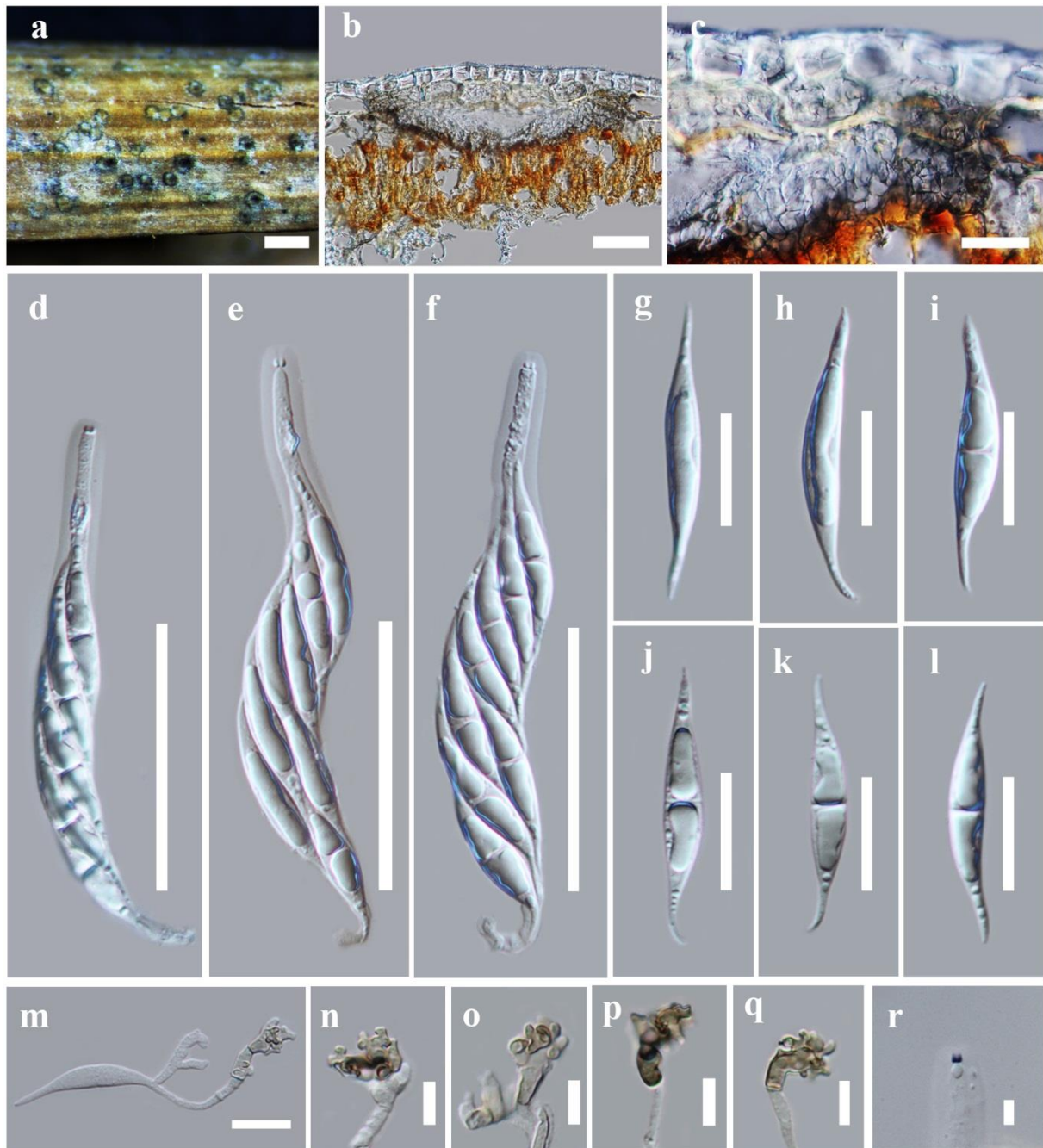


Fig. 7 – *Oxydothis palmicola* (holotype) a. Ascomata on host substrate. b. Section of ascus. c. Peridium. d–f. Asci. g–l. Ascospores. m. Germinating ascospore. n–q. Appressoria. r. J+ reaction of apical ring in Melzer's reagent. Scale bars: a = 500 μm , b = 50 μm , c = 20 μm , d–f = 50 μm , g–l = 20 μm , r = 5 μm .

Material examined – THAILAND, Chiang Rai, on dead petiole of *Rhapis excelsa* (*Areaceae*), 29 July 2014, S. Konta, DTCR03 (MFLU 15-0002, holotype); *Ibid.* (HKAS 95035, isotype); ex-type living culture, MFLUCC 14-0616.

Notes – *Oxydothis rhapsidicola* is morphologically similar to *O. asiatica* J. Fröhl. & K.D. Hyde. and *O. parvula* (H. Syd. & P. Syd.) Petr. in ascomata and ascospore shape. *Oxydothis rhapsidicola* however, differs from both species in having a single large guttule in each of the cells of the ascospores, with small guttules near each end, while *O. asiatica* and *O. parvula* lack these. Phylogenetic analyses indicate that *O. rhapsidicola* is closely related to *O. inaequalis* with moderate support (66% ML, 0.94 PP), but *O. rhapsidicola* differs from *O. inaequalis* in having 2–3-seriate ascospores in the asci, with a large guttules in each ascospore cell. Asci and ascospores are smaller than *O. inaequalis*.

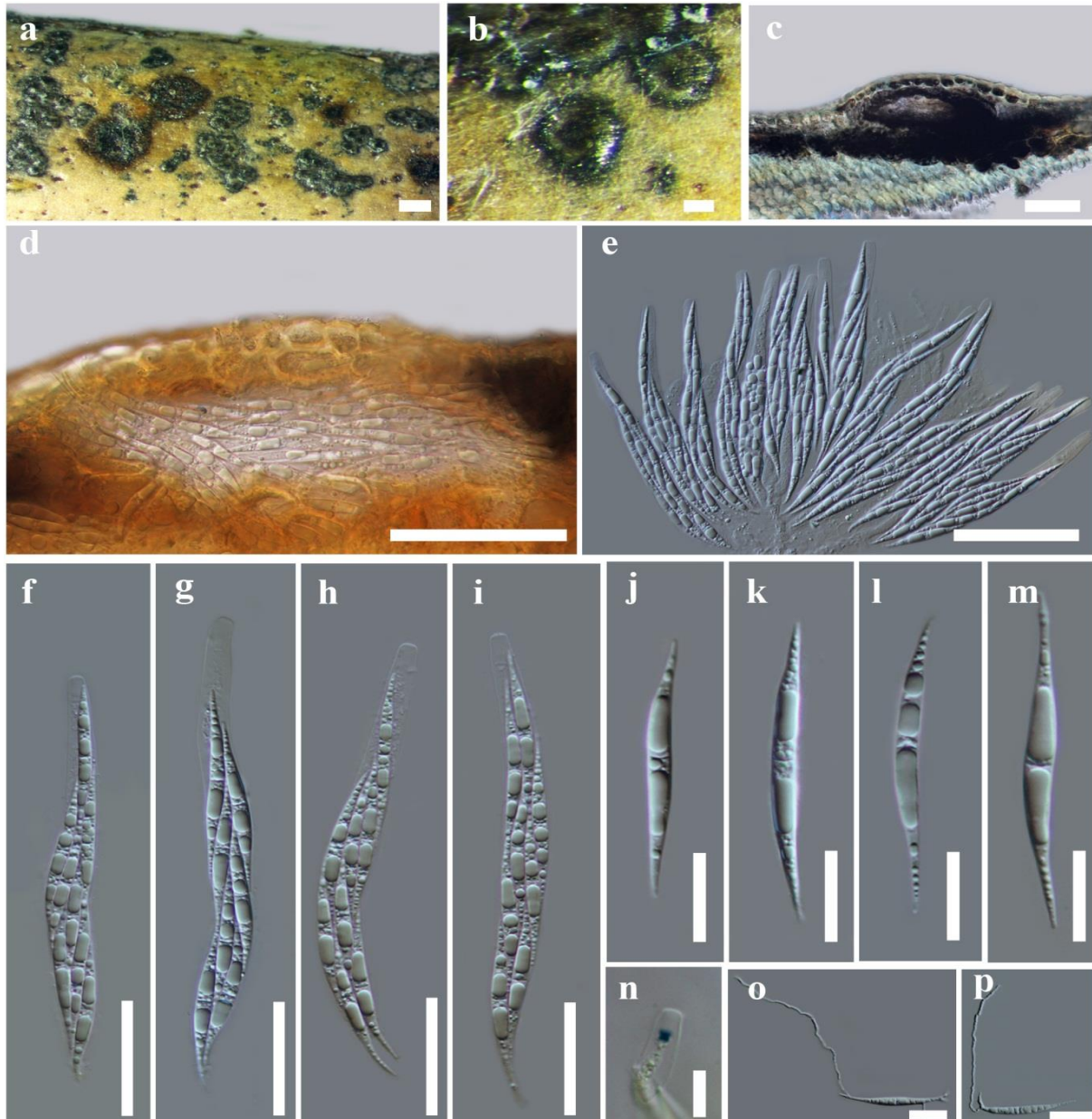


Fig. 8 – *Oxydothis rhapsidicola* (holotype) a. Ascomata on host substrate. b. Close up of ascomata. c–d. Sections of ascomata. f–i. Asci. j–m. Ascospores. n. J+ reaction of apical ring. o–p. Germinating ascospores. Scale bars: a = 500 μ m, b = 100 μ m, c–e = 50 μ m, f–i = 20 μ m, j–m = 10 μ m, n = 5 μ m, o–p = 20 μ m.

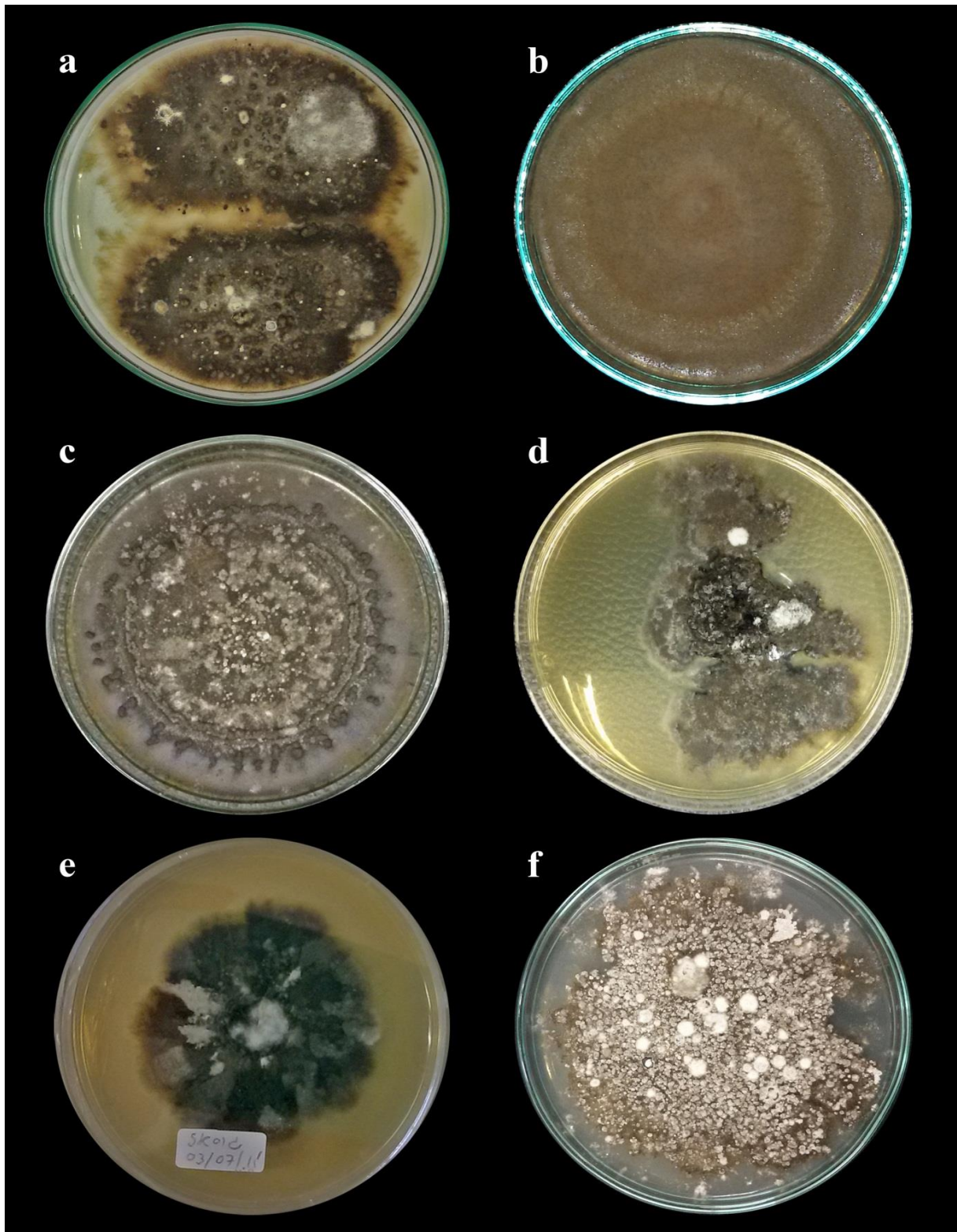


Fig. 9 – *Oxydothis* species on MEA. a. *O. calamicola* from *Calamus* L. b. *O. garethjonesii* from *Eleais* Jacq. c. *O. metroxylo nicola* from *Metroxylon sagu* Rottb. d. *O. metroxylonis* from *Metroxylon sagu* Rottb. e. *O. palmicola* from *Eleais guineensis* Jacq. f. *O. rhapsidicola* from *Rhaps excelsa* (Thunb.) Rehder.

Discussion

Oxydothis is a distinct genus of *Xylariales* that occurs mostly on rachides, petioles and leaves of *Arecaceae* (60 species), *Pandanaceae* (2 species), *Liliaceae* (1 species) and *Poaceae* (3 species). Most previous studies used stromata, orientation of ascomata and ascospore characters as

a basis for distinguishing genera and species. Müller & von Arx (1962) used the ascomata as the most important characteristic to distinguish *Oxydothis* from *Leiosphaerella*. Hyde (1994) mention that the ascoma orientation in species of *Oxydothis* is species dependent, with some having ascomata that are parallel to the host surface (e.g. *O. grisea*), while in others it can be oblique or perpendicular (e.g. *O. nypae* K.D. Hyde & Nakagiri). The ascospore is a unique and reliable characteristic when distinguishing species of *Oxydothis*, especially their shape (Hyde 1993b, 1994, Shenoy et al. 2005). Most *Oxydothis* species are saprobic on leaves, rachides and petioles, while a single species is pathogenic (Fröhlich & Hyde 1994) and some species are endophytes (Hyde 1994, Fröhlich et al. 2000). *Oxydothis* species may also be host-specific (Zhou & Hyde 2001).

Oxydothis was placed in the order *Xylariales* (Sordariomycetes) based on phylogenetic analyses and morphological characteristics (Kang et al. 1998, 1999, 2002, Smith et al. 2003, Hidayat et al. 2006). In this paper, phylogenetic analyses of combined ITS, LSU and SSU sequence data (Fig 1) indicate that *Oxydothis* can be placed in *Xylariales* (Fig. 1) with moderate support. *Oxydothis* species cluster as a monophyletic group and are placed within the new family *Oxydothidaceae* in *Xylariales*. The low bootstrap values in some cases are probably because we did not obtain ITS sequence data for *O. Chiangraiensis* and *O. calamicola*.

An asexual morph has only been reported for a single *Oxydothis* species (Samuels & Rossman 1987). Most *Oxydothis* species have not produced an asexual morph in culture, except *O. selenosporellae* Samuels & Rossman. Hyde (1994) could not find the asexual morph of *Oxydothis* on media, although mature ascomata were produced. In our study, we also could not obtain the asexual morph and found only ascomata in culture in some strains.

In this study, we observed that appressoria were produced by germinating ascospores in four of the six species, even though the ascospores were from apparent saprobes. Appressoria are penetration structures produced in pathogenic taxa and aid infection of the host (Xu & Hamer 1996, Bechinger et al. 1999) and have rarely been observed in saprobic fungi (Phukhamsakda et al. 2016). They are specialized infection structures for penetrating plants that are differentiated from the tips of fungal hyphae when they come into contact the plant surface (Xu & Hamer 1996). The appressoria produced in this study were the irregular type with rigid melanin-pigmented cell walls. It is remarkable that saprobic taxa produce appressoria and this suggests that they may be adaptations for an endophytic life style. It is thought that endophytes live asymptotically within the tissues of host plants and become active at host senescence and become the first colonizers of dead material (Hyde et al. 2007, Promputtha et al. 2007). *Oxydothis* species are the initial colonizers of dead palm material (K.D. Hyde, pers obs). Our observations therefore suggest that as *Oxydothis* species produce appressoria, they can infect healthy plants as endophytes and then become early colonizers of dead material as saprobes.

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