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Establishment of Zygosporiaceae *fam. nov.* (Xylariales, Sordariomycetes) based on rDNA sequence data to accommodate *Zygosporium*

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Abstract

Zygosporium species are widespread on hosts and usually associated with monocotyledonous plants including Arecaceae (palms) and Pandanaceae. *Zygosporium* forms colonies as darkened spots on the host surface. This study recovers two *Zygosporium* species from China and Thailand and makes an attempt to clarify the systematic placement of *Zygosporium* based on morphological characteristics and phylogenetic analyses based on rDNA sequence data. Phylogenetic analyses of a combined LSU and ITS sequence data generated under Maximum Likelihood and Bayesian criteria indicate that *Zygosporium* species form a strongly supported monophyletic lineage basal to the Coniocessiaceae and not to the Microdochiaceae as previously assumed. Given the distinct morphological features of *Zygosporium* and its strongly supported monophyletic nature, a new family, Zygosporiaceae is introduced within the Xylariales.

Key words – Asexual fungi – hyphomycetes – multigene phylogeny – new family

Introduction

Zygosporium (Xylariales, Sordariomycetes) species are usually found as saprobes occurring on various angiosperms and some gymnosperms with a cosmopolitan distribution from temperate to tropical regions (Photita et al. 2001, Whitton et al. 2002, Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011, Pratibha et al. 2012, Taheriyan et al. 2014, Farr & Rossman 2017). Currently there are 16 described species (Wijayawardene et al. 2017). Sequence data of

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Zygosporium gibbum (Sacc., M. Rousseau & E. Bommer) S. Hughes is available in GenBank. *Zygosporium majus* Piroz. has been reported as a parasite on leaves of *Brillantaisia patula* while *Z. gibbum* was found as a hyperparasite on *Coleosporium plumeriae*, the latter causing *Plumeria* (frangipani) rust (Ellis 1976, Abbas et al. 2011, Manimohan & Mannethody 2011). *Zygosporium masonii* has been reported as a fungal antagonist, which produces some unidentified secondary metabolites that are effective against plant pathogens and multi-drug resistant bacteria (Kanoh et al. 2008, Ajith & Lakshmidevi 2012).

Zygosporium was first described by Montagne (1842) with the type species *Zygosporium oscheoides* Mont. The genus is known only from its hyphomycetous asexual morph and is characterized by setiform conidiophores, dark pigmented, incurved vesicular cells that give rise to 2–4 ampulliform conidiogenous cells and single-celled conidia that are typically ellipsoid or globose and smooth or variously ornamented. Only one conidium is produced per conidiogenous cell (Mason 1941, Hughes 1951, Meredith 1962, Photita et al. 2001, Whitton et al. 2002, Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011).

Mason (1941) reviewed the genus and referred to the vesicular portion of the conidiophore as a 'falx', and the setiform conidiophores that gives rise to the vesicular cells as the 'falciphore'. Some authors have used these terms with respect to *Zygosporium* taxonomy (Hughes 1951, Barron 1968, Pirozynski 1972, Thakur & Udipi 1976, Subramanian & Bhat 1987), but there is really little need for such terms whose definitions appear to be rather vague (Photita et al. 2001, Whitton et al. 2002, Manoharachary et al. 2006). *Zygosporium* has usually been described as lacking true setae, with the setiform structures being described as conidiophores or setiform conidiophores (Montagne 1842, Mason 1941, Hughes 1951, Ellis 1971, 1976, Photita et al. 2001, Whitton et al. 2002, Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011). However, it has been very confusing to use these morphological features to segregate species. For example, *Z. deightonii* M.B. Ellis (Ellis 1976) and *Z. echinosporum* Bunting & E.W. Mason (Hughes 1951) produced setiform structures without vesicles and these have been referred to the true setae group (Whitton et al. 2002). In contrast, *Zygosporium* species which produce vesicles from the side of the setiform structures referred as setiform conidiophores include *Z. geminatum* S. Hughes, *Z. majus*, *Z. minus* S. Hughes and *Z. oscheoides* (Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011). The differences of setiform conidiophores and vesicular conidiophores was discussed following a terminology more or less utilized by Ellis (1971, 1976) and discussed by Whitton et al. (2002), Manoharachary et al. (2006), McKenzie et al. (2007) and Abbas et al. (2011).

Zygosporium species have mostly been identified using only morphological characteristics given the paucity of DNA sequence data and few species are being encountered and described in the field. To date, there are 16 described species which include 27 epithets listed in Index Fungorum (2017) but the genus is poorly represented with sequence data in GenBank. The placement of the genus is uncertain and is listed in Ascomycota genera *incertae sedis* (Wijayawardene et al. 2012, 2017, Index Fungorum 2017). However Hernández-Restrepo et al. (2017) showed that the genus belongs to the Xylariales based on analysis of LSU sequence data.

During our investigations in China and Thailand, we recovered two *Zygosporium* species, which are described and illustrated herein. Phylogeny of these taxa, together with *Z. gibbum* was analysed based on a combined LSU and ITS sequence data. A new family Zygosporiaceae, is introduced herein to accommodate *Zygosporium*.

Materials & Methods

Isolation and identification

The fungal species were collected from dead leaves of *Ptychosperma macarthurii* (H. Wendl. ex H. J. Veitch) H. Wendl. ex Hook. f. (Arecaceae) in Thailand and from *Pandanus* sp. (Pandanaceae) in China, during the years 2014–2017. Samples were returned to the laboratory for examination and description of morphological characters. The specimens were observed under a Motic SMZ 168 series dissecting stereo-microscope. Macro-morphological structures were

photographed using a Discovery V.8 stereo-microscope fitted with a CARL ZEISS Axio Cam ERc5S microscope camera. The conidial structures were picked up with a surgical needle and transferred into 10% lacto-glycerol on a clean slide and examined using a Nikon Eclipse 80*i* compound microscope and photographs taken with a Canon 600D digital camera using DIC microscopy. Tarosoft® Image Frame Work version 0.9.0.7 and Adobe Photoshop CS5 Extended version 10.0 software (Adobe Systems, USA) were used for measurements and compiling the photographic plates respectively. The species were described following the terminologies of subsequent authors to avoid taxonomic confusion (Whitton et al. 2002, Manoharachary et al. 2006, McKenzie et al. 2007, Abbas et al. 2011).

Single spore isolation was carried out to obtain pure cultures after a few conidiophores with conidia were picked from the substrate using a sterilized needle and placed in few drops of sterilized distilled water mounted on a sterilized cavity slide. Conidiophores with conidia were stirred to separate the conidia and obtain the conidia suspension. The suspension was inoculated on to fresh malt extract agar (MEA) or potato dextrose agar (PDA) plates and incubated at room temperature. Germinating conidia were transferred aseptically to PDA and MEA plates and grown at 25–30°C in alternating day and night light conditions. The characters of fungal colonies were observed and recorded after one week and at weekly intervals. The specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), Yunnan, China. Living cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Institute of Botany Culture Collection (KUMCC). Faces of Fungi numbers and Index Fungorum numbers are registered (Jayasiri et al. 2015, Index Fungorum 2017). Establishment of the new family followed guidelines as described in Liu et al. (2016).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia and individual fungal conidia were scraped off and placed in a 1.5 ml the sterilized micro-centrifuge tube for DNA extraction as outlined by Jeewon et al. (2002). The Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China) was used to extract fungal genomic DNA from mycelium and the Omega DNA Extraction Kit (OMEGA® E.Z.N.A.) was used to extract fungal genomic DNA from conidia, following the protocols in the manufacturer's instructions. DNA amplification was performed by polymerase chain reaction (PCR) using the nuclear large subunit ribosomal RNA gene (28S, LSU) and the internal transcribed spacer (ITS). Respective primers LROR and LR5 (Vilgalys & Hester 1990) were used to amplify the partial ribosomal RNA for the LSU gene; ITS5 and ITS4 primer pairs were used to amplify the ITS and 5.8S regions of the rDNA gene (White et al.1990) following the protocols as described in Li et al. (2017). Purification and sequencing of PCR products were carried out at Shanghai Majorbio Biopharm Technology Co., China.

Sequence alignment and phylogenetic analyses

Sequences generated from this study were analyzed together with related taxa in the order Xylariales, which were obtained from GenBank and derived from Maharachchikumbura et al. (2015, 2016) and Senanayake et al. (2014, 2015) (Table 1). Consensus sequences were obtained from both forward and reverse directions using Geneious Pro.v4.8.5. Sequence alignments were automatically aligned with MAFFT v.6.864b (Katoh & Standley 2013; http://mafft.cbrc.jp/alignment/server/) and manually aligned wherever necessary in MEGA version 6.0 (Tamura et al. 2013). The sequence datasets were combined using BioEdit v.7.2.3 (Hall 1999). The individual gene alignments were initially analysed for comparing the tree topologies and the combined LSU and ITS sequence data was analysed and conducted the phylogenetic tree based upon the Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian Inference (BI) analyses.

The evolutionary model of nucleotide substitution for BI and RAxML were selected independently for each locus using MrModeltest 2.2 (Nylander 2004), implemented in PAUP v.

4.0b10 under the Akaike Information Criterion (AIC).RAxML was performed by using RAxMLGUI v. 0.9b2 (Stamatakis 2006, 2014, Stamatakis et al. 2008, Silvestro & Michalak 2010) with 1,000 rapid bootstrap replicates using the GTR + GAMMAI model of nucleotide substitution.

Bayesian Inference analysis (BI) (Huelsenbeck et al. 2001) was conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov chain Monte Carlo sampling (BMCMC). Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation and 10,000 trees were obtained. The first 2,000 trees, representing the burn-in phase of the analyses, were discarded, the remaining 8,000 trees were used for calculating posterior probabilities in the majority rule consensus tree (critical value for the topological convergence diagnostic is 0.01) (Cai et al. 2006).

The phylograms were represented in Treeview X (Page 1996), drawn in Microsoft Power Point and converted to jpeg file in Adobe Photoshop version CS5 (Adobe Systems, USA). The newly generated sequences were submitted to GenBank (Table 1). The alignment was deposited in TreeBASE (2017) under the accession number 21406.

Table 1 Taxa used in the phylogenetic analysis and their corresponding GenBank numbers. The newly generated sequences are indicated in red bold, while the type strains are in black bold.

Species	**Culture collection**	**GenBank accession numbers**	
		LSU	**ITS**
Apiosordaria verruculosa	**F-152365**	**AY346258**	**–**
Apiospora bambusae	ICMP 6889	DQ368630	–
Apiospora setosa	ICMP 4207	DQ368631	–
Arecophila bambusae	**HKUCC 4794**	**AF452038**	**–**
Arthrinium hyphopodii	**MFLUCC 15-0003**	**KY356093**	**KY356088**
Arthrinium phaeospermum	HKUCC 3395	AY083832	–
***Arthrinium* sp.**	**MFLUCC 15-0002**	**KU863135**	**KU940148**
Arthrinium subglobosa	**MFLUCC 11-0397**	**KR069113**	**KR069112**
Atrotorquata spartii	**MFLUCC 13-0444**	**KP325443**	**–**
Barrmaelia moravica	**CBS 142769**	**MF488987**	**MF488987**
Barrmaelia rappazii	**CBS 142771**	**MF488989**	**MF488989**
Barrmaelia rhamnicola	**CBS 142772**	**MF488990**	**MF488990**
Beltrania rhombica	**CPC 27482**	**KX306778**	**KX306749**
Beltraniella carolinensis	**DQ810262**	**DQ810233**	**–**
Cainia anthoxanthis	**MFLUCC 15-0539**	**KR092777**	**NR138407**
Cainia graminis	**CBS 136.62**	**AF431949**	**–**
Cainia graminis	MFLUCC 15-0540	KR092781	KR092793
Camillea obularia	ATCC 28093	KY610429	KY610384
Ciferriascosea uniseptata	MFLUCC 15-0542	KR092776	KR092786
Ciferriascosea wavyseptata	**MFLUCC 15-0541**	**KR092778**	**KR092789**
Coniocessia anandra	**Co108**	**GU553349**	**GU553338**
Coniocessia cruciformis	Co113	GU553348	GU553337
Coniocessia cruciformis	Co115	GU553346	GU553335
Coniocessia cruciformis	Co116	GU553347	GU553336
Coniocessia maxima	**Co117**	**GU553344**	**GU553332**
Coniocessia minima	Co111	GU553345	GU553334
Coniocessia nodulisporioides	**CBS 281.77**	**AJ875224**	**GU553342**
Coniocessia nodulisporioides	**Co106**	**GU553351**	**GU553343**
Coniocessia nodulisporioides	Co107	GU553350	GU553339
Coniocessia nodulisporioides	Co126	GU553352	GU553333
Creosphaeria sassafras	**CBS119001**	**DQ840056**	**–**

Table 1 Continued.

Species	Culture collection	GenBank accession numbers	
		LSU	ITS
Daldinia concentric	CBS 113277	KT281895	AY616683
Diatrype disciformis	**MFLUCC 15-0538**	**KR092784**	**KR092795**
Diatrype palmicola	**MFLUCC 11-0018**	**KP744481**	**KP744439**
Diatrype whitmanensis	**ATCC MYA-4417**	**FJ430587**	**FJ430595**
Eutypa flavovirens	**MFLUCC 13-0625**	**KR092774**	**KR092798**
Eutypa lata	**CBS 208.87**	**DQ836903**	**DQ006927**
Graphostroma platystoma	**CBS 270.87**	**DQ836906**	**JX658535**
Hyponectria buxi	**UME 31430**	**AY083834**	**–**
Hypoxylon fragiforme	MUCL 51264	KM186295	KC477229
Idriella lunata	**CBS 204.56**	**KP858981**	**KP859044**
Idriella lunata	CBS 177.57	KP858980	KP859043
Immersidiscosia eucalypti	**KT 2091**	**AB593722**	**AB594790**
Immersidiscosia eucalypti	**KT 2115**	**AB593723**	**AB594791**
Iodosphaeria ongrenensis	**MFLU 15-0393**	**KR095283**	**KR095282**
Jackrogersella multiforme	CBS 119016	KT281893	KC477234
Kretzschmaria deusta	CBS 163.93	KT281896	KT281901
Lopadostoma americanum	**CBS 133211**	**KC774568**	**NR 132027**
Lopadostoma dryophilum	**CBS 107.39**	**KC774573**	**KC774573**
Lopadostoma fagi	**MFLUCC 15-0008**	**KU820973**	**KU820972**
Lopadostoma quercicola	**CBS 134633**	**KC774610**	**NR 132035**
Lopadostoma turgidum	**CBS 133207**	**KC774618**	**KC774618**
Melogramma campylosporum	**MBU**	**JF440978**	**JF440978**
Microdochium fisheri	**CBS 242.91**	**KY777594**	**KY777595**
Microdochium phragmitis	**CBS 285.71**	**KP858949**	**KP859013**
Microdochium tainanense	**CBS 269.76**	**KP858945**	**KP859009**
Oxydothis garethjonesii	**MFLUCC 15-0287**	**KY206762**	**KY206773**
Oxydothis metroxylonicola	**MFLUCC 15-0281**	**KY206763**	**KY206774**
Oxydothis metroxylonis	**MFLUCC 15-0283**	**KY206764**	**KY206775**
Oxydothis palmicola	**MFLUCC 15-0806**	**KY206765**	**KY206776**
Phlogicylindrium eucalyptorum	**CBS 111689**	**KF251708**	**KF251205**
Phlogicylindrium uniforme	**CBS 131312**	**JQ044445**	**JQ044426**
Podosordaria tulasnei	CBS 128.80	KT281897	KT281902
Poronia punctata	CBS 656.78	KT281900	KT281904
Pseudomassaria carolinensis	**PC1**	**DQ810233**	**–**
Pseudomassaria chondrospora	MFLUCC 15-0545	KR092779	KR092790
Pseudomassaria sepincoliformis	**CBS129022**	**JF440984**	**JF440984**
Sarcoxylon compunctum	CBS 359.61	KT281898	KT281903
Seynesia erumpens	SMH 1291	AF279410	–
Sordaria fimicola	**HKUCC 3714**	**AF132330**	**AY681188**
Vialaea mangifia	**MFLUCC 12-0808**	**KF724975**	**KF724974**
Vialaea minutella	BRIP 56959	KC181924	KC181926
Xylaria hypoxylon	**CBS 122620**	**KY610495**	**KY610407**
Xylaria polymorpha	MUCL 49884	KT281899	KY610408
Zygosporium gibbum	**CBS 137306**	**KY853546**	**KY853482**
Zygosporium minus	**HKAS99625**	**MF621586**	**MF621590**
Zygosporium oscheoides	**MFLUCC 14-0402**	**MF621585**	**MF621589**

Abbreviations: ATCC: American Type Culture Collection, Virginia, U.S.A.; **BRIP**: Plant Pathology Herbarium, Department of Agriculture, Fisheries and Forestry, GPO Box 267, Brisbane, Queensland 4001, Australia; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **Co**: Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP), 25-28 rue du Docteur Roux 75724 Paris Cedex 15 France; **CPC**: Culture collection of Pedro Crous, housed at CBS, Utrecht, The Netherlands; **F**: Fernandez, F.A., The Field Museum of Natural History, 1400 S. Lake Shore Dr., Chicago, IL 60605-2496, USA; **GKM**: G.K. Mugambi; **HKAS**: The Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Kunming, China. **HKUCC**: Ecology & Biodiversity, University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China; **ICMP**: International Collection of Micro-organisms from Plants, Landcare Research, Private Bag 92170, Auckland, New Zealand; **IFO**: Institute for Fermentation, Osaka, Yodogawa-ku, Osaka, Japan; **KT**: K. Tanaka; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **MUCL**: (Agro) industrial fungi & yeasts collection, Mycotheque de l'Universite catholique de Louvain Place Croix du Sud 3,B-1348 Louvain-la-Neuve, Belgium; **PC**: P.W. Crous; **SMH**: S.M. Huhndorf; **UME**: The herbarium of the Department of Ecology and Environmental Science of Umeå University, Umeå, Sweden.

Results

Taxonomy

The family Zygosporiaceae is introduced to accommodate a single genus *Zygosporium*. The family is justified based on morphologically differences from other taxa in Xylariales and is phylogenetically supported. Detailed descriptions, illustrations of macro-and micro-morphological characteristics of two representative *Zygosporium* species are provided.

Zygosporiaceae J.F. Li, Phookamsak & K.D. Hyde, fam. nov.

Synonym: Zygosporaceae Locq., Mycol. gén. struct. (Paris): 202. 1984. (nom. inval., Art 39.1, Melbourne Code).

Index Fungorum number: IF10473; Facesoffungi number: FoF03760

Saprobic on plant litter, especially on monocotyledons. *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Setae* or setiform conidiophores with sphaerical apex. *Conidiophores* macronematous, mononematous, solitary or in small groups, pale brown, thin-walled, unbranched, septate, smooth, bearing swollen dark brown, thick-walled vesicles. *Conidiogenous cells* holoblastic, discrete, hyaline or light brown, smooth, sphaerical to ellipsoid, borne in groups of 2–4 conidiogenous cells on the vesicular cell. *Conidia* solitary, aseptate, hyaline or pale brown, globose or ellipsoid, thin- or thick-walled.

Notes – Zygosporiaceae was invalidly published as 'Zygosporaceae' (Nom. inval., Art. 39.1, Melbourne) by Locquin (1984) to accommodate the genus *Zygosporium* (Index Fungorum 2017, MycoBank 2017). However, the taxonomic placement of *Zygosporium* is still ambiguous due to its unique morphological characters of setae/setiform or vesicular conidiophores, with a sphaerical apex and distinct vesicles which are different from other hyphomycetous taxa. Lack of molecular data to clarify its phylogenetic position is also a problem. In this study, two representative species, *Z. oscheoides* and *Z. minus* were collected and molecular data was obtained. Phylogenetically, *Zygosporium* forms a distinct lineage separated from other taxa in Xylariales. Therefore, the new family Zygosporiaceae is validly introduced herein based on the morphological and phylogenetic support.

Type genus – *Zygosporium* Mont.

Zygosporium Mont., in Sagra, *Annls Sci. Nat.*, Bot., sér. 2 17: 120 (1842)

Facesoffungi number: FoF03761

Saprobic on plant litter. *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Setae*/*setiform conidiophores* with a sphaerical apex. *Conidiophores* macronematous, mononematous, pale brown, thin-walled, unbranched, septate, smooth, bearing swollen dark brown, thick-walled vesicles on the base, solitary or in small groups. *Conidiogenous cells* usually on the base of conidiophores, born on

the apex of thick-walled dark vesicles, holoblastic, thin-walled, smooth, discrete, sphaerical to ellipsoid, borne in groups of 2–4 conidiogenous cells on the vesicular cell. *Conidia* solitary, aseptate, hyaline or pale brown, globose or ellipsoid, smooth to minutely verruculose or verruculose, thin- or thick-walled.

Notes – Montagne (1842) established *Zygosporium* and designated *Z. oscheoides* as the type species. Morphologically, the genus has a set of unique characters consisting of darkly pigmented, incurved vesicular cells usually born from the side of setiform conidiophores and the vesicles give rise to ampulliform conidiogenous cells that produce aseptate, globose or ellipsoid, smooth or variously ornamented conidia (Mason 1941, Hughes 1951).

Type species – *Zygosporium oscheoides* Mont., Annls Sci. Nat., Bot., sér. 2, 17: 121 (1842)

Zygosporium oscheoides Mont., Annls Sci. Nat., Bot., sér. 2, 17: 121 (1842) Fig. 1

Facesoffungi number: FoF03285

Saprobic on dead leaves of *Ptychosperma macarthurii* (Arecaceae). **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* effuse, white to light pink. *Mycelium* immersed or partly superficial, composed of smooth, thin-walled, white or light pink hyphae. *Conidiophores* setiform portion 24–45.5 µm long × 2–4 µm diam. ($\overline{x}$ = 35.4 × 2.7 µm, n = 10), macronematous, mononematous, consisting of a setiform portion with a vesicle borne on a short cell near its base, light brown to pale brown, thin-walled, smooth, solitary or in small groups; *Conidiogenous cells* (6.6–)8.2–15.5 µm long × 4.7–10 µm diam. ($\overline{x}$ = 11.5 × 6.8 µm, n = 20), on the apex of vesicular conidiophores, holoblastic, thick-walled in the dark vesicular cell, light brown to dark brown, smooth, solitary. *Conidia* (8.6–)9.9–10.6 × 9.2–10 µm diam. ($\overline{x}$ = 10.5× 9.6 µm, n = 10), smooth to minutely verruculose, solitary, aseptate, hyaline to pale brown, globose to ellipsoid, thin-walled.

Cultural characteristics – Conidia germinating on MEA within 10 hours, producing a single germ tube, colonies reaching 5 mm diam. within 20 days at 30ºC, cottony, white to pale pink. Mycelium partly superficial, partly immersed, slightly effuse, with regular edge, white to light pink hyphae; sexual or asexual spores not formed within 60 days.

Material examined – THAILAND, Chiang Rai Province, Mae Fah Luang University, on dead leaves of *Ptychosperma macarthurii* H. Wendl. & H.J. Veitch (Arecaceae), 9 January 2014, J.F. Li, H-17 (MFLU 16-0273, reference specimens designated here), living culture MFLUCC 14-0402, KUMCC 17-0172.

Known hosts (not molecular based) – *Agave fourcroydes* Lem., *Areca* sp., Arecaceae, *Carica papaya* Linn., *Chamaedorea* sp., *Cocos nucifera* Linn., *Ficus carica* Linn., *Ficus* sp., *Jasminum sambac* (L.) Aiton, *Musa sapientum* (L.) O. Ktze., *Pinus elliottii* Engelm., *Podocarpus* sp., *Pucciniopsis caricae* Earle, *Rhapis flabelliformis* L'Hert, *Sabal palmetto* (Walt.) Lodd., *Saccharum officinarum* Linn., *Thuja* sp., *Tillandsia* sp., *Ptychosperma macarthurii* Linn. (Montagne 1842, Mason 1941, Hughes 1951, Ellis 1971, 1976, Whitton et al. 2002, Farr & Rossman 2017).

Distribution – distributed in tropical and temperate regions.

Notes: *Zygosporium oscheoides* is unique in having a vesicular cell produced from the side of the setiform conidiophores. Apical cell sub-hyaline of the setiform conidiophores is smooth, acute or narrowly clavate. The conidia are sphaerical, globose to ellipsoid, hyaline to pale brown, smooth to minutely verruculose. Our isolate (MFLU 16-0273) is morphologically typical *Z. oscheoides* and was collected from different host and location with holotype. Therefore, MFLU 16-0273 is assigned here as the reference specimen following the procedures described in Ariyawansa et al. (2014).

Zygosporium minus S. Hughes, Mycological Papers 44: 6 (1951) Fig. 2

Facesoffungi number: FoF03762

Saprobic on fallen dead and decaying leaves of *Pandanus* sp. Sexual morph: Undetermined. **Asexual morph**: *Colonies* effuse to compact, forming a thin layer, spread on the substrate surface, numerous. *Mycelium* mostly superficial, consisting of cylindrical, dark brown to black, smooth, septate, branched hyphae with slightly thickened walls. *Conidiophores* setiform portion 43–78.6

µm long × 2.9–6.4 µm diam. ($\overline{x}$ = 56.6 × 4.5 µm, n = 20), macronematous, mononematous, scattered, wide towards the base, 3–4-septate, erect, straight or slightly flexuous, smooth, unbranched, dark brown at the base with connecting the conidiophores with each other, sub-hyaline narrowing towards the apex, swollen vesicles on short stalk arising from the side of the first cell of the conidiophores, vesicular cylindrical, brown, smooth. *Conidiogenous cells* 13.1–27.7 × 6.4–10.4 µm ($\overline{x}$ = 20 × 8.3 µm, n = 20), holoblastic, discrete, terminal, ellipsoidal to ampulliform, upwardly curved, smooth, pale brown, apex obtuse, thin-walled, borne in pairs, arising directly from the vesicular cell. *Conidia* 5.3–10 × 5.3–10.7 µm ($\overline{x}$ = 7.4 × 6.9 µm, n = 40), solitary, aseptate, verruculose, hyaline, globose to broadly ellipsoid, thick-walled.

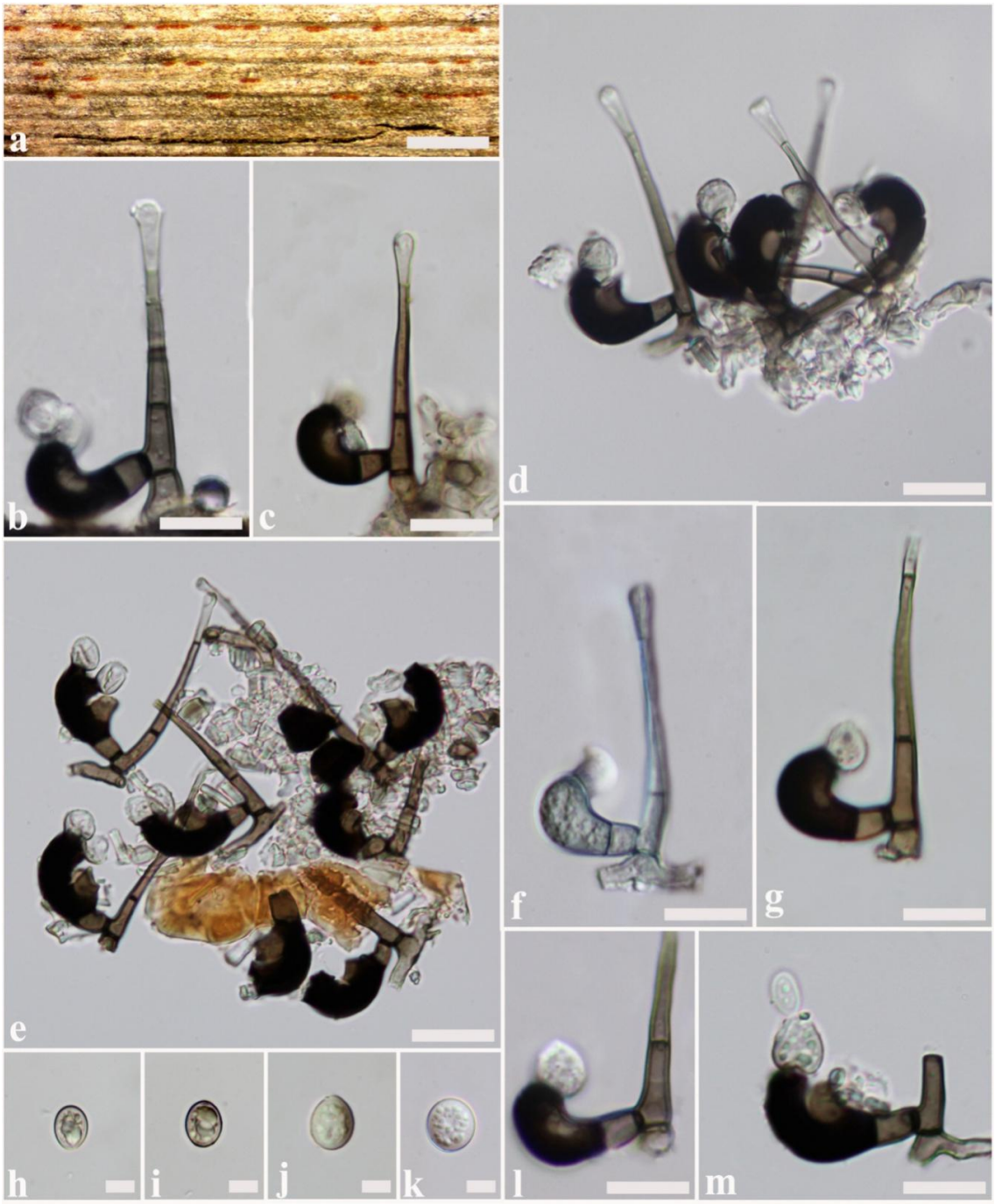

Figure 1 – *Zygosporium oscheoides* (MFLU 16-0273) a Appearance of the fungus on dead leaves of *Ptychosperma macarthurii*. b–g Conidiophores with vesicles. h–k Conidia. l–m Vesicles and conidiogenous cells. *Scale bars* a = 200 µm, b–g = 10 µm, h–m = 5 µm.

Material examined – CHINA, Yunnan, Xishuangbanna Tropical Botanical Garden, on fallen dead and decaying leaves of *Pandanus* sp. (Pandanaceae), 28 April 2017, R. Phookamsak and N. I. de Silva, XTBG17 (HKAS99625, reference specimens designated here).

Hosts (not based on molecular data) – *Polyalthia longifolia* Sonn., *Pandanus* sp. (Hughes 1951, Whitton et al. 2002).

Distribution – China, Cuba, Ghana, Great Britain, Myanmar, Sierra Leone, Tamil Nadu (India), Tanzania, Venezuela, West Bengal (India), Zambia.

Notes – Hughes (1951) introduced *Zygosporium minus* based on its morphology having subulate conidiophores, with thickened at the base, bearing a single vesicle laterally on a short stalk and sphaerical, hyaline to very pale brown, verruculose conidia. This species can be found on *Pandanus* spp. worldwide (Whitton et al. 2002). This is first record of *Z. minus* from China. DNA sequence analyses indicate that *Z. oscheoides* and *Z. minus* cluster together with very high support (100% ML/0.99 PP; Fig 3). Due to same species found on different host and location with holotype, HKAS99625 is assigned here as the reference specimen following the procedures described in Ariyawansa et al. (2014).

Phylogenetic analyses

The combined LSU and ITS sequence data comprised 78 taxa with *Apiosordaria verruculosa* (F-152365) and *Sordaria fimicola* (HKUCC 3714) selected as the outgroup taxa. The best scoring RAxML tree (Final ML Optimization Likelihood: -21750.480571) is chosen to represent the phylogenetic relationships of *Zygosporium* spp. with other genera in Xylariales (Sordariomycetes) (Fig. 3). The phylogenetic trees obtained from maximum likelihood and Bayesian Inference analyses gave essentially similar topologies with regards to the position of *Zygosporium* within the Xylariales and did not differ significantly. *Zygosporium oscheoides* (MFLUCC 14-0402), *Z. minus* (HKAS99625) and *Z. gibbum* (CBS 137306) isolates sequenced and analysed constitute a strongly supported distinct clade basal to the Coniocessiaceae (76% ML, 0.98 PP; Fig. 3).

Discussion

The taxonomy of *Zygosporium* has always been a contentious issue especially with regards to its taxonomic position. Only a few species have been described so far with limited DNA sequence data, which make it almost impossible to refer them to an appropriate familial position. In addition, its prevalence only in its asexual morphs (with no known sexual morphs to date) makes it also difficult to ascertain to which group of fungi it might be closely related to and hence DNA sequence data is essential to clarify taxonomic relationships. It is noteworthy to mention that *Zygosporium* species are characterized by unique and peculiar morphological characters, in particular vesicular cells usually born from the side of setiform conidiophores and the vesicles give ampulliform conidiogenous cells that set it apart from other known asexual morphs of the Xylariales.

Whitton et al. (2002) reviewed the taxonomy of the genus *Zygosporium* while describing two new species and provided key information on species differences which was later updated by Manoharachary et al. (2006), McKenzie et al. (2007) and Abbas et al. (2011). There was no DNA sequence data for any species until Hernández-Restrepo et al. (2017) recovered *Z. gibbum* from unidentified dead leaves in Spain. The latter analysed rDNA sequence data and reported a close affinity of *Z. gibbum* to the family Microdochiaceae, but this relationship received no support at all. The authors also reported that *Zygosporium* constitutes a separate and independent lineage within the Xylariales and referred it to an uncertain position due to sparse taxon sampling.

In this study, two additional species, viz *Zygosporium oscheoides* and *Z. minus* collected from Thailand and China, are analysed phylogenetically to provide a better taxonomic interpretation. All three species of *Zygosporium* analysed herein constitute a strong monophyletic group with high support and basal to the family Coniocessiaceae, as compared to a sister relationship to the family Microdochiaceae as proposed by Hernández-Restrepo et al. (2017). It should be pointed out that *Zygosporium* is characterized by asexual morphs that are quite distinct

from both families. Microdochiaceae species are characterized by globose, erumpent stromata of minute, hyaline cells, small papillate conoid conidiogenous cells and solitary, fusiform to subfalcate, hyaline conidia (Hernández-Restrepo et al. 2016). *Zygosporium* species are morphologically distinguished from species in Coniocessiaceae as *Zygosporium* species have dark setae and setiform conidiophores with a sphaerical head and distinct vesicles. On the other hand, Coniocessiaceae species lack setae, have hyaline, macronematous or micronematous conidiophores with integrated or discrete conidiogenous cells that bear conidia on conspicuous denticles (Asgari & Zare 2010). No sexual morph is known for *Zygosporium*, but Coniocessiaceae species have distinct ascomata, asci and ascospores (Asgari & Zare 2010).

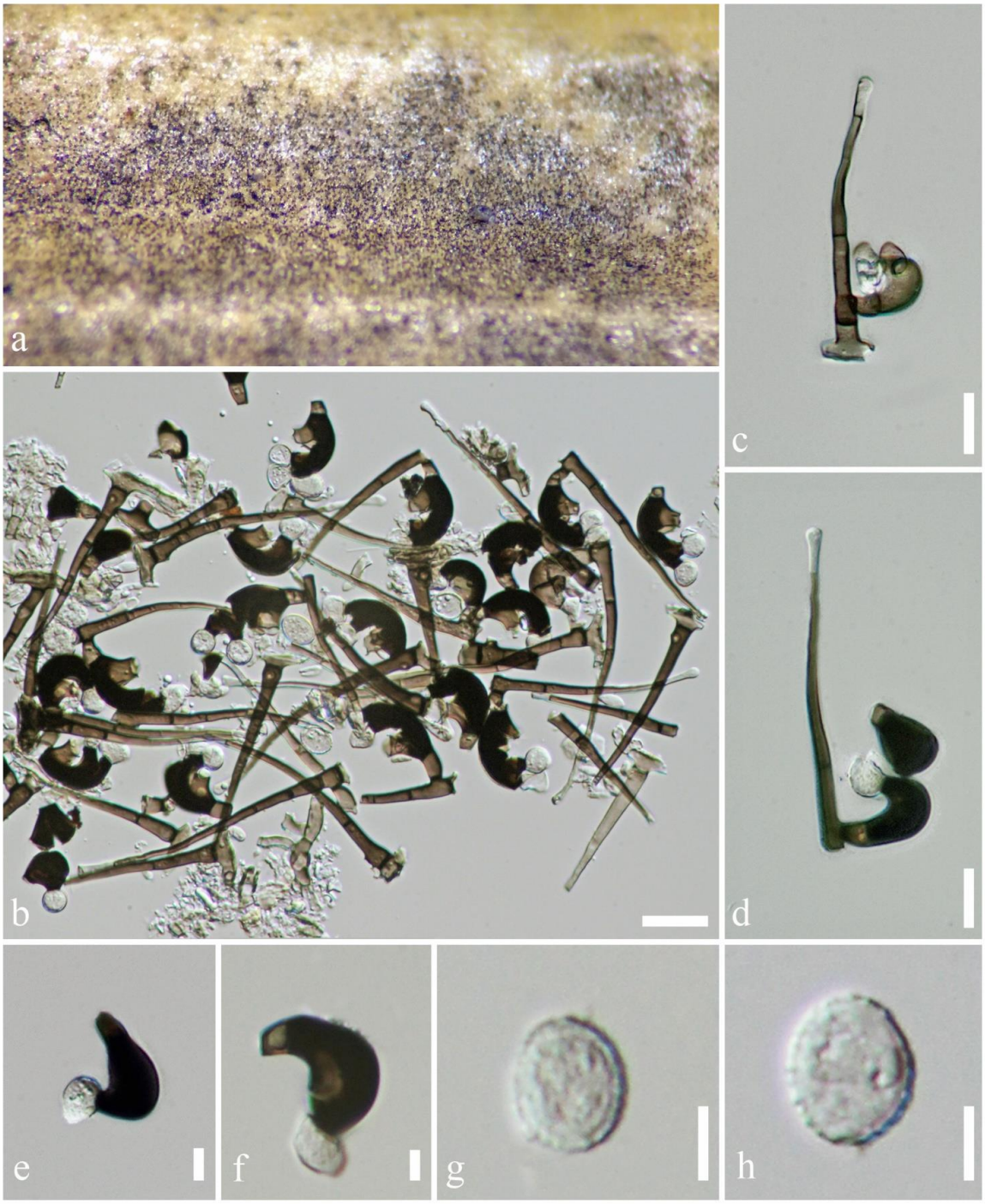

Figure 2 – *Zygosporium minus* (HKAS99625). a Appearance of the fungus on dead leaves of *Pandanus* sp. b–d Conidiophores, vesicles and conidiogenous cells. e–f Vesicles and conidiogenous cells. g–h Conidia. Scale bars: b = 20 µm, c–d = 10 µm, e–h = 5 µm.

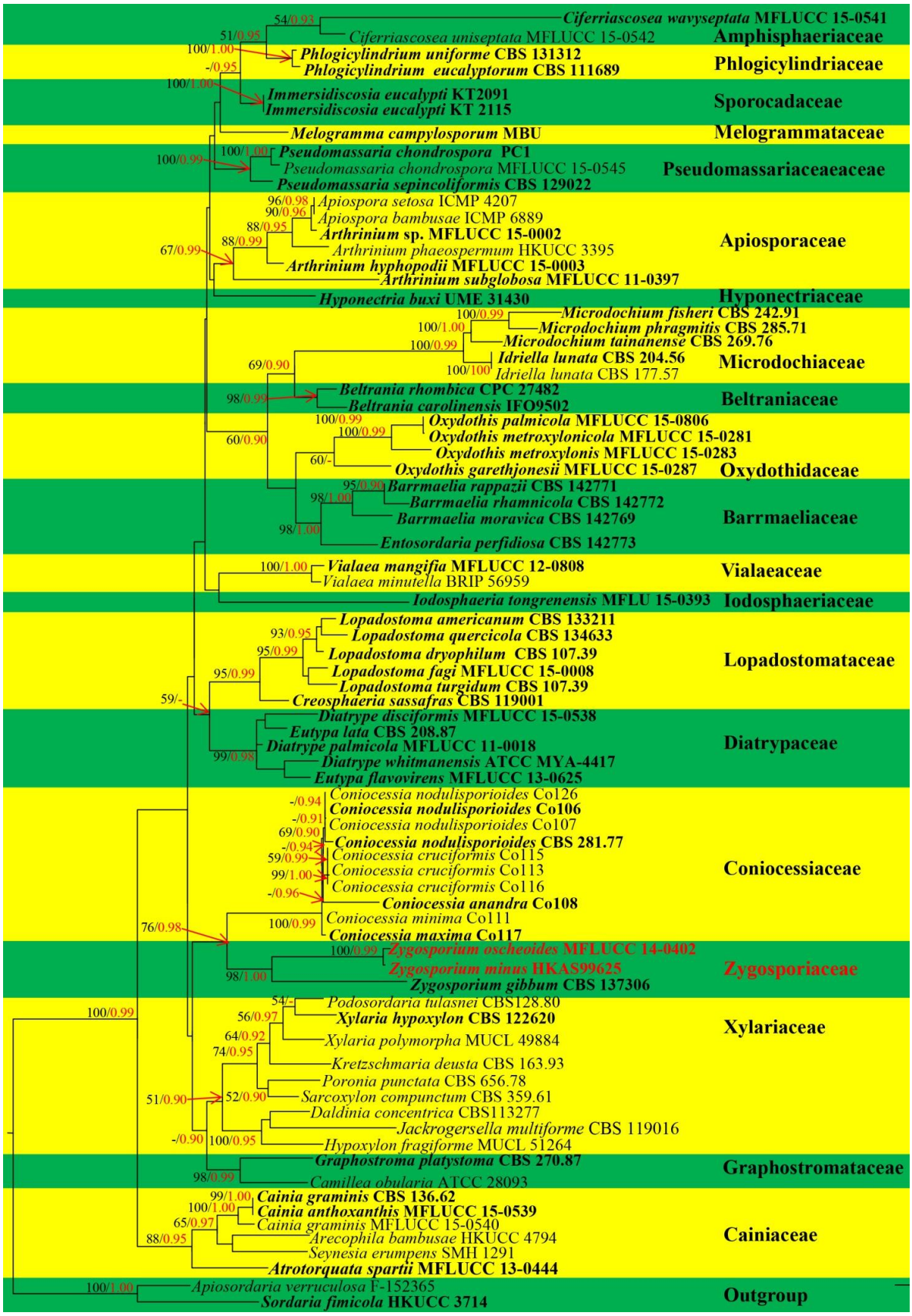

Figure 3 – Phylogenetic tree constructed from RAxML analysis based on a combined dataset of LSU and ITS DNA sequences. RAxML bootstrap support values equal or greater than 50% and Bayesian posterior probabilities equal or higher than 0.90 PP (red) are provided below or above the node. The tree is rooted to *Apiosordaria verruculosa* (F-152365) and *Sordaria fimicola* (HKUCC 3714). Newly generated strains are indicated in red. Type strains are in bold.

Taking into account the robustness of our phylogeny herein and more taxon sampling, a new family, Zygosporiaceae is being proposed to accommodate *Zygosporium* species. At the species level, our phylogeny herein also depicts a close relationship between *Z. oscheoides* and *Z. minus* with high support (Fig. 3). However these two taxa are recognized as morphologically distinct species as *Z. oscheoides* is characterized by setiform conidiophores, apical cell subhyaline, smooth, acute or narrowly clavate and conidia not sphaerical, smooth or minutely verruculose, ellipsoid, hyaline to pale-brown, smooth to minutely verruculose whereas *Z. minus* possesses setiform conidiophores bearing a single vesicle laterally on a short stalk and sphaerical and conidia hyaline to very pale brown, verruculose (Figs 1, 2; Montagne 1842, Hughes 1951, Whitton et al. 2002). *Zygosporium gibbum* on the other hand is morphologically distinct from the above two with darkly pigmented, incurved vesicular cells usually born from the side of setiform conidiophores; the vesicles may be stalked or sessile, and give rise to 2–4 ampulliform conidiogenous cells that produce aseptate, ellipsoid or globose, smooth or variously ornamented conidia (Mason 1941, Hughes 1951). In addition, all these species have been described from different hosts. Even though Zygosporiaceae was originally invalidly published (Art. 39.1, Melbourne) by Locquin (1984), the Zygosporiaceae is being introduced herein as a new family to accommodate *Zygosporium* with morphologically distinct characters coupled with phylogenetic support.

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