Cryptogamie, Bryologie, 2015, 36 (3): 301-317

Zeloasperisporiales ord. nov., and two new species of *Zeloasperisporium*

Sinang HONGSANAN[a,b,c], Qing TIAN[a,b,c], Ali H. BAHKALI[c], Jun-Bo YANG[e], Eric H.C. MCKENZIE[f], Putarak CHOMNUNTI[c] & Kevin D. HYDE[a,b,c,d]*

[a]World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, China

[b]Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Lanhei Road No 132, Panlong District, Kunming, Yunnan Province, 650201, PR China

[c]Centre of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand

[d]Botany and Microbiology Department, College of Science, King Saud University, Riyadh, KSA 11442, Saudi Arabia

[e]Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, China

[f]Landcare Research, Private Bag 92170, Auckland, New Zealand

Abstract – *Neomicrothyrium* is an epiphytic genus that forms small black dots on the surface of living or dead fallen leaves, although it rarely causes any damage to the host. Based on its flattened thyriothecia, it was considered that *Neomicrothyrium* belongs in the order Microthyriales, a group of fungi that is relatively poorly studied. "Microthyriaceae"-like taxa appearing as small black dots on leaves were collected in Chiang Rai Province, Thailand, and studied using morphological characterization and phylogenetic analyses. As a result of molecular and morphological study, we established that *Neomicrothyrium* is linked to the asexual genus *Zeloasperisporium* and that we had collected two new taxa. Two new species, *Zeloasperisporium ficusicola* and *Z. wrightiae* are therefore introduced in this study based on morphology and phylogeny. *Neomicrothyrium* is linked to *Zeloasperisporium* and is therefore synonymized under the older name *Zeloasperisporium*. Phylogenetic analyses of combined LSU and SSU rDNA sequence data indicate that *Zeloasperisporiaceae* belong in the class Dothideomycetes, but clusters with Natipusillales in a distinct lineage from Microthyriales. We therefore introduce a new order, Zeloasperisporiales to accommodate the family *Zeloasperisporiaceae*. The life cycle of *Zeloasperisporium* species is remarkable. The sexual morph produces ascomata on the leaf surface and appears to lack any other structures and it is unclear how the ascomata obtain nutrients. The asexual morph produces conidia which can be found on the surface of plants, or in air, but it is also unclear how conidia obtain their nutrients. In this study, isolates from the sexual morph produced asexual morphs in culture.

Dothideomycetes / Microthyriales / *Neomicrothyrium* / Phylogeny / Taxonomy

* Corresponding author: kdhyde3@gmail.com

doi/10.7872/cryb/v36.iss3.2015.301

INTRODUCTION

The order Microthyriales comprises the families *Micropeltidaceae* and *Microthyriaceae*. Species in this order are epiphytes, pathogens or saprobes found on leaves or stems, and have a worldwide distribution (Schoch *et al*., 2009; Li *et al*., 2011; Wu *et al*., 2011; Hyde *et al*., 2013; Hongsanan *et al.*, 2014; Wijayawardene *et al.*, 2014). Microthyriales species are characterized by small black dots on host plants, which are the flattened, ostiolate thyriothecia; they lack any other external structures such as hyphopodia. The thyriothecia are poorly developed at the base, asci are bitunicate with fissitunicate dehiscence, and ascospores are hyaline to brown and one to multi-septate (Arnaud, 1918; Luttrell, 1973; von Arx & Müller, 1975; Barr, 1987; Kirk *et al*., 2008; Wu *et al.*, 2011; Hyde *et al.*, 2013; Hongsanan *et al.*, 2014). *Microthyriaceae* is the type family of Microthyriales and differs from *Micropeltidaceae* in having thyriothecia with radiating cells, a prominent central ostiole, fusiform to cylindrical asci, and 1-septate ascospores, often with ciliate appendages (Doidge, 1942; Müller & von Arx, 1962; Luttrell, 1973; Hofmann & Piepenbring, 2006; Hofmann, 2010; Wu *et al.*, 2011, 2014; Hyde *et al.*, 2013; Hongsanan *et al.*, 2014). Species of *Micropeltidaceae* are characterized by black to blue, or greenish to black, flattened thyriothecia with central ostioles and are poorly developed at the base, with walls comprising interwoven hyphae, and hyaline ascospores with one to several transverse septa (Clements & Shear, 1931; Batista, 1959; von Arx & Müller, 1975; Barr, 1987; Wu *et al.*, 2011). Taxa of Microthyriales have been poorly studied and there is little molecular data in GenBank (Wu *et al.*, 2011; Hongsanan *et al.*, 2014). *Neomicrothyrium* was introduced as a monotypic genus by Wu *et al.* (2011), with the type species *N. siamense* Boonmee *et al.* This genus is typical of *Microthyriaceae* in having flattened thyriothecia with radiating cells, however, it differs in lacking an ostiole (Wu *et al.*, 2011). Wu *et al.* (2011) noted that the genus can probably be placed in Microthyriales based on its phylogeny and the morphology of the sexual morph.

Zeloasperisporium was introduced by Castañeda Ruíz *et al*. (1996) to accommodate *Zeloasperisporium hyphopodioides* R.F. Castañeda, a species isolated from air in Cuba, but was not placed in any family. *Zeloasperisporium hyphopodioides* was placed in *Venturiaceae* by Crous *et al*. (2007) as it clustered basal to the family *Venturiaceae*. A second species, *Zeloasperisporium eucalyptorum* Cheew. & Crous was introduced by Cheewangkoon *et al*. (2009) from leaves of *Eucalyptus tectifica*. Phylogenetic analysis placed the species in *Venturiaceae* (Cheewangkoon *et al*., 2009). A third species, *Zeloasperisporium cliviae* Crous, was isolated from leaves of *Clivia* sp. and was introduced by Crous *et al*. (2015) and placed in a new family *Zeloasperisporiaceae*, comprising the genera *Neomicrothyrium* and *Zeloasperisporium*. Both of these genera had not previously been linked. *Zeloasperisporiaceae* was poorly resolved in molecular analysis in Crous *et al*. (2015) and appears related to freshwater taxa in the order Natipusillales, an order with which it has no obviously similar morphological characters (Ferrer *et al.*, 2011; Wu *et al.*, 2011). In this study we collected two further species of *Neomicrothyrium* which produced *Zeloasperisporium* states in culture. We therefore consider these to be the same genera and combine them under the older name *Zeloasperisporium*.

In this study, we introduce the two new species as *Zeloasperisporium ficusicola* and *Z. wrightiae*. A combination of morphological and phylogenetic analyses shows the new taxa to differ from other species in *Zeloasperisporiaceae*. *Neomicrothyrium* is synonymized under *Zeloasperisporium* based on morphology of the sexual and asexual morphs, as well as phylogenetic analyses.

MATERIAL AND METHODS

Collections, morphology and isolation

Specimens were collected in Chiang Rai Province, northern Thailand. Gross morphology was observed under a stereomicroscope, and photographed. Sections of ascomata were made free-hand. Various specimens were used to observe the asci and ascospore characters and slides were preserved in lactoglycerol. Morphological characters were observed under a compound microscope (Nikon 80i), and measurements were determined using Tarosoft (R) Image Frame Work v. 0.9.7. Single spore isolation was carried out using a sterile needle to remove ascospores from 5 to 10 ascomata, which were then placed in a drop of sterile water on a glass slide. A spore suspension was obtained, and transferred with a sterile pipette onto the surface of a PDA (potato dextrose agar) plate. The plate was left 12 h of light/12 h at 25-28°C to allow spores to germinate and thereafter observed every 12 hours. Germinated ascospores were transferred onto fresh PDA media, and incubated at 25-28°C (Chomnunti *et al.*, 2011, 2014). Type specimens of the new species are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand, and ex-type cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), and in Kunming Institute of Botany (KIB). Faces of fungi numbers and Index Fungorum numbers are as explained in Jayasiri *et al.* (2015) and Index Fungorum (2015).

DNA isolation, amplification and sequencing

Fungal isolates were grown on 2% PDA for 20 days at 25°C. Genomic DNA was extracted from the growing mycelium using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux®, P.R. China); following the instructions of the manufacturer (Hangzhou, P.R. China). Polymerase chain reaction (PCR) was carried out using known primer pairs LROR and LR5 to amplify a region spanning the large subunit rDNA (White *et al*., 1990). The amplification was performed following the instructions, and were set up for initial denaturation of 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 45 s at 52°C and 90 s at 72°C, and a final extension period of 10 min at 72°C. PCR-products were checked on 1% agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were done by Majorbio Co., China. DNA sequence data were obtained from the large subunit rDNA (LSU) and the small subunit rDNA (SSU). Primer sequences and database are available in GenBank.

Phylogenetic analysis

Sequences data were downloaded from GenBank to supplement the dataset (Table 1) and aligned with those newly obtained using Clustal X 2.0.11 (Thompson *et al*., 1997), and then checked manually using Bioedit (Hall, 2004). *Schismatomma decolora* was selected as outgroup.

Maximum likelihood analysis was performed by using raxmlGUIv.0.9b2 (Silvestro & Michalak, 2012). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTRGAMMAI model of nucleotide substitution. The number of replicates was inferred using the stopping criterion (Pattengale *et al.*, 2009). Maximum likelihood bootstrap values equal or greater than 70% are given as the first set of numbers above the nodes (Fig. 1). The model of evolution was carried out using MrModeltest 2.2 (Nylander *et al.*, 2008). 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). 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 were discarded. The remaining 8,000 trees were performed for calculating posterior probabilities (Cai *et al.*, 2006, 2008, 2009). Bayesian posterior probabilities (BYPP) equal or greater than 0.95 are given as the second set of numbers above the nodes (Fig. 1).

Table 1. Taxa used in the phylogenetic analysis and GenBank accession numbers (LSU and SSU) and species voucher/culture numbers

Species	*Voucher/ culture numbers*	*Accession numbers*	
		LSU	*SSU*
Apiosporina collinsii	CBS 118973	GU301798	GU296135
Asterina phenacis	TH589	GU586217	GU586211
Asterina weinmanniae	TH 592	GU586218	GU586212
Asterina zanthoxyli	TH 561	GU586219	GU586213
Chaetothyriothecium elegans	CPC 21375	KF268420	–
Fusicladium pini	CBS 463.82	EU035436	–
Fusicladium ramoconidii	CBS 462.82	EU035439	–
Micropeltis zingiberacicola	IFRDCC 2264	JQ036227	JQ036222
Microthyrium microscopicum	CBS 115976	GU301846	GU296175
Natipusilla decorospora	L_A236_1A	HM196369	HM196376
Natipusilla limonensis	L_AF286_1A	HM196370	HM196377
Natipusilla naponensis	L_AF217_1A	HM196371	HM196378
Paramicrothyrium chinensis	IFRDCC2258	KF636760	JQ036224
Phaeotrichum benjaminii	CBS 541.72	GU357788	AY016348
Schimatomma decolorans	DUKE 0047570	NG 027622	–
Stomiopeltis betulae	CBS 114420	GU214701	GU214701S
Sympoventuria capensis	CPC 12840	DQ885904	–
Sympoventuria capensis	CBS 120136	DQ885906	–
Trichodelitschia bisporula	CBS 262.69	GU348996	GU296202
Trichodelitschia munkii	CBS 118232	DQ384096	DQ384070
Venturia inaequalis	CBS 815.69	GU301878	GU296204
Venturia inaequalis	CBS 594.70	GU301879	KF156093
Zeloasperisporium cliviae	CPC 25145	KR476781	KR476748
Zeloasperisporium eucalyptorum	CBS:14603	GQ303329	–
Zeloasperisporium ficusicola	MFLUCC 15-0221	**KT387733**	**KT387734**
Zeloasperisporium ficusicola	MFLUCC 15-0222	**KT387735**	**KT387736**
Zeloasperisporium hyphopodioides	CBS 218.95	EU035442	–
Zeloasperisporium siamemse	IFRDCC 2194	JQ036228	JQ036223
Zeloasperisporium wrightiae	MFLUCC 15-0210	**KT387739**	**KT387743**
Zeloasperisporium wrightiae	MFLUCC 15-0214	**KT387741**	**KT387745**
Zeloasperisporium wrightiae	MFLUCC 15-0224	**KT387740**	**KT387744**
Zeloasperisporium wrightiae	MFLUCC 15-0225	**KT387737**	**KT387738**

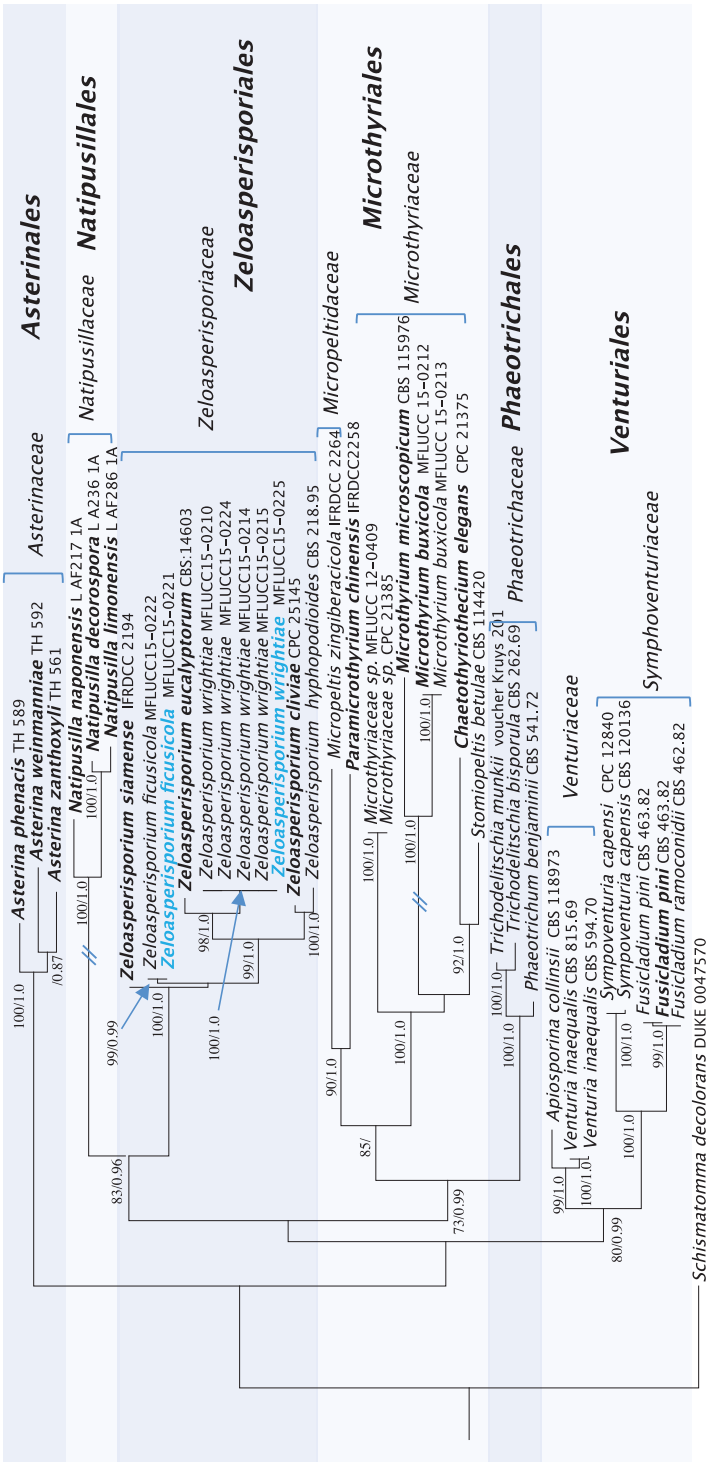

Fig. 1. RAxML maximum likelihood phylogenetic tree (LSU and SSU). The first set of numbers above the nodes are RAxML bootstrap values expressed from 1,000 repetitions with values above 70% shown. The second set of numbers above the nodes are Bayesian posterior probabilities, with values above 0.95 shown. Strain numbers are indicated after species names. New sequence data are in blue bold, and other types are in black bold.

RESULTS

Molecular phylogeny

LSU and SSU sequence data of *Zeloasperisporium ficusicola* and *Z. wrightiae* were blasted in GenBank to find the most closely related strains. Sequence data from taxa in Asterinales, Microthyriales, Natipusillales, Phaeotrichales, and Venturiales were downloaded from GenBank to supplement the dataset (Table 1). Phylogenetic analyses used LSU and SSU sequence data (Fig. 1). The Asterinales clade comprises three representative members of *Asterinaceae* (100% ML, 0.1 PP support). The *Natipusillaceae* clade contains three strains of freshwater species in *Natipusilla* (100% ML, 0.1 PP support), and clustered as a sister group to the *Zeloasperisporiaceae* clade (83% ML, 0.96 PP support). However, *Zeloasperisporiaceae* species are not morphologically similar to species of *Natipusillaceae*, and form a distinct lineage from Microthyriales in the class Dothideomycetes. Thus, *Zeloasperisporiaceae* represents a new order in Dothideomycetes. *Zeloasperisporium ficusicola* and *Z. wrightiae* cluster within the clade of *Zeloasperisporiaceae* (100% ML, 0.1 PP support), and are distinct species. The *Microthyriaceae* clade contains six strains from *Microthyriaceae* (100% ML, 0.1 PP support), and includes *Stomiopeltis betulae* (92% ML, 1.0 PP support). The *Micropeltidaceae* clade contains *Micropeltis zingiberacicola* from *Micropeltidaceae* (90% ML, 1.0 PP support). *Paramicrothyrium chinensis* which belongs to *Microthyriaceae* in Wu *et al.* (2001), nests between *Micropeltidaceae* and *Microthyriaceae* clade (90% ML, 1.0 PP support). Three strains of *Phaeotrichaceae* cluster in the Phaeotrichales clade (100% ML, 0.1 PP support). The Venturiales clade comprises three strains of *Venturiaceae*, and five strains of *Sympoventuriaceae* (80% ML, 0.99 PP support), which probably had the same ancestor as Microthyriales.

TAXONOMY

Zeloasperisporiales Hongsanan & K.D. Hyde, **ord. nov.**

Facesoffungi number: FoF 00900; *Index Fungorum number:* IF 551336.

Epiphytic on the surface of living and dead fallen leaves, appearing as small black dots, or in air. *Superficial hyphae* absent. *Sexual morph: Thyriothecia* superficial, solitary, circular, flattened, brown to dark brown, easy removed, base poorly developed, ostiole lacking. *Upper wall* composed of ellipsoid angular cells, radiating from the center to the outer rim. *Pseudoparaphyses* not observed. *Asci* 8-spored, bitunicate, fissitunicate, globose to ovoid or clavate, apedicellate, with an apical ocular chamber. *Ascospores* 2-3-seriate, obovoid to clavate, 1-septate, slightly constricted at the septum, widest in upper cell, hyaline, smooth-walled or verrucose, surrounded by a thin mucilaginous sheath, or without mucilaginous sheath in some species (Wu *et al*., 2011; Crous *et al*., 2015). *Asexual morph: Hyphae* branched, septate, and swollen by constrictions at the septa, pale brown to brown, occasionally hyaline, smooth-walled, micronematous conidiogenous present or absent. *Conidiophores* reduced to conidiogenous cells, arising as lateral hyphal branches, cylindrical to subcylindrical, straight or slightly curved, unbranched, slightly tapering

towards the apex, brown, slightly thick-walled. *Conidial proliferation* sympodial, with one to several conidiogenous loci, mostly crowded at the apex, protuberant of conidial scars thickened-refractive. *Conidia* fusiform to obclavate or cylindrical, straight to curved, 1-3-septate, distinctly or slightly constricted at the septum, tapered towards the apex, toward a protruding scar, somewhat thickened and darkened-refractive, pale brown to brown, smooth-walled or verrucose.

Notes: Phylogenetic analyses using LSU and SSU indicates that *Zeloasperisporiaceae* is a distinct lineage from Microthyriales in the class Dothideomycetes, and appears to form a distinct sister relationship to Natipusillales which is freshwater family. However, morphologically Natipusillales and *Zeloasperisporiaceae* are very different, their habitats are also distinct and thus *Zeloasperisporiaceae* represents a new order.

Ordinal type: ***Zeloasperisporiaceae*** Crous., Persoonia 34: 167-266 (2015)

Monotypic, characters same as for Zeloasperisporiales.

Family type: ***Zeloasperisporium*** R.F. Castañeda, Mycotaxon 60: 284 (1996).

Zeloasperisporium R.F. Castañeda, Mycotaxon 60: 284 (1996)

= ***Neomicrothyrium*** Boonmee, H.X. Wu & K.D. Hyde, in Wu, Schoch, Boonmee, Bahkali, Chomnunti & Hyde, Fungal Diversity 51(1): 217 (2011).

Notes: Zeloasperisporium was established by Castañeda (1996), with the type species *Z. hyphopodioides*. The genus was placed as genera *incertae sedis* based on its morphology differing from other hyphomycetes. Crous *et al*. (2007) re-examined the type culture of *Z. hyphopodioides* and found that *Zeloasperisporium* clustered basal to the family *Venturiaceae* in their phylogenetic tree. Cheewangkoon *et al*. (2009) introduced a *Zeloasperisporium* species from *Eucalyptus tectifica* which was closely related to the type species in phylogenetic tree, therefore the placement of *Zeloasperisporium* was supported in *Venturiaceae*. Crous *et al*. (2015) introduced a new family *Zeloasperisporiaceae* to accommodate two genera, *Neomicrothyrium* and *Zeloasperisporium.* In this paper we synonymize *Neomicrothyrium* under *Zeloasperisporium*.

Type species: ***Zeloasperisporium hyphopodioides*** R.F. Castañeda, in Castañeda Ruíz *et al.*, Mycotaxon 60: 285 (1996).

Zeloasperisporium siamense (Boonmee *et al.*) Hongsanan & K.D. Hyde, **comb. nov.**

Facesoffunginumber: FoF 00901; *Index Fungorum number:* IF 551338.

≡ *Neomicrothyrium siamense* Boonmee, H.X. Wu & K.D. Hyde, in Wu, Schoch, Boonmee, Bahkali, Chomnunti & Hyde, Fungal Diversity 51(1): 217 (2011).

Epiphyte on the surface of dead fallen leaves, appearing as small black dots. *Superficial hyphae* absent. *Sexual morph: Thyriothecia* superficial, solitary, circular, flattened, brown to dark brown, easy removed, base poorly developed, ostiole lacking. *Upper wall* composed of ellipsoid angular cells, radiating from the center to the outer rim. *Pseudoparaphyses* not observed. *Asci* 8-spored, bitunicate, fissitunicate, globose to ovoid or clavate, apedicellate, with an apical ocular chamber. *Ascospores* 2-3-seriate, obovoid to clavate, 1-septate, slightly constricted at the septum, widest in upper cell, hyaline, smooth-walled, surrounded by a thin mucilaginous sheath (Wu *et al*., 2011). *Asexual morph:* Undetermined (Wu *et al*., 2011).

Notes: Neomicrothyrium was introduced as a monotypic genus based on morphology of sexual morph and phylogenetic analyses by Boonmee *et al.* (in Wu *et al.*, 2001), with the type species *N. siamense.* Species of *Zeloasperisporium* was described and introduced by asexual characters (Crous *et al*., 2007; Cheewangkoon *et al.*, 2008; Crous *et al.*, 2015). Crous *et al*. (2015) included *Neomicrothyrium* and *Zeloasperisporium* in *Zeloasperisporiaceae* based on phylogenetic analyses, although these two genera occurred as different morphs. Moreover, there was no evidence linking the sexual and asexual morphs, and little molecular data was available in GenBank. Phylogenetic analyses of LSU and SSU from the two new species in this paper demonstrate that *Zeloasperisporium ficusicola* is closely related to *Neomicrothyrium siamense*. Furthermore, sexual characters of *Z. ficusicola* are also typical of *N. siamense* in having flattened thyriothecia, lacking an ostiole, and in having hyaline, 1-sepate ascospores. The asexual morph of *Z. ficusicola* is however, identical to *Zeloasperisporium* in having conidiophores reduced to conidiogenous cells, sympodial proliferation, mostly crowded at the apex of conidiogenous cells, and 1-2-septate, hyaline conidia. Thus, *Neomicrothyrium* should be synonymized under the older name *Zeloasperisporium* based on its sexual and asexual state morphology, as well as phylogenetic evidence.

New species of *Zeloasperisporium*

Zeloasperisporium ficusicola Hongsanan & K.D. Hyde, **sp. nov.** **Figs 2-3**

Facesoffungi number: FoF 00886; *Index Fungorum number:* IF 551314.
Etymology: ficusicola referring to the host on which the taxon was found.
Holotype: MFLU 15-1306.

Epiphytic on the upper and lower surface of dead leaves, appearing as very small black dots. *Superficial hyphae* absent. *Sexual morph: Thyriothecia* 170-215 µm diam. ($\bar{x}$ = 192 µm; n = 5), superficial, solitary, circular or subcircular, flattened, brown to dark brown, with a 20-24 µm wide darkened rim, rounded at the margin, easy removed, base poorly developed, ostiole lacking. *Upper wall* comprising dark brown cells of *textura angularis*, radiating from the center to the outer rim. *Pseudoparaphyses* not observed. *Asci* 15-17 × 18-20 µm ($\bar{x}$ = 15.5 × 19 µm; n = 10), 8-spored, bitunicate, fissitunicate, globose to subglobose, apedicellate, rounded at apex, with an ocular chamber. *Ascospores* 7-8 × 3-4 µm ($\bar{x}$ = 7.6 × 3 µm; n = 10), 2-3-seriate, ellipsoid to fusiform, or obovoid, 1-septate, slightly constricted at the septum, upper cell wider than lower cell, rounded to narrow ends, hyaline, smooth-walled, lacking a mucilaginous sheath. *Asexual morph: Hyphae* 2-4 µm diam., branched, septate, and swollen by constrictions at the septa, pale brown to brown, occasionally hyaline, smooth-walled, micronematous conidiogenous cells lacking. *Conidiophores* 11-14 × 3-4 µm ($\bar{x}$ = 13 × 3.5 µm; n = 10), reduced to conidiogenous cells, arising as lateral hyphal branches, cylindrical to subcylindrical, straight or slightly curved, unbranched, slightly tapering towards the apex, brown, slightly thick-walled. *Conidial proliferation* sympodial, with one to several conidiogenous loci, mostly crowded at the apex, protuberant of conidial scars thickened-refractive. *Conidia* 14-16 × 3-5 µm ($\bar{x}$ = 15 × 4.5 µm; n = 10), fusiform to obclavate or cylindrical, straight to curved, 1-septate, slightly constricted at the septum, tapered towards the apex, toward a protruding scar, somewhat thickened and darkened-refractive, pale brown to brown, smooth-walled.

Culture characters: Ascospores germinating on PDA at 25-28°C for 12 h of light/12 h of dark, germ tubes appearing from each end of the ascospores, septate,

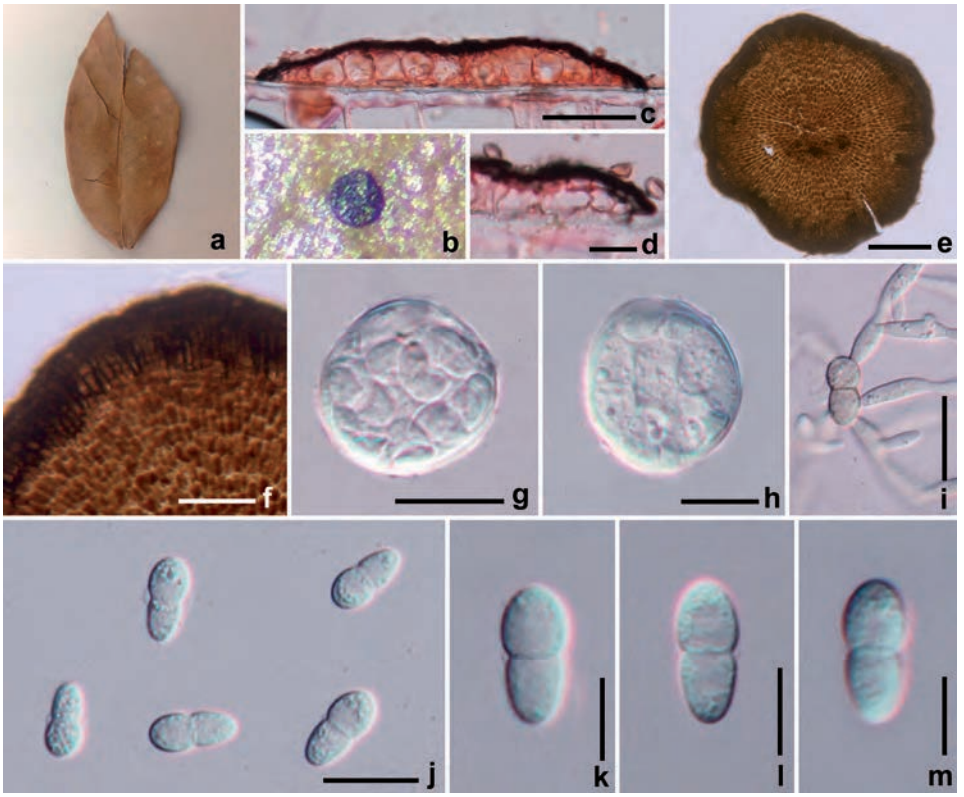

Fig. 2. *Zeloasperisporium ficusicola* (holotype). **a.** Specimen. **b.** Thyriothecium on the upper surface of leaves. **c, d.** Section through thyriothecium and close-up of the margin. **e.** Thyriothecium viewed in squash mount. **f.** Upper wall of thyriothecium viewed in squash mount. **g, h.** Globose asci with 8 ascospores. **i.** Ascospore germinating. **j-m.** Ascospores. Scale bars: c, e = 50 µm, d, f = 20 µm, g-j = 10 µm, k-m = 5 µm.

constricted at the septum, hyaline to brown but becoming brown to dark brown, or black, olivaceous green, reverse iron gray later. Colonies reaching 2 cm diam. after 7 days on PDA at 25-28°C, colony superficial to erumpent, surface smooth, velvety, easily removed, asexual structures was produced in PDA after 5 days incubation (Fig. 3).

Material examined: THAILAND, Chiang Rai, Tasud, Mae Fah Luang University, near S7 building, on leaves of *Ficus benjamina* L. (*Moraceae*), 24 January 2015, S. Hongsanan S7 (MFLU 15-1306, **holotype**); ex-type living culture, **MFLUCC 15-0221**, MFLUCC 15-0222, KIB.

Notes: The sexual morph of *Zeloasperisporium ficusicola* is most similar to *Z. siamense* (Boonmee *et al*.) Hongsanan & K.D. Hyde., but differs in having larger, darker brown thyriothecia, with a thick, darkened rim, globose to subglobose asci and ascospores with rounded to narrow ends. *Zeloasperisporium siamense* has thyriothecia with a thin, darkened rim, globose to subglobose or clavate to ovoid asci and ascospores surrounded by a thin mucilaginous sheath (Fig. 2). *Zeloasperisporium ficusicola* produces an asexual morph in media which is

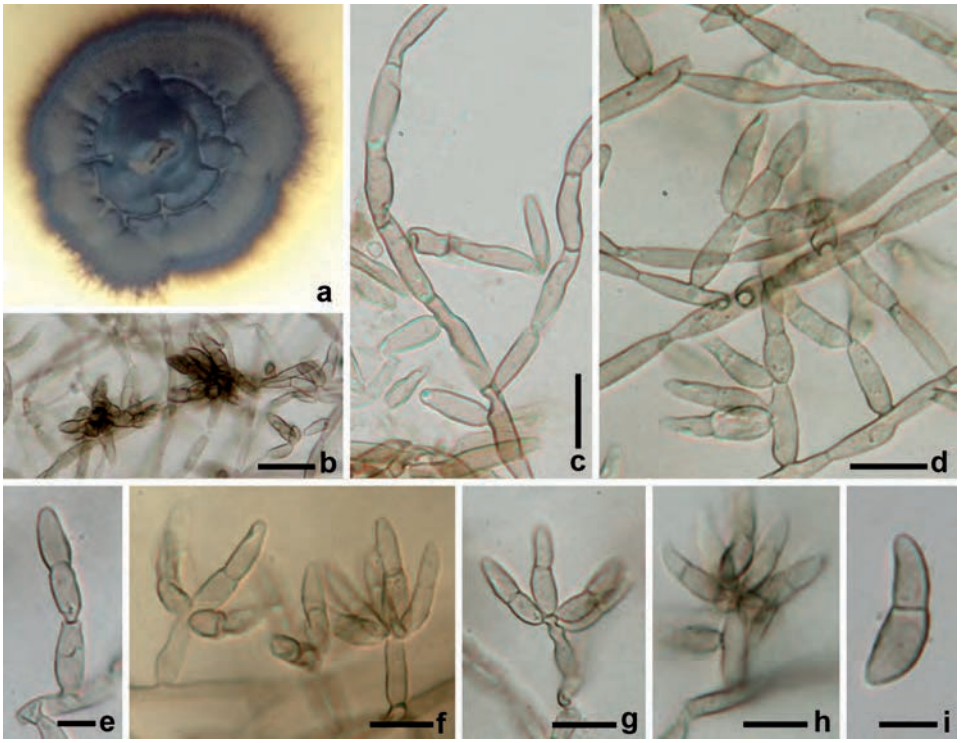

Fig. 3. *Zeloasperisporium ficusicola* (asexual morph). **a.** Colony on PDA. **b.** Crowded conidia on conidiogenous cells. **c.** Septate hyphae. **d, f-h.** Conidia on conidiogenous cell with sympodial proliferation. **e.** Conidiogenous cell. **i.** Conidia with 1 septum. Scale bars: b = 20 µm, c, d, f-h = 10 µm, e, i = 5 µm.

morphologically similar to *Z. cliviae* Crous *et al*. (2015) (Fig. 3). There is no report concerning the asexual morph in *Z. siamense*. Molecular analyses indicate that *Z. ficusicola* is closely related to *Z. siamense*, but is a distinct species.

Zeloasperisporium wrightiae Hongsanan & K.D. Hyde, **sp. nov.** **Figs 4-7**

Facesoffungi number: FoF 00887; *Index Fungorum number:* IF 551313.
Etymology: wrightiae referring to the host on which the taxon was found.
Holotype: MFU 15-1308.

Epiphytic on the upper surface of living leaves, rarely on the lower surface, appearing as small black dots. *Superficial hyphae* absent. ***Sexual morph:*** *Thyriothecia* 225-600 µm diam. ($\bar{x}$ = 530 µm; n = 5), superficial, solitary, circular, flattened, dark brown, with 8 × 10 µm wide darkened rim, rounded at the margin, easy removed, but difficult to remove at the margin, base poorly developed, ostiole lacking. *Upper wall* comprising dark brown cells of *textura angular*, radiating from the center to the outer rim. *Pseudoparaphyses* not observed. *Asci* 15-18 × 19-22 µm ($\bar{x}$ = 16 × 21 µm; n = 10), 8-spored, bitunicate, fissitunicate, globose to subglobose, or broadly clavate, apedicellate, rounded at apex, with an ocular chamber. *Ascospores* 8-10 × 3-4 µm ($\bar{x}$ = 9 × 4 µm; n = 10), 2-3-seriate, ellipsoid to fusiform, or obovoid,

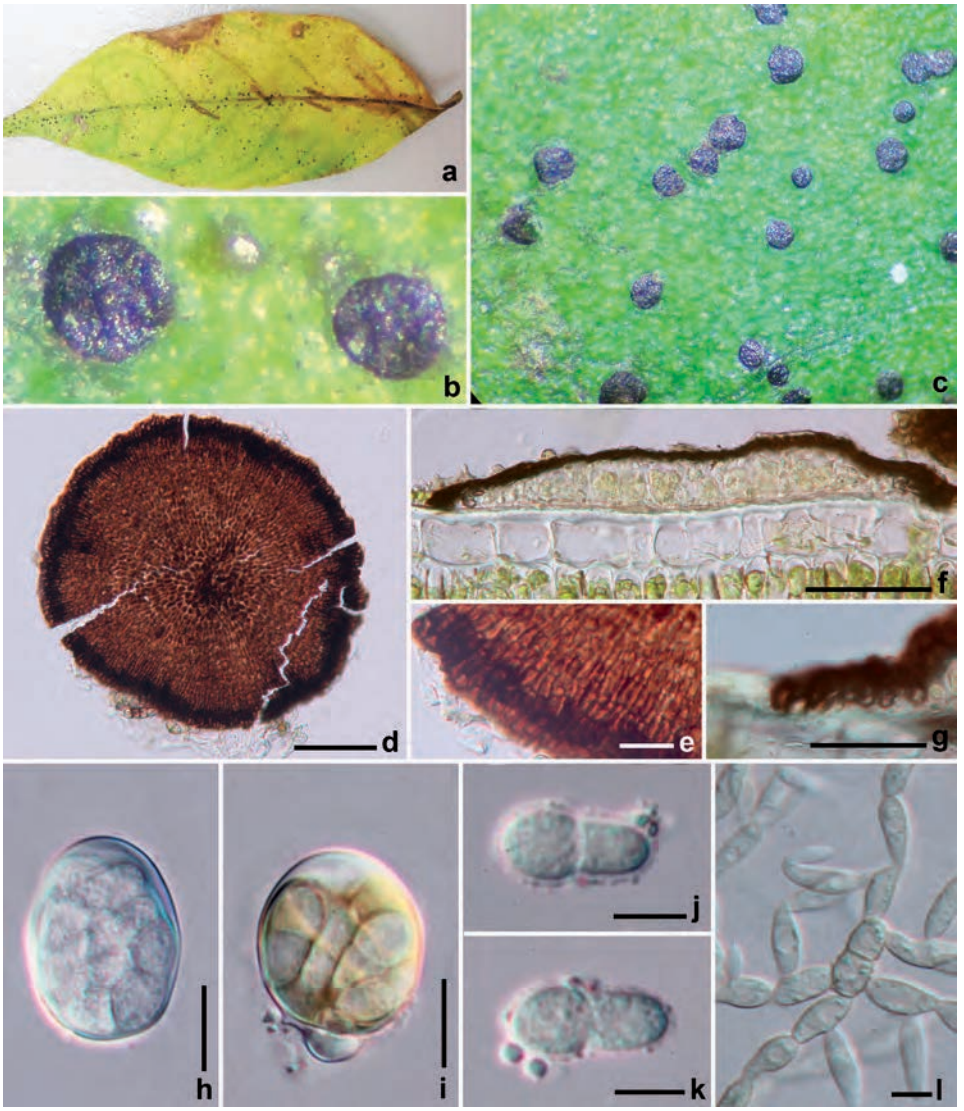

Fig. 4. *Zeloasperisporium wrightiae* (holotype). **a.** Substrate. **b, c.** Thyriothecia on surface of leaves. **d.** Thyriothecium in squash mount. **e.** Upper wall of thyriothecium in squash mount. **f, g.** Section through thyriothecium and close-up of the margin. **h.** Globose asci with 8 ascospores. **i.** Ascus in Melzer's reagent. **j, k.** Ascospores surrounded by a thin sheath. **l.** Ascospore germinating, with monilioid hyphae. Scale bars: d, f = 50 µm, e, g-i = 10 µm, j-l =5 µm.

1-septate, slightly constricted at the septum, upper cell wider than lower cell, rounded ends, hyaline, smooth-walled to slightly verrucose, surrounded by a thin mucilaginous sheath. ***Asexual morph:*** *Hyphae* 2-4 µm diam., branched, septate, and swollen by constrictions at the septa, pale brown to brown, occasionally hyaline, smooth-walled, micronematous conidiogenous present 3-4 × 1-2 µm ($\bar{x}$ = 3.4 × 1.5 µm; n = 10).

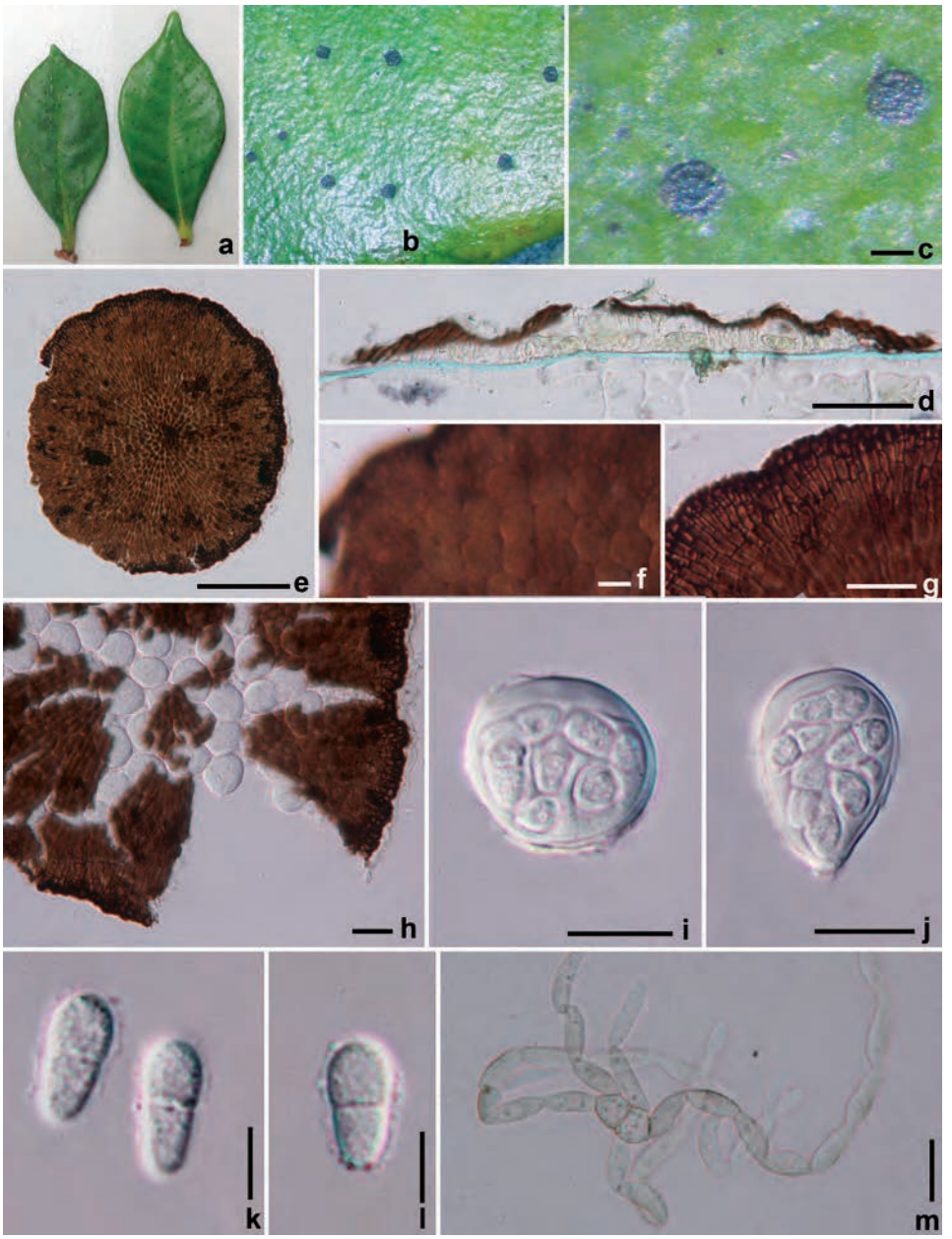

Fig. 5. *Zeloasperisporium wrightiae* (MFLU 15-1309). **a.** Substrate. **b, c.** Thyriothecia on surface of leaves. **d.** Section through thyriothecium. **e-h.** Thyriothecia in squash mounts and close-up of asci arrangement. **i, j.** Asci with 8 spores. **k, l.** Ascospores surrounded by a thin sheath. **m.** Ascospore germinating, with monilioid hyphae. Scale bars: c = 500 µm, d = 50 µm, e = 100 µm, f-h = 20 µm, i, j, m = 10 µm, k, l = 5 µm.

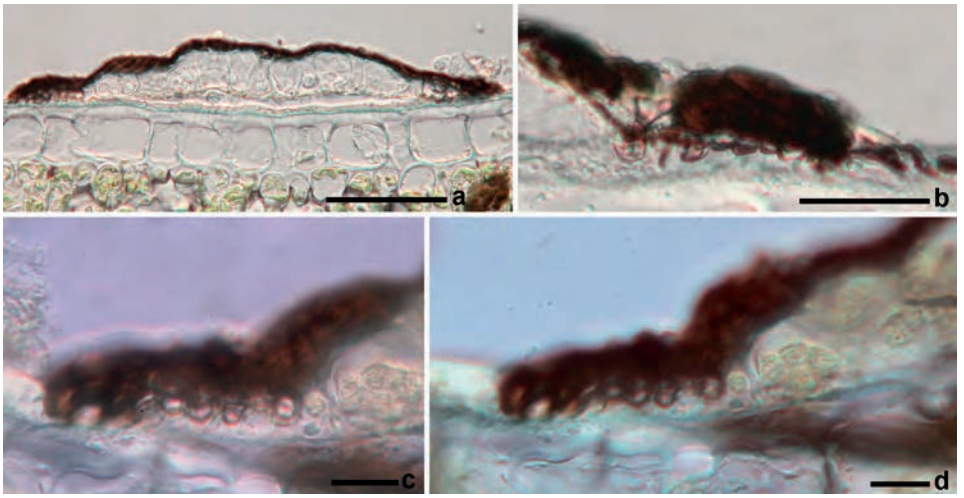

Fig. 6. The thyriothecial margin of *Zeloasperisporium wrightiae* where it is attached to the host. **a.** Section through thyriothecium. **b-d.** The thyriothecial margin with attachment organs. Scale bars: a = 50 µm, b = 20 µm, c, d = 10 µm.

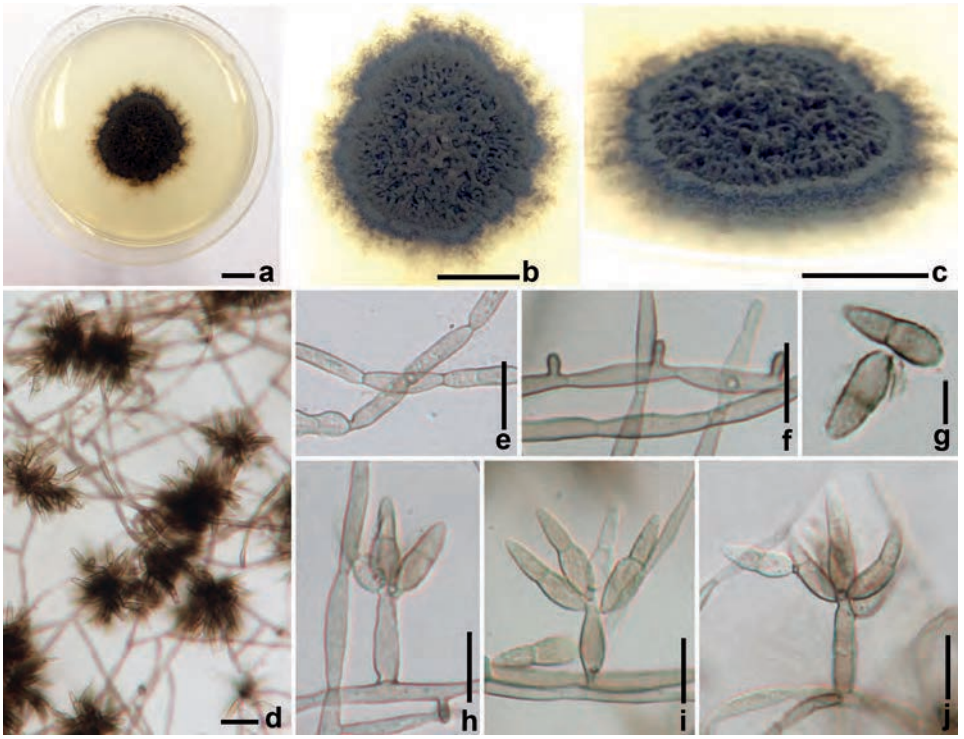

Fig. 7. *Zeloasperisporium wrightiae* (asexual morph). **a-c.** Colonies on PDA. **d.** Crowded conidia on conidiogenous cells. **e.** Septate hyphae. **f.** Micronematous conidiogenous cells. **g.** Conidia with 1 septum. **h-j.** Conidia on conidiogenous cell with sympodial proliferation. Scale bars: a-c = 1 cm, d-f, h, i = 10 µm, g = 5 µm.

Conidiophores 14-16 × 3-4 μm ($\bar{x}$ = 15 × 3.8 μm; n = 10), reduced to conidiogenous cells, arising as lateral hyphal branches, cylindrical to subcylindrical, straight or slightly curved, unbranched, slightly tapering towards the apex, brown, slightly thick-walled. *Conidial proliferation* sympodial, with one to several conidiogenous loci, mostly crowded at the apex, protuberant of conidial scars thickened-refractive. *Conidia* 14-17 × 4-5 μm ($\bar{x}$ = 15 × 4.5 μm; n = 10), fusiform to obclavate or cylindrical, straight to curved, 1-septate, slightly constricted at the septum, tapered towards the apex, toward a protruding scar, somewhat thickened and darkened-refractive, pale brown to brown, smooth-walled.

Culture characters: Ascospores germinating on PDA at 25-28°C under 12 h of light/12 h of dark, hyphae emerging from both cells of the ascospores, septate, strongly constricted at the septa forming monilioid cells in culture, hyaline to brown initially, becoming dark brown to black, bluish reverse iron gray later. Colonies reaching 2 cm diam. after 4 days on PDA at 25-28°C, colony superficial to erumpent, surface verrucose, velvety, easily removed, powder-like, asexual morph structures was produced in PDA after 3 days incubation (Fig. 7).

Material examined: THAILAND, Chiang Rai, Tasud, Mae Fah Luang University, on leaves of *Wrightia religiosa* Benth. (*Apocynaceae*), 15 January 2015, S. Hongsanan MOK01 (MFLU 15-1308, **holotype**); ex-type living culture, **MFLUCC 15-0225**, MFLUCC 15-0210, KIB; Chiang Rai, Tasud, House No. 496, on leaves of *W. religiosa*(*Apocynaceae*), 12 February 2015, S. Hongsanan MOK02 (MFLU 15-1307), MFLUCC 15-0224, KIB; Chiang Rai, Tasud, Mae Fah Luang University, STK resort, on leaves of undetermined tree, 21 January 2015, S. Hongsanan STK08 (MFLU 15-1309); MFLUCC 15-0214, MFLUCC 15-0215, KIB.

Notes: Zeloasperisporium wrightiae is similar to *Z. siamense*, but it was found on living leaves, while *Z. siamense* was found on dead fallen leaves. The thyriothecia are relatively larger and darker in *Z. wrightiae*, while smaller and brown in *Z. siamense* (Figs 4, 5). *Zeloasperisporium wrightiae* differs from *Z. ficusicola* in having larger and darker thyriothecia (Fig. 6), and ascospores surrounded by a thin mucilaginous sheath. Molecular analyses indicate that *Z. wrightiae* is closely related to *Z. eucalyptorum* Cheew. & Crous, but is distinct species within the genus *Zeloasperisporium*.

DISCUSSION

The two new species introduced in this study show that sexual characters of *Zeloasperisporium* are identical to *Neomicrothyrium*, thus the genus *Neomicrothyrium* is synnonymized under *Zeloasperisporium*; this is also well-supported in our phylogenetic analyses. *Zeloasperisporium ficusicola* and *Z. wrightiae* are similar to *Z. siamense* in having flattened thyriothecia, lacking obvious ostioles, possessing an upper peridial wall comprising cells radiating from the centre, and a poorly developed base (Wu *et al.*, 2011). *Zeloasperisporium ficusicola* differs from *Z. siamense* in having large thyriothecia with thick, darkened rims. *Zeloasperisporium siamense* is characterized by a thin, darkened rim, small, pale brown to brown thyriothecia, and rounded ends to the ascospores. *Zeloasperisporium wrightiae* is distinct from *Z. ficusicola* and *Z. siamense* as it was found on living leaves, it has larger and darker thyriothecia, is difficult to remove at the ascomatal margin, and it has slightly verrucose ascospores. The phylogenetic analyses of LSU and SSU

rDNA sequence data indicate that *Z. ficusicola* is related to, but well-resolved from *Z. siamense* and *Z. wrightiae*, and well-resolved from *Z. eucalyptorum*. *Zeloasperisporium* is placed in the phylogenetic tree as a distinct lineage from Microthyriales in the class Dothideomycetes, but with a sister relationship to Natipusillales. The latter is a family of freshwater taxa with a very different morphology, thus, we introduce a new order Zeloasperisporiales to accommodate *Zeloasperisporium*. After ascospore germination on agar, both new species produce distinctive hyphae that are strongly constricted at the septa.

Species of *Zeloasperisporium* can be found on dead and living leaves in Thailand, and are frequently found on plants during the cold season (December to February). *Zeloasperisporium siamense* (Wu *et al*., 2011) was also collected during this period. After February, we were unable to find this fungus even in the locations where we previously collected them.

The life cycle of *Zeloasperisporium* species are remarkable. The sexual morph produce ascomata on the leaf surface and appear to lacks any other structures and it is unclear how they obtain their nutrients. Attempts to observe any attachment and absorption organs in *Zeloasperisporium ficusicola* and *Z. wrightiae* using free-hand section (stained in Melzer's reagent, cotton blue reagent, and Congo red) did not reveal any penetration of the host. *Zeloasperisporium wrightiae* attaches on the surface of host plants at the ascomatal margin thus making it difficult to remove ascomata. Thus, the sexual morphs of *Zeloasperisporium* found on living leaves may obtain nutrients from plant cells at the margin of the thyriothecia (Fig. 6).

The asexual state on the other hand, have been isolated from the air and leaves which may obtain nutrients from plant cells using appressorium-like, inflated hyphopodia which are slightly warted to lobed at the apex (Castañeda *et al*., 1996). However, Crous *et al*. (2007) recognized this structure as conidiogenous cells of a synanamorph forming a second conidial type.

Acknowledgements. The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this Prolific Research group (PRG-1436-09). Kevin D. Hyde thanks the Chinese Academy of Sciences, project number 2013T2S0030, for the award of Visiting Professorship for Senior International Scientists at Kunming Institute of Botany.

REFERENCES

ARNAUD G., 1918 — Lés Asterinées. *Annals d'École National d'Agriculture de Montpellier Série* 2, 16: 1-288.

ARX J.A. VON & MÜLLER E., 1975 — A re-evaluation of the bitunicate ascomycetes with key to families and genera. *Studies in Mycology* 9: 1-159.

BARR M.E., 1987 — New taxa and combinations in the Loculoascomycetes. *Mycotaxon* 29: 501-505.

BATISTA A.C., 1959 — Monografia dos fungos *Micropeltaceae*. *Publicações Instituto de Micologia da Universidade do Recife* 56: 1-519.

CAI L., GUO X.Y. & HYDE K.D., 2008 — Morphological and molecular characterization of a new anamorphic genus *Cheirosporium*, from freshwater in China. *Persoonia* 20: 53-58.

CAI L., JEEWON R. & HYDE K.D., 2006 — Phylogenetic investigations of *Sordariaceae* based on multiple gene sequences and morphology. *Mycological Research* 110: 137-150.

CAI L., WU W.P. & HYDE K.D., 2009 — Phylogenetic relationships of *Chalara* and allied species inferred from ribosomal DNA sequences. *Mycological Progress* 8 (2): 133-143.

CASTAÑEDA R.F., FABRE D.E., PARRA M., PEREZ M. & GUARRO J., 1996 — Some airborne conidial fungi from Cuba. *Mycotaxon* 60: 283-290.

CHEEWANGKOON R., GROENEWALD J.Z., SUMMERELL B.A., HYDE K.D., TO-ANUN C. & CROUS P.W., 2008 — *Myrtaceae*, a cache of fungal biodiversity. *Persoonia* 23: 55-85.

CHOMNUNTI P., HONGSANAN S., HUDSON B.A., TIAN Q., PERŠOH D., DHAMI M.K., ALIAS A.S., XU J., LIU X., STADLER M. & HYDE K.D., 2014 — The sooty moulds. *Fungal Diversity* 66: 1-36.

CHOMNUNTI P., SCHOCH C.L., AGUIRRE-HUDSON B., KO-KO T.W., HONGSANAN S., JONES E.B.G., KODSUEB R., PHOOKAMSAK R., CHUKEATIROTE E., BAHKALI A.H. & HYDE K.D., 2011 — *Capnodiaceae*. *Fungal Diversity* 51: 103-134.

CLEMENTS F.E. & SHEAR C.L., 1931 — *Genera of fungi 2 Vol. i-vii*. H.W. Wilson, USA, New York, 496 p.

CROUS P.W., SCHUBERT K., BRAUN U., HOOG DE G.S., HOCKING A.D., SHIN H-D. & GROENEWALD J.Z., 2007 — Opportunistic, human-pathogenic species in the *Herpotrichiellaceae* are phenotypically similar to saprobic or phytopathogenic species in the *Venturiaceae*. *Studies in Mycology* 58: 185-217.

CROUS P.W., WINGFIELD M.J., GUARRO J., RESTREPO-HERNÁNDEZ M., SUTTON D.A., ACHARYA K., BARBER P.A., BOEKHOUT T., DIMITROV R.A., DUEÑAS M., DUTTA A.K., GENÉ J., GOULIAMOVA D.E., GROENEWALD M., LOMBARD L., MOROZOVA O.V., SARKAR J., SMITH M.TH., STCHIGEL A.M., WIEDERHOLD N.P., ALEXANDROVA A.V., ANTELMI I., ARMENGOL J., BARNES I., CANO-LIRA J.F., CASTAÑEDA RUIZ R.F., CONTU M., COURTECUISSE PR.R., DA SILVEIRA A.L., DECOCK C.A., GOES A.DE., EDATHODU J., ERCOLE E., FIRMINO A.C., FOURIE A., FOURNIER J., FURTADO E.L., GEERING A.D.W., GERSHENZON J., GIRALDO A., GRAMAJE D., HAMMERBACHER A., HE X-L., HARYADI D., KHEMMUK W., KOVALENKO A.E., KRAWCZYNSKI R., LAICH F., LECHAT C., LOPES U.P., MADRID H., MALYSHEVA E.F, MARÍN-FELIX Y., MARTÍN Y.P, MOSTERT L., NIGRO F., PEREIRA O.L, PICILLO B., PINHO D.B., POPOV E.S., RODAS PELÁEZ C.A., ROONEY-LATHAM S., SANDOVAL-DENIS M., SHIVAS R.G., SILVA V., STOILOVA-DISHEVA M.M., TELLERIA M.T., ULLAH C., UNSICKER S.B., VAN DER MERWE N.A., VIZZINI A., WAGNER H-G., WONG P.T.W., WOOD A.R., GROENEWALD J.Z., 2015 — Fungal Planet description sheets: 320-370. *Persoonia* 34: 167-266.

DAI D.Q., BOONMEE S., TIAN Q., XIA Y.P., BHAT D.J., CHUKEATIROTE E., ALIA S.A. & ZHAO R.L., WANGI Y. & HYDE K.D., 2014 — *Englerulaceae* (Dothideomycetes). *Phytotaxa* 176 (1):139-155.

DISSANAYAKE A.J., JAYAWARDENA R.S., BOONMEE S., THAMBUGALA K.M., TIAN Q., MAPOOK A., SENANAYAKE I.C., YAN J., LI Y.M., LI X., CHUKEATIROTE E. & HYDE K.D., 2014 — The status of *Myriangiaceae* (Dothideomycetes). *Phytotaxa* 176 (1): 219-237.

DOIDGE E.M., 1942 — South African *Microthyriaceae*. *Bothalia* 4: 273-344.

FERRER A., MILLER A.N. & SHEARER C.A., 2011 *Minutisphaera* and *Natipusilla*: two new genera of freshwater Dothideomycetes. *Mycologia* 103: 411-423.

HALL T.A., 2004 — BioEdit. Ibis Therapeutics, Carlsbad, CA, 92008, USA. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). [18 March 2005]

HOFMANN T.A., 2010 — *Plant parasitic Asterinaceae and Microthyriaceae from the Neotropics* (*Panama*). PhD thesis, The Faculty of Biological Sciences at the J.W. Goethe-University Frankfurt am in, Germany, 408 p.

HOFMANN T.A. & PIEPENBRING M., 2006 — New records and host plants of fly-speck fungi from Panama. *Fungal Diversity* 22: 55-70.

HONGSANAN S., CHOMNUNTI P., CROUS P.W., CHUKEATIROTE E. & HYDE K.D., 2014 — Introducing *Chaetothyriothecium*, a new genus of Microthyriales. *Phytotaxa* 161 (2): 157-164.

HUELSENBECK J.P. & RONQUIST F., 2001 — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17 (8): 754-755.

HYDE K.D., JONES E.B.G., LIU J.K., ARIYAWANSA H., BOEHM E., BOONMEE S., BRAUN U., CHOMNUNTI P., CROUS P., DAI D.Q., DIEDERICH P., DISSANAYAKE A., DOILOM M., DOVERI F., HONGSANAN S., JAYAWARDENA R., LAWREY J.D., LI Y.M., LIU. Y.X., LÜCKING R., MONKAI J., NELSON M.P., PHOOKAMSAK R., MUGGIA L., PANG K.L., SENANAYAKE I., SHEARER C.A., WIJAYAWARDENE N., WU H.X., THAMBUGALA M., SUETRONG S., TANAKA K., WIKEE S., ZHANG Y., HUDSON B.A., ALIAS S.A., APTROOT A., BAHKALI A.H., BEZERRA L.J., BHAT J.D., CAMPORESI E., CHUKEATIROTE E., HOOG S.D., GUEIDAN C., HAWKSWORTH D.L., HIRAYAMA K., KANG J.C., KNUDSEN K., LI W.J., LIU Z.Y., MCKENZIE E.H.C., MILLER A.N., NADEESHAN D., PHILLIP A.J.L., MAPOOK A., RAJA H.A., TIAN Q., ZHANG M., SCHEUER C., SCHUMM F., TAYLOR J., YACHAROEN S., TIBPROMMA S., WANG Y., YAN J. & LI X., 2013 — Families of Dothideomycetes. *Fungal Diversity* 63: 1-313.

JAYAWARDENA R.S., ARIYAWANSA H.A., SINGTRIPOP C., LI Y.M., YAN J., LI X., NILTHONG S. & HYDE K.D., 2014 A re-assessment of *Elsinoaceae* (Myriangiales, Dothideomycetes).

KIRK P.M., CANNON P.F., MINTER D.W. & STALPERS J.A., 2008 — *Ainsworth & Bisby's dictionary of the fungi*, 10th edition. CAB International, Wallingford, UK, 428 p.

LI Y.M., HAIXIA, WU-CHENG H. & HYDE K.D., 2011 — Morphological studies in Dothideomycetes: *Elsinoë* (*Elsinoaceae*), *Butleria* and three excluded genera. *Mycotaxon* 115: 507-520.

LUTTRELL E.S., 1973 — Loculoascomycetes. *In*: Ainsworth G.C., Sparrow F.K. & Sussman A.S. (eds) *The fungi. An advanced treatise*. Academic Press, New York and London, pp. 135-219.

MÜLLER E. & ARX J.A. VON., 1962 — Die Gattungen der didymosporen Pyrenomyceten. *Beiträge zur Kryptogamenflora der Schweiz* 11 (2): 1-922.

NYLANDER J.A.A., WILGENBUSCH J.C., WARREN D.L. & SWOFFORD D.L., 2008 — AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581-583.

PATTENGALE N.D., ALIPOUR M., BININDA-EMONDS O.R.P., MORET B.M.E. & STAMATAKIS A., 2009 — How many bootstrap replicates are necessary? *Lecture Notes in Computer Science* 5541: 184-200.

RANNALA B. & YANG Z., 1996 — Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* 43: 304-311.

SCHOCH C.L., CROUS P.W., GROENEWALD J.Z., BOEHM E.W., BURGESS T.I., DE GRUYTER J., DE HOOG G.S., DIXON L.J., GRUBE M., GUEIDAN C., HARADA Y., HATAKEYAMA S., HIRAYAMA K., HOSOYA T., HUHNDORF S.M., HYDE K.D., JONES E.B., KOHLMEYER J., KRUYS A., LI Y.M., LUCKING R., LUMBSCH H.T., MARVANOVA L., MBATCHOU J.S., MCVAY A.H., MILLER A.N., MUGAMBI G.K., MUGGIA L., NELSON M.P., NELSON P., OWENSBY C.A., PHILLIPS A.J., PHONGPAICHIT S., POINTING S.B., PUJADE-RENAUD V., RAJA H.A., PLATA E.R., ROBBERTSE B., RUIBAL C., SAKAYAROJ J., SANO T., SELBMANN L., SHEARER C.A., SHIROUZU T., SLIPPERS B., SUETRONG S., TANAKA K., VOLKMANN-KOHLMEYER B., WINGFIELD M.J., WOOD A.R., WOUDENBERG J.H., YONEZAWA H., ZHANG Y. & SPATAFORA J.W., 2009 — A class-wide phylogenetic assessment of Dothideomycetes. *Studies in Mycology* 64: 1-15.

SILVESTRO D. & MICHALAK I., 2012 — RaxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335-337.

THOMPSON J.D., GIBSON T.J., PLEWNIAK F., JEANMOUGIN F. & HIGGINS D.G., 1997 — alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

WHITE T.J., BRUNS T., LEE S. & TAYLOR J., 1990 — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: *PCR Protocols: a guide to methods and applications*. (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, New York, USA: 315-322.

WIJAYAWARDENE N.N., CROUS P.W., KIRK P.M., HAWKSWORTH D.L., BOONMEE S., BRAUN U., CHOMNUNTI P., DAI D.Q., D'SOUZA M.J., DIEDERICH P., DISSANAYAKE A., DOILOM M., HONGSANAN S., JONES E.B.G., GROENEWALD J.Z., JAYAWARDENA R., LAWREY J.D., LIU J.K., LÜCKING R., MADRID H., MANAMGODA D.S., MUGGIA L., NELSON M.P., PHOOKAMSAK R, SUETRONG S, TANAKA K, THAMBUGALA K.M., WIKEE S., ZHANG Y., APTROOT A., ARIYAWANSA H.A., BAHKALI A.H., BHAT J.D., GUEIDAN C., DE HOOG G.S., KNUDSEN K., MCKENZIE E.H.C., MILLER A.N., MORTIMER P.E., WANASINGHE D.N., PHILLIPS A.J.L., RAJA H.A., SLIPPERS B., SHIVAS R.S., TAYLOR J.E., WANG Y., WOUDENBERG J.H.C., PIATEK M., CAI L., JAKLITSCH W.M. & HYDE K.D., 2014 — Naming and outline of Dothideomycetes-2014. *Fungal Diversity* 69 (1): 1-55.

WU X., SCHOCH C.L., BOONMEE S., BAHKALI A.H., CHOMNUNTI P. & HYDE K.D., 2011 — A reappraisal of Microthyriaceae. *Fungal Diversity* 51 (1): 189-248.

WU X., TIAN Q., LI W.J. & HYDE K.D., 2014 — A reappraisal of *Microthyriaceae*. *Phytotaxa* 176 (1): 201-212.

ZHAXYBAYEVA O. & GOGARTEN J.P., 2002 — Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. *BMC Genomics* 3: 4.