

Taxonomic and phylogenetic placement of *Phaeodimeriella* (*Pseudoperisporiaceae*, Pleosporales)

Ausana MAPOOK^{a,b,c,d}, Saranyaphat BOONMEE^c, Jian-Kui LIU^e,
E. B. Gareth JONES^f, Ali H. BAHKALIF^f & Kevin D. HYDE^{a,b,c,f*}

^aKey Laboratory for Plant Diversity and Biogeography of East Asia,
Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201,
Yunnan, People's Republic of China

^bWorld Agroforestry Centre, East and Central Asia, Kunming 650201,
Yunnan, People's Republic of China

^cCenter of Excellence in Fungal Research, Mae Fah Luang University,
Chiang Rai. 57100, Thailand

^dSchool of Science, Mae Fah Luang University, Chiang Rai. 57100, Thailand

^eGuizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy
of Agricultural Sciences, Guiyang 550006, Guizhou, China

^fBotany and Microbiology Department, College of Science,
King Saud University, Riyadh, 1145, Saudi Arabia

Abstract – *Phaeodimeriella* is an epiphytic or pathogenic genus that occurs on plant surfaces associated with other fungi and belongs in the family *Pseudoperisporiaceae* (Dothideomycetes family, *incertae sedis*). The taxonomy of *Phaeodimeriella* has been problematic because of lack of molecular data and as some of the important morphological characters having not been reported. The present study clarifies the phylogenetic placement of *Phaeodimeriella* using fresh collections from northern Thailand. Two *Phaeodimeriella* species are characterized based on combined analyses of LSU, SSU, TEF and RPB2 sequence datasets. In the phylogenetic analyses, the two *Phaeodimeriella* species form a distinct lineage, adjacent to the *Lentitheciaceae* and *Bambusicolaceae*, in the order Pleosporales. The genus *Phaeodimeriella* is re-described and illustrated using modern concepts from fresh collections and provided with molecular data. The new species *Phaeodimeriella cissampeli* and *P. dilleniae* are introduced based on morphological characters, and molecular data is provided. The genus *Lizonia* is placed in *Didymellaceae* based on phylogenetic analysis.

Dothideomycetes / epiphytes / *Lizonia* / morphology / phylogeny

* Corresponding author: kdhyde3@gmail.com

INTRODUCTION

Pseudoperisporiaceae, a family of uncertain position in the class Dothideomycetes (Lumbsch & Huhndorf, 2007; Hyde *et al.* 2013; Wijayawardene *et al.* 2014) was introduced by Toro in Seaver & Chardón (1926) with *Lasiostemma* as the type genus (Barr 1987, 1997; Hyde *et al.* 2013) and *Lasiostemma melioloides* (= *Pseudoperisporium erigeronicola* F. Stevens) as the type species (Barr 1997). The asexual morph is the coelomycete genus *Chaetosticta*, which is related to *Lasiostemma* and *Nematostoma* (Kirk *et al.* 2008; Hyde *et al.* 2011, 2013). Based on morphology, Barr (1997) accepted nine genera in the family. Lumbsch & Huhndorf (2007, 2010) and Hyde *et al.* (2013) listed 22 genera in the family, in an uncertain position in the class Dothideomycetes, while 24 genera were listed in the family by Wijayawardene *et al.* (2014). Stenroos *et al.* (2010) provided sequence data from five genes for *Bryochiton*, *Epibryon* and *Lizonia*, genera which have been assigned to *Pseudoperisporiaceae* (Barr 1997; Stenroos *et al.* 2010). Their analyses clearly showed that *Lizonia* belongs in the order *Pleosporales*, while *Epibryon* was placed in the subclass Chaetothyriomycetidae (*Chaetothyriales*) and *Bryochiton* was placed in *Capnodiales* with strong phylogenetic support (Wali *et al.* 2014). However, most genera in *Pseudoperisporiaceae* lack molecular data and are presently poorly documented.

Phaeodimeriella was introduced by Spegazzini (1908). The genus was lectotypified by *P. occulta* (Theissen 1912) and the name has been used by Müller & von Arx (1962). *Phaeodimeriella occulta* was introduced by Raciborski (1900) under the name, *Dimerosporium occultum*. The name was a combination of *Dimerium occultum* (Saccardo & Sydow 1905), *Phaeodimeriella occulta* (Spegazzini 1908) and *Phaeodimeris occulta* (Clem. & Shear 1931). Index Fungorum (2016) list 37 species epithets for the genus, however, eight species have been transferred to other genera and families.

This study reappraises the taxonomic and phylogenetic position of *Phaeodimeriella* and its relationships with other families within *Pleosporales* by using multi-loci analyses. Descriptions, illustrations and phylogenetic placement are provided. Gene character comparisons and morphological characteristics are used for determine distinct species.

MATERIALS AND METHODS

Collection, examination, and isolation of fungi

Fresh material was collected from different sampling sites in Chiang Mai Province at Muang and Mae Teang Districts during 2013-2014. Fungal micromorphology studies follow Chomnunti *et al.* (2014). Fresh materials were used for the molecular study. Type and ex-type specimens are deposited in the Herbaria MFLU (Herb. MFLU) with duplicates in Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (HKAS), China. Index Fungorum (2016) and Faces of fungi (Jayasiri *et al.* 2015) numbers are provided.

DNA extraction, PCR amplification and sequencing

DNA was extracted directly from the ascomata using an E.Z.N.A.[®] Forensic DNA Kit (Omega Bio-Tek, Inc., United States) following the manufacturer's protocol. The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5 (Vilgalys and Hester 1990). The partial small subunit nuclear rDNA (SSU) was amplified with primer pairs NS1 and NS4 (White *et al.* 1990). The translation elongation factor 1-alpha gene (TEF1) was amplified by using primers EF1-983F and EF1-2218R (Rehner 2001). The partial RNA polymerase second largest subunit (RPB2) was amplified by using primers fRPB2-5F and fRPB2-7cR (Liu *et al.* 1999).

Methods for PCR amplification and sequencing follow the study of Mapook *et al.* (2016).

Phylogenetic analysis

The phylogenetic tree was generated by analysing a combined LSU, SSU, TEF1 and RPB2 sequence data. Seven families close to *Pseudoperisporiaceae* were selected to build the phylogenetic tree i.e., *Bambusicolaceae*, *Didymellaceae*, *Didymosphaeriaceae*, *Lentitheciaceae*, *Massarinaceae*, *Morosphaeriaceae*, and *Trematosphaeriaceae*. The tree was rooted to *Melanomma pulvis-pyrius* (CBS 124080). The closest matched taxa were determined through nucleotide blast searches in GenBank. The sequences used for analyses are deposited in GenBank, with details given in Table 1. All sequence data were aligned using MAFFT (v7.110) online program (<http://mafft.cbrc.jp/alignment/server/>) (Katoh and Standley 2013). The alignments were checked and uninformative gaps minimized manually where necessary in BioEdit 7.0.1 (Hall 2004). Maximum Likelihood (ML) and Bayesian Inference (BI) were used in the analyses with individual data from each partition in addition to the combined aligned dataset.

Maximum Likelihood (ML) analysis was run in RAxML (Stamatakis 2006) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak 2010) with 1000 rapid bootstrap replicates using the GTR+ GAMMA model of nucleotide substitution. Maximum Likelihood bootstrap values (MLBP) equal or greater than 70% are given above each node (Fig. 1).

The model of nucleotide substitution for each gene along with the combined aligned dataset was performed using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996; Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Four simultaneous Markov chains were run for 5000000 generations and trees were sampled every 100th generations with a resulting in 50000 total trees. The first 20% trees were discarded as the burn in phase and the remaining trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (the standard deviation of split frequencies were reached to 0.01) (Ariyawansa *et al.* 2013b; Udayanga *et al.* 2015). Bayesian Posterior Probabilities (BYPP) with those equal or greater than 0.95 are given above or below the nodes and branches (Fig. 1). Phylogenetic trees were drawn using Treeview v. 1.6.6 (Page 1996).

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

Taxon	Culture accession no. ¹	GenBank accession no. ²			
		LSU	SSU	TEF	RPB2
<i>Ascochyta pisi</i>	CBS 126.54	DQ678070	DQ678018	DQ677913	DQ677967
<i>Asteromassaria pulchra</i>	CBS 124082 ^T	GU301800	GU296137	GU349066	GU371772
<i>Bambusicola bambusae</i>	MFLUCC 110389 ^T	JX442035	JX442039	–	–
<i>Bambusicola irregularispora</i>	MFLUCC 110614 ^T	JX442036	JX442040	–	–
<i>Bambusicola massarinia</i>	MFLUCC 110439 ^T	JX442037	JX442041	–	–
<i>Bambusicola splendida</i>	MFLUCC 11-0175 ^T	JX442038	JX442042	–	–
<i>Bimuria novae-zelandiae</i>	CBS 107.79 ^T	AY016356	AY016338	DQ471087	DQ470917
<i>Byssothecium circinans</i>	CBS 675.92 ^T	GU205217	GU205235	GU349061	–
<i>Corynespora olivacea</i>	CBS 114450 ^T	GU301809	–	GU349014	–
<i>Didymella bryoniae</i>	CBS 133.96	GU301863	–	–	GU371767
<i>Falciformispora lignatilis</i>	BCC 21118 ^T	GU371827	GU371835	GU371820	–
<i>Falciformispora lignatilis</i>	BCC 21117 ^T	GU371826	GU371834	GU371819	–
<i>Halomassarina thalassiae</i>	JK 5385B ^T	GU479804	–	GU479853	–
<i>Halomassarina thalassiae</i>	JK 5262D ^T	GU301816	–	GU349011	–
<i>Helicascus nypae</i>	BCC 36752 ^T	GU479789	GU479755	GU479855	GU479827
<i>Kalmusia scabrispora</i>	NBRC 106237/K ^T 2202 ^T	AB524594	AB524453	AB539107	AB539094
<i>Karstenula rhodostoma</i>	CBS 690.94 ^T	GU301821	GU296154	GU349067	GU371788
<i>Katunotoa bambusicola</i>	MAFF 239641 ^T	AB524595	AB524454	AB539108	AB539095
<i>Keissleriella cladophila</i>	MFLUCC 110437 ^T	GU301822	GU205241	–	GU371735
<i>Lentithecium aquaticum</i>	CBS 123099 ^T	GU301823	GU296156	GU349068	GU371789
<i>Lentithecium arundinaceum</i>	CBS 619.86 ^T	GU301824	GU296157	–	FJ795473
<i>Lentithecium fluviale</i>	CBS 122367 ^T	GU301825	GU296158	GU349074	–
<i>Leptosphaerulina argentinensis</i>	CBS 569.94	GU301829	–	GU349008	–
<i>Leptosphaerulina australis</i>	CBS 317.83	GU301830	GU296160	GU349070	GU371790
<i>Lizonia sexangularis</i>	M222 ^T	EU940134	EU940061	–	EU940347

<i>Lizonia sexangularis</i>	M179 ^T	EU940122	EU940049	–	EU940334
<i>Macroventuria anomochaeta</i>	CBS 525.71	GU456315	GU238208	GU456262	GU456346
<i>Massarina cisti</i>	CBS 266.62 ^T	FJ795447	FJ795490	–	FJ795464
<i>Massarina eburnea</i>	CBS 473.64 ^T	GU301840	GU296170	GU349040	GU371732
<i>Massarina igniaria</i>	CBS 845.96 ^T	FJ795452	FJ795494	–	GU371793
<i>Melanomma pulvis-pyrus</i>	CBS 124080 ^T	GU456323	GU456302	GU456265	GU456350
<i>Monascostroma innumerosum</i>	CBS 345.50	GU301850	GU296179	GU349033	–
<i>Montagnula opulenta</i>	CBS 168.34 ^T	NG_027581	JN673013	–	–
<i>Morosphaeria ramunculicola</i>	JK 5304B ^T	GU479794	GU479760	–	GU479831
<i>Neottiosporina paspali</i>	CBS 331.37 ^T	EU754172	EU754073	GU349079	GU371779
<i>Ophiosphaerella sasitcola</i>	MAFF 239644 ^T	AB524599	AB524458	AB539111	AB539098
<i>Palmiascoma gregariascomum</i>	MFLUCC 110437 ^T	KP744495	KP753958	–	KP998466
<i>Paraphaeosphaeria michotii</i>	CBS 591.73 ^T	GU456326	GU456305	GU456267	GU456352
<i>Paraconiothyrium minitans</i>	CBS 122788 ^T	EU754173	EU754074	GU349083	GU371776
<i>Peyronellaea glomerata</i>	CBS 528.66	EU754184	EU754085	GU349081	GU371781
<i>Peyronellaea zeae-maydis</i>	CBS 588.69	EU754192	EU754093	GU349082	GU371782
<i>Phaeodimeriella dilleniae</i>	MFLU 14-0013 ^T	KU746805	KU746807	KU746811	KU746809
<i>Phaeodimeriella cissampeli</i>	MFLU 16-0558 ^T	KU746806	KU746808	KU746812	KU746810
<i>Phaeodothis winteri</i>	CBS 182.58 ^T	GU301857	GU296183	–	–
<i>Phoma complanata</i>	CBS 268.92	EU754180	EU754081	GU349078	GU371778
<i>Phoma exigua</i>	CBS 431.74	EU754183	EU754084	GU349080	GU371780
<i>Phoma herbarum</i>	CBS 276.37	DQ678066	DQ678014	DQ677909	DQ677962
<i>Saccothecium sepincola</i>	CBS 278.32	GU301870	GU296195	GU349029	GU371745
<i>Stagonospora macropycnidia</i>	CBS 114202 ^T	GU301873	GU296198	GU349026	–
<i>Trematosphaeria pertusa</i>	CBS 122368 ^T	FJ201990	FJ201991	KF015701	FJ795476
<i>Trematosphaeria pertusa</i>	CBS 122371 ^T	GU301876	FJ201993	KF015702	GU371801

¹BCC: Belgian Coordinated Collections of Microorganisms; CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; JK: J. Kohnmeier; MFLU: Mae Fah Luang University Herbarium Collection; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; T: ex-type/ex-epitype strain; S: working collection of William Quaedvlieg; ²LSU: 28S large subunit of the mRNA gene, SSU: 18S small subunit of the mRNA gene, TEF: translation elongation factor 1-alpha gene and RPB2: RNA polymerase second largest subunit.

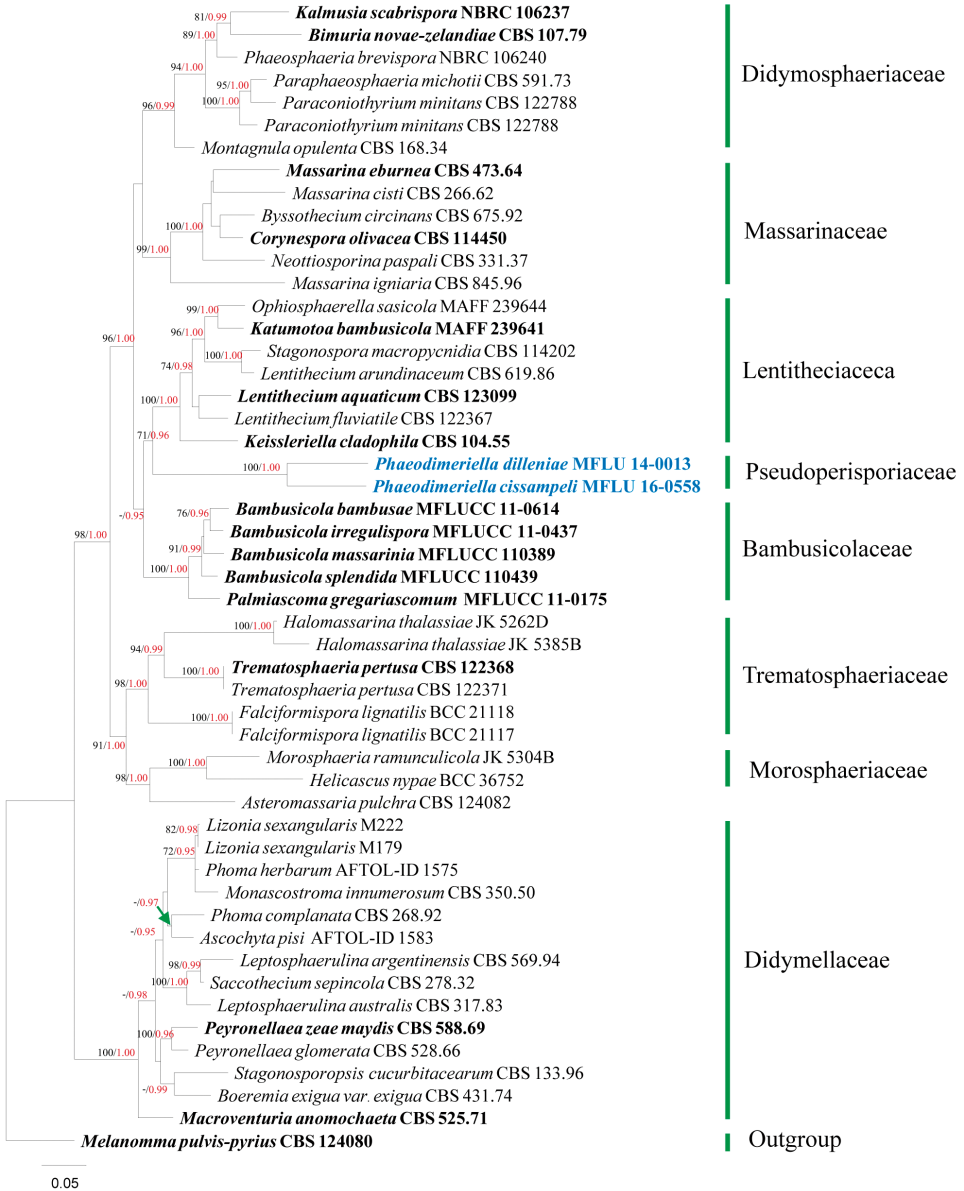


Fig. 1. Selected families of Pleosporales and phylogram generated from RAxML based on combined LSU, SSU, TEF and RPB2 sequenced data for *Phaeodimeriella* species and related taxa. Maximum likelihood (ML, black) bootstrap support values greater than 70% are indicated above or below the nodes and branches with Bayesian posterior probabilities (PP, red) equal to or greater than 0.95 are indicated above or below the nodes and branches. The ex-type strains and reference strains are indicated in bold. New isolates are in blue. The tree is rooted to *Melanomma pulvis-pyrius* CBS 124080.

RESULTS

Phylogenetic analysis of combined LSU, SSU, TEF and RPB2 sequence data

The combined dataset includes LSU, SSU, TEF and RPB2 sequence data that were analyzed using Maximum Likelihood (ML) and Bayesian analyses. All trees were similar in topology and did not differ significantly (data not shown).

The combined sequence alignment comprised 51 taxa, including our new strains, with *Melanomma pulvis-pyrius* (CBS 124080) selected as the outgroup taxon. A best scoring tree obtained by RAxML analysis based on a combined aligned dataset of LSU, SSU, TEF and RPB2 sequence data is shown in Fig. 1. The phylogenetic tree (Fig. 1) shows that two *Phaeodimeriella* isolates formed a monophyletic clade within the suborder Massarineae (Pleosporales) and form a sister clade with *Lentitheciaceae* and also a close phylogenetic relationship with *Bambusicolaceae*. A pairwise comparison of TEF and RPB2 gene characters of the two *Phaeodimeriella* collections significantly differentiated the species. Table 2 shows that they differ in 76 and 132 base pair positions respectively. Furthermore, the two isolates of *Lizonia* clustered in *Didymellaceae* (Fig. 1).

TAXONOMY

Pseudoperisporiaceae Toro, in Seaver & Palacios Chardon, Scient. Surv. P. Rico 8(1): 40 (1926)

Epiphytes on living leaves, or associated with other fungi on leaves, in tropical regions. *Sexual morph*: *Ascomata* superficial, solitary or scattered, coriaceous, globose to subglobose, surrounded by brown mycelium at the base. *Ostiole* central, with or without dark brown setae, tapering to subacute apex. *Peridium* comprising thin-walled, brown to dark brown cells of *textura angularis*. *Hamathecium* comprising cylindrical to filiform, branched, septate pseudoparaphyses, in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, oblong-clavate to oval-ellipsoid, slightly curved, sessile or with knob-like pedicel, with a small ocular chamber. *Ascospores* 2-3-seriate, hyaline, becoming brownish at maturity, oblong to broadly fusiform, 1-septate, slightly constricted at the septum, wall minutely verrucose. *Asexual morph*: Coelomycetous. *Conidiomata* pycnidial, superficial, globose to subglobose, with dark brown, long, aseptate, hook-like setae, with blunt apex. *Peridium* thin, comprising brown cells of *textura angularis*. *Conidiogenous cells* enteroblastic, monophialidic. *Conidia* fusiform, hyaline, aseptate, guttulate.

Type: Lasiostemma Theiss., Syd. & P. Syd., in Sydow & Sydow, Annl. mycol. 15(3/4): 218 (1917)

Synonymy (from Index Fungorum 2016)

Chaetyllis Clem., in Clements & Shear, Gen. fung., Edn 2 (Minneapolis): 253 (1931)

Epiploca Kleb., Haupt- und Nebenfruchtformen der Ascomyzeten (Leipzig) 1: 167 (1918)

Lasiostemmella Petr., Sydowia 4(1-6): 376 (1950)

Neodimerium Petr., Sydowia 4(1-6): 341 (1950)

Pseudoperis Clem. & Shear, Gen. fung., Edn 2 (Minneapolis): 272 (1931)

Pseudoperisporium Toro, Scient. Surv. P. Rico 8(1): 41 (1926)

Table 2. TEF and RPB2 gene character comparisons of *Phaeodimeriella* species used in this study

Taxon/Character	TEF																													
	29	33	35	58	66	75	102	103	104	108	109	151	152	153	160	162	174	201	206	207	213	216	225	228	231	237	240			
<i>P. dilleniae</i> MFLU 14-0013	A	A	C	G	C	G	C	C	G	T	A	C	A	T	G	G	G	C	G	C	G	C	C	C	C	C	G	G		
<i>P. cissampeli</i> MFLU 16-0558	G	G	-	C	G	C	C	G	T	C	A	G	T	C	A	T	C	C	G	T	T	C	T	T	T	T	T	C	C	
Taxon/Character	TEF																													
	246	258	267	276	285	312	321	324	327	330	336	339	342	351	363	388	389	396	414	438	447	465	468	471	489	504	522			
<i>P. dilleniae</i> MFLU 14-0013	C	C	C	C	G	G	G	G	T	G	C	C	C	G	G	T	C	C	G	C	C	G	C	C	C	T	A	G		
<i>P. cissampeli</i> MFLU 16-0558	T	T	T	T	C	C	C	T	C	C	T	T	C	A	C	T	T	C	A	G	T	C	A	G	T	T	G	G	C	
Taxon/Character	TEF																		RPB2											
	531	537	543	549	555	564	573	607	627	630	636	639	681	687	705	723	747	750	753	756	759	771	9	12	15	18	24			
<i>P. dilleniae</i> MFLU 14-0013	G	C	T	C	C	G	G	T	C	G	C	C	G	C	C	G	C	C	C	C	C	T	T	G	A	C	G	A		
<i>P. cissampeli</i> MFLU 16-0558	C	T	C	T	T	C	T	C	T	C	T	T	G	T	C	A	T	T	T	T	T	C	C	T	G	G	C	G	C	
Taxon/Character	RPB2																													
	27	36	39	42	61	75	81	90	100	105	114	117	138	141	142	144	147	156	159	177	180	183	195	198	201	202	204			
<i>P. dilleniae</i> MFLU 14-0013	G	T	A	G	C	T	T	C	C	T	A	G	T	C	T	G	C	T	C	T	C	C	A	T	C	A	C	C		
<i>P. cissampeli</i> MFLU 16-0558	A	C	G	T	T	C	G	T	T	G	G	A	C	G	C	A	G	C	T	C	T	A	G	C	T	G	C	T	G	G

RPB2	
Taxon/Character	210 219 222 225 252 261 267 270 282 288 291 300 306 309 333 336 342 345 363 369 372 381 390 393 396 417 429
<i>P. ditleniiae</i> MFLU 14-0013	C C G C C C T C T C C G G C C G A G C C T T C G C A C C
<i>P. cissampeli</i> MFLU 16-0558	T T A T A T C G C T A A T T G C G A T A C T C T T T T
RPB2	
Taxon/Character	432 435 459 462 471 480 485 486 501 503 507 513 516 526 528 531 537 540 546 550 552 561 567 576 582 591 603
<i>P. ditleniiae</i> MFLU 14-0013	C A A A C G A G C A T C C G C C A G C C G T T G C A C T
<i>P. cissampeli</i> MFLU 16-0558	G C C G T A G A T G C T T T G T G A T T C C A G T T C
RPB2	
Taxon/Character	604 606 612 619 621 625 636 639 672 675 678 687 693 699 708 714 718 719 723 726 732 733 738 741 750 753 762
<i>P. ditleniiae</i> MFLU 14-0013	T G T A T T C A A C G G C C A C C A A A G A C G C G
<i>P. cissampeli</i> MFLU 16-0558	C C C G G C T G C G T A A G A G T T G C G A G T T A
RPB2	
Taxon/Character	774 775 780 783 789 792 798 804 807 813 822 823 825 828 831 837 846 849 855
<i>P. ditleniiae</i> MFLU 14-0013	C G C C C T T G C C T C T C T T C T A G
<i>P. cissampeli</i> MFLU 16-0558	T C T T T C C A T G A T A C T C C C A

Phaeodimeriella Speg., *Revta Mus. La Plata* 15(2): 13 (1908)

Faces of fungi number: FoF 01927

Possible synonyms (from Index Fungorum 2016)

Acanthostoma Theiss., *Beih. bot. Zbl.*, Abt. 2 29: 46, 69 (1912)

Chaetostigmella Syd. & P. Syd., *Annls mycol.* 15(3/4): 199 (1917)

Phaeodimeriella Theiss., *Beih. bot. Zbl.*, Abt. 2 29: 46 (1912)

Phaeodimeris Clem. & Shear, *Gen. fung.*, Edn 2 (Minneapolis): 251 (1931)

Epiphytes on living leaves, or associated with other fungi on leaves. *Sexual morph*: *Ascomata* superficial, solitary or scattered, coriaceous, globose to subglobose, dark brown, surrounded by brown to dark brown mycelium at the base. *Ostiole* at the apex, with or without dark brown setae. *Peridium* 7-13 µm wide, comprising brown to dark brown cells of *textura angularis*. *Hamathecium* comprising 1.5-2 µm wide, cylindrical to filiform, hyaline, branched, septate pseudoparaphyses. *Asci* 8-spored, bitunicate, oval to ellipsoid, straight or slightly curved, smooth-walled, apically round, short pedicellate, with a small ocular chamber. *Ascospores* 2-3-seriate, hyaline when immature and pale brown to brown at maturity, oblong to broadly fusiform, 1-septate, constricted at the septum, guttulate, with a mucilaginous sheath. *Asexual morph*: Coelomycetous. *Conidiomata* pycnidial, superficial, globose to subglobose, with long, hook-like, aseptate, dark brown setae, with blunt apex. *Peridium* thin, comprising brown cells of *textura angularis*. *Conidiogenous cells* enteroblastic, monophialidic. *Conidia* fusiform, hyaline, aseptate, guttulate.

Type species: ***Phaeodimeriella occulta*** (Racib.) Speg.

Phaeodimeriella occulta (Racib.) Speg., *Revta Mus. La Plata* 15(2): 13 (1908)

Figs 2, 3

Synonyms

Dimerosporium occultum Racib., *Parasit. Alg. Pilze Java's* (Jakarta) 3: 32 (1900)

Dimerium occultum (Racib.) Sacc. & P. Syd., *Syll. fung.* (Abellini) 17: 537 (1905)

Phaeodimeris occulta (Racib.) Clem. & Shear, *Gen. fung.*, Edn 2 (Minneapolis): 251 (1931)

Phaeodimeriella parvula (Cooke) Hansf., *Mycol. Pap.* 15: 64 (1946)

Epiphytes on living leaves, or associated with *Asterina* on leaves of *Trema tomentosa* var. *viridis* Planch. & Hewson. *Sexual morph*: *Ascomata* 90-100 µm high × 85-110 µm diam. (\bar{x} = 95 × 98 µm, n = 5), superficial, solitary or scattered, coriaceous, globose to subglobose, dark brown, surrounded by brown to dark brown mycelium at the base. *Ostiole* protruding, with dark brown setae (35-)40-50 × 4.5-6(-7) µm (\bar{x} = 42 × 6 µm, n = 10), long, hook-like, aseptate, with a blunt apex. *Peridium* 9-13 µm wide, comprising brown cells of *textura angularis*. *Hamathecium* comprising 1.5-2.5 µm wide, cylindrical to filiform, hyaline, branched, septate pseudoparaphyses. *Asci* (35-)40-55 × 10-15 µm (\bar{x} = 45 × 13 µm, n = 10), 8-spored, bitunicate, oval to ellipsoid, straight or slightly curved, short pedicellate, apically round, with a small ocular chamber. *Ascospores* 13-23 × 3-6 µm (\bar{x} = 18 × 4.5 µm, n = 20), 2-3-seriate, hyaline when immature, greyish brown at maturity, oblong to broadly fusiform, 1-septate, constricted at the septum, guttulate. *Asexual morph*: Coelomycetous. *Conidiomata* pycnidial 75-100 × 80-100 µm (\bar{x} = 88 × 90 µm, n = 5), superficial, globose to subglobose, with long, hook-like, aseptate, dark brown setae (35-)40-50 × 4-5 µm (\bar{x} = 42 × 4.5 µm, n = 10), with a blunt apex. *Peridium* thin, comprising brown cells of *textura angularis*. *Conidiogenous cells* enteroblastic,

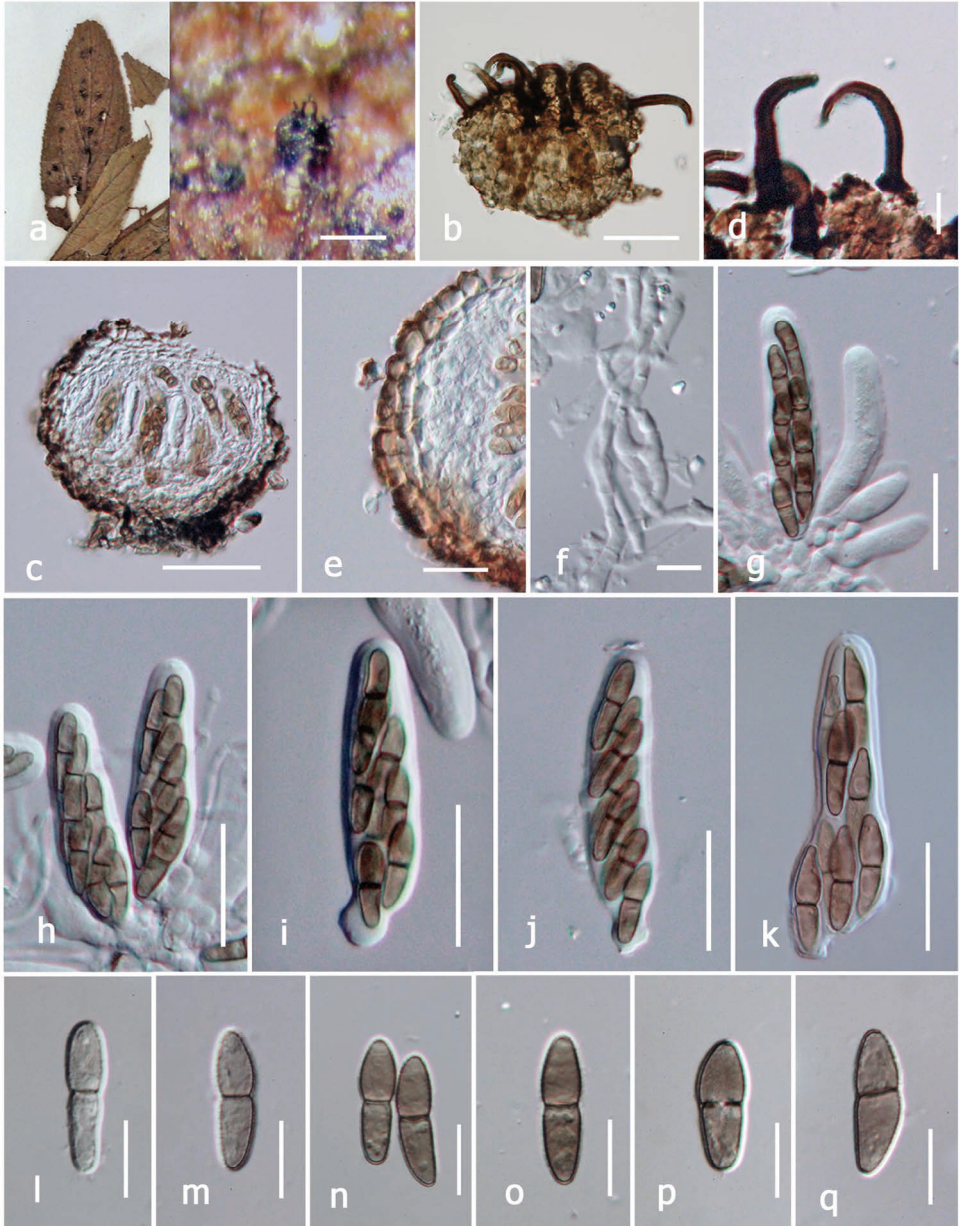


Fig. 2. *Phaeodimeriella occulta* (K(M) 181461, holotype) **a, b**. Superficial ascomata on host leaf. **c**. Section through of ascoma. **d**. Setae. **e**. Peridium. **f**. Pseudoparaphyses. **g-k**. Immature and mature asci. **l-q**. Immature and mature ascospores. Scale bars: a = 100 μ m, b, c = 40 μ m, d, e, l-q = 10 μ m, f = 5 μ m, g-k = 20 μ m.

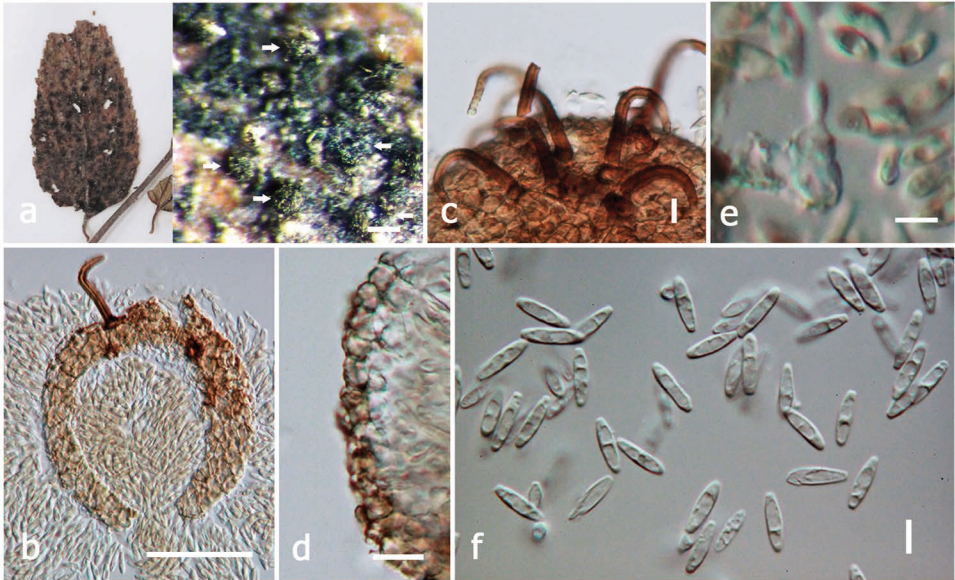


Fig. 3. *Phaeodimeriella occulta* (K(M) 181461, **holotype**) **a.** Asexual morph on substrate. **b.** Section through of conidioma. **c.** Setae. **d.** Peridium. **e.** Conidiogenous cell. **f.** Conidia. Scale bars: a = 100 μ m, b = 50 μ m, c, d = 40 μ m, e, f = 5 μ m.

monophialidic. *Conidia* 9-12 \times 2.5-3 μ m (\bar{x} = 10.5 \times 3 μ m, n = 20), fusiform, hyaline, aseptate, guttulate.

Material examined: AUSTRALIA, Queensland, on leaves of *Trema tomentosa* var. *viridis* (Cannabaceae). Coll. Bailey 902, Det. A. Sivanesan, (K(M) 181461, **holotype**).

Phaeodimeriella dilleniae Mapook, Boonmee & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF551963, *Facesoffunginumber:* FoF 01928

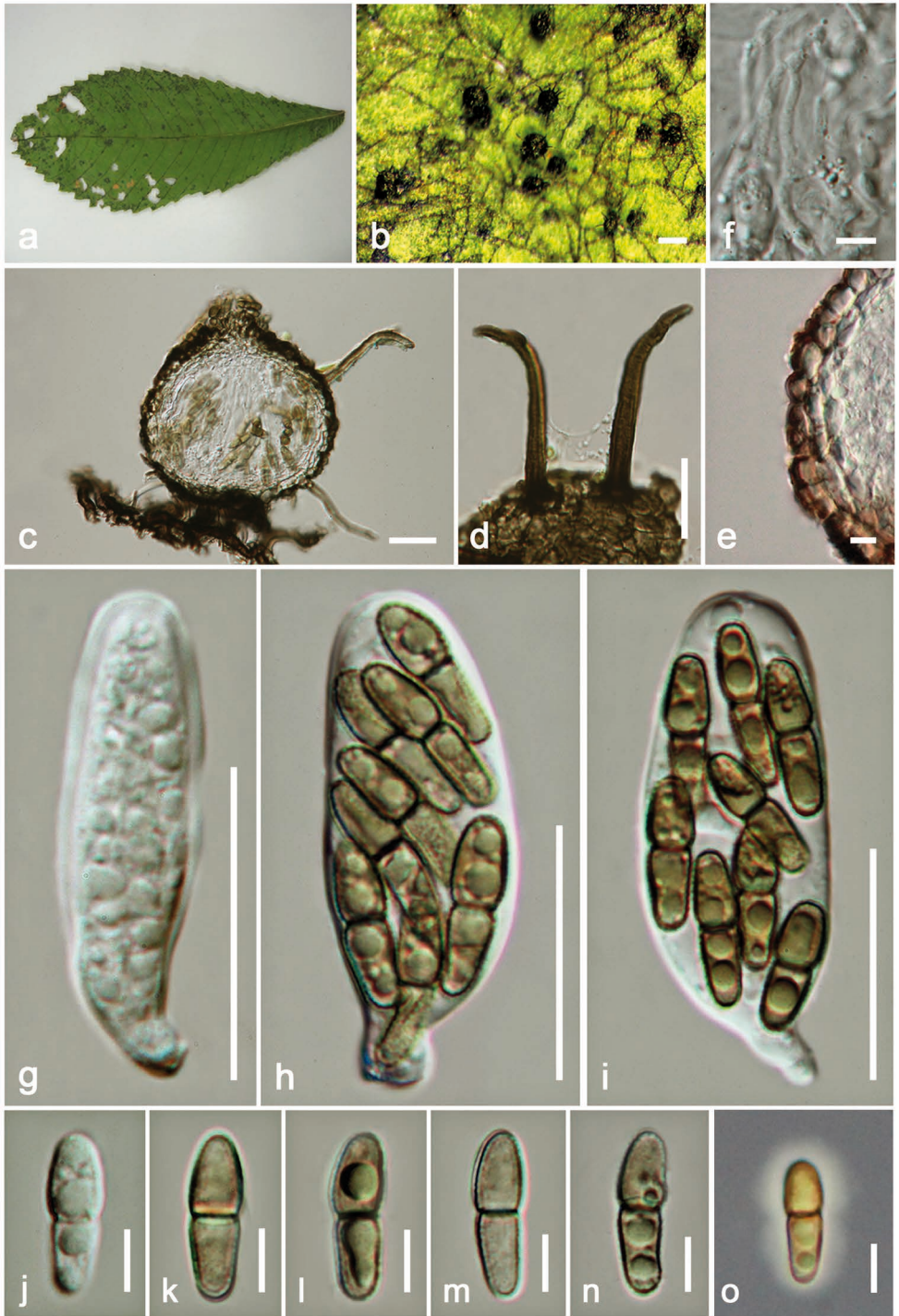
Figs 4, 5

Etymology: Named after the host genus *Dillenia*, from which this species was collected.

Holotype: MFLU 14-0013.

Epiphytic on living leaves surface of *Dillenia* sp. *Sexual morph:* *Ascomata* 70-95 μ m high \times 75-95 μ m diam. (\bar{x} = 80 \times 85 μ m, n = 5), superficial, solitary or scattered, coriaceous, globose to subglobose, dark brown, surrounded by brown to dark brown mycelium at the base. *Ostiolar* neck protruding, with dark brown setae 40-55(-70) \times 4-6 μ m (\bar{x} = 55 \times 5 μ m, n = 10), long, hook shape, aseptate, with blunt apex. *Peridium* 8-10 μ m wide, comprising brown to dark brown cells of *textura angularis*. *Hamathecium* comprising 1.5-2 μ m wide, cylindrical to filiform, hyaline

Fig. 4. *Phaeodimeriella dilleniae* (**holotype**) **a, b.** Superficial ascomata on substrate. **c.** Section through of ascoma. **d.** Setae. **e.** Peridium. **f.** Pseudoparaphyses. **g-i.** Asci at immature and mature. **j-n.** Ascospores at immature and mature. **o.** Ascospores surrounded by hyaline gelatinous sheath in Indian ink. Scale bars: b = 100 μ m, c-d, g-i, p = 20 μ m, e, f, j-o = 5 μ m.



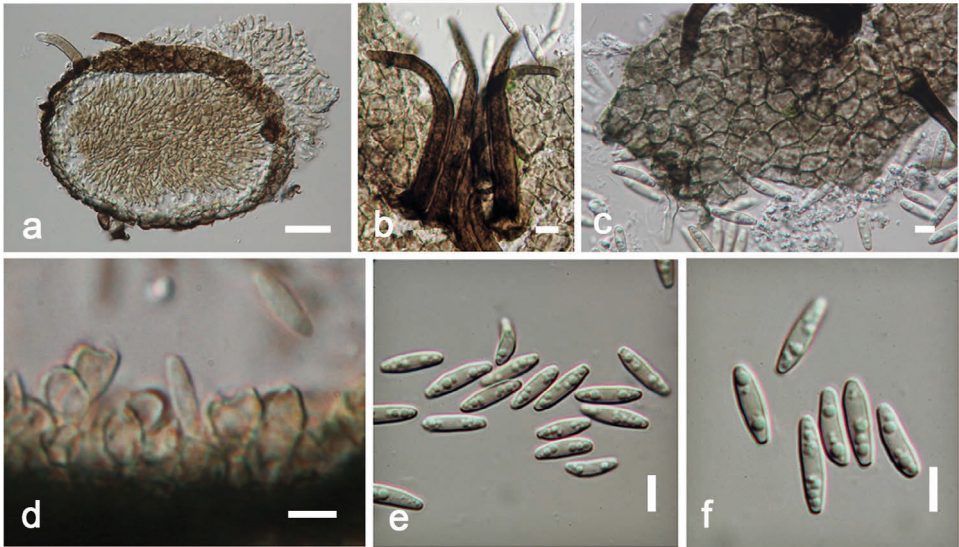


Fig. 5. *Phaeodimeriella dilleniae* (holotype) **a.** Section through conidioma. **b.** Setae. **c.** Peridium texture. **d.** Conidiogenous cells. **e, f.** Conidia. Scale bars: a = 20 μm , c = 10 μm , b, d-f = 5 μm .

pseudoparaphyses. *Asci* (32–)35–40 \times (10–)14–16 μm (\bar{x} = 37 \times 13 μm , n = 10), 8-spored, bitunicate, oval to ellipsoid, straight or slightly curved, short pedicellate, apically round, with a small ocular chamber. *Ascospores* (11–)13–14(–15) \times 4–5 μm (\bar{x} = 13 \times 4 μm , n = 10), irregularly arranged, oblong to broadly fusiform, hyaline when immature and dark greenish brown when mature, 1-septate, constricted at the septum, guttulate, with gelatinous sheath, observed clearly when mounted in Indian ink. *Asexual morph*: Coelomycetous. *Conidiomata* pycnidial 65–70 \times 90–95 μm (\bar{x} = 70 \times 90 μm , n = 5), superficial, globose to subglobose, with hook-like, aseptate, dark brown setae, 45–55 \times 4–5(–6) μm (\bar{x} = 50 \times 5 μm , n = 10) with a blunt apex. *Peridium* thin, comprising brown cells of *textura angularis*. *Conidiogenous cells* enteroblastic, monophialidic. *Conidia* (8–)9–10 \times 2.5–3 μm (\bar{x} = 10 \times 2.5 μm , n = 20), fusiform, hyaline to pale brown, aseptate, guttulate.

Material examined: THAILAND, Chiang Mai, Mushroom Research Center, on living leaves of *Dillenia* sp. (Dilleniaceae). 27 September 2013. Coll. X. Zeng, Det. A. Mapook (MFLU 14-0013, **holotype**) (**isotype** in HKAS, under the code of HKAS 92520).

Notes: Our collection is morphologically similar to *Phaeodimeriella occulta* (Figs 2, 3) in having similar associated sexual and asexual morphs, but our collection differs from *P. occulta* in the shape and size of ascomata, asci and ascospores. The ascospores in *P. dilleniae* are smaller, dark greenish brown and surrounded by gelatinous sheath (Table 3). Furthermore, our collection was found only as epiphytic on leaves, but *P. occulta* was associated with an *Asterina* species as a putative pathogen. Thus, we consider our taxon is a new species based on morphology.

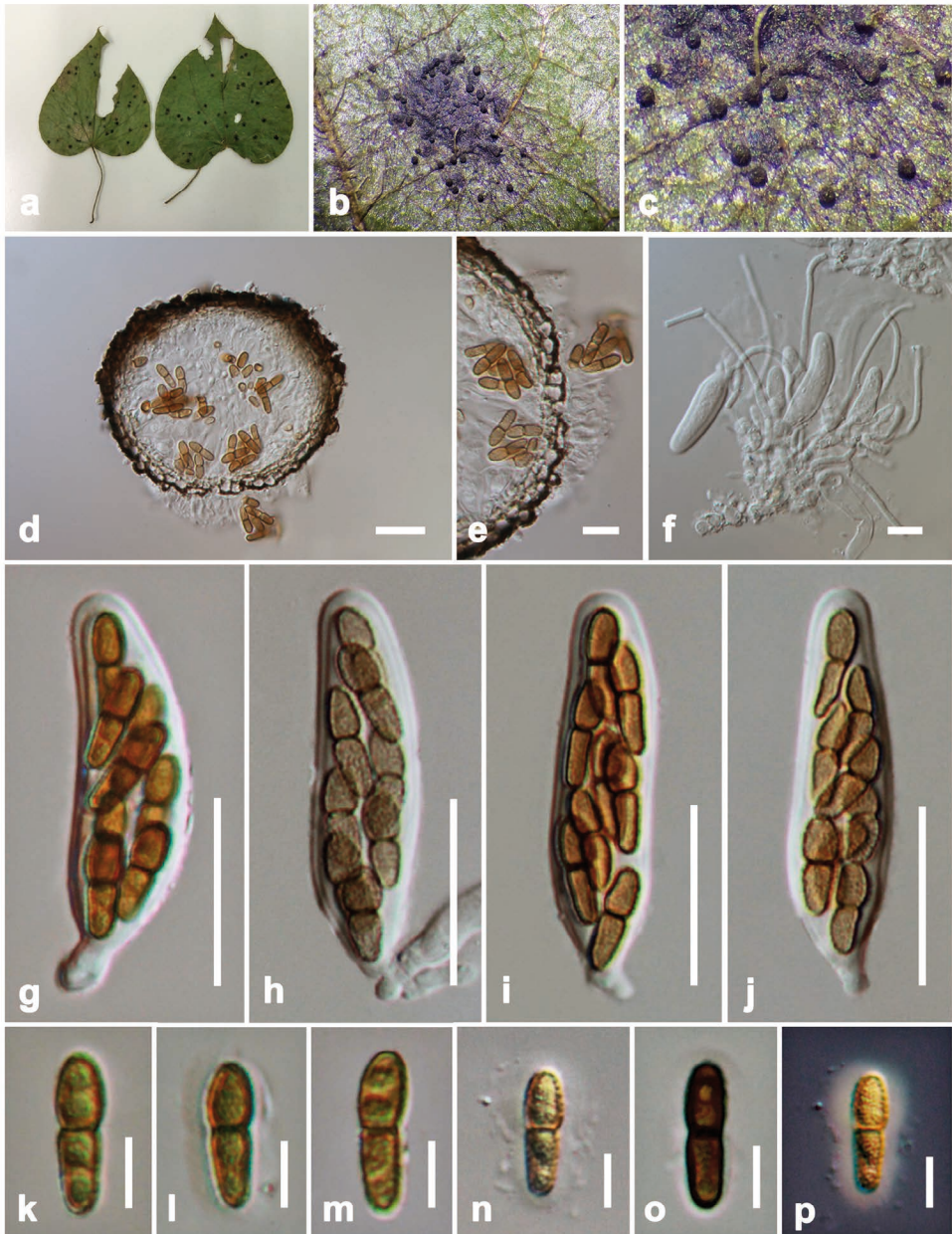


Fig. 6. *Phaeodimeriella cissampeli* (holotype) a-c. Superficial ascomata on substrate. d. Section through ascoma. e. Peridium. f. Pseudoparaphyses and immature asci. g-j. Asci. k-o. Ascospores. p. Ascospores surrounded by hyaline gelatinous sheath in Indian ink. Scale bars: e, f = 10 μ m, d, g-j = 20 μ m, k-p = 5 μ m.

Phaeodimeriella cissampeli Mapook, Boonmee & K.D. Hyde, *sp. nov.**Index Fungorum number:* IF551962, *Facesoffunginumber:* FoF 01929

Fig. 6

Etymology: Named after the host genus *Cissampelos*, from which this species was collected.*Holotype:* MFLU 16-0558.

Epiphytic on living leaves surface of *Cissampelos* sp. *Sexual morph:* *Ascomata* 70-120 μm high \times 100-150 μm diam. (\bar{x} = 95 \times 125 μm , n = 5), superficial, solitary or scattered, coriaceous, globose to subglobose, dark brown, surrounded by brown to dark brown mycelium at the base. *Ostiolar* neck protruding, without dark brown setae. *Peridium* 7-11 μm wide, comprising of brown to dark brown cells of *textura angularis*. *Hamathecium* comprising 1.5-2 μm wide, cylindrical to filiform, hyaline pseudoparaphyses. *Asci* (35-)40-45(-50) \times 7-12 μm (\bar{x} = 40 \times 10 μm , n = 10), 8-spored, bitunicate, oval to ellipsoid, straight or slightly curved, short pedicellate, apically round, with a small ocular chamber. *Ascospores* 10-13 \times 2-3.5 μm (\bar{x} = 12 \times 3 μm , n = 20), irregularly arranged, reddish brown, oblong to broadly fusiform, 1-septate, constricted at the septum, guttulate, surrounded by hyaline gelatinous sheath observed clearly when mounted in Indian ink. *Asexual morph:* Undetermined.

Material examined: THAILAND, Chiang Mai, Doi Suthep, on living leaves of *Cissampelos* sp. (Menispermaceae). 11 August 2014. Coll. & Det. A. Mapook (MFLU 16-0558, **holotype**) (**isotype** in HKAS, under the code of HKAS 92521).

Notes: *Phaeodimeriella cissampeli* is epiphytic on living leaves surface of *Cissampelos* sp. This fungus is most similar to *Phaeodimeriella occulta* in the shape of asci and ascospores, but differs in having larger ascomata and lacking setae, and smaller asci and reddish brown ascospores surrounded by a gelatinous sheath (Table 3). *Phaeodimeriella cissampeli* is also similar to *Phaeodimeriella asterinae* in having ascomata without dark brown setae, but differs in having larger ascomata, and smaller asci and ascospores (Table 3). Thus, we consider this taxon to be a new species based on morphology supported with phylogenetic analysis (Fig. 1).

Table 3. Synopsis of *Phaeodimeriella* species with similar morphological features discussed in this study

<i>Name</i>	<i>Ascomata/Conidiomata</i> (μm)	<i>Asci</i> (μm)	<i>Ascospore/Conidia</i> (μm)
<i>Phaeodimeriella occulta</i> K(M) 181461	90-100 \times 85-110	(35-)40-55 \times 10-15	13-23 \times 3-6, greyish brown
Asexual morph	75-100 \times 80-100	–	9-12 \times 2.5-3
<i>Phaeodimeriella dilleniae</i> (MFLU 14-0013)	70-95 \times 75-95	(32-)35-40 \times (10-) 14-16	(11-)13-14(-15) \times 4-5, dark greenish brown
Asexual morph	65-70 \times 90-95	–	(8-)9-10 \times 2.5-3
<i>Phaeodimeriella cissampeli</i> (MFLU **)	70-120 \times 100-150	(35-)40-45(-50) \times 7-12	10-13 \times 2-3.5, reddish brown
<i>Phaeodimeriella asterinae</i> (Ahn & Crane 2004)	60-130 \times 60-110	44-60 \times 12-20	15.5-19 \times 3-3.5, yellow brown

DISCUSSION

Phaeodimeriella is a ubiquitous genus that was introduced by Spegazzini (1908) with *P. occulta* as a type species and reported as parasitic on *Asterina* (Raciborski 1900; Spegazzini 1908). The genus comprises epiphytic species on living leaves, which may be associated with other fungi and may be parasitic on *Asterinaceae* and *Meliolaceae*. Whether species are parasitic or associated with these other fungi needs to be confirmed, as our new species were not associated with other fungi. Molecular data for *Phaeodimeriella* was previously lacking (Hyde *et al.* 2013; Stenroos *et al.* 2010; Wäli *et al.* 2014). Two new *Phaeodimeriella* species with molecular data are introduced in this study, based on morphological comparisons. In addition, we found ascospores surrounded by gelatinous sheath, which can be observed clearly when mounted in Indian ink; this character has not been reported previously for the genus.

In phylogenetic studies, two *Phaeodimeriella* isolates formed a monophyletic clade within the suborder Massarineae (Pleosporales) and form a sister clade with *Lentitheciaceae* and also show close phylogenetic relationship with *Bambusicolaceae* with high bootstrap supported. This suggests that the family *Pseudoperiariaceae* is the eighth well-resolved family of Massarineae (Li *et al.* 2016). Furthermore, the two isolates of *Lizonia* clustered in *Didymellaceae* although *Lizonia* was assigned to *Pseudoperisporiaceae* in previous studies (Barr 1997; Stenroos *et al.* 2010). Stenroos *et al.* (2010) also obtained similar result.

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