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Lamproconiaceae *fam. nov.* to accommodate *Lamproconium desmazieri*

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Abstract

The genus *Lamproconium* comprises species that are endophytes, saprobes and pathogens on a wide variety of plants. This genus is currently placed in Diaporthales genera *incertae sedis*. Fresh specimens of *Lamproconium* were collected in Russia and studied to provide morphological and phylogenetic data. Phylogenetic analyses of single spore isolates generated from maximum likelihood, maximum parsimony and Bayesian inference analyses using combined ITS and LSU sequence data, place *L. desmazieri* in the order Diaporthales. *Melanconis desmazieri* is synonymized under *Lamproconium desmazieri*, and Lamproconiaceae is introduced as a new family to accommodate *L. desmazieri* and *Hercospora tiliae*, based on morphology and phylogenetic analyses.

Key words: Foliar pathogens, genera *incertae sedis*, morphological data, phylogenetic analyses, synonym

Introduction

Lamproconium (Grove) Grove was introduced as a monotypic genus by Grove (1937) to accommodate *L. desmazieri* (Berk. & Broome) Grove, and was placed in genera *incertae sedis* in the order Diaporthales by Cannon & Minter (2014). This genus is apparently a saprobe with an endophytic life style, or vice versa. Some authors, however, have considered a few species in this genus to be plant pathogens (Grove 1937, Cannon & Minter 2014, Sutton 1980).

The type species of *Lamproconium*, originally described as *Discella desmazieri* Berk. & Broome, was collected from twigs of lime (*Citrus aurantiifolia*) in the UK (Berkeley & Broome 1850). Grove (1918) transferred the species to *Melanconium* in the subgenus *Lamproconium* as *Melanconium desmazieri* (Berk. & Broome) Sacc. Subsequently, Grove (1937) found that *M. desmazieri* differed from the type species of *Melanconium* (*M. atrum* Link) in having bluish to glistening or dark blue, but not brownish black, 1-septate conidia and accordingly re-circumscribed the species. *Melanconium desmazieri* differed from species of *Discella* by the absence of a true peridium, whereas *Discella* species have a proliferous stratum at the base. Grove (1937) therefore considered *Discella desmazieri* and *Melanconium desmazieri* as conspecific and introduced a new genus *Lamproconium* to accommodate this taxon (Grove 1937, Sutton 1980).

Melanconium has been reported as the asexual morph of *Melanconis* Tul. & C. Tul. (Sutton 1980). The genus *Melanconis* belongs in the family Melanconidaceae in Diaporthales, Sordariomycetes (Maharachchikumbura *et al.* 2015, 2016). Castlebury *et al.* (2002) used LSU sequence data from *M. stilbostoma* (Fr.) Tul. & C. Tul., the type species of *Melanconis*, plus *M. alni* Tul. & C. Tul. and *M. marginalis* (Peck) Wehm. in a phylogenetic analysis and showed that *Melanconis desmazieri* Petr. clustered with *Hercospora tiliae* Tul. & C. Tul. However, the group clustered distantly from the family Melanconidaceae. Thus, Castlebury *et al.* (2002) placed *Melanconis desmazieri* and *Hercospora tiliae* in *Melanconis sensu lato* as genera *incertae sedis* in Diaporthales. This taxonomic treatment was followed by Voglmayr *et al.* (2012), Voglmayr & Jaklitsch (2014) and Maharachchikumbura *et al.* (2015, 2016).

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Hercospora tiliae differs from *Melanconis desmazieri* in having ellipsoidal to cylindric-ellipsoidal, aseptate, hyaline conidia, while the sexual morph has ellipsoidal to cylindrical, hyaline, 1-septate ascospores (Tulasne & Tulasne 1863, Petrak 1938, Sutton 1980). In *M. desmazieri* conidia are oblong-fusoid, straight, at first acute, and later becoming obtuse at one or both ends (Petrak 1938, Castlebury *et al.* 2002, Voglmayr *et al.* 2012, Voglmayr & Jaklitsch 2014).

In this study, we provide morphological and phylogenetic data for *Lamproconium* based on five specimens collected in Russia. Furthermore, *Melanconis desmazieri* is synonymized under *Lamproconium desmazieri* based on morphological characters and multi-locus phylogenetic analyses. These taxa form a distinct lineage in Diaporthales and therefore we introduce the family Lamproconiaceae to accommodate them.

Material and methods

Sample collection and examination of specimens

The samples were collected from dead branches of *Tilia cordata* Mill. in the Rostov region, Russia, in May 2014. The specimens were returned to the laboratory in small paper bags, examined, identified and described following Norphanphoun *et al.* (2015). Micro-morphological characters were studied using a Motic SMZ 168 dissecting microscope for fungal fruiting bodies. Hand sections of the fruiting structures were mounted in water and examined for morphological details. Fungi were also examined using a Nikon Ni compound microscope and photographed with a Canon EOS 600D digital camera fitted to the microscope. Photo-plates were made by using Adobe Photoshop CS6 Extended version 13.0 × 64 (Adobe Systems, USA), while Tarosoft (R) Image Frame Work program v. 0.9.7 was used for measurements. The contents inside the conidiomata, which comprised conidiophores, conidiogenous cells, conidia and paraphyses, were removed with a sterile needle and soaked in sterile water in a glass container prior to examination.

Cultures were obtained by single spore isolation as described in Chomnunti *et al.* (2014). Spore germination was observed and photographed using a Nikon Ni compound microscope fitted with Canon EOS 600D digital camera. Geminated spores were transferred aseptically to fresh malt extract agar (MEA) and incubated at room temperature (18−25°C). Colony characters were observed and measured after one week and also one month.

The Herbarium specimens are deposited in the Mae Fah Luang University Herbarium, Chiang Rai, Thailand (MFLU) and duplicated in New Zealand Fungarium (PDD). Living cultures are deposited at Mae Fah Luang University Culture Collection (MFLUCC) and Kunming Culture Collection (KUMCC). Facesoffungi and Index Fungorum numbers are registered (Jayasiri *et al.* 2015, Index Fungorum 2016).

DNA extraction, PCR amplification and sequencing

DNA extraction was performed from fresh fungal mycelia growing on MEA at room temperature (18−25°C) for 3 weeks. The genomic DNA was obtained using a E.Z.N.A.TM Fungal DNA MiniKit (Omega Biotech, CA, USA) following the manufacturer's instructions.

Polymerase chain reactions (PCR) were carried out using primer pairs of ITS5 and ITS4 to amplify the internal transcribed spacer region (ITS1-5.8S-ITS2) (White *et al.* 1990), and the large subunit rDNA (28S, LSU) was amplified with primers LROR and LR5 (Vilgalys & Hester 1990). The amplification reaction was performed in 50 µl reaction volume containing 2 µl of DNA template, 2 µl of each forward and reverse primers, 25 µl of 2 × Bench Top™Taq Master Mix (mixture of Taq DNA Polymerase (recombinant): 0.05 units/µL, $MgCl_2$: 4 mM, and dNTPs (dATP, dCTP, dGTP, dTTP): 0.4 mM) and 19 µl of double-distilled water (ddH_2O) (sterilized water). The PCR thermal cycle program for ITS gene amplification were set as: initially 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 51 °C for 1 min, elongation at 72 °C for 45 s, and final extension at 72 °C for 10 min. The PCR thermal cycle program for LSU gene amplification were provided as: initially 95 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 30 s, annealing at 51 °C for 50 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 10 min. The quality of PCR products were checked by using 1% agarose gel electrophoresis stained with ethidium bromide. Purification and sequencing of PCR product were carried out at Life Biotechnology Co., Shanghai, China.

Phylogenetic analysis

Blast searches were made to identify the closest matches in GenBank and recently published sequences in of Castlebury *et al.* (2002), Voglmayr *et al.* (2012), Voglmayr & Jaklitsch (2014) and Maharachchikumbura *et al.* (2015, 2016).

Combined analyses of ITS and LSU sequence data of 62 taxa (Table 1) from Diaporthales were downloaded from GenBank and *Magnaporthe salvinii* (CBS 243.76) and *M. grisea* (GAD1) were used as outgroup taxa. Additionally, the datasets were optimized manually as detailed in Castlebury *et al.* (2002), Voglmayr *et al.* (2012), Voglmayr & Jaklitsch (2014) and Maharachchikumbura *et al.* (2015, 2016). The combined sequence alignments were obtained from MEGA7 version 7.0.14 (Kumar *et al.* 2015) and ambiguously aligned regions were excluded, gaps were treated as missing data which performed in BioEdit v. 7.2 (Hall 1999). Phylogenetic trees were inferred with maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI).

TABLE 1. GenBank accession numbers of the sequences used in phylogenetic analyses.

Species	Strain	ITS	LSU
Anisogramma anomala	529478	EU683064	EU683066
Anisogramma virgultorum	529479	EU683062	EU683065
Cainiella johansonii	**Kruys 731 (UPS)**	**-**	**JF701920**
Chapeckia nigrospora	**AR 3809**	**-**	**EU683068**
Cryphonectria parasitica	CMW 7048	JN942325	JN940858
Cryptodiaporthe aesculi	AFTOL-ID 1238	-	DQ836905
Cryptodiaporthe salicella	AR3455	-	AF408345
Cryptometrion aestuescens	CMW 18790	GQ369458	HQ730869
Cryptometrion parasitica	**ATCC 38755**	**AY141856**	**EU199123**
Cryptosporella hypodermia	**AR3552**	**EU199181**	**AF408346**
Cryptosporella hypodermia	AFTOL-ID 2124	-	DQ862028
Cytospora elaeagni	CFCC 89633	KF765677	KF765693
Cytospora parasitica	**MFLUCC 14-1055**	**KT459408**	**KT459409**
Cytospora tanaitica	**MFLUCC 14-1057**	**-**	**-**
Diaporthales sp.	YMJ 1364	JX570889	JX570891
Diaporthales sp.	BCC00200	-	EF622231
Diaporthe eres	AFTOL-ID 935	DQ491514	-
Diaporthe eres	**AR3538**	**-**	**AF408350**
Diaporthe detrusa	AR3424	-	AF408349
Ditopella ditopa	**AR3423**	**-**	**AF408360**
Gnomonia gnomon	**CBS 199.53**	**AY818956**	**-**
Hapalocystis occidentalis	WU 24705	-	AY616231
Harknessia australiensis	CPC 15029	JQ706085	JQ706211
Harknessia ellipsoidea	CPC 17111	JQ706087	JQ706213
Harknessia eucalypti	**CBS 342.97**	**AY720745**	**AF408363**
Harknessia pseudohawaiiensis	CPC 17379	JQ706111	JQ706234
Hercospora tiliae	AR3526	-	AF408365
Lamproconium desmazieri	**MFLUCC 14-1047**	**KX430132**	**KX430133**
Lamproconium desmazieri	MFLUCC 15-0870	KX430134	KX430135
Lamproconium desmazieri	MFLUCC 15-0871	KX430136	KX430137
Lamproconium desmazieri	MFLUCC 15-0872	KX430138	KX430139

...Continued on next page

TABLE 1. (Continued)

Species	Strain	ITS	LSU
Lamproconium desmazieri	MFLUCC 15-0873	KX430140	KX430141
Leucostoma niveum	AR 3413	JX438624	NG_027590
Luteocirrhus shearii	**CBS 130776**	KC197021	KC197019
Magnaporthe grisea	GAD1	-	JQ920470
Magnaporthe salvinii	**CBS 243.76**	**KM484861**	**DQ341498**
Melanconiella ellisii	BPI 843491	JQ926268	JQ926268
Melanconiella spodiaea	**MSH**	**JQ926298**	**JQ926298**
Melanconiella spodiaea	**SPOD**	**JQ926300**	**-**
Melanconis alni	AR3500	-	AF408371
Melanconis alni	AR3748	EU199195	EU199130
Melanconis desmazieri	AR3525	-	AF408372
Melanconis desmazieri	AR3827	JX522735	-
Melanconis desmazieri	CBS 109780	JX522736	-
Melanconis marginalis	AR3442	-	AF408373
Melanconis stilbostoma	**AR3501**	**-**	**AF408374**
Ophiovalsa betulae	AR3524	-	AF408375
Ophiovalsa suffusa	**AR3496**	**-**	**AF408376**
Phragmoporthe conformis	**AR3632**	**-**	**AF408377**
Pilidiella castaneicola	**CBS 143.97**	**-**	**AF408378**
Pilidiella diplodiella	STE-U 3708	AY339323	AY339284
Pilidiella wangiensis	CPC 19397	JX069873	JX069857
Plagiostoma euphorbiae	**CBS 340.78**	**EU199198**	**AF408382**
Pseudoplagiostoma eucalypti	**CBS 124807**	**GU973512**	**GU973606**
Pseudoplagiostoma oldii	CBS 124808	GU973534	GU973609
Pseudoplagiostoma variabile	CBS 113067	GU973536	GU973611
Pseudovalsa longipes	AR3541	-	EU683072
Pseudovalsa modonia	AR 3558	-	EU683073
Rossmania ukurunduensis	**AR 3484**	**-**	**EU683075**
Schizoparme straminea	**CBS 149.22**	**-**	**AF362569**
Schizoparme straminea	STE-U 3932	AY339348	AY339296
Stegonsporium protopyriforme	**CBS 117041**	**NR_126119**	**-**
Stilbospora macrosperma	**CBS 121883**	**JX517290**	**JX517299**
Stilbospora macrosperma	**CBS 121695**	**JX517288**	**JX517297**
Sydowiella fenestrans	**AR 3777**	**-**	**EU683078**
Thailandiomyces bisetulosus	**BCC00018**	**-**	**EF622230**
Tirisporella beccariana	**BCC36737**	**-**	**JQ655450**

Note. The ex-type strains are in bold.

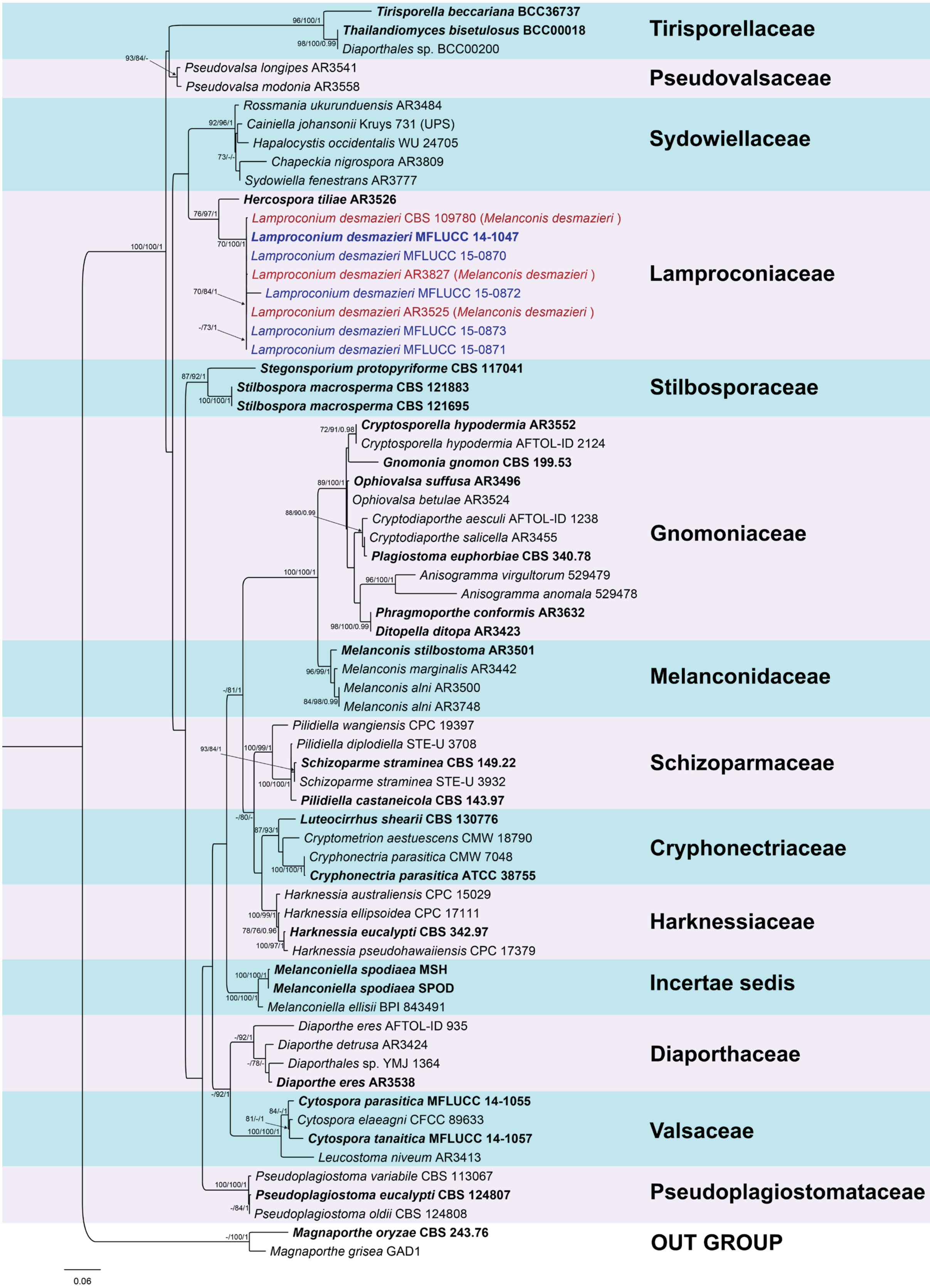

FIGURE 1. Maximum likelihood (ML) majority rule consensus tree of combined ITS and LSU sequence data based on MP, ML and Bayesian analyses. Values above the branches indicate maximum parsimony and maximum likelihood bootstrap ≥70%, (MPBS/MLBS). Values at the third positions, respectively, above or below the branches represent posterior probabilities (BI PP ≥ 0.95) from Bayesian inference analysis. The tree is rooted to *Magnaporthe salvinii* and *M. grisea*. Strain numbers are given following the taxon names. The new sequences resulting from this study are in blue. Synonyms are in red. Ex-type strains are in black bold.

Maximum-likelihood (ML) analysis was performed in RAxML (Stamatakis 2006) implemented in raxmlGUI v.1.3 (Silvestro & Michalak 2012). The 1000 rapid bootstrap replicates were run with generalized time reversible GTRGAMMA model of nucleotide substitution and searches for model selected for ML were applied. Trees were visualized using FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/, Rambaut 2012).

Maximum parsimony (MP) analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). The trees were inferred using the heuristic search option with tree bisection-reconnection (TBR) as the branch swapping algorithm and 1000 random sequence additions. Maxtrees were setup to 5000, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC] and homoplasy index [HI] were calculated for the Maximum Parsimonious Tree (MPT). The robustness of the most parsimonious trees were evaluated by 1000 bootstrap replications, each with ten replicates of random stepwise addition of taxa (Felsenstein 1985). The Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed to determine whether the trees were significantly different. Trees were viewed in TreeView v.1.6.6 (Page 1996).

Bayesian inference (BI) analysis was performed using the Markov Chain Monte Carlo (MCMC) method with MrBayes 3.2.2 (Ronquist *et al.* 2012). The best-fit nucleotide substitution models for each dataset were separately determined using MrModeltest version 2.2 (Nylander 2004). GTR+I+G were selected as best-fitting models for the ITS and LSU datasets. The Markov Chain Monte Carlo sampling (MCMC) analyses, with four chains, were run, started from random tree topology and lasted 5,000,000 generations and sampled every 100 generations (Nylander *et al.* 2008). The Tracer v. 1.5.0 program was used to check the effective sampling sizes (ESS) that should be above 200, the stable likelihood plateaus and burn–in value (Rambaut & Drummond 2009). The first 5000 generations were excluded as burn-in and tree were visualized using FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/, Rambaut 2012).

The phylograms are visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/, Rambaut 2012) and made in Adobe Illustrator CS6 and Adobe Photoshop CS6 Extended version 13.0 × 64. Sequences data from this study are deposited in GenBank.

Results

Phylogenetic analyses

The phylogenetic tree based on combined analysis of ITS and LSU sequence data was used to resolve the relationships in *Lamproconium* in Diaporthales. The phylogenetic analyses were obtained from maximum likelihood (ML), maximum parsimony (MP) and Bayesian analyses. The alignment comprised 67 taxa and 1534 total characters including gaps. Parsimony analyses indicate that 920 characters were constant, 141 variable characters were parsimony uninformative and 473 characters were parsimony informative. The parsimony analysis of the data matrix resulted in two equally parsimonious trees and the first tree (TL = 518, CI = 0.530, RI = 0.801, RC = 0.425, HI = 0.470) is shown in Fig. 1. The Bayesian analysis resulted in the same topology as the MP trees. The phylogenetic results from Fig. 1 are discussed in the notes.

Five isolates (MFLUCC 14-1047, MFLUCC 15-0870, MFLUCC 15-0871, MFLUCC 15-0872 and MFLUCC 15-0873) grouped together with three strains of *Melanconis desmazieri* (AR3525, AR3827 and CBS 109780) in the combined phylogeny with 100% ML, 70% MP bootstrap support and 1.0 PP support (Fig. 1). Our isolate grouped close to *Hercospora tiliae*, but as a distinct lineage with 97% ML, 76% MP support and 1.0 PP in the combined phylogeny (Fig. 1). There are two genera with nine strains constituting the family Lamproconiaceae, based on the multi-gene phylogeny and with support from morphological observations.

Taxonomy

Lamproconiaceae C. Norphanphoun, T.C. Wen & K.D. Hyde, ***fam. nov.***
Index Fungorum number: IF552187, *Facesoffungi number*: FoF 02248

Pathogen and *saprobe* on dying twigs and branches. *Sexual morph:* Undetermined. *Asexual morph: Conidiomata* pycnidial, solitary, partly immersed in host tissue, uniloculate, multiloculate or convoluted, dark blue (*Lamproconium*), dark blackish brown (*Hercospora*), erumpent in the centre. *Pycnidium* thick-walled, thin at inner layer, hyaline (*Lamproconium*), dark brown (*Hercospora*), comprising wall cells of *textura angularis* (*Lamproconium*) or *textura intricata* (*Hercospora*). *Ostiole* absent, dehiscence irregular. *Paraphyses* interspersed with conidiophores. *Conidiophores* filiform or cylindrical, pale bluish or hyaline, septate, branched, smooth-walled, formed at the base of conidiomatal wall. *Conidiogenous cells* holoblastic, cylindrical to subcylindrical, each forming a single conidium at the conidiophore apex, or annellidic, colourless to olivaceous, smooth-walled. *Conidia* fusiform, ellipsoid, thick-walled, contents granular, aseptate, bluish to glistening dark blue (*Lamproconium*), hyaline (*Hercospora*), smooth-walled, produced in mucilage but without a distinct mucilaginous envelope or appendage.

Type genus:—*Lamproconium* (Grove) Grove.

Notes:—The order Diaporthales comprises 12 families, viz. Cryphonectriaceae Gryzenh. & M.J. Wingf., Diaporthaceae Höhn. ex Wehm., Gnomoniaceae G. Winter, Harknessiaceae Crous, Melanconidaceae G. Winter, Pseudoplagiostomataceae Cheew., M.J. Wingf. & Crous, Pseudovalsaceae M.E. Barr, Schizoparmaceae Rossman, D.F. Farr & Castl., Stilbosporaceae Link, Sydowiellaceae Lar.N. Vassiljeva, Tirisporellaceae Suetrong *et al.* and Valsaceae Tul. & C. Tul. (Maharachchikumbura 2015, 2016).

The family Lamproconiaceae is established to accommodate *Lamproconium* and *Hercospora* and is introduced based on morphology and phylogenetic analyses. Lamproconiaceae forms a robust clade basal to Sydowiellaceae and Stilbosporaceae in the combined ITS and LSU phylogeny (Fig. 1). It is morphologically different in conidial form from the asexual morphs of Sydowiellaceae and Stilbosporaceae.

Species of Sydowiellaceae have been reported as *Melanconis*-like and is allied with *Hercospora*, as shown in the earlier studies (Castlebury *et al.* 2002, Rossman *et al.* 2007). Nevertheless, *Hercospora* is a distinct genus in that the ostioles from individual fruiting bodies converging within the stroma and emerge as one ostiole. *Hercospora tiliae*, with its unusual asexual morph groups with *Melanconis desmazieri*, also from *Tilia* (Castlebury *et al.* 2002, Rossman *et al.* 2007, Petrak 1938, Castlebury *et al.* 2002, Voglmayr *et al.* 2012, Voglmayr & Jaklitsch 2014, Maharachchikumbura *et al.* 2015, 2016). Thus *Hercospora* falls outside Sydowiellaceae and belongs in Lamproconiaceae.

The asexual species of Stilbosporaceae and Lamproconiaceae are coelomycetes. The conidia of Lamproconiaceae species are aseptate, fusiform or ellipsoid, with granular contents, and hyaline or bluish to glistening dark blue, while in Stilbosporaceae conidia are cylindrical, clavate to pyriform, eu- or distoseptate, with or without oblique or longitudinal septa and brown (Maharachchikumbura *et al.* 2016).

Lamproconium (Grove) Grove, British Stem- and Leaf-Fungi (Coelomycetes) (Cambridge) 2: 321 (1937)

Melanconium sect. *Lamproconium* Grove, Bull. Misc. Inf., Kew: 161 (1918)

Type species:—*Lamproconium desmazieri* (Berk. & Broome) Grove.

Lamproconium desmazieri (Berk. & Broome) Grove [as '*desmazieri*'], British Stem- and Leaf-Fungi (Coelomycetes) (Cambridge) 2: 321 (1937) Figs. 2–4

Discella desmazieri Berk. & Broome, Ann. Mag. nat. Hist., Ser. 2 5: 377 (1850)
Melanconium desmazieri (Berk. & Broome) Sacc., Michelia 2(no. 7): 355 (1881)
Melanconis desmazieri Petr., Annls. mycol. 36(1): 55 (1938)
Facesoffungi number: FoF02249

Pathogen causing canker on branches or twigs of lime trees (*Tilia* spp.). Lime cankers associated with *L. desmazieri*, produced splitting and longitudinal breakage of the outer branches, the symptom will appear as localized, sunken, slightly discolored, dark blue to black lesions on branches discoloration and necrosis of the branches. Branch/top dieback associated with *L. desmazieri* in having black terminal dead shoots, apex downwards initially discoloration; becoming wilted, with brown to dark brown discoloration at the base, midrib, and finally becoming dry and dead. *Sexual morph*: Undetermined. *Asexual morph*: *Conidiomata* 800–1000 × 400–550 µm diam., pycnidial, solitary, partly immersed in host tissue, uniloculate, dark blue, with a raised centre. *Pycnidium* 50–70 µm, with multi-layered wall, thin at inner layer, hyaline, comprising wall cells of *textura angularis*. *Paraphyses* interspersed within conidiophores. *Conidiophores* 30–120 µm, arising from the outermost wall layer at the basal of pycnidium, filiform or cylindrical, pale bluish to hyaline, septate, branched, smooth-walled. *Conidiogenous cells* cylindrical to subcylindrical, annellidic, with flared periclinal thickenings in the collarette zone, colourless to olivaceous, smooth-walled. *Conidia* 22–28.5 ×

8–10 µm ($\bar{x}$ = 25.25 × 9 µm, n = 30), fusiform, ellipsoid, infrequently slightly curved, aseptate, initially hyaline, bluish to glistening dark blue at maturity, narrowly rounded at ends, smooth-walled.

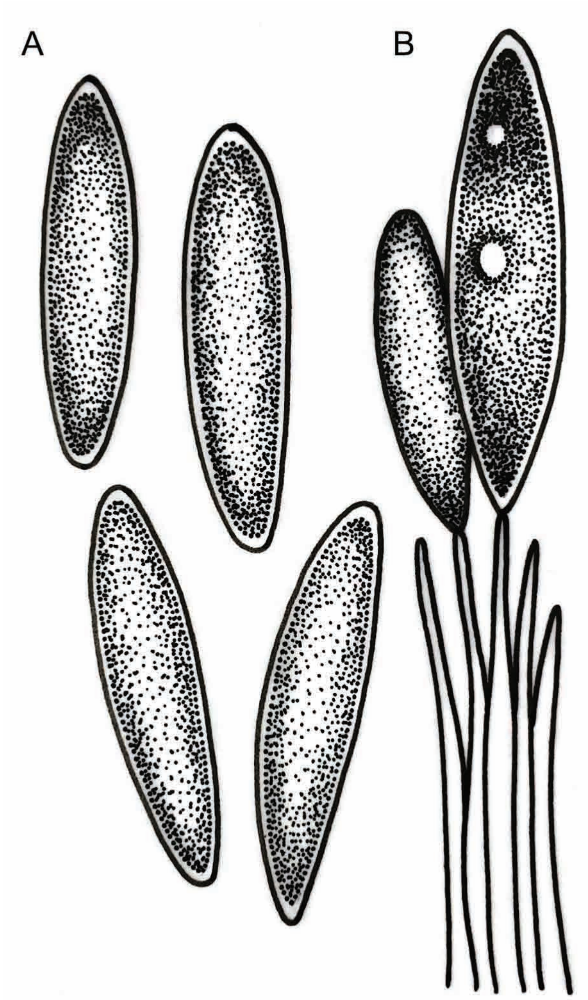

FIGURE 2. *Lamproconium desmazieri* (= *Melaconium desmazieri*) (redrawn from Grove 1918). **A.** Conidia. **B.** Conidiophores and developing conidia.

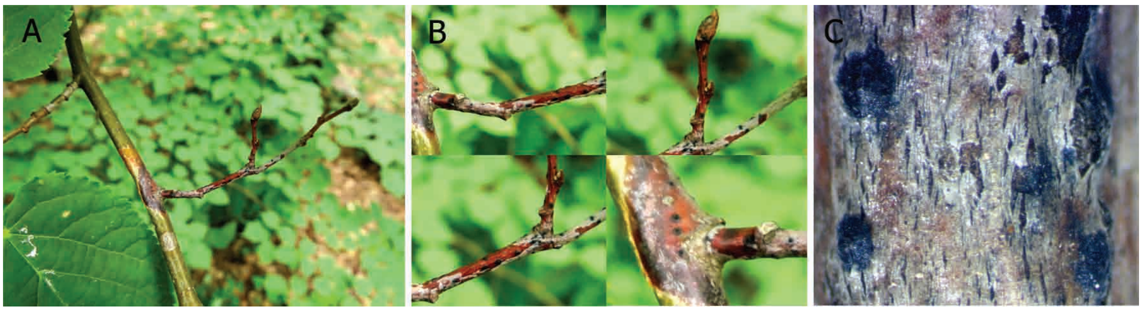

FIGURE 3. Dieback disease caused by *Lamproconium desmazieresi* (MFLU 14-0780, reference specimen). **A, B.** *Tilia cordata* with conidiomata on twigs and branches. **C.** Immersed conidiomata on branch.

Material examined:—RUSSIA. Rostov region: Krasnosulinsky district, Donskoye forestry, artificial forest, on dead branches of *Tilia cordata* Mill. (Tiliaceae), 21 May 2014, T. Bulgakov (MFLU 14-0780, **reference specimen designated here**, PDD); living culture, MFLUCC 14-1047, KUMCC. RUSSIA. Rostov region: Shakhty city, Central urban microdistrict, Central Park, parkland, on dying brunches (necrotrophic) of *T. tomentosa* Moench, 9 July 2015, T. Bulgakov (MFLU 15-1940, PDD); living culture, MFLUCC 15-0870, KUMCC. RUSSIA. Rostov region: Krasnosulinsky district, Donskoye forestry, ravine forest, on dead branches of *T. cordata*, 18 June 2015, T. Bulgakov (MFLU 15-2037, PDD); living culture, MFLUCC 15-0871, KUMCC. RUSSIA. Rostov region: Rostov-on-Don city, territory of Southern Federal University, parkland, on dead and dying branches of *T. cordata*, 23 April

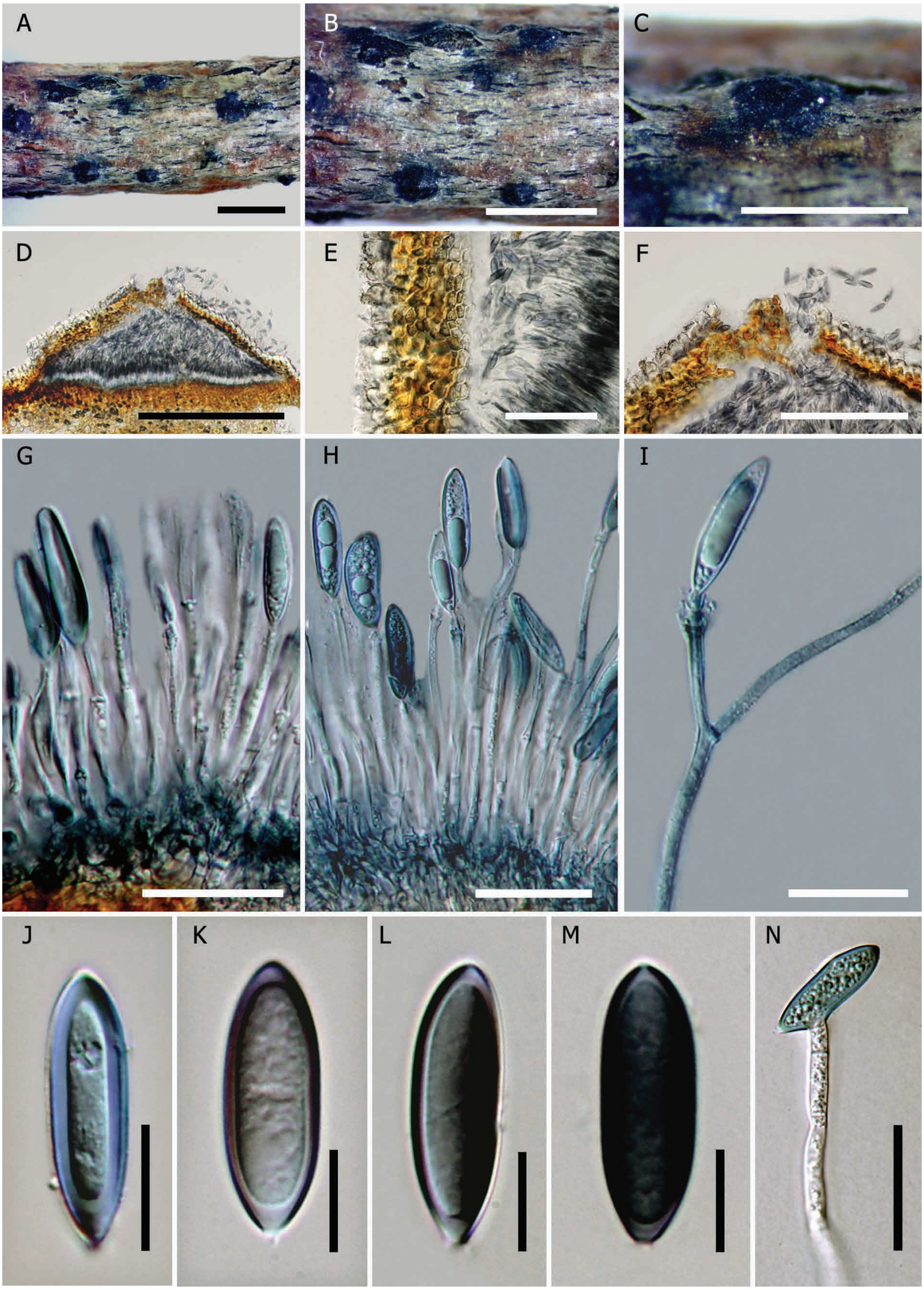

FIGURE 4. *Lamproconium desmazieresi* (MFLU 14-0780, reference specimen). **A–C.** Conidiomata on host. **D.** Cross section of a conidioma. **E.** Peridium and raised host tissue. **F.** Apex of conidioma. **G–I.** Conidiogenous cells with attached conidia (note: annellations at the tip of the conidiogenous cell). **J.** Immature conidium. **K–M.** Mature conidia. **N.** Germinating conidium. Scale bars: A, B = 2 mm, C = 1 mm, D = 500 µm, E = 100 µm, F = 200 µm, G**,** H = 30 µm, I = 20 µm, J–M = 10 µm, N = 30 µm.

2015, T. Bulgakov (MFLU 15-2111, PDD); living culture, MFLUCC 15-0872, KUMCC. RUSSIA. Rostov region: Krasnosulinsky district, Donskoye forestry, ravine forest, on dying branches of *T. cordata*, 18 June 2015, T. Bulgakov (MFLU 15-2192, PDD); living culture, MFLUCC 15-0873, KUMCC.

Notes:—*Lamproconium* was introduced as a section of *Melanconium* by Grove (1918) and as a subgenus (Index Fungorum 2016), with *Melanconium desmazieri* as the type species. The taxon with bright coloured spores was collected on living twigs of *Tilia* sp. in the UK. Grove (1937) had raised the subgenus to generic rank. In this study, we have determined our collections as having fusiform, ellipsoid, infrequently slightly curved, aseptate and glistening, dark blue conidia, with narrowly rounded ends (22–28.5 × 8–10 µm). The morphology of our collections is similar to *Lamproconium desmazieri* (Table 2). Therefore, we introduce our collections as belonging to the genus *Lamproconium*.

Petrak (1938) reported *Melanconis desmazieri* as the sexual morph of *Melanconium desmazieri*, also from *Tilia* sp. In the phylogenetic study of Castlebury *et al.* (2002), based on LSU sequence data, *Melanconis desmazieri* fell outside Melanconidaceae *sensu stricto* and grouped with *Hercospora tiliae*. These taxa were therefore placed in Diaporthales genera *incertae sedis* (Castlebury *et al.* 2002, Voglmayr *et al.* 2012, Voglmayr & Jaklitsch 2014). Phylogenetic analyses in this study generated from maximum likelihood, maximum parsimony and Bayesian analyses using combined ITS and LSU sequence data from 67 taxa (including our new strains), indicate that *L. desmazieri* belongs with *Hercospora tiliae* as a distinct lineage of Diaporthales (Fig. 1). Hence, we synonymize *M. desmazieri* under *L. desmazieri* and designate one of our collections as a reference specimen for *L. desmazieri*.

Hercospora Fr., Syst. orb. veg. (Lundae) 1: 119 (1825)

Possible synonyms (See Index Fungorum 2016)
Facesoffungi number: FoF02250

Saprobic on branches and twigs of temperate trees. *Sexual morph*: *Stromatic* tissues prosenchymatous around perithecia, delimited externally by blackened dense pseudoparenchymatous zone, interior whitish, composed of interwoven hyphae mixed with substrate cells. *Ascomata* perithecial, few, small, circinate, beaks converging, becoming united and erumpent through stroma surface as single large opening. *Asci* 8-spored, unitunicate, broadly cylindrical. *Ascospores* hyaline, broadly ellipsoid, one septate, wall smooth, without gelcoating, with narrow terminal and median appendages in some species. *Asexual morph*: *Rabenhorstia* sp., *Stromata* prosenchymatous. *Conidiomata* pycnidial, uniloculate, ostiolate, ostiole surrounded by a superficial cap of sterile tissues. *Conidiophores* elongate. *Conidia* hyaline ovoid to ellipsoid, one-celled.

Type species:—*Hercospora tiliae* (Pers.) Tul. & C. Tul.

Notes:—Fries (1825) listed *Sphaeria tiliae* Pers., and *Sphaeria atrovirens* Alb. & Schwein., as two of the species in *Hercospora*. However morphologically *S. tiliae* has hyaline ascospores while *S. atrovirens* comprising opaque ascospores. Tulasne and Tulasne (1863) accepted *H. tiliae* as the type species of *Hercospora*. Petrak (1938) and Ruhland (1900) implicated *Rabenhorstia tiliae* (Pers.) Fr., as the asexual morph of *Hercospora tiliae*. Fourteen species listed under *Hercospora* (Index Fungorum, 2016).

Hercospora tiliae (Pers.) Tul. & C. Tul., Select. fung. carpol. (Paris) 2: 154 (1863) Figs. 5–6

Possible synonyms (See Index Fungorum 2016)
Facesoffungi number: FoF02452

Saprobic on branches and twigs of *Tilia* sp. *Sexual morph*: *Stromata* 700–800 µm wide, prosenchymatous around perithecia, delimited externally by greenish-blackened dense pseudoparenchymatous zone, interior whitish, composed of interwoven hyphae mixed with substrate cells, 3–5 ascomata in a stromata. *Ascomata* 1–1.05 mm high, 0.24–0.34 mm diam., ($\bar{x}$ = 1.02 × 334 µm, n = 10), perithecial, small, aggregated, scattered, globose to subglobose, light brown to dark brown, coriaceous, ostiolate, papillate. *Papilla* 625–645 µm high, 190–290 µm diam., ($\bar{x}$ = 640 × 250 µm, n = 10), converging and erumpent through stroma surface as single, large opening, wide at the top, narrowing towards the base, dark brown region around base of papilla. *Peridium* 10–20 µm wide ($\bar{x}$ = 16 µm, n = 10), comprises light brown, compressed, cells of *textura angularis*. *Asci* 140–175 µm × 17–24 µm diam., ($\bar{x}$ = 160 × 21 µm, n = 10), 8-spored, unitunicate, cylindrical, short-stalked, J- apical apparatus. *Ascospores* 20–25 µm × 9–11 µm diam., ($\bar{x}$ = 23 ×

10 µm, n = 10), uniseriate, broadly ellipsoid, 1-septate, not or lightly constricted at the septa, hyaline, smooth. *Asexual morph*: *Stromata* prosenchymatous. *Conidiomata* pycnidial, uniloculate, ostiolate, ostiole surrounded by a superficial cap of sterile tissues. *Conidiophores* elongate. *Conidia* 14–16.5 × 4.5–6.5 µm ($\bar{x}$ = 15 × 5 µm, n = 10), hyaline ovoid to ellipsoid, one-celled.

FIGURE 5. *Hercospora tiliae* (F148711, reference specimen). **A.** Packet of herbarium specimen. **B.** Herbarium specimens. **C.** Cross section of ascomata. **D.** Peridium. **E.** Papilla. **F–H.** Asci in water. **I–K.** Ascospores. Scale bars: C = 200 µm, D, F–H = 40 µm, E = 100 µm.

Material examined:—SWEDEN. Uppland: Upl. Stockholm: Roslagstull Stockholm, on bark of *Tilia* sp., L. Romell, 1 April 1887, F148711 (S).

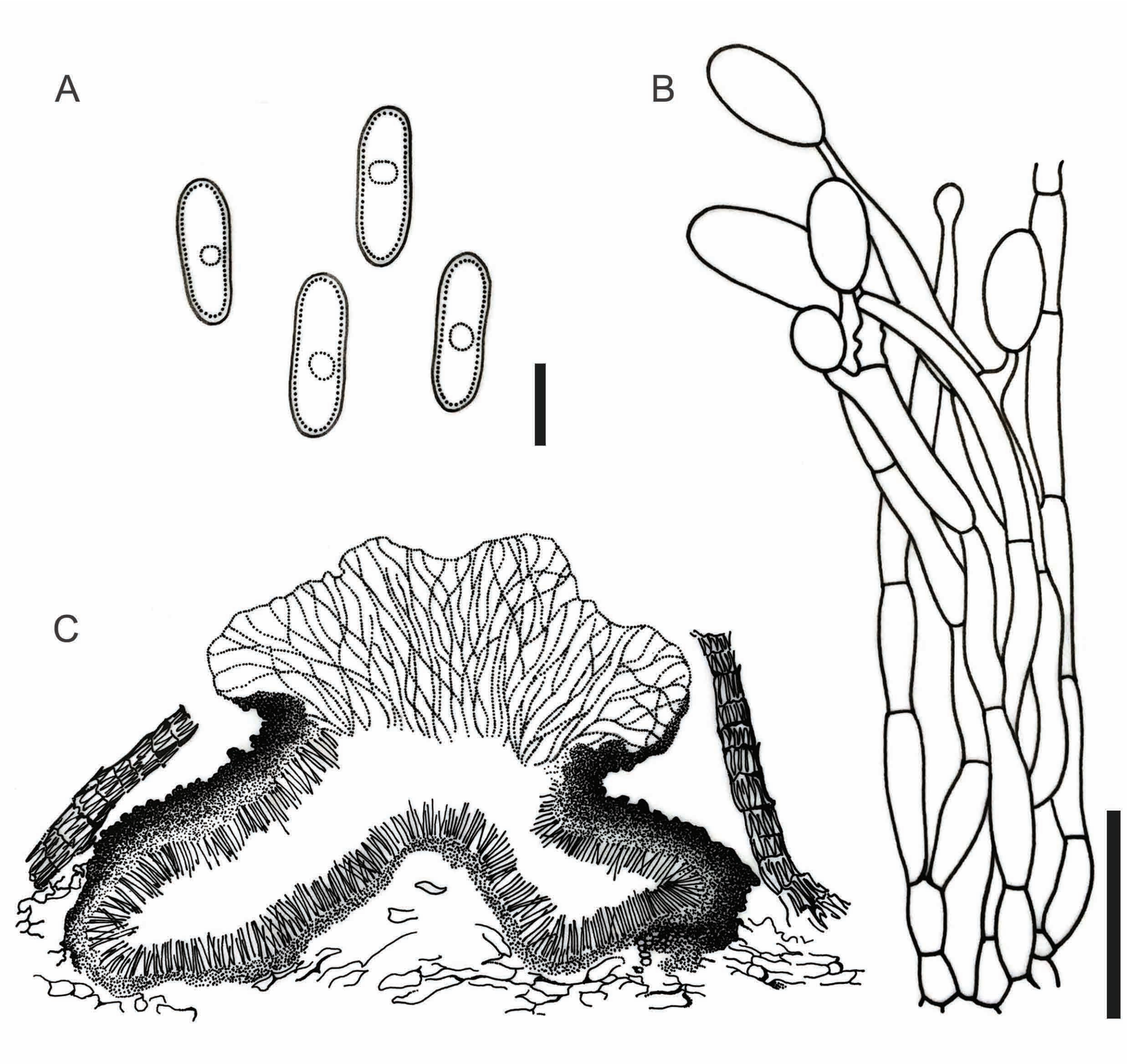

FIGURE 6. *Hercospora tiliae* (redrawn from Sutton 1980) **A.** Conidia. **B.** Conidiophores and developing conidia. **C.** Conidioma. Scale bars: A = 10 µm, B = 20 µm.

Notes:—*Hercospora* Fr. comprises 15 species (Index Fungorum 2016) with *Hercospora tiliae* as the type. The characters of the genus include eustromatic, immersed, subepidermal, dark blackish brown, separate, uniloculate, multiloculate or convoluted and thick-walled conidiomata; conidiophores branched extensively at the base, less so above, hyaline, septate, smooth, often developing in mucilage, formed at the base and sides of the conidiomatal wall; and ellipsoid, thick-walled, hyaline, aseptate conidia (18–20 × 6–7.5 µm) (Petrak 1938, Sutton 1980).

Phylogenetic studies (Castlebury *et al.* 2002, Rossman *et al.* 2007, Voglmayr *et al.* 2012, Voglmayr & Jaklitsch 2014) based on LSU sequence data, placed *H. tiliae* in Diaporthales, genera *incertae sedis*, where it grouped with *Melanconis desmazierii*. In the present study based on maximum likelihood, maximum parsimony and Bayesian analyses of combined ITS and LSU sequence data, *Lamproconium desmazierii* (= *Melanconis desmazieri*) and *Hercospora tiliae*, clustered in Lamproconiaceae fam. nov.

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