



New higher taxa in the lichen family Graphidaceae (lichenized Ascomycota: Ostropales) based on a three-gene skeleton phylogeny

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Abstract

We provide an updated skeleton phylogeny of the lichenized family Graphidaceae (excluding subfamily Gomphilloideae), based on three loci (mtSSU, nuLSU, *RPB2*), to elucidate the position of four new genera, *Aggregatorygma*, *Borinquenotrema*, *Corticorygma*, and *Paratopeliopsis*, as well as the placement of the enigmatic species *Diorygma erythrellum*, *Fissurina monilifera*, and *Redingeria desseiniiana*. Based on the resulting topology, in addition to three tribes described previously, we recognize four further tribes in the subfamily Graphidoideae: *Acanthothecieae* Lumbsch, Kraichak & Lücking, *Diploschisteae* (Zahlbr.) Lumbsch, Kraichak & Lücking, *Leptotremateae* Lumbsch, Kraichak & Lücking, and *Wirthiotremateae* Lumbsch, Kraichak & Lücking. The phylogenetic position of *Aggregatorygma* and *Borinquenotrema* was not resolved with support, whereas *Corticorygma* forms part of *Acanthothecieae*, supported sister to *Acanthothecis*, and *Paratopeliopsis* belongs in *Thelotremateae*, unsupported sister to *Leucodecton*. *Diorygma erythrellum* is confirmed as a member of the *Diorygma-Thalloloma* clade, while *Fissurina monilifera*, in spite of its myriotremoid ascomata, belongs in *Fissurina* s.str. *Redingeria desseiniiana*, although resembling the genus *Phaeographopsis*, is supported sister to *R. glaucoglyphica*. *Topeliopsis darlingtonii* forms the sister group to *Gintarasia megalophthalma*. Consequently, *T. darlingtonii* and the closely related *T. elixii* are recombined in *Gintarasia* as *Gintarasia darlingtonii* (Frisch & Kalb) Lumbsch, Kraichak & Lücking, and *G. elixii* (Frisch & Kalb) Lumbsch, Kraichak & Lücking.

Key words: Brazil, classification, Diploschistaceae, Puerto Rico, *Xalocoa*.

Introduction

The lichenized fungal family Graphidaceae now includes the previously separated families Graphidaceae, Thelotremataceae, Gomphillaceae, and Solorinellaceae (Rivas Plata *et al.* 2012a). Molecular data support four distinct clades within the emended family, classified as subfamilies Fissurinoideae, Gomphilloideae, Graphidoideae, and Redonographoideae (Rivas Plata *et al.* 2012a; Lücking *et al.* 2013). These do not correspond to

the previously separated families: while Gomphillaceae, Asterothyriaceae, and Solorinellaceae are now united in Gomphilloideae, elements of both the former Graphidaceae and Thelotremataceae are found in the two large subfamilies Fissurinoideae and Graphidoideae, and Redonographoideae only contains graphidoid species. Fissurinoideae, Graphidoideae, and Redonographoideae can therefore be considered the core Graphidaceae, encompassing both species with rounded and species with lirellate ascomata.

Within the previously separated Graphidaceae and Thelotremataceae, genus-level classification was for a long time based on gross ascoma morphology and ascospore septation and pigmentation, recognizing between 13 and 14 genera. With the revisionary classifications put forward by Staiger (2002) and Frisch *et al.* (2006) and further developed in subsequent studies (Rivas Plata *et al.* 2008, 2012a, b, 2013; Parnmen *et al.* 2012a, 2013; Lücking *et al.* 2013), genera within the family have been redefined using a phylogeny-based approach and a combination of morphological, anatomical, and chemical features. Thus, at the time of the monographic revisions by Staiger (2002) and Frisch *et al.* (2006), 40 genera were recognized in the group, 21 graphioid (with lirellate ascomata) and 19 thelotremoid (with rounded ascomata). By the time of the publication of the treatments for *Flora of Australia* (Archer 2009; Mangold *et al.* 2009), 44 genera were distinguished. Rivas *et al.* (2012a) presented a summary classification, then with a total of 57 genera in the core group and introducing three tribes in Graphidoideae. Since then, further ten genera have been established, viz. *Astrochapsa* Parnmen *et al.* (2012a: e51392), *Crutarndina* Parnmen *et al.* (2012a: e51392), *Gintarasia* Kraichak *et al.* (2014: 470), *Mangoldia* Lücking *et al.* (2012: 3), *Myriochapsa* M. Cáceres, Lücking & Lumbsch in Parnmen *et al.* (2013: 128), *Nitidochapsa* Parnmen *et al.* (2013: 128), *Pseudochapsa* Parnmen *et al.* (2012a: e51392), *Pseudotopeliopsis* Parnmen *et al.* (2012a: e51392), *Redonographa* Lücking *et al.* (2013: 846), and *Xalocoa* Kraichak *et al.* (2014: 472), bringing the total to 67. These newly recognized genera mostly represent segregates of previously circumscribed, broader taxa, whereas only few entirely novel lineages were discovered, such as *Heiomasia* Nelsen *et al.* (2010: 744) and *Mangoldia*.

In this paper, we present an updated three-gene skeleton phylogeny based on a previous analysis (Lücking *et al.* 2013). We use a similar approach in selecting representatives of each generic lineage to elucidate the phylogenetic position of four novel genus-level lineages discovered in recent inventories in tropical America, specifically Puerto Rico and Brazil (Cáceres *et al.* 2014; Mercado-Díaz *et al.* 2014). All have unique morphological and/or chemical features, supporting their taxonomic distinction. In addition, we use the updated skeleton phylogeny as a framework to introduce four new tribes and to highlight the placement of further new species described in this volume (Mercado-Díaz *et al.* 2014; Van den Broeck *et al.* 2014).

Material and Methods

A total of eleven new sequences of the mtSSU and nuLSU rDNA were obtained from seven species. In addition, 220 ingroup sequences and four outgroup sequences representing further 97 species were used from our previous analysis (Lücking *et al.* 2013; Table 1).

New sequences were generated for this study using the Sigma REDEExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, SA) for DNA isolation following the manufacturer's instructions, except that 40 μ L of extraction buffer and 40 μ L dilution buffer were used. DNA dilutions (5x) were used in PCR reactions of the genes coding for the nuLSU, mtSSU and RPB2, respectively. Primers for amplification were: (a) for nuLSU: AL2R (Mangold *et al.* 2008a), and nu-LSU-1125-3' (= LR6) (Vilgalys & Hester 1990), (b) for mtSSU: mr-SSU1 and mr-SSU3R (Zoller *et al.* 1999), and (c) for RPB2: fRPB2-7cF and fRPB2-11aR (Liu *et al.* 1999). PCR reactions contained 5.0 μ L R4775 Sigma REDEExtract-N-Amp™ PCR ReadyMix, 0.5 μ L of each primer (10 μ M), 2 μ L genomic DNA extract and 2 μ L distilled water for a total of 10 μ L. Thermal cycling parameters were: (1) for nuLSU: initial denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 95°C, 30 s at 58°C, 1 min at 72°C, and a final elongation for 10 min at 72°C; (2) for mtSSU: initial denaturation for 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 1 min at 50°C, 1 min 30 s at 72°C, and a final elongation for 10 min at 72°C; and (3) for RPB2: initial denaturation for 3 min at 95°C, then 1 min at 95°C, and 37 cycles of 1 min at 57°C, 1 min at 58°C, 1 min at 59°C, 1 min at 60°C, 1 min at 61°C, 1 min at 62°C, 1 min at 63°C, 1 min at 64°C and 1.5 min at 72°C, and a final elongation for 10 min at 72°C. Samples were visualized on a 1% ethidium bromide-stained agarose gel under UV light and bands were gel extracted, heated at 70° C for 5 minutes, cooled to 45° C for 10 minutes, treated with 1 μ L GELase (Epicentre Biotechnologies, Madison, WI, USA) and incubated at 45° C for at least 24 hours. The 10 μ L cycle sequencing

reactions consisted of 1–1.5 µl of Big Dye version 3.1 (Applied Biosystems, Foster City, California, U.S.A.), 2.5–3 µl of Big Dye buffer, 6 µM primer, 0.75–2 µl gelated PCR product and water. Samples were sequenced with PCR primers. The cycle sequencing conditions were as follows: 96° C for 1 minute, followed by 25 cycles of 96° C for 10 seconds, 50° C for 5 seconds and 60° C for 4 minutes. Samples were precipitated and sequenced using Applied Biosystems 3730 DNA Analyzer (Foster City, California, U.S.A.), sequences were assembled in SeqMan 4.03 (DNASTAR) and submitted to GenBank (Table 1).

TABLE 1. Genbank numbers and voucher information of taxa used in this study.

Species	Voucher	mtSSU	nuLSU	RPB2
<i>Acanthothecis hololeuroides</i> (Nyl.) Staiger & Kalb		JX420952	JX421423	JX420938
<i>Acanthothecis pepliphora</i> (M. Wirth & Hale) E.A. Tripp & Lendemer		JX420953	JX421424	[—]
<i>Acanthotrema brasilianum</i> (Hale) Frisch		JX420958	JX421429	JX420876
<i>Aggregatorygma triseptatum</i> M. Cáceres <i>et al.</i>	isotype	KJ440979	KJ440939	[—]
<i>Ampliotrema amplius</i> (Nyl.) Kalb ex Kalb		JF828958	JF828973	[—]
<i>Ampliotrema</i> sp.		JX420963	JX421432	JX420900
<i>Asteristion platycarpum</i> (Tuck.) Leight.		JX421007	JX421460	[—]
<i>Astrochapsa astroidea</i> (Berk. & Broome) Parmen <i>et al.</i>		JX420974	JX421441	JX420859
<i>Borinquenotrema soredicarpum</i> Mercado-Díaz <i>et al.</i>	isotype	KJ440940	[—]	[—]
<i>Carbacanthographis stictica</i> Staiger & Kalb		JF828974	JX421435	JX420875
<i>Chapsa alborosella</i> (Nyl.) Frisch		JX420972	JX421439	JX420936
<i>Chroodiscus argillaceus</i> (Müll. Arg.) Lücking & Papong		HQ639585	JX421468	JX420863
<i>Clandestinotrema stylothecium</i> (Vain.) Rivas Plata <i>et al.</i>		HQ639597	JX421470	[—]
<i>Coenogonium luteum</i> (Dicks.) Kalb & Lücking		AY584699	AF279387	[—]
<i>Compositrema cerebriforme</i> J. Hern. & Lücking		JX421017	JX421471	JX420901
<i>Corticorygma stellatum</i> M. Cáceres <i>et al.</i>	isotype	KJ440981	KJ435136	[—]
<i>Creographa intricans</i> (Nyl.) comb. ined.		JX421254	JX421602	JX420924
<i>Cruentotrema cruentatum</i> (Mont.) Rivas Plata <i>et al.</i>		HQ639587	HQ639660	[—]
<i>Crutarndina petractoides</i> (P. M. Jørg. & Brodo) Parmen <i>et al.</i>		JX421383	JX421664	JX420891
<i>Diorygma erythrellum</i> (Mont. & Bosch) Kalb <i>et al.</i>	Australia, Kalb <i>s.n.</i>	KJ440982	[—]	[—]
<i>Diorygma poitaei</i> (Fée) Kalb <i>et al.</i>		HQ639596	HQ639627	JF828942
<i>Diploschistes cinereocaesius</i> (Sw.) Vain.		DQ912306	DQ883799	DQ883755
<i>Dyplolabia afzelii</i> (Ach.) A. Massal.		JX421027	JX421483	[—]
<i>Ectographis sculpturata</i> (Ach.) Trevis.		JN127364	[—]	[—]
<i>Fibrillithecis gibbosa</i> (H. Magn.) Rivas Plata & Lücking		EU075573	EU075621	[—]
<i>Fissurina aggregatula</i> Common & Lücking		JX421036	JX421490	JX420871
<i>Fissurina astroisidiata</i> Herrera-Camp. & Lücking		JX421039	JX420843	[—]
<i>Fissurina insidiosa</i> C. Knight & Mitt.		DQ972995	DQ973045	DQ973083
<i>Fissurina monilifera</i> Mercado-Díaz <i>et al.</i>	isotype	KJ440980	KJ440941	[—]
<i>Fissurina nigrolabiata</i> Rivas Plata <i>et al.</i>		JF828961	JF828976	JF828943
<i>Glaucotrema glaucophaenum</i> (Kremp.) Rivas Plata & Lumbsch		JX421061	JX421501	JX420862
<i>Glyphis cicatricosa</i> Ach.		HQ639610	JX421505	[—]
<i>Graphis librata</i> C. Knight		HQ639621	HQ639636	JF828945
<i>Graphis ruiziana</i> (Fée) A. Massal.		DQ431985	DQ431945	[—]
<i>Gyalecta jenensis</i> (Batsch) Zahlbr.		AF431956	AF465450	[—]
<i>Gyrotrema wirthii</i> Rivas Plata <i>et al.</i>		JX421071	[—]	[—]
<i>Halegrapha chimera</i> Rivas Plata & Lücking		JF505933	[—]	[—]
<i>Heiomasia sipmanii</i> (Aptroot, Lücking & Rivas Plata) Nelsen <i>et al.</i>		GU395552	[—]	[—]
<i>Hemithecium chlorocarpum</i> (Fée) Trevis.		HQ639595	[—]	JF828946
<i>Hemithecium implicatum</i> (Fée) Staiger		DQ431978	HQ639654	JF828947

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TABLE 1. (Continued)

Species	Voucher	mtSSU	nuLSU	RPB2
<i>Leiorreuma hypomelaenum</i> (Müll. Arg.) Staiger		DQ431971	DQ431933	[—]
<i>Leptotrema wightii</i> (Tayl.) Müll. Arg.		EU075574	JF828977	JF828948
<i>Leucodecton expallescens</i> (Nyl.) Rivas Plata & Lücking		[—]	AY605072	[—]
<i>Leucodecton occultum</i> (Eschw.) Frisch		HQ639611	HQ639657	JF828949
<i>Malmographina plicosa</i> (C. F. W. Meissn.) M. Cáceres <i>et al.</i>		HQ639590	[—]	[—]
<i>Mangoldia australiana</i> Lücking, Parnmen & Lumbsch		[—]	JX421519	[—]
<i>Melanotopelia rugosa</i> (Kantvilas & Vězda) Lumbsch & Mangold		HQ639615	[—]	[—]
<i>Melanotrema lynceodes</i> (Nyl.) Rivas Plata <i>et al.</i>		JX421088	JX421520	JX420907
<i>Myriotrema album</i> Fée		JX421090	[—]	[—]
<i>Myriotrema laeviusculum</i> (Nyl.) Hale		JX421094	JX421528	JX420920
<i>Myriotrema olivaceum</i> Fée		JX421095	EU126645	[—]
<i>Myriotrema peninsulae</i> R. C. Harris		HQ639616	[—]	JF828950
<i>Nadvornikia hawaiiensis</i> (Tuck.) Tibell		EU075581	JX421533	[—]
<i>Nitidochapsa leprieurii</i> (Mont.) Parnmen <i>et al.</i>		JX420991	JX421451	JX420930
<i>Ocellularia albocincta</i> (Hale) Divakar & Mangold		JX421113	JX421543	JX420873
<i>Ocellularia allosporoides</i> (Nyl.) Patw. & C. Kulk.		JX421118	JX421544	JX420925
<i>Ocellularia cavata</i> (Ach.) Müll. Arg.		DQ384878	DQ431935	[—]
<i>Ocellularia dolichotata</i> (Nyl.) Zahlbr.		JX421146	JX421554	[—]
<i>Ocellularia domingensis</i> (Fée ex Nyl.) Müll. Arg.		JX421151	JX421560	JX420918
<i>Ocellularia eumorpha</i> (Stirt.) Hale		DQ384885	JX421561	[—]
<i>Ocellularia inturgescens</i> (Müll. Arg.) Mangold		EU075577	EU075625	[—]
<i>Ocellularia laeviusculoides</i> Sipman & Lücking		JX421167	JX421569	JX420896
<i>Ocellularia microstoma</i> (Müll. Arg.) Mangold		JX421140	JX421576	JX420823
<i>Ocellularia percolumellata</i> Sipman		JX421180	[—]	JX420888
<i>Ocellularia praestans</i> (Müll. Arg.) Hale		JX421192	JX421581	JX420892
<i>Ocellularia profunda</i> (Stirt.) Mangold <i>et al.</i>		JX421198	JX421585	JX420825
<i>Ocellularia psorbarroensis</i> Sipman		JX421202	JX421588	JX420874
<i>Ocellularia pyrenuloides</i> Zahlbr.		DQ384896	[—]	[—]
<i>Ocellularia wirthii</i> Mangold, Elix & Lumbsch		JX421228	JX421599	[—]
<i>Pallidogramme chlorocarpoides</i> (Nyl.) Staiger <i>et al.</i>		DQ431969	DQ431932	JF828951
<i>Paratopeliopsis caribica</i> Mercado-Díaz <i>et al.</i>	isotype	KJ440983	[—]	[—]
<i>Phaeographis dendritica</i> (Ach.) Müll. Arg.		JX421247	[—]	[—]
<i>Phaeographis lecanographa</i> (Nyl.) Staiger		JX421280	JX421609	[—]
<i>Phaeographis lobata</i> (Eschw.) Müll. Arg.		DQ431984	DQ431944	[—]
<i>Phaeographopsis palaeotropica</i> Kalb & Frisch		JX644423	[—]	[—]
<i>Phlegographa leprieurii</i> (Mont.) A. Massal.		JN127363	JN127365	[—]
<i>Platygramme caesiopruinosa</i> (Fée) Fée		HQ639599	JX421611	[—]
<i>Platygramme impudica</i> (A. W. Archer) A. W. Archer		JX421288	JX421612	JX420926
<i>Platythecium grammitis</i> (Fée) Staiger		JX421293	[—]	[—]
<i>Pseudochapsa dilatata</i> (Müll. Arg.) Parnmen <i>et al.</i>		JX420981	JX421446	JX420906
<i>Pseudotopeliopsis laceratula</i> (Müll. Arg.) Parnmen <i>et al.</i>		JX420988	JX421448	JX420831
<i>Pycnotrema pycnoporellum</i> (Nyl.) Rivas Plata & Lücking		JX421295	JX421615	[—]
<i>Redingeria desseiniiana</i> Van den Broeck <i>et al.</i>	holotype	KJ145246	KJ145245	[—]
<i>Redingeria glaucoglyphica</i> (Sipman) Frisch		JX421296	JX421618	[—]
<i>Redonographa chilensis</i> (Zahlbr.) Lücking		JX890304	JX890301	JX890306
<i>Redonographa saxiseda</i> (Zahlbr.) Bungartz		[—]	JX890300	JX890305
<i>Reimnitzia santensis</i> (Tuck.) Kalb		HQ639622	HQ639664	JF828952

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TABLE 1. (Continued)

Species	Voucher	mtSSU	nuLSU	RPB2
<i>Rhabdodiscus isidiiferus</i> (Hale) Rivas Plata <i>et al.</i>		JX421302	JX421623	JX420908
<i>Sarcographa fenicis</i> (Vain.) Zahlbr.		DQ431967	DQ431931	[—]
<i>Sarcographa glyphiza</i> (Nyl.) Kr.P. Singh & D.D. Awasthi		DQ431972	DQ431934	[—]
<i>Sarcographa labyrinthica</i> (Ach.) Müll. Arg.		JF828969	[—]	JF828953
<i>Schistophoron tenue</i> Stirt.		EU544933	EU544932	[—]
<i>Schizotrema schizolomum</i> (Müll. Arg.) Mangold & Lumbsch		FJ708500	FJ708492	[—]
<i>Stegobolus wrightii</i> (Hale) Rivas Plata <i>et al.</i>		JX421334	JX421636	JX420913
<i>Thalloloma hypoleptum</i> (Nyl.) Staiger		JF828970	[—]	[—]
<i>Thecaria montagnei</i> (Bosch) Staiger		JX644422	HQ639666	[—]
<i>Thecaria quassicola</i> Fée		HQ639617	HQ639667	[—]
<i>Thelotrema bicinctulum</i> Nyl.		EU075598	EU075642	JF828955
<i>Thelotrema lepadinum</i> (Ach.) Ach.		JX421366	JX421653	JX420934
<i>Thelotrema leucophthalmum</i> Nyl.		JX421374	JX421658	JX420830
<i>Topeliopsis darlingtonii</i> Frisch & Kalb		DQ384924	[—]	[—]
<i>Topeliopsis muscigena</i> (Stizenb.) Kalb		EU075611	EU126655	JF828957
<i>Wirthiotrema trypaneoides</i> (Nyl.) Rivas Plata & Lücking		JX421422	JX421681	JX420916
<i>Xalocoa ocellata</i> (Vill.) Kraichak, Lücking & Lumbsch		KF688505	AY605077	DQ366253

Sequences were arranged into multiple sequence alignments (MSA) for each gene using BIOEDIT 7.09 (Hall 1999) and automatically aligned with MAFFT using the -auto option (Kato & Toh 2005). The unaligned MSA for the mtSSU and nuLSU gene partitions were also submitted to the GUIDANCE web server at <http://guidance.tau.ac.il> to assess alignment confidence scores for each site (Penn *et al.* 2010a, b). GUIDANCE uses a MAFFT alignment and returns a colored MSA that allows delimiting ambiguously aligned portions of the MSA. These were then excluded from further analysis. Introns were deleted from the nuLSU gene partition because of their random occurrence but kept in the mtSSU partition if consistent within species or species groups. This resulted in alignments of 941 sites for the mtSSU, 981 sites for the nuLSU, and 904 for RPB2, for a total of 2,826 sites in the combined dataset. After testing for supported topological conflicts (Mason-Gamer & Kellogg 1996; Miadlikowska & Lutzoni 2000; Kauff & Lutzoni 2002), the three genes were combined into a single supermatrix. Individual datasets and the combined supermatrix were subjected to maximum likelihood search using the RAxML-HPC BlackBox 7.3.2, with parametric bootstrapping generating 600 replicates as automatically determined by RAxML using a saturation criterion, and to Bayesian analysis using MrBAYES 3.2.2, with two independent runs with one million generations each, resampling every 1000 trees, and 25% burnin. Both analyses were run on the Cipres Gateway server (Huelsenbeck & Ronquist 2001; Stamatakis *et al.* 2005, 2008; Stamatakis 2006; Miller *et al.* 2010; <http://www.phylo.org/portal2/login!input.action>). The universal GTR-Gamma model was chosen for both analyses.

Results and Discussion

Except for a few details, the overall topology obtained with this dataset (Fig. 1) mirrors the results presented by Lücking *et al.* (2013), with Fissurinoideae being sister to Redonographoideae plus Graphidoideae. The three major tribes in Graphidoideae, Graphideae, Ocellularieae, and Thelotremateae, were recovered with similar resolution and support as in the previous study. In addition to these tribes, the following further, supported clades are recognized: the *Leptotrema* clade, including *Leptotrema* Mont. & Bosch in Miquel (1855: 483) and *Reimnitzia* Kalb (2001: 325); the *Acanthothecis* clade, comprising *Acanthothecis* Clements (1909: 59), *Gintarasia*, *Topeliopsis darlingtonii* Frisch & Kalb (2006: 39), and the newly described *Corticorygma* Cáceres *et al.* (2014); and the *Carbacanthographis-Topeliopsis-Wirthiotrema* clade, including *Asteristion* Leighton (1869: 163), *Carbacanthographis* Staiger & Kalb in Staiger (2002: 98), *Melanotopelia* Lumbsch & Mangold in Mangold *et al.* (2008b: 43), *Nadvornikia*, *Schizotrema* Mangold & Lumbsch in Mangold *et al.* (2009: 657), *Topeliopsis* Kantvilas & Vězda (2000: 347), and *Wirthiotrema* Rivas Plata *et al.* (2010: 198), as well as 'orphaned' species currently

classified in *Acanthothecis*, *Leucodecton* Massalongo (1860: 325), *Myriotrema* Fée (1825: xlix, 103), and *Thelotrema* Acharius (1803: 130). In addition to *Corticorygma*, supported sister to *Acanthothecis*, we also recognize *Aggregatoryma* Cáceres *et al.* (2014) unsupported sister to tribe Graphideae, *Borinquenotrema* Mercado-Díaz *et al.* (2014), unsupported sister to tribe Ocellularieae, and *Paratopeliopsis* Mercado-Díaz *et al.* (2014), unsupported sister to *Leucodecton* in Thelotremateae. Other recovered relationships that agree with previous results (Lücking *et al.* 2013; Rivas Plata *et al.* 2013), such as the *Melanotopelia-Schizotrema-Topeliopsis* and the *Acanthotrema-Diploschistes-Phaeographopsis* clades, the latter containing *Acanthotrema* Frisch in Frisch *et al.* (2006: 77), *Diploschistes*, and *Phaeographopsis* Sipman in Aptroot *et al.* (1997: 129), remain unsupported.

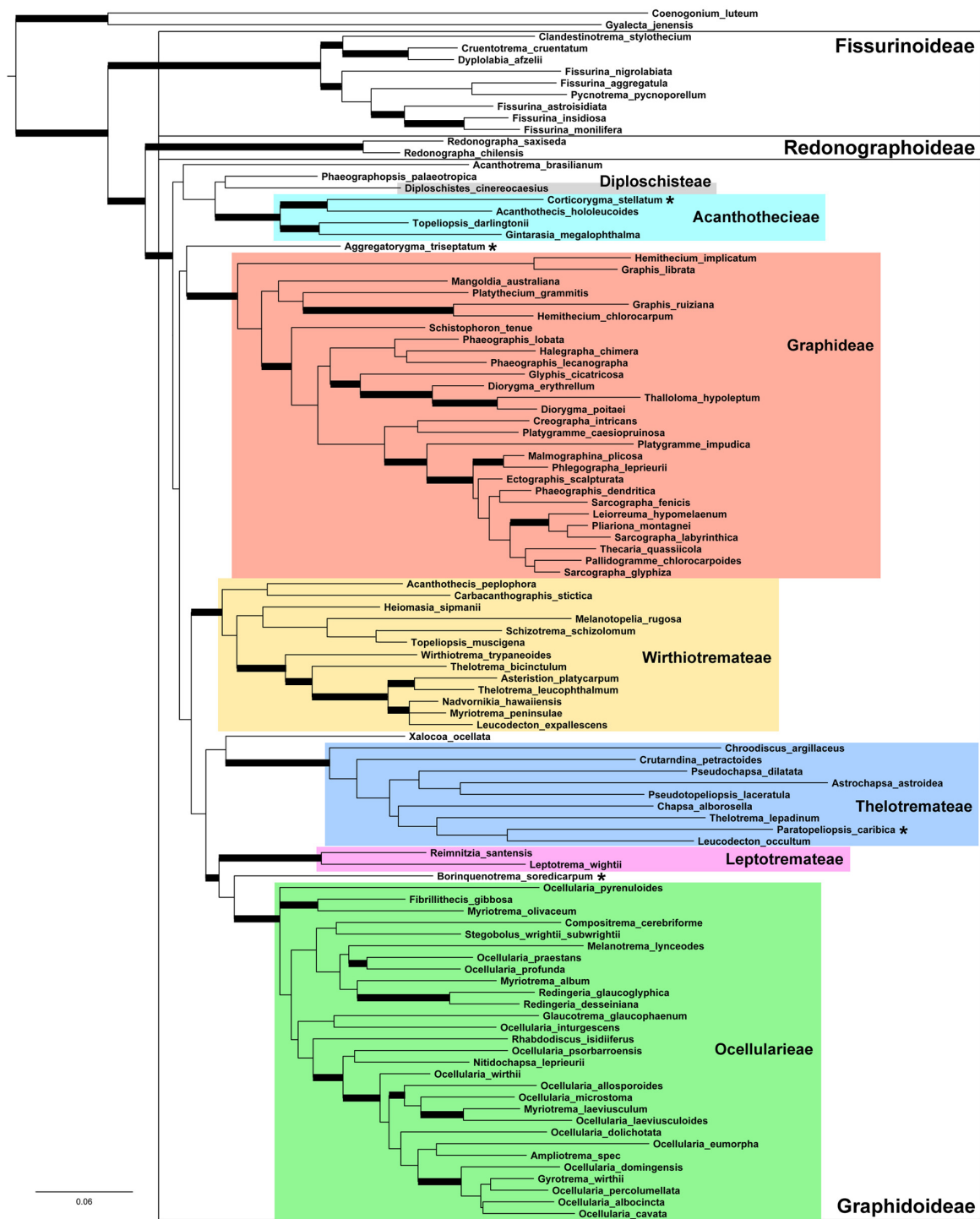


FIGURE 1. Best-scoring maximum-likelihood tree based on a three-gene skeleton dataset of the core Graphidaceae. Thick branches indicate bootstrap support 70% or higher and/or posterior probabilities 95% or higher. Major lineages are highlighted. Novel genus lineages are marked with an asterisk.

The analysis also clarified the placement of three enigmatic species, two of them newly described in this

volume, with unique morphological characters. Thus, *Diorygma erythrellum* (Mont. & Bosch) Kalb *et al.* (2004: 150), with previously unpublished molecular sequence data mentioned in Kalb *et al.* (2004), was confirmed as forming part of a clade containing *Diorygma* Eschweiler (1824: 13, 25) and *Thallooloma* Trevisan (1853: 13). *Fissurina monilifera* Mercado-Díaz *et al.* (2014), a species closely resembling the genus *Myriotrema*, is the supported sister to *F. insidiosa* Knight & Mitten (1860: 102) in this analysis. *Redingeria desseiniiana* Van den Broeck *et al.* (2014), a species first believed to belong in the genus *Phaeographopsis*, is supported sister to *R. glaucoglyphica* (Sipman) Frisch in Frisch *et al.* (2006: 409).

The four newly recognized genera, which are formally described in parallel in separate papers (Cáceres *et al.* 2014; Mercado-Díaz *et al.* 2014) feature unique combinations of characters. Thus, *Aggregatorygma* resembles *Diorygma* but has very small ascospores and a compact, corticate thallus. *Corticorygma* resembles *Anonomorpha* Nyl. ex Hue (1891: 167) but has large, muriform, non-amyloid ascospores and produces unknown chemical compounds in addition to stictic acid. *Paratopeliopsis* has small, *Topeliopsis*-like ascomata with layered excipulum but differs from *Topeliopsis* in the very small, 3-septate, brown ascospores. A further novel genus, *Borinquenotrema*, is characterized by ascomata formed beneath soralia. Two of these genera, *Corticorygma* and *Paratopeliopsis*, are supported in larger clades, whereas the other two, *Aggregatorygma* and *Borinquenotrema*, represent novel lineages with unresolved placement within the family.

Based on the results from this analysis and from previous analyses (Lücking *et al.* 2013; Rivas Plata *et al.* 2013), we formally recognize the following further, small tribes within Graphidoideae: tribe Acanthothecieae (*Acanthothecis* clade), tribe Diploschisteeae (*Diploschistes*), tribe Leptotremateae (*Leptotrema* clade), and tribe Wirthiotremateae (*Carbacanthographis-Topeliopsis-Wirthiotrema* clade). In addition, we propose to include the species *Topeliopsis darlingtonii* and *T. elixii* in the recently established genus *Gintarasia*.

Taxonomic Treatment

Acanthothecieae Lumbsch, Kraichak & Lücking, *trib. nov.*

Mycobank # 807538

A new tribe in Graphidaceae subfamily Graphidoideae. Ascomata rounded to more rarely elongate. Excipulum uncarbonized. Hamathecium and asci non-amyloid. Ascospores hyaline, generally non-amyloid. Secondary chemistry variable but generally with substances of the stictic and protocetraric acid chemosyndromes.

Type:—*Acanthothecis* Clem.

Thallus usually thin, mostly thinly corticate. Ascomata rounded to more rarely elongate, erumpent to prominent or more rarely sessile, often chroodiscoid or topeliopsidoid. Excipulum uncarbonized; periphysoids absent or present. Hamathecium and asci non-amyloid; paraphyses apically smooth or spinulose. Ascospores transversely septate to submuriform, hyaline, generally non-amyloid and with thin walls and septa, lumina more or less rectangular. Secondary chemistry variable but generally with substances of the stictic and protocetraric acid chemosyndromes.

Genera included in tribe:—*Acanthothecis* Clem. (Fig. 2A), *Corticorygma* M. Cáceres, Feuerstein, Aptroot & Lücking (Fig. 2B), *Gintarasia* Kraichak, Lücking & Lumbsch (Fig. 2C–D).

Remarks:—This tribe is here recognized for the first time formally, although it was recovered in part in previous analyses (Lücking *et al.* 2013; Rivas Plata *et al.* 2013). Its members mostly resemble species of *Chapsa* s.lat. or *Topeliopsis*, but are not closely related to either of these genera, suggesting that these morphotypes are possibly plesiomorphic in subfamily Graphidoideae. The type genus, *Acanthothecis*, is characterized by apically spinulose paraphyses, a feature also known from unrelated lineages (*Acanthotrema*, *Fissurina*), and not all species currently classified in *Acanthothecis* belong here: *A. peplophora* (M. Wirth & Hale) E. A. Tripp & Lendemer in Tripp *et al.* (2010: 62) belongs in tribe Wirthiotremateae (see below). The other two genera recognized in tribe Acanthothecieae have been recently established (Cáceres *et al.* 2014; Kraichak *et al.* 2014); each has unique morphological and/or chemical features but the three genera have no obvious synapomorphies that would indicate their phylogenetic relationships.

Since the two species *Topeliopsis darlingtonii* and *T. elixii* are not true *Topeliopsis* species but come out as the

supported sister to *Gintarasia megalophthalma* (Müll. Arg.) Kraichak *et al.* (2014: 470), agreeing in morphology and chemistry with that genus (Fig. 2C–D), we propose formal combination in *Gintarasia* here:

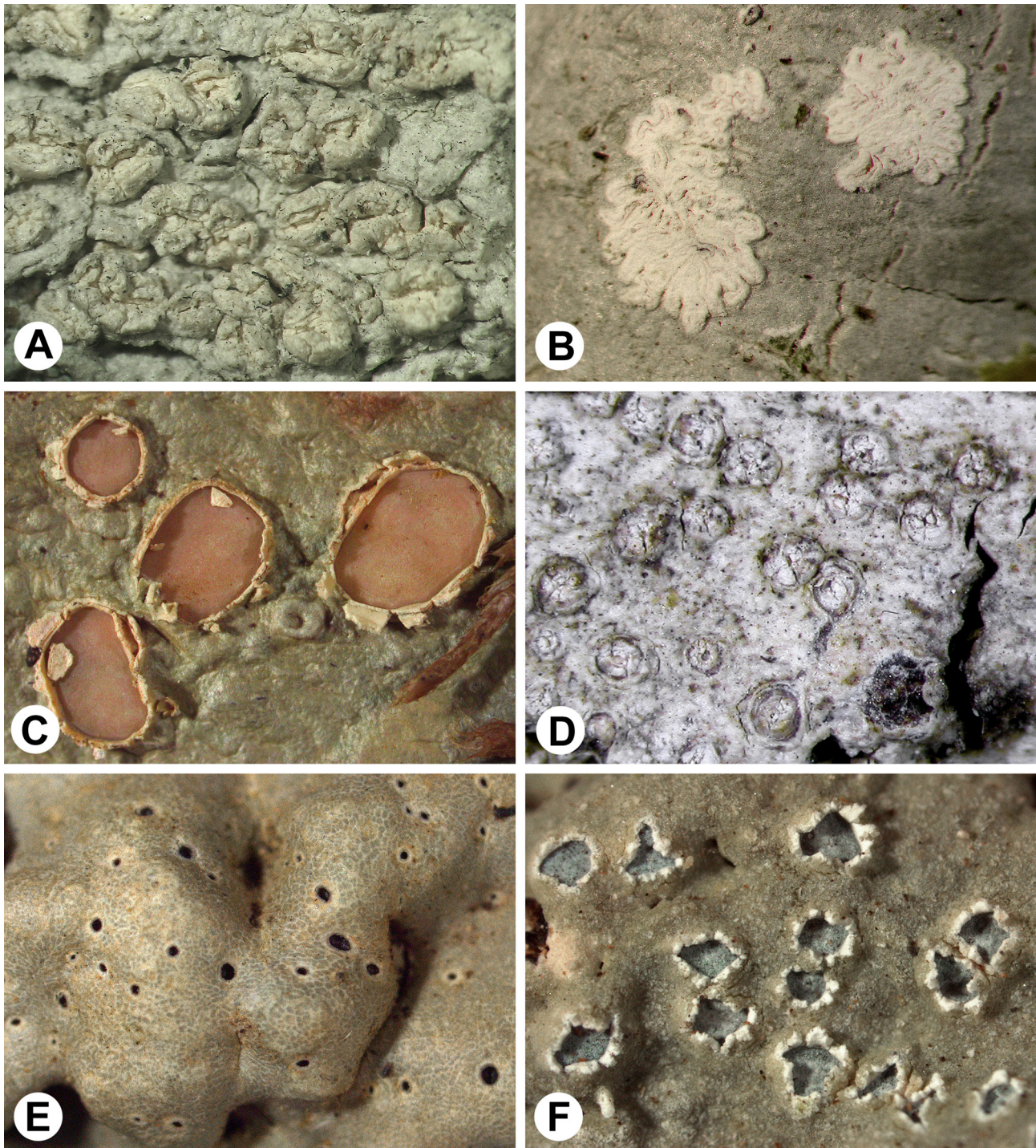


FIGURE 2. A. *Acanthothecis hololeucoides*, thallus with ascomata (lectotype of *Acanthothecium pachygraphoides*). B. *Corticorygma stellatum*, thallus with ascomata (isotype). C. *Gintarasia megalophthalma*, thallus with ascomata (Elix 30220). D. *Gintarasia darlingtonii*, thallus with ascomata (holotype). E. *Leptotrema wightii*, thallus with ascomata (Elix 34790). F. *Reimnitzia santensis*, thallus with ascomata (Nelsen *s.n.*).

Gintarasia darlingtonii (Frisch & Kalb) Lumbsch, Kraichak & Lücking, *comb. nov.* [Mycobank #808666]; *Topeliopsis darlingtonii* Frisch & Kalb, *Lichenologist* 38: 39. 2006. Holotype: Australia, Kalb 33979A (WIS ex hb. Kalb). Remarks:

The type of this species was given the same number as the type of *Topeliopsis elixii* and is here corrected to 33979/1 (Klaus Kalb and Andreas Frisch, pers. comm. 2014).

***Gintarasia elixii* (Frisch & Kalb) Lumbsch, Kraichak & Lücking, comb. nov.** [Mycobank #808667]; Frisch & Kalb, *Lichenologist* 38: 40. 2006. Holotype: Australia, Kalb 33979B (WIS ex hb. Kalb). Remarks: The type of this species was given the same number as the type of *Topeliopsis darlingtonii* and is here corrected to 33979/2 (Klaus Kalb and Andreas Frisch, pers. comm. 2014).

***Diploschisteeae* (Zahlbr.) Lumbsch, Kraichak & Lücking, comb. et stat. nov.**

MycoBank # 807541

Diploschistaceae Zahlbr. in Engler & Prantl, *Nat. Pflanzenfam.* 1(1): 121. 1905; Engler, *Syllabus* 2: 44. 1898 [as 'Diplochistaceae'; nom. nud.]; Zahlbruckner, *Sber. Akad. Wiss. Wien* 111: 393. 1902 [nom. nud.].

Type and only genus included in tribe:—*Diploschistes* Norman.

Remarks:—We recognize this tribe for the single genus *Diploschistes*, based on its isolated phylogenetic position and its unique ecology within the family, featuring a chlorococcoid photobiont and being mostly found on soil and rock in subtropical semi-arid and tropical montane regions (Lücking *et al.* 2014).

***Leptotremateae* Lumbsch, Kraichak & Lücking, trib. nov.**

MycoBank # 807539

A new tribe in Graphidaceae subfamily Graphidoideae. Ascumata rounded to angular or lobate. Excipulum uncarbonized. Hamathecium and asci non-amyloid. Ascospores brown, non-amyloid. Secondary chemistry variable but generally lacking substances.

Type:—*Leptotrema* Mont. & Bosch.

Thallus usually thick or inflated with hollow base, with columnar clusters of calcium oxalate crystals; isidia sometimes present. Ascumata rounded to more rarely angular to lobate, immersed to erumpent, myriotrema or chroodiscoid. Excipulum uncarbonized, usually brown; periphysoids absent. Hamathecium and asci non-amyloid; paraphyses apically smooth. Ascospores small muriform, becoming dark brown and often ornamented, non-amyloid, with thickened walls and septa, lumina more or less rounded. Secondary chemistry variable but generally lacking substances.

Genera included in tribe:—*Leptotrema* Mont. & Bosch. (Fig. 2E), *Reimnitzia* Kalb (Fig. 2F).

Remarks:—The two genera included in this tribe were previously included in tribe Ocellularieae but do not cluster with support within the tribe and hence this small clade is here recognized as a separate tribe.

***Wirthiotremateae* Lumbsch, Kraichak & Lücking, trib. nov.**

MycoBank # 807540

A new tribe in Graphidaceae subfamily Graphidoideae. Ascumata rounded to rarely lirellate or mazaediate. Excipulum uncarbonized or carbonized. Hamathecium and asci non-amyloid. Ascospores hyaline, rarely dark brown, generally non-amyloid. Secondary chemistry variable but generally with substances of the stictic acid chemosyndrome.

Type:—*Wirthiotrema* Rivas Plata, Kalb, Frisch & Lumbsch.

Thallus usually well-developed, mostly corticate. Ascumata rounded to rarely lirellate, immersed prominent to sessile, myriotrema, lepadinoid, topeliopsidoid or graphidoid. Excipulum uncarbonized or carbonized; periphysoids absent or present but indistinct. Hamathecium and asci non-amyloid; paraphyses apically smooth or spinulose. Ascospores transversely septate to submuriform, mostly hyaline, dark brown only in mazaediate ascumata, generally non-amyloid and with thin to thickened walls and septa, lumina more or less rectangular to lens-shaped or rounded. Secondary chemistry variable but mostly with substances of the stictic acid chemosyndrome.

Genera included in tribe:—*Asteristion* Leight. (Fig. 3A), *Carbacanthographis* Staiger & Kalb (Fig. 3B),

Heiomasia Nelsen (Fig. 3C), Lücking & Rivas Plata, *Melanotopelia* Lumbsch & Mangold (Fig. 3D), *Nadvornikia* Tibell (Fig. 3E), *Schizotrema* Mangold & Lumbsch, *Topeliopsis* Kantvilas & Vězda, *Wirthiotrema* Rivas Plata, Kalb, Frisch & Lumbsch (Fig. 3F).

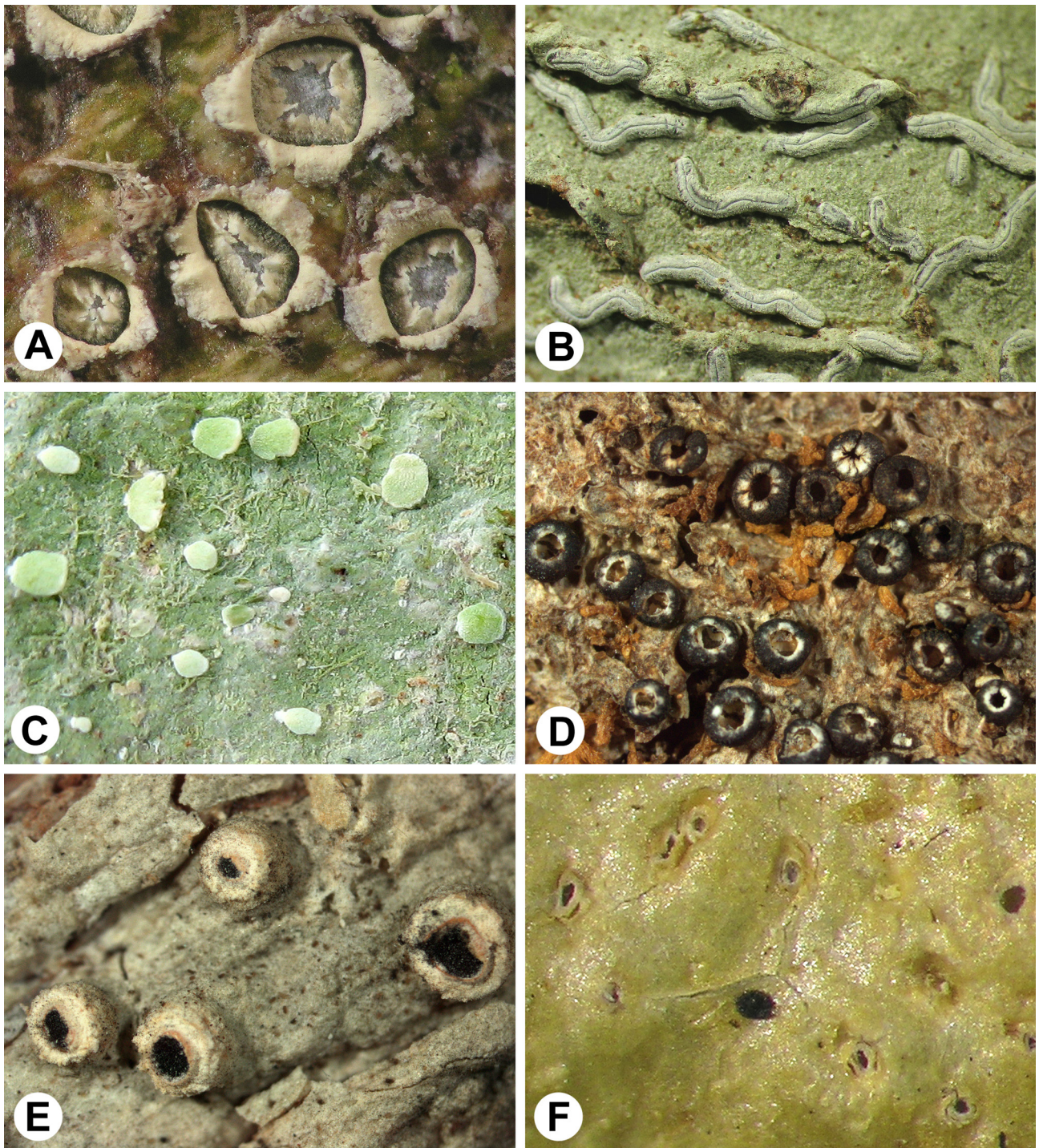


FIGURE 3. A. *Asteristion platycarpum*, thallus with ascomata (Lücking 26575). B. *Carbacanthographis marcescens*, thallus with ascomata (Cáceres 168). C. *Heiomasia sipmanii*, thallus with disc-shaped isidia (holotype). D. *Melanotopelia rugosa*, thallus with ascomata (holotype). E. *Nadvornikia hawaiiensis*, thallus with ascomata (Tibell 12673). F. *Wirthiotrema glaucopallens*, thallus with ascomata (lectotype).

Remarks:—This mid-sized tribe includes species with very variable morphology, mostly previously included in the genera *Myriotrema* and *Thelotrema*, but also the mazediate *Nadvornikia*, the graphidoid *Carbacanthographis*, and the sterile *Heiomasia*. There are no known synapomorphies that would characterize this

tribe as a whole, but tendencies to have an internal anatomy, ascospores, and chemistry similar to tribe Thelotremateae are apparent. Most of the genera included in this tribe were previously assigned to tribe Thelotremateae s.lat., but further analyses showed that they are not closely related (Lücking *et al.* 2013; Rivas Plata *et al.* 2013).

Generic delimitation in this tribe, including resurrection of the genus *Asteristion*, is currently under revision and will be dealt with in a forthcoming publication.

Acknowledgements

Data obtained for this study were gathered as part of several projects funded by the National Science Foundation: *TICOLICHEN* (DEB 0206125 to The Field Museum; PI Robert Lücking), *Phylogeny and Taxonomy of Ostropalean Fungi, with Emphasis on the Lichen-forming Thelotremataceae* (DEB 0516116 to The Field Museum; PI H. T. Lumbsch; Co-PI R. Lücking), *Neotropical Epiphytic Microlichens—An Innovative Inventory of a Highly Diverse yet Little Known Group of Symbiotic Organisms* (DEB 715660 to The Field Museum; PI R. Lücking), and *ATM—Assembling a taxonomic monograph: The lichen family Graphidaceae* (DEB-1025861 to The Field Museum; PI T. Lumbsch, CoPI R. Lücking).

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