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Malmographina, a new genus for *Graphina malmei* (Ascomycota: *Ostropales*: *Graphidaceae*)

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Abstract: The new genus *Malmographina* Cáceres, Rivas Plata & Lücking is introduced for *Opegrapha plicosa* Meissn. (syn. *Graphis malmei* Redinger), with the new combination *Malmographina plicosa* (Meiss.) Cáceres, Rivas Plata & Lücking. The taxon is characterized by prominent, striate, orange-pigmented, non-carbonized lirellae, a more or less clear hymenium, and large, muriform, hyaline ascospores. *Malmographina* is most similar to *Hemithecium* in lirellae morphology and anatomy, but in a molecular phylogenetic analysis falls within a clade containing *Phaeographis* and other genera with usually inspersed hymenium and brown ascospores. Within that clade, it is thus far the only taxon that has persistently hyaline ascospores. It is otherwise similar to *Pallidogramme* but does not cluster within that genus. A preliminary phylogenetic analysis of the *Phaeographis* clade confirms monophyly of the genera *Halegrapha*, *Pallidogramme*, and *Platygramme*, and suggests that *Phaeographis* should be divided into several generic lineages and that *Leiorreuma* and *Sarcographa* perhaps be merged.

Key words: Amazonia, Brazil, *Creographa*, *Ectographis*, lichens, Peru, *Phlegographa*, Surinam

Introduction

Until most recently, genera within the *Graphidaceae* with lirellate ascomata were distinguished based on ascospore pigment and septation (hyaline versus grey-brown, transversely septate versus muriform), as well as ascoma organization [solitary versus (pseudo-)stromatic] into eight genera: *Graphis*, *Phaeographis*, *Graphina*, *Phaeographina*, *Glyphis*, *Medusulina*, *Sarcographa* and *Sarcographina* (Zahlbruckner 1923; Wirth & Hale 1963, 1978). Staiger (2002) challenged this highly artificial concept and provided new genus circumscriptions based on a combination of morphological, anatomical, and chemical features, distinguishing 22 genera. This concept was subsequently refined and tested with molecular data (Kalb *et al*. 2004; Archer 2006, 2009; Staiger *et al*. 2006; Lücking 2007; Lücking *et al*. 2007; Lücking & Rivas Plata 2008; Mangold *et al*. 2008). These studies confirmed many of the taxa proposed by Staiger (2002) but challenged others, such as *Graphis*, which actually contains two unrelated lineages, and *Hemithecium*, which splits up into at least four separate lineages (Rivas Plata *et al*. 2011). One species, *Hemithecium plicosum* (Meissn.) Lücking & Aptroot (syn. *Graphina malmei* Redinger), did not cluster with either *Graphis* or *Hemithecium* but fell within the clade containing *Phaeographis* and other grey-brown-spored genera (Rivas Plata *et al*. 2011), even if its ascospores are persistently hyaline. This species (as *Graphina malmei*) was said to have uncertain affinities by Staiger (2002), but was subsequently included in *Hemithecium* by Lücking *et al*. (2008).

Here, we present a more detailed study of the *Phaeographis* clade to elucidate the exact position of *Hemithecium plicosum* and, based

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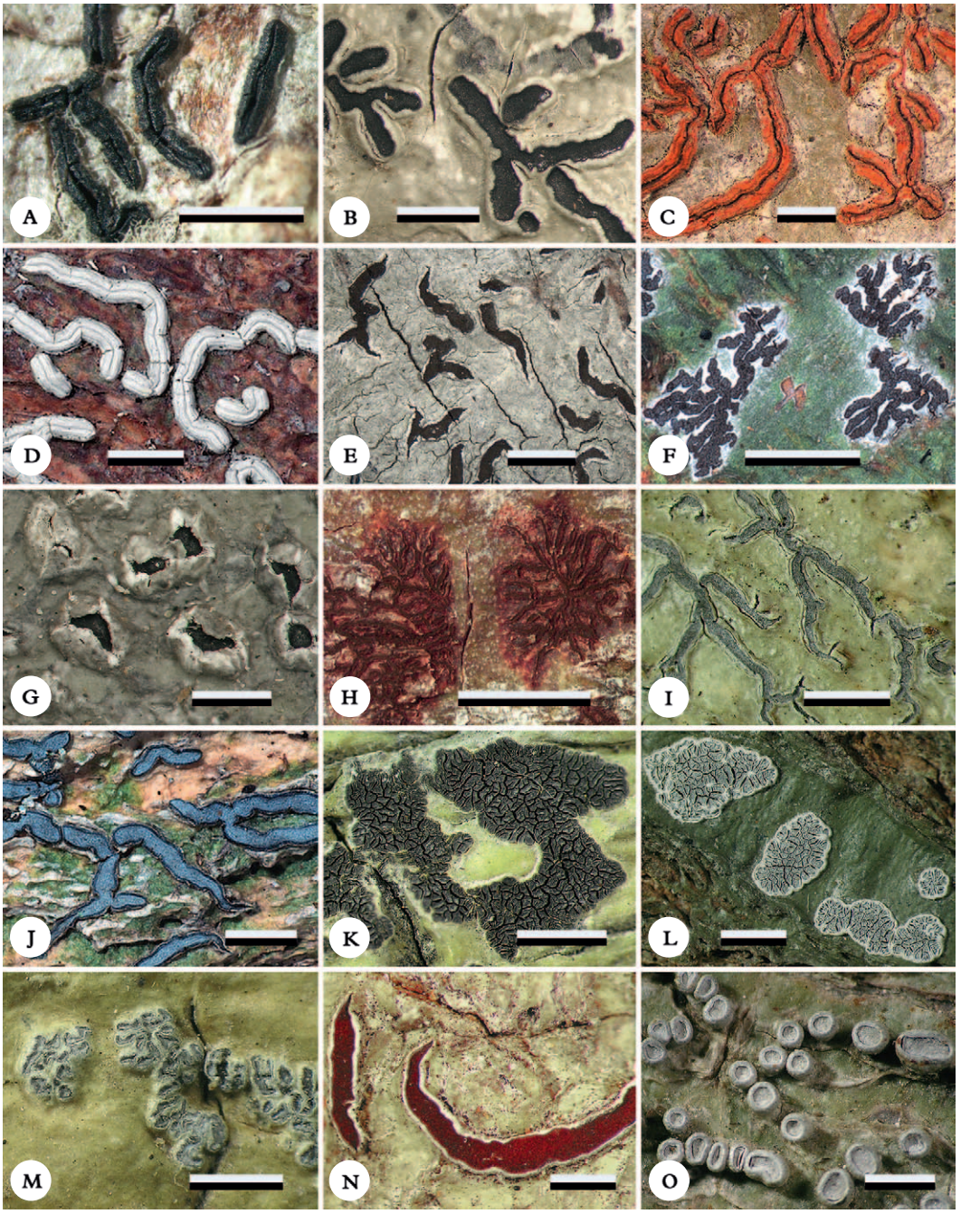
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TABLE 1. *GenBank accession numbers and voucher information (for newly generated sequences) for taxa used in this study.*

Genus	Species	mtSSU	nuLSU	Origin	Voucher
Diorygma	*poitaei*	HQ639596	HQ639627		
Glyphis	*cicatricosa*	HQ639610	HQ639630		
Thalloloma	*hypoleptum*	HQ639609			
Halegrapha	*chimera*	JF505933			
Halegrapha	*chimera*	JF505934			
Hemithecium	*plicosum*	HQ639590			
Leiorreuma	*hypomelaenum*	DQ431971	DQ431933		
Pallidogramme	*chlorocarpoides*	JN127362		Philippines	*Rivas Plata* 2008b (F)
Pallidogramme	*chrysenteron*	JN127361		Philippines	*Rivas Plata* 1157D (F)
Phaeographis	*brasiliensis*	DQ431958			
Phaeographis	*caesioradians*	DQ431968			
Phaeographis	*dendritica*	HQ639592			
Phaeographis	*intricans*	DQ431960			
Phaeographis	*lecanographa*	DQ431983	DQ431943		
Phaeographis	*leprieurii*	JN127363	JN127365	Brazil	*Cáceres s.n.* (F)
Phaeographis	*lobata*	DQ431984	DQ431944		
Phaeographis	*scalpturata*	JN127364		Peru	*Rivas Plata* 106A (F)
Platygramme	*australiensis*	DQ431970			
Platygramme	*caesiopruinosa*	DQ431973			
Platygramme	*caesiopruinosa*	EU075593	EU075639		
Sarcographa	*fenicis*	DQ431967	DQ431931		
Sarcographa	*glyphiza*	DQ431972	DQ431934		
Sarcographa	*labyrinthica*	JF828969			
Sarcographa	*ramificans*	DQ431981	DQ431942		
Thecaria	*montagnei*	HQ639605			
Thecaria	*quassiicola*	JF828971			
Thecaria	*quassiicola*	HQ639617	HQ639667		

on our findings, introduce the new genus *Malmographina* for this taxon.

Material and Methods

To elucidate the phylogenetic position of *Hemithecium plicosum*, we assembled a dataset consisting of the small subunit of the mitochondrial ribosomal DNA (mtSSU) and the large subunit of the nuclear ribosomal DNA (nuLSU) of selected species of the *Phaeographis* clade (Fig. 1), with *Diorygma poitaei*, *Glyphis cicatricosa* and *Thalloloma hypoleptum* as outgroup taxa (Table 1). Most sequences were downloaded from Genbank, but five sequences (see Table 1) were newly generated following the protocol outlined in Rivas Plata *et al.* (2011). The sequences were aligned using MAFFT (Katoh & Toh 2005) and the alignment was subjected to maximum likelihood search using RAxML 7.2.6 (Stamatakis *et al.* 2005; Stamatakis 2006), with parametric bootstrapping using 1000 replicates under the GTRGAMMA model.

Malmographina Cáceres, Rivas Plata & Lücking gen. nov.

MycoBank No: MB 563384

Genus novum familiae *Graphidacearum*. Thallus laevigatus, viridis. Lirellae proeminentes, striatae, aurantiacae. Excipulum crenatum, non-carbonizatum. Hymenium non inspersum. Ascosporae muriformes,

FIG. 1. Taxa of the *Phaeographis* clade analyzed for this study. A, *Halegrapha chimaera* (holotype); B, *Leiorreuma exaltatum* (Dominica, *Imshaug* 32934); C, *Malmographina plicosa* (Peru, *Rivas Plata* FM-09); D, *Pallidogramme chrysenteron* (Costa Rica, *Lücking* 15287t); E, *Phaeographis dendritica* (Argentina, *Ferraro s.n.*); F, *Phaeographis intricans* (Costa Rica, *Lücking* 15252e); G, *Phaeographis kalbii* (Costa Rica, *Trest* 1584i); H, *Phaeographis leprieurii* (Brazil, *Cáceres s.n.*); I, *Phaeographis scalpturata* (Brazil, *Cáceres* 523); J, *Platygramme caesiopruinosa* (Costa Rica, *Lücking* 15060k); K, *Sarcographa fenicis* (Brazil, *Cáceres* 12); L, *Sarcographa labyrinthica* (Brazil, *Cáceres* 21); M, *Sarcographina glyphiza* (Philippines, *Merrill* 9045); N, *Thecaria montagnei* (Trinidad and Tobago, *Imshaug* 32437); O, *Thecaria quassicola* (Florida, *Lücking* 26626).

hyalinae, leviter amyloideae. Acidi lichenum desunt sed lirellae antraquinonae continentes.

Typus: *Malmographina plicosa* (Meissn.) Cáceres, Rivas Plata & Lücking, comb. nov. [Mycobank MB 563385].— *Opegrapha plicosa* Meissn., *Bot. Zeitung* **13:** 422 (1855);—*Hemithecium plicosum* (Meissn.) Lücking & Aptroot, *Fieldiana, Botany* **46:** 65 (2008); lectotype (fide Lücking *et al*. 2008: 65): Surinam, *Kegel* 1419 (L!). = *Graphina malmei* Redinger, *Ark. Bot*. **26A**(1)**:** 49 (1933) [holotypus—Brazil, *Malme* 3508 (S!)].

(Fig. 1C)

Thallus smooth, olive-green (becoming yellowish in the herbarium), in section with dense, prosoplectenchymatous cortex and irregular photobiont layer intermingled with scattered clusters of calcium-oxalate crystals.

Lirellae erumpent to prominent, finely but distinctly striate, with orange to cinnabar-red pigment. *Disc* concealed. *Excipulum* distinctly crenate, orange-brown and becoming dark brown to carbonized in inner apical parts; old, compressed layers of hymenia between the excipular striae becoming dark brown to almost carbonized as well. *Hymenium* clear; epithecium with clusters of dark brown to blackish granules. *Ascospores* muriform, hyaline, non-amyloid.

Chemistry. No lichen substances detected in the thallus but lirellae with anthraquinones (tetrahydroxyanthrachinon-1,3,6,8 and other, unidentified pigments). For a more detailed description, see Staiger (2002: 452, as *Graphina malmei*).

Notes. Staiger (2002) discussed the systematic position of *Graphina malmei* as uncertain. Lücking *et al*. (2008) subsequently found an earlier name for this species, *Opegrapha plicosa* Meissn., and proposed the combination *Hemithecium plicosum* (syn. *Graphina malmei*), because of the prominent lirellae with well-developed labia and uncarbonized excipulum, and the hyaline ascospores. In a molecular phylogenetic analysis, however, *Hemithecium* was shown to be polyphyletic: one group including *H. implicatum* (Fée) Staiger clustered with *Graphis* Adans. s. str., another group including *H. chlorocarpum* (Fée) Trevis. clustered with *Allographa* Chevall., a segregate of *Graphis*, while *H. rufopallidum* (Vain.) Staiger and *H. plicosum* formed separate lineages (Rivas Plata *et al*. 2011). Surprisingly, in spite of the hyaline ascospores, *Hemithecium plicosum* fell within a clade formed by *Phaeographis* and allied genera, all characterized by grey-brown ascospores and a usually inspersed hymenium with inspersion of the *Phaeographis* type (Lücking 2009). Within that clade, *Hemithecium plicosum* is morphologically most similar to species of *Pallidogramme*, which also have *Hemithecium*-like, prominent, striate lirellae (and were included in *Hemithecium* by Staiger 2002) but differ in the grey-brown ascospores and usually inspersed hymenium (Staiger 2002; Lücking *et al*. 2008).

Our emended phylogenetic analysis of the *Phaeographis* clade (Fig. 2), although preliminary since only few species of this clade have been sequenced so far, confirms several of the genera distinguished by Staiger (2002) and in subsequent studies (Lücking *et al*. 2008, 2011), such as *Halegrapha*, *Pallidogramme*, *Platygramme*, and *Thecaria* s. str., as independent, monophyletic lineages. *Phaeographis* as circumscribed by Staiger (2002) is polyphyletic and forms several clades that are morphologically well distinguished: the *P. lecanographa* clade with *Chapsa*-like ascomata, the *P. intricans* clade (*Creographa*) with pseudostromatic ascomata and clear hymenium, the *P. leprieurii* clade (*Phlegographa*) including *Glyphis*-like species with nearly stromatic, heavily carbonized ascomata, the *P. scalpturata* clade (*Ectographis*) which contains the bulk of corticate species with pruinose disc, and *Phaeographis* s. str., a small clade characterized by ecorticate, whitish thalli and ascomata with chocolate-brown disc. Other oddball taxa within the *Phaeographis* clade also form separate lineages: *Sarcographa fenicis* with completely flattened, stromatic ascomata, *Sarcographa glyphiza*, whose generic position has been unclear, and *Hemithecium plicosum*, for which the new genus *Malmographina* is established here. The phylogeny also suggests that *Leiorreuma* and *Sarcographa* should be merged, but must await molecular analysis of the type species of

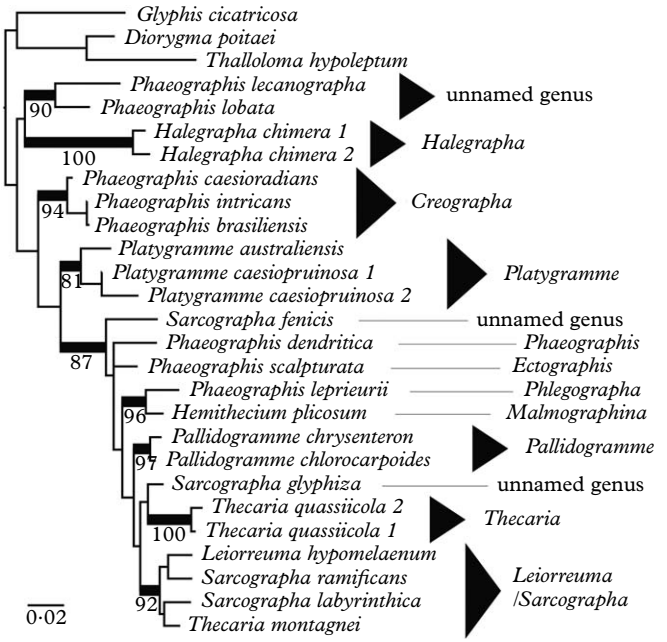

FIG. 2. Phylogeny of the *Phaeographis* clade derived by RAxML maximum likelihood analysis of a combined mtSSU-nuLSU dataset, using *Diorygma poitaei*, *Glyphis cicatricosa*, and *Thalloloma hypoleptum* as outgroup. Numbers below branches indicate bootstrap values.

Sarcographa, *S. cinchonarum*, before further conclusions can be drawn, as this species differs from the bulk of *Sarcographa* species centred around the common *S. labyrinthica*. It should also be noted that *Thecaria montagnei* clusters with the *Leiorreuma-Sarcographa* clade and not with *T. quassiicola*. This is not surprising as *T. quassiicola* is a morphologically unique species somewhat resembling *Glyphis scyphulifera*, whereas *T. montagnei* is very similar to *Leiorreuma* species except for the muriform ascospores and pigmented disc and hymenium.

Based on our results, it is appropriate to introduce a new genus, *Malmographina*, for *Opegrapha plicosa* (syn. *Graphina malmei*). *Malmographina* is distinguished from all other genera of *Graphidaceae* by the combination of *Hemithecium*-like (prominent, striate, uncarbonized) but pigmented lirellae, a clear hymenium, and hyaline, non-amyloid ascospores. Critical revision of the characters found in *Malmographina* suggests that the placement within the clade including *Phaeographis* and allied genera might not be that surprising after all. Lack of inspersion is found in a few lineages within this clade, including the *Phaeographis intricans* group and even a species of *Pallidogramme*, *P. chapadana* (Redinger) Staiger, Kalb & Lücking (Staiger 2002). In species of *Phaeographis* and other genera with large, muriform ascospores, the ascospores often remain hyaline for a long time but eventually become at least pale grey-brown. In species of *Pallidogramme* with striate excipulum, such as *P. chrysenteron* (Mont.) Staiger, Kalb & Lücking, mature ascospores become light gold-brown but the compressed, old hymenia between the excipular striae become very dark brown due to heavy pigmentation of collapsed, dead ascospores (Staiger 2002). We can therefore assume that *Malmographina plicosa*, which also shows heavy dark brown pigmentation of compressed old hymenia, has the potential to produce ascospore pigment but this only becomes expressed in postmature, collapsed and dead ascospores within excipular remnants. This is supported by the lack of iodine reaction of

the ascospores which is more similar to that found in *Phaeographis* and allies than that typical of *Graphis* and *Hemithecium*.

Malmographina plicosa appears to be restricted to the Amazon basin. It is thus far known from Surinam (type of *Opegrapha plicosa*), Amazonian Peru (several collections from Madre de Dios province by Rivas Plata), Amazonian Brazil (several collections from Rondônia by Cáceres), and adjacent Mato Grosso (the type of *Graphina malmei* and collections made by Kalb). It prefers semi-exposed to exposed microsites mostly on bark of larger trees, where it can be found associated with other lichens having similar preferences, such as *Glyphis* spp., *Phaeographis* spp., and *Trypetheliaceae*.

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