



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

H. THORSTEN LUMBSCH¹, EKAPHAN KRAICHAK¹, SITTIPORN PARNMEN², EIMY RIVAS PLATA¹, ANDRÉ APTROOT³, MARCELA E. S. CÁCERES⁴, DAMIEN ERTZ⁵, SHIRLEY CUNHA FEUERSTEIN⁶, JOEL A. MERCADO-DÍAZ⁷, BETTINA STAIGER⁸, DRIES VAN DEN BROECK⁵ & ROBERT LÜCKING¹

¹Science & Education, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois 60605-2496, U.S.A.; email: rlücking@fieldmuseum.org, mvonkonrat@fieldmuseum.org

²Toxicology and Biochemistry Section, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000 Thailand; email: sparnmen@gmail.com

³ABL Herbarium, G.v.d. Veenstraat 107, NL-3762 XK Soest, The Netherlands; email: andreaptroot@gmail.com

⁴Departamento de Biociências, Universidade Federal de Sergipe, CEP: 49500-000, Itabaiana, Sergipe, Brazil; email: mscaceres@hotmail.com

⁵Department of Bryophytes-Thallophytes, National Botanic Garden of Belgium, domein van Bouchout, Nieuwelaan 38, 1860 Meise, Belgium; email: damien.ertz@br.fgov.be, dries.van.den.broeck@br.fgov.be

⁶Universidade Federal do Paraná, Departamento de Botânica, Laboratório de Liqueenologia, Caixa Postal 19031, 81531970 Curitiba, PR, Brasil; email: shirleycunha_@hotmail.com

⁷International Institute of Tropical Forestry, USDA Forest Service, Ceiba St. 1201, Jardín Botánico Sur, Río Piedras; email: joel_pr19@hotmail.com

⁸University of Regensburg, Institute for Botany, Universitätsstr. 31, D-93040 Regensburg, Germany

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 desseiniana*. Based on the resulting topology, in addition to three tribes described previously, we recognize four further tribes in the subfamily Graphidoideae: *Acanthotheciae* Lumbsch, Kraichak & Lücking, *Diploschistiae* (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 *Acanthotheciae*, supported sister to *Acanthothecis*, and *Paratopeliopsis* belongs in *Thelotremateae*, unsupported sister to *Leucodection*. *Diorygma erythrellum* is confirmed as a member of the *Diorygma-Thalloloma* clade, while *Fissurina monilifera*, inspite of its myriotremoid ascomata, belongs in *Fissurina* s.str. *Redingeria desseiniana*, although resembling the genus *Phaeographopsis*, is supported sister to *R. glaucoglypica*. *Topeliopsis darlingtonii* forms the sister group to *Gintarasia megalopthalma*. 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

tribe as a whole, but tendencies to have an internal anatomy, ascospores, and chemistry similar to tribe Thelotremaeae are apparent. Most of the genera included in this tribe were previously assigned to tribe Thelotremaeae 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 Ostopalean Fungi, with Emphasis on the Lichen-forming Thelotremaeae* (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).

References

- Acharius, E. (1803) *Methodus qua omnes detectos lichenes secundum organa carpomorpha ad genera, species et varietates redigere atque observationibus illustrare tentavit*. F.D.D. Ulrich, Stockholm.
<http://dx.doi.org/10.5962/bhl.title.79411>
- Aptroot, A., Diederich, P., Sérusiaux, E. & Sipman, H.J.M. (1997) Lichens and lichenicolous fungi from New Guinea. *Bibliotheca Lichenologica* 64: 1–220.
<http://dx.doi.org/10.1017/s0024282997000613>
- Archer, A.W. (2009) Graphidaceae. *Flora of Australia* 57 (Lichens 5): 84–194.
- Caceres, M.E.S., Aptroot, A., Parnmen S. & Lücking, R. (2014) Remarkable diversity of the lichen family *Graphidaceae* in the Amazon rain forest of Rondônia, Brazil. *Phytotaxa* 189(1): 87–136.
<http://dx.doi.org/10.11646/phytotaxa.189.1.8>
- Clements, F.E. (1909) *The Genera of Fungi*. New York.
<http://dx.doi.org/10.5962/bhl.title.54501>
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
<http://dx.doi.org/10.1186/1471-2148-7-214>
- Eschweiler, F.G. (1824) *Systema lichenum, genera exhibens rite distincta, pluribus novis adiecta*. Nürnberg, pp. 1–26.
- Fée, A.L.A. (1825) *Essai sur les cryptogames des écorces exotiques officinales*. Paris. vii.xiv, 1–167 pp.
- Frisch, A. & Kalb, K. (2006b) The lichen *Topeliopsis*, additions and corrections. *Lichenologist* 38: 37–45.
<http://dx.doi.org/10.1017/s0024282905005530>
- Frisch, A., Kalb, K. & Grube, M. (2006) Contributions towards a new systematics of the lichen family Thelotremaeae. *Bibliotheca Lichenologica* 92: 1–556.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hue, A. (1891) Lichenes exóticos a professore W. Nylander descriptos vel recognitos et in herbario musei Parisiense pro maxima parte asservatos in ordine systematico depositus. *Nouvelles Archives du Muséum d'Histoire Naturelle* [Sér. 3] 3: 33–120.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Kalb, K. (2001) The lichen genus *Topeliopsis* in Australia and remarks on Australian Thelotremaeae. *Mycotaxon* 79: 319–328.
- Kalb, K., Staiger, B. & Elix, J.A. (2004) A monograph of the lichen genus *Diorygma* - a first attempt. *Symbolae Botanicae Upsalienses* 34(1): 133–181.
- Kantvilas, G. & Vězda, A. (2000) Studies on the lichen family Thelotremaeae in Tasmania. The genus *Chroodiscus* and its relatives. *Lichenologist* 32: 325–357.
<http://dx.doi.org/10.1006/lich.2000.0274>
- Katoh, K. & Toh, M. (2005) MAFFT Version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33: 511–518.
<http://dx.doi.org/10.1093/nar/gki198>

- Kauff, F. & Lutzoni, F. (2002) Phylogeny of Gyalectales and Ostropales (Ascomycota, Fungi): among and within order relationships based on nuclear ribosomal RNA small and large subunits. *Molecular Phylogenetics and Evolution* 25: 138–156.
[http://dx.doi.org/10.1016/s1055-7903\(02\)00214-2](http://dx.doi.org/10.1016/s1055-7903(02)00214-2)
- Knight, C. & Mitten, W. (1860) Contribution to the Lichenographia of New Zealand; being an Account, with Figures, of some New Species of Graphidaceae and allied Lichens. *Transactions of the Linnean Society, London* 23: 101–106.
<http://dx.doi.org/10.1111/j.1096-3642.1860.tb00124.x>
- Kraichak, E., Parnmen, S., Lücking, R., Rivas Plata, E., Aptroot, A., Cáceres, M.E.S., Ertz, D., Mangold, A., Mercado-Díaz, J.A., Papong, K., Van den Broeck, D., Weerakoon, G. & Lumbsch, H.T. (2014) Revisiting the phylogeny of Ocellulariae, the second largest tribe within Graphidaceae (lichenized Ascomycota: Ostropales). *Phytotaxa* 189(1): 52–81.
<http://dx.doi.org/10.11646/phytotaxa.189.1.6>
- Leighton, W.A. (1869) The Lichens of Ceylon, collected by G. H. K. Thwaites. *Transactions of the Linnean Society, London* 27: 161–185.
<http://dx.doi.org/10.1111/j.1096-3642.1870.tb00210.x>
- Liu, Y.J., Whelen, S. & Hall, B.D. (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.
<http://dx.doi.org/10.1093/oxfordjournals.molbev.a026092>
- Lücking, R., Parnmen, S. & Lumbsch, H.T. (2012) *Mangoldia*, a new lichen genus in the family Graphidaceae (Ascomycota: Ostropales). *Phytotaxa* 69: 1–5.
- Lücking, R., Tehler, A., Bungartz, F., Rivas Plata, E. & Lumbsch, H.T. (2013) Journey from the West: Did tropical Graphidaceae (lichenized Ascomycota: Ostropales) evolve from a saxicolous ancestor along the American Pacific coast? *American Journal of Botany* 100: 844–856.
<http://dx.doi.org/10.3732/ajb.1200548>
- Lücking, R., Aptroot, A., Boonpragob, K., Cáceres, M.E.S., Ertz, D., Harris, R.C., Jia, Z.-F., Kalb, K., Kraichak, E., Lendemer, J.C., Mangold, A., Manoch, L., Mercado-Díaz, J., Moncada, B., Mogkulsuk, P., Papong, K., Parnmen, S., Peláez, R., Poengsunoen, V., Rivas-Plata, E., Saipunkaew, W., Sipman, H.J.M., Sutjaritturakan, J., van den Broeck, D., von Konrat, M., Weerakoon, G. & Lumbsch H.T. (2014) One hundred and seventy five new species of Graphidaceae: closing the gap or a drop in the bucket? *Phytotaxa* 189(1): 7–38.
<http://dx.doi.org/10.11646/phytotaxa.189.1.4>
- Mangold, A., Martin, M.P., Lücking, R. & Lumbsch, H.T. (2008a) Molecular phylogeny suggests synonymy of Thelotremae within Graphidaceae (Ascomycota: Ostropales). *Taxon* 57: 476–486.
- Mangold, A., Martín, M. P., Kalb, K., Lücking, R. & Lumbsch, H.T. (2008b) Molecular data show that Topeliopsis (Ascomycota, Thelotremae) is polyphyletic. *Lichenologist* 40: 39–46.
<http://dx.doi.org/10.1017/s0024282908007366>
- Mangold, A., Elix, J.A. and Lumbsch, H.T. (2009) Thelotremae. *Flora of Australia Volume* 57: 195–420.
- Mason-Gamer, R.J. & Kellogg, E.A. (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology* 45: 524–545.
<http://dx.doi.org/10.2307/2413529>
- Massalongo, A.B. (1860) Esame comparativo di alcuni generi dei Licheni. *Atti del Reale Istituto Veneto di Scienze, Lettere et Arti, Series* 3(5): 247–276, 313–337.
- Miadlikowska, J. & Lutzoni, F. (2000) Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota) based on morphological, chemical and large subunit nuclear ribosomal DNA data. *International Journal of Plant Science* 161: 925–968.
<http://dx.doi.org/10.1086/317568>
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*: 1–8. New Orleans.
<http://dx.doi.org/10.1109/gce.2010.5676129>
- Miquel, F.A.W. (1855). *Plantae Junghuhnianae*. Leyden.
- Müller, J. (1887) Lichenologische Beiträge 26. *Flora* 70: 268–273, 283–288, 316–322, 336–338, 396–402, 423–429.
- Nelsen, M., Lücking, R., Rivas Plata, E. & Mbatchou, J.S. (2010) *Heiomasia*, a new genus in the lichen-forming family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales) with disjunct distribution in Southeastern North America and Southeast Asia. *Bryologist* 113: 742–751.
<http://dx.doi.org/10.1639/0007-2745-113.4.742>
- Norman, J.M. (1853) Conatus praemissus redactionis novae generum nunnulorum Lichenum. *Nytt Magazin for Naturvidenskapene* 7: 213–252.
- Parnmen, S., Lücking, R. & Lumbsch, H.T. (2012) Phylogenetic classification at generic level in the absence of distinct phylogenetic patterns of phenotypical variation: A case study in Graphidaceae (Ascomycota). *PLOS One* 7(12): 1–13.
<http://dx.doi.org/10.1371/journal.pone.0051392>
- Parnmen, S., Cáceres, M.E.S., Lücking, R. & Lumbsch, H.T. (2013) *Myriochapsa* and *Nitidochapsa*, two new genera in Graphidaceae (Ascomycota: Ostropales) for chroodiscoid species in the *Ocellularia* clade. *The Bryologist* 116: 127–133.
<http://dx.doi.org/10.1639/0007-2745-116.2.127>

- Penn, O., Privman, E., Landan, G., Graur, D. & Pupko, T. (2010a) An alignment confidence score capturing robustness to guide-tree uncertainty. *Molecular Biology and Evolution* 27: 1759–67.
<http://dx.doi.org/10.1093/molbev/msq066>
- Penn, O., Privman, E., Ashkenazy, H., Landan, G., Graur, D. & Pupko, T. (2010b) GUIDANCE: a web server for assessing alignment confidence scores. *Nucleic Acids Research* 38: W23–W28.
<http://dx.doi.org/10.1093/nar/gkq443>
- Rivas Plata, E., Lücking, R. & Lumbsch, H.T. (2008) When family matters: an analysis of Thelotremaeae (Lichenized Ascomycota: Ostropales) as bioindicators of ecological continuity in tropical forests. *Biodiversity and Conservation* 17: 1319–1351.
<http://dx.doi.org/10.1007/s10531-007-9289-9>
- Rivas Plata, E., Lücking, R., Sipman, H.J.M., Mangold, A., Kalb, K. & Lumbsch, H.T. (2010) A world-wide key to the thelotremoid *Graphidaceae*, excluding the *Ocellularia*-*Myriotrema*-*Stegobolus* clade. *Lichenologist* 42: 139–185.
<http://dx.doi.org/10.1017/s0024282909990491>
- Rivas Plata, E., Lücking, R. & Lumbsch, H.T. (2012a) A new classification for the family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). *Fungal Diversity* 52: 107–121.
<http://dx.doi.org/10.1007/s13225-011-0135-8>
- Rivas Plata, E., Lücking, R. & Lumbsch, H.T. (2012b) Molecular phylogeny and systematics of the *Ocellularia* clade (Ascomycota: Ostropales: Graphidaceae). *Taxon* 61: 1161–1179.
- Rivas Plata, E., Parnmen, S., Staiger, B., Mangold, A., Frisch, A., Weerakoon, G., Hernández M.J.E., Cáceres, M.E.S., Kalb, K., Sipman, H.J.M., Common, R.S., Nelsen, M.P., Lücking, R. & Lumbsch, H.T. (2013) A molecular phylogeny of Graphidaceae (Ascomycota, Lecanoromycetes, Ostropales) including 428 species. *MycoKeys* 6: 55–94.
<http://dx.doi.org/10.3897/mycokeys.6.3482>
- Staiger, B. (2002) Die Flechtenfamilie Graphidaceae. Studien in Richtung einer natürlicheren Gliederung. *Bibliotheca Lichenologica* 85: 1–526.
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum-Likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–90.
<http://dx.doi.org/10.1093/bioinformatics/btl446>
- Stamatakis, A., Ludwig, T. & Meier, H. (2005) RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21: 456–463.
<http://dx.doi.org/10.1093/bioinformatics/bti191>
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A fast bootstrapping algorithm for the RAxML web-Servers. *Systematic Biology* 57: 758–771.
<http://dx.doi.org/10.1080/10635150802429642>
- Tibell, L. (1984) A reappraisal of the taxonomy of Caliciales. *Beiheft zur Nova Hedwigia* 79: 597–713.
- Trevisan, V. (1853) *Spighe e Paglie* 1. Padova. 64 pp.
- Tripp, E.A., Lendemer, J.C. & Harris, R.C. (2010) Resolving the genus *Graphina* Müll. Arg. in North America: new species, new combinations, and treatments for *Acanthothecis*, *Carbacanthographis*, and *Diorygma*. *Lichenologist* 42: 55–71.
<http://dx.doi.org/10.1017/s0024282909990296>
- Van den Broeck, D., Lücking, R. & Ertz, D. (2014) Three new species of Graphidaceae from tropical Africa. *Phytotaxa* 189(1): 325–330.
<http://dx.doi.org/10.11646/phytotaxa.189.1.23>
- Vilgalys, R. & Hester, M. (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. *Journal of Bacteriology* 172: 4238–4246.
- Zoller, S., Scheidegger, C. & Sperisen, C. (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* 31: 511–516.
<http://dx.doi.org/10.1017/s0024282999000663>