The monotypic genus *Bulborrhizina* belongs to *Bulbothrix* sensu lato (Parmeliaceae, Ascomycota)

Author(s): Paul M. Kirika, Steven D. Leavitt, Pradeep K. Divakar, Ana Crespo, Grace W. Gatheri, George Mugambi, and H. Thorsten Lumbsch
Source: The Bryologist, 118(2):164-169.
Published By: The American Bryological and Lichenological Society, Inc.
URL: http://www.bioone.org/doi/full/10.1639/0007-2745-118.2.164

The monotypic genus *Bulborrhizina* belongs to *Bulbothrix* sensu lato (Parmeliaceae, Ascomycota)

Paul M. Kirika[1,2], Steven D. Leavitt[3,4], Pradeep K. Divakar[5], Ana Crespo[5], Grace W. Gatheri[1], George Mugambi[6] and H. Thorsten Lumbsch[3,7]

[1] *Department of Plant Sciences, Kenyatta University, P. O Box 43844-00100, Nairobi, Kenya;* [2] *Botany Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya;* [3] *Science & Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, U.S.A.;* [4] *Committee on Evolutionary Biology, University of Chicago, 1025 E. 57th Street, Chicago, IL 60637, U.S.A;* [5] *Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid 28040 Spain;* [6] *Department of Biological Sciences, Karatina University, P. O. Box 1957-10101, Karatina, Kenya*

Abstract. The phylogenetic position of the monotypic genus *Bulborrhizina* is studied using molecular data from three loci (mtSSU, nuLSU and ITS) aligned with sequences from 95 other samples of parmelioid lichens. *Bulborrhizina africana* clusters within the predominantly paleotropical *Bulbothrix* 'clade II' with strong support. However, its relationships within this clade remain uncertain. The morphological characters used to circumscribe *Bulborrhizina* are interpreted as adaptations to the habitat of this species in semi-arid regions of eastern Africa. A formal synonymy of the genus *Bulborrhizina* with either *Bulbothrix* or *Parmelinella* is postponed until sequences of the type species of *Bulbothrix* become available that will allow us to identify which of the two clades of species currently placed in *Bulbothrix* represents *Bulbothrix* s.str.

Keywords. Generic concept, lichens, molecular systematics, parmelioid lichens, phylogeny, taxonomy.

◆ ◆ ◆

The classification of genera in parmelioid lichens has been thoroughly revised on the basis of phylogenetic hypotheses inferred from molecular data resulting in a broad consensus system (Crespo et al. 2010; Thell et al. 2012). This is remarkable given that the generic classification of parmelioid lichens has been vigorously debated (Hale 1984; Hawksworth 1994; Nimis 1998; Rambold & Triebel 1999). Despite the progress in understanding phylogenetic relationships among clades of parmelioid lichens, there are a number of remaining questions, especially regarding the delimitations of some of the mostly tropical genera in the *Parmelia* and *Parmelina* clades (Crespo et al. 2010). Additionally, a few genera of parmelioid lichens have not yet been studied using molecular markers, including *Bulborrhizina* Kurok. and *Parmotremopsis* Elix & Hale. The genus *Pseudoparmelia* Lynge had not been included in molecular phylogenetic studies; however, recently we were able to elucidate that *Pseudoparmelia* Lynge is a distinct genus related to *Relicina* (Hale & Kurok.) Hale and *Relicinopsis* Elix & Verdon (Buaruang et al. 2015).

Bulborrhizina africana was described as a new genus and species for a single collection from semi-arid regions of eastern Mozambique (Kurokawa 1994) and until recently was only known from the type locality. The species occurs at the base of shrubs and on soil in semi-arid habitats. It has loosely adnate, divaricate thalli composed of linear lobes which are canaliculate below and with marginal bulbate appendages. The genus was considered to be closely related to *Cetrariastrum* Sipman (including *Everniastrum* Hale ex Sipman), a genus currently classified as a subgenus of *Hypotrachyna* Hale (Divakar et al. 2013), since both genera have linear elongate lobes. *Bulborrhizina* was said to differ in having a pale straw-yellow lower surface in contrast to a black to brown or rarely pale lower surface in *Cetrariastrum*; further, *Bulbothrix* Hale was said to

[7] Corresponding author's e-mail: tlumbsch@fieldmuseum.org
DOI: 10.1639/0007-2745-118.2.164

0007-2745/15/$0.75/0

have marginal bulbate rhizines in contrast to the slender rhizines found in *Cetrariastrum*. Kurokawa (1994) also noted the similarities to the bulbate appendages in *Bulborrhizina* with bulbate cilia found in *Bulbothrix* and *Relicina*, but the structures were interpreted as fundamentally different since the cilia in the two latter genera are not anchoring the thallus. Molecular data have shown that *Bulbothrix* and *Relicina* are only distantly related, with *Bulbothrix* belonging to the *Parmelina* clade, whereas *Relicina* belongs to the *Parmelia* clade (Crespo et al. 2010). Further, *Bulbothrix* was found to be non-monophyletic, falling into two separate clades, one of them (clade II) being sister to *Parmelinella* Elix & Hale (Divakar et al. 2006, 2010).

On a recent field trip in southeastern Kenya, we collected fresh material of the monotypic genus *Bulborrhizina*, representing a new record of the species for Kenya and only the second known population of *B. africana*. With the fresh material of *Bulborrhizina* available, we generated DNA sequence data from three loci to investigate whether or not *Bulborrhizina* is a distinct lineage and to identify the closest relatives of this enigmatic lichen—a species with linear elongate lobes, currently classified in *Hypotrachyna* subg. *Cetrariastrum* (Sipman) Divakar et al., or species with bulbate cilia as found in the two *Bulbothrix* clades or the genus *Relicina*.

Materials and Methods

Taxon sampling. We included a total of 96 specimens representing all major groups in the *Hypotrachyna* and *Parmelina* clades (Crespo et al. 2010; **Supplementary Table S1**). In summary, representatives of the following genera were included: *Bulbothrix* sensu lato (Divakar et al. 2006), *Hypotrachyna,* including representatives for each subgenus (Divakar et al. 2013), *Myelochroa*, *Parmelina*, *Parmelinopsis* and *Remototrachyna* (Divakar et al. 2010). *Bulborrhizina africana* was sampled from a robust population recently found in southeastern Kenya at Yambyu dam area, Mwingi County, Eastern Province, 0°51′S, 38°05′E, 980m, *P. Kirika 4819 & H.T. Lumbsch*, (EA, F, MAF), where it was found growing on soil, intermixed with tufts of grasses on sandstone on a rocky inselberg in dry *Acacia/Commiphora* shrubland.

DNA extraction and PCR amplification. DNA was extracted from a small piece of *Bulborrhizina africana* thallus free from visible damage or contamination using the USB PrepEase Genomic DNA Isolation Kit (USB, Cleveland, OH) and following the manufacturer's recommendations. We generated sequence data from two nuclear ribosomal markers, the internal transcribed spacer region (ITS), and a fragment of the large subunit (nuLSU), in addition to a fragment of the mitochondrial small subunit (mtSSU). PCR amplifications were performed using Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA) using primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) for the ITS region, AL2R (Mangold et al. 2008) and LR3 (Larena et al. 1999) for nuLSU rDNA, and mrSSU1 and mrSSU3R (Zoller et al. 1999) for mtSSU rDNA. PCR conditions were as described previously (Leavitt et al. 2012). Products were visualized on 1% agarose gel and cleaned using ExoSAP-IT (USB, Cleveland, OH, USA). Cycle sequencing of complementary strands was performed using BigDye v3.1 (Applied Biosystems, Foster City, CA, USA) and the same primers used for PCR amplifications. Sequenced PCR products were run on an ABI 3730 automated sequencer (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, Chicago, IL, USA.

Sequence editing and alignment. New *Bulborrhizina africana* sequences were assembled and edited using Sequencher v4.10 (Gene Codes Corporation, Ann Arbor, MI). Multiple sequence alignments for each locus were performed using the program MAFFT v7 (Katoh et al. 2005; Katoh & Toh 2008). For the nuLSU sequences, we used the G-INS-i alignment algorithm and '200PAM/K=2' scoring matrix, with an offset value of 0.3, and the remaining parameters were set to default values. ITS sequences were aligned using the L-INS-i alignment algorithm and '200PAM/K=2' scoring matrix, with an offset value of 0.6, and the remaining parameters were set to default values. We used the E-INS-i alignment algorithm and '200PAM/K=2' scoring matrix, with the remaining parameters were set to default values for the mtSSU sequences. We used the program Gblocks v0.91b (Talavera & Castresana 2007) to delimit and remove ambiguous alignment nucleotide positions from the final alignments using the online web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), implementing the options for a less stringent selection of ambiguous nucleotide positions.

Phylogenetic analyses. Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI). Exploratory phylogenetic analyses of individual gene topologies showed no evidence of well-supported (≥70% bootstrap values) topological conflict, and relationships were estimated from a concatenated, three-locus (ITS, nuLSU and mtSSU) data matrix using a total-evidence approach (Wiens 1998). We used the program RAxML v8.1.11 (Stamatakis 2006; Stamatakis et al. 2008) to reconstruct the concatenated ML gene-tree using the CIPRES Science Gateway server (http://www.phylo.org/portal2/). We implemented the 'GTRGAMMA' model, used locus-specific model partitions treating all loci as separate partitions, and evaluated nodal support using 1000 bootstrap pseudoreplicates. Exploratory analyses using alternative partitioning schemes resulted in identical topologies and highly similar bootstrap support values. We also reconstructed phylogenetic relationships from the concatenated multi-locus data matrix under BI using the program BEAST v1.8.0 (Drummond & Rambaut 2007). We ran two independent Markov Chain Monte Carlo (MCMC) chains for 50 million generations, implementing a relaxed lognormal clock, a constant coalescent speciation process prior. The most appropriate model of DNA sequence evolution was selected for each marker using the program jModeltest v2.1.7 (Darriba et al. 2012). The first 12.5 million generations were discarded as burn-in. Chain mixing and convergence were evaluated in Tracer v1.5 (Rambaut & Drummond 2009), considering ESS values >200 as a good indicator. Posterior trees from the two independent runs were combined using the program LogCombiner v1.8.0 (Drummond et al. 2012), and the final maximum clade credibility (MCC) tree was estimated from the combined posterior distribution of trees.

Results and Discussion

We generated a new DNA sequence of each marker (mtSSU, nuLSU and ITS) representing *Bulborrhizina africana* for this study (**Supplementary Table S1**). The matrix of the combined data set included 2079 unambiguously aligned nucleotide position characters (784 mtSSU, 849 nuLSU and 446 ITS). In the combined data set, 1308 positions were constant and 583 of the 771 variable characters were parsimony informative. The topologies of the single locus phylogenies did not show any conflicts and hence a concatenated data set was analysed (data not shown). The ML and BI analyses were identical in their topology and therefore here only the ML tree with support values of both analyses is shown (**Fig. 1**). In the phylogenetic tree, *Bulborrhizina africana* clusters within *Bulbothrix* 'clade II', which includes the predominantly paleotropical species of *Bulbothrix* s.l. (Divakar et al. 2006, 2010). However, the relationships within this clade remain uncertain, since the topology is unsupported in this part of the tree.

Our results confirm the polyphyly of *Bulbothrix* as currently circumscribed, with a predominantly neotropical clade and a predominantly paleotropical clade being sister to *Parmelinella.* Currently, it is unknown to which of the two clades the type species, *B. semilunata* (Lynge) Hale belongs. However, the type was collected in Brazil (Hale 1974, 1976) and hence it is likely that clade I represents *Bulbothrix* s.str. Further, it remains to be seen whether the *Bulbothrix* spp. of clade II are congeneric with *Parmelinella* or not. If they should be kept separate, *Bulborrhizina* would be an available generic name for species of clade II currently placed in *Bulbothrix.* Alternatively, all species of clade II could be transferred to an emended genus *Parmelinella.* We want to wait until additional species of *Parmelinella* are available for sequencing and molecular data of the type species of *Bulbothrix* or its close relative *B. schiffneri* become available before making a proposal on the generic delimitation in the *Parmelina* clade. However, our study demonstrates that *Bulborrhizina africana* is not a phylogenetically isolated species and also not related to the morphologically similar species of *Hypotrachyna* subg. *Cetrariastrum* and subg. *Everniastrum* (Hale ex Sipman) Divakar et al., but belongs to clade II of *Bulbothrix.* Within different clades of parmelioid lichens, terrestrial species have evolved that differ morphologically from their saxicolous or corticolous relatives, such as vagrant species in the genus *Xanthoparmelia* (Elix et al. 1986; Hale 1990), and some of them have been placed in separate genera. However, phylogenetic analyses demonstrated that they actually are not separate isolated lineages. Examples include the genera *Chondropsis* Nyl. ex Cromb. and species of the genus *Xanthomaculina* Hale, both of them currently placed in *Xanthoparmelia* (Blanco et al. 2004; Esslinger 1981; Hale 1985; Hawksworth & Crespo 2002; Thell et al. 2006). Morphological

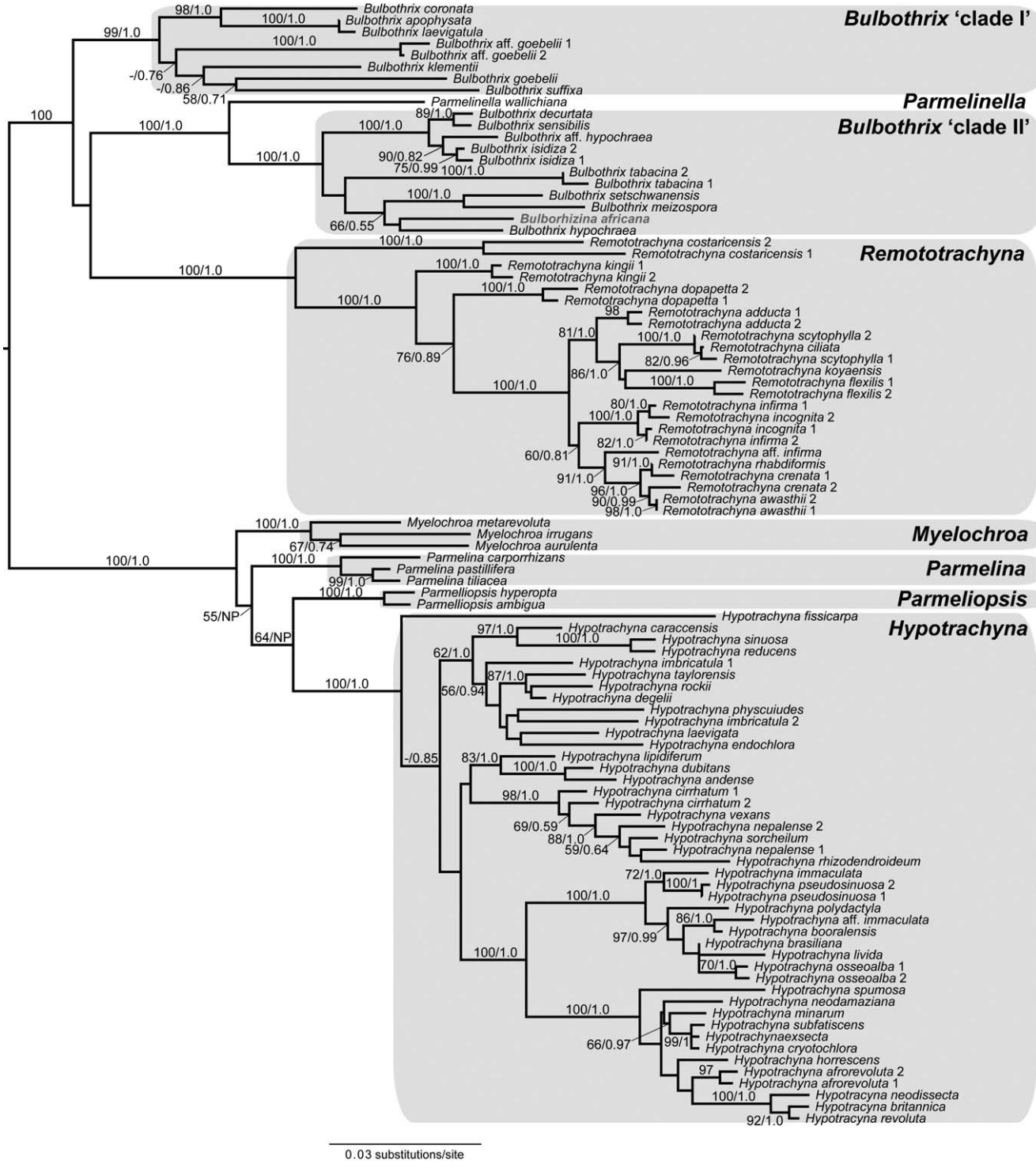

Figure 1. Phylogenetic placement of *Bulborrhizina africana* based on a maximum-likelihood (ML) analysis of a concatenated, three-marker dataset (mtSSU, nuLSU and ITS). ML and Bayesian inference topologies were identical, and only the ML topology is reported. Values at each node indicate non-parametric bootstrap support (BS)/posterioir probability values, with support indices >50 BS/0.50 PP are indicated. *Bulbothrix* 'clade 1' and 'clade 2' correspond to previously recognized clades in this polyphyletic genus (Divakar et al. 2006).

differences of terrestrial species in semi-arid areas often include lobes being narrower and canaliculate, sometimes also more richly branched—the former two traits are found in *Bulborrhizina*.

The bulbate appendages found in *Bulborrhizina* support the placement of the species in the *Bulbothrix* clade and suggest that the distinction of cilia and rhizines as used by Kurokawa (1994) resulted in a misinterpretation of phylogenetic relationships. Morphological characters in lichen-forming fungi are variable and distinction based of structures that do not take into account their development are prone to typological characterizations (Beltman 1978). This is especially true for lichen-forming fungi without tissues and with remarkable regenerative abilities (Honegger 1993, 1996).

Acknowledgments

Newly obtained DNA sequences were generated in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum. This study was supported by a grant of the IDP/The Field Museum Africa Training Fund and the Spanish Ministerio de Ciencia e Innovación (CGL2013-42498-P).

Literature Cited

Beltman, H. A. 1978. Vegetative Strukturen der Parmeliaceae und ihre Entwicklung. Bibliotheca Lichenologica 11: 1–193.

Blanco, O., A. Crespo, J. A. Elix, D. L. Hawksworth & H. T. Lumbsch. 2004. A molecular phylogeny and a new classification of parmelioid lichens containing *Xanthoparmelia*-type lichenan (Ascomycota: Lecanorales). Taxon 53: 959–975.

Buaruang, K., K. Scharnagl, P. K. Divakar, S. D. Leavitt, A. Crespo, T. H. Nash III, L. Manoch, R. Lucking & H. T. Lumbsch. 2015. Molecular data support *Pseudoparmelia* as a distinct lineage related to *Relicina* and *Relicinopsis* (Ascomycota, Lecanorales). Lichenologist 47: 43–49.

Crespo, A., F. Kauff, P. K. Divakar, G. Amo, A. Arguello, O. Blanco, P. Cubas, R. del Prado, J. A. Elix, T. L. Esslinger, Z. Ferencova, D. L. Hawksworth, F. Lutzoni, A. M. Millanes, M. C. Molina, S. Perez-Ortega, M. Wedin, T. Ahti, F. Bungartz, S. Calvelo, A. Aptroot, E. Barreno, M. Candan, M. Cole, D. Ertz, B. Goffinet, L. Lindblom, R. Lücking, J. E. Mattsson, M. I. Messuti, J. Miadlikowska, M. Piercey-Normore, V. Rico, H. J. M. Sipman, I. Schmitt, T. Spribille, A. Thell, G. Thor & H. T. Lumbsch. 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. Taxon 59: 1735–1753.

Darriba, D., G. L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

Divakar, P. K., A. Crespo, O. Blanco & H. T. Lumbsch. 2006. Phylogenetic significance of morphological characters in the tropical *Hypotrachyna* clade of parmelioid lichens (Parmeliaceae, Ascomycota). Molecular Phylogenetics and Evolution 40: 448–458.

Divakar, P. K., A. Crespo, J. Núñez-Zapata, A. Flakus, H. J. M. Sipman, J. A. Elix & H. T. Lumbsch. 2013. A molecular perspective on generic concepts in the *Hypotrachyna* clade (Parmeliaceae, Ascomycota). Phytotaxa 132: 21–38.

Divakar, P. K., Z. Ferencova, R. Del Prado, H. T. Lumbsch & A. Crespo. 2010. *Remototrachyna*, a new tropical lineage in hypotrachynoid lichens (Parmeliaceae, Ascomycota): a multigene and morphological approach. American Journal of Botany 97: 579–590.

Drummond, A. & A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Drummond, A. J., M. A. Suchard, D. Xie & A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29: 1969–1973.

Elix, J. A., J. Johnston & P. M. Armstrong. 1986. A revision of the lichen genus *Xanthoparmelia* in Australasia. Bulletin of the British Museum (Natural History), Botany Series 15: 163–362.

Esslinger, T. L. 1981. *Almbornia*, a new lichen genus from South Africa. Nordic Journal of Botany 1: 125–127.

Gardes, M. & T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - Application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Hale, M. E. Jr. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae). Smithsonian Contributions to Botany 74: 1–250.

Hale, M. E. Jr. 1974. *Bulbothrix, Parmelina, Relicina*, and *Xanthoparmelia*, four new genera in the Parmeliaceae. Phytologia 28: 479–490.

Hale, M. E. Jr. 1976. A monograph of the lichen genus *Bulbothrix* Hale (Parmeliaceae). Smithsonian Contributions to Botany 32: 1–29.

Hale, M. E. Jr. 1984. An historical review of the genus concept in lichenology. Beiheft zur Nova Hedwigia 79: 11–23.

Hale, M. E. Jr. 1985. *Xanthomaculina* Hale, a new lichen genus in the Parmeliaceae (Ascomycotina). Lichenologist 17: 255–265.

Hawksworth, D. L. 1994. Ascomycete Systematics. Problems and Perspectives in the Nineties. NATO Advanced Science Institute Series, 269. Plenum Press, NY.

Hawksworth, D. L. & A. Crespo. 2002. Proposal to conserve the name *Xanthoparmelia* against *Chondropsis* nom. cons. (Parmeliaceae). Taxon 51: 807.

Honegger, R. 1993. Tansley Review No. 60. Developmental biology of lichens. New Phytologist 125: 659–677.

Honegger, R. 1996. Experimental studies of growth and regenerative capacity in the foliose lichen *Xanthoria parietina*. New Phytologist 133: 573–581.

Katoh, K., K.-i. Kuma, H. Toh & T. Miyata. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518.

Katoh, K. & H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298.

Kurokawa, S. 1994. *Bulborrhizina africana*, a new genus and species of the Parmeliaceae. Acta Botanica Fennica 150: 105–107.

Larena, I., O. Salazar, V. González, M. C. Julián & V. Rubio. 1999. Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. Journal of Biotechnology 75: 187–194.

Leavitt, S. D., T. L. Esslinger, P. K. Divakar & H. T. Lumbsch. 2012. Miocene and Pliocene dominated diversification of the lichen-forming fungal genus *Melanohalea* (Parmeliaceae, Ascomycota) and Pleistocene population expansions. BMC Evolutionary Biology 12: 176.

Mangold, A., M. P. Martin, R. Lücking & H. T. Lumbsch. 2008. Molecular phylogeny suggests synonymy of Thelotremataceae within Graphidaceae (Ascomycota : Ostropales). Taxon 57: 476–486.

Nimis, P. L. 1998. A critical appraisal of modern generic concepts in lichenology. Lichenologist 30: 427–438.

Rambold, G. & D. Triebel. 1999. Generic concepts in lichenized and lichenicolous ascomycetes since 1950 - a historical approach. Symbolae Botanicae Upsalienses 32: 123–164.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Stamatakis, A., P. Hoover & J. Rougemont. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. Systematic Biology 57: 758–771.

Talavera, G. & J. Castresana. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577.

Thell, A., A. Crespo, P. K. Divakar, I. Kärnefelt, S. D. Leavitt, H. T. Lumbsch & M. R. D. Seaward. 2012. A review of the lichen family Parmeliaceae - history, phylogeny and current taxonomy. Nordic Journal of Botany 30: 641–664.

Thell, A., T. Feuerer, J. A. Elix & I. Kärnefelt. 2006. A contribution to the phylogeny and taxonomy of *Xanthoparmelia* (Ascomycota, Parmeliaceae). Journal of the Hattori Botanical Laboratory 100: 797–807.

White, T. J., T. D. Bruns, S. B. Lee & J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322. In: M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White (eds.), PCR Protocols. Academic Press, NY.

Wiens, J. J. 1998. Combining data sets with different phylogenetic histories. Systematic Biology 47: 568–581.

Zoller, S., C. Scheidegger & C. Sperisen. 1999. PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist 31: 511–516.

manuscript received March 27, 2015; accepted May 1, 2015.

Supplementary documents online:

Supplementary Table S1. Specimens used in the study, including collection details (locality and voucher information), and GenBank accession numbers.