ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/317244448

The genus Relicinopsis is nested within Relicina (Parmeliaceae, Ascomycota)

Article *in* The Lichenologist · May 2017

DOI: 10.1017/S0024282916000748

CITATIONS
7

READS
170

8 authors, including:

Paul M. Kirika
National Museums of Kenya
43 PUBLICATIONS **465** CITATIONS

SEE PROFILE

Pradeep K Divakar
Complutense University of Madrid, Faculty of Pharmacy
315 PUBLICATIONS **8,850** CITATIONS

SEE PROFILE

Steven D. Leavitt
Brigham Young University - Provo Main Campus
170 PUBLICATIONS **6,751** CITATIONS

SEE PROFILE

Kawinnat Buaruang
Ramkhamhaeng University
15 PUBLICATIONS **210** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Flora of Kenya Project (FOKP) View project

Evolutionary Genomic of lichen forming fungi (Parmeliaceae) View project

All content following this page was uploaded by Thorsten Lumbsch on 15 February 2019.

The user has requested enhancement of the downloaded file.

The Lichenologist **49**(3): 189–197 (2017) © British Lichen Society, 2017
doi:10.1017/S0024282916000748

The genus *Relicinopsis* is nested within *Relicina* (*Parmeliaceae*, Ascomycota)

Paul M. KIRIKA, Pradeep K. DIVAKAR, Steven D. LEAVITT, Kawinnat BUARUANG, Ana CRESPO, George MUGAMBI, Grace W. GATHERI and H. Thorsten LUMBSCH

Abstract: Macro-morphological features traditionally used to segregate genera in *Parmeliaceae* have been shown to be highly plastic, placing limits on their taxonomic value. Here we aim to elucidate the evolutionary relationships of the genera *Relicina* and *Relicinopsis* and reassess the phenotypic features traditionally used to separate these genera. To this end, we gathered ribosomal DNA sequences of ITS, nuLSU and mtSSU and analyzed them in a phylogenetic framework. *Relicina* was recovered as paraphyletic, with *Relicinopsis* nested within, and three different clades were identified within *Relicina*. Alternative hypothesis tests significantly rejected the monophyly of *Relicina*. Our results indicate that the presence or absence of bulbate cilia is of limited taxonomic value in this clade. Based on differences in conidia, however, we propose to accept *Relicinopsis* as a subgenus within *Relicina* as *Relicina* subgen. *Relicinopsis* (Elix & Verdon) Kirika, Divakar & Lumbsch. It is proposed that five new combinations of species previously classified in *Relicinopsis* be placed in *Relicina*.

Key words: generic circumscription, integrative taxonomy, lichenized fungi, molecular systematics, parmelioid lichens

Accepted for publication 8 *September* 2016

Introduction

Phenotype-based circumscriptions of genera have repeatedly been challenged in different groups of lichenized fungi. In the hyperdiverse family *Parmeliaceae*, many genera that were chiefly separated based on vegetative traits have not been supported as monophyletic clades in molecular phylogenetic reconstructions (reviewed in Lumbsch 2007; Printzen 2010; Crespo *et al.* 2011; Thell *et al.* 2012; Divakar & Crespo 2015). The frequent incongruence between traditional circumscriptions of genera in *Parmeliaceae* and monophyletic evolutionary lineages highlights the necessity to carefully evaluate generic circumscriptions within an evolutionary context.

Currently in *Parmeliaceae c.* 80 genera are accepted based on phenotypic features and analyses of multilocus sequence data (Thell *et al.* 2012; Divakar *et al.* 2015). The largest group within the family is the parmelioid core, to which the genera *Relicina* (Hale & Kurok.) Hale and *Relicinopsis* Elix & Verdon belong (Crespo *et al.* 2010; Divakar *et al.* 2015). The evolutionary relationships of these two genera have only been partially explored. The genus *Relicinopsis* was segregated from *Pseudoparmelia* Lynge based on morphological features, such as the presence of simple marginal cilia, fusiform conidia and usnic acid as a cortical extrolite (Elix *et al.* 1986). This genus includes a total of five

P. M. Kirika and G. W. Gatheri: Department of Plant Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.
P. M. Kirika: Botany Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya.
P. K. Divakar and A. Crespo: Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid 28040, Spain.
S. D. Leavitt and H. T. Lumbsch (corresponding author): Science & Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA. Email: tlumbsch@fieldmuseum.org
K. Buaruang: Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkhen, Bangkok, 10900 Thailand.
G. Mugambi: Department of Biological Sciences, School of Pure and Applied Sciences, Meru University of Science and Technology, P.O. Box 972-60200, Meru, Kenya.

Downloaded from https:/www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

species, which are widely distributed in South-East Asia and Australasia (Hale 1976; Elix 1993, 1994; Divakar & Upreti 2005). The genus *Relicina* was segregated from *Parmelia* Ach. s. lat. (Hale 1974) based on having bulbate marginal cilia and bifusiform conidia, and containing usnic acid in the upper cortex. This genus includes *c.* 54 species (Thell *et al.* 2012) with a centre of distribution in South-East Asia and Australasia (Hale 1975; Elix 1993). In a recent study, *Relicina* and *Relicinopsis* formed a well-supported sister-group relationship, although the taxon sampling was limited and monophyly was not supported in an mtSSU single locus phylogeny (Buaruang *et al.* 2015). Moreover, the distinction of the two genera was supported in the '1GENE' data analysis by Crespo *et al.* (2010). In the present study we used an extended taxon sampling to 1) examine the monophyly of *Relicina* and *Relicinopsis* and 2) evaluate the taxonomic significance of phenotypic features in these two genera.

Materials and Methods

Taxon sampling

Data matrices of 36 samples including four of the five described species of *Relicinopsis* and six species of *Relicina* were analyzed, including eight new samples of these genera collected from East Africa. We assembled a multilocus DNA matrix comprised of nuLSU, ITS and mtSSU rDNA to infer evolutionary relationships. The multilocus data set included 74 sequences from a previous study (Buaruang *et al.* 2015) and 14 sequences generated for this study. Three species of *Notoparmelia* were used as the outgroup since the genus has been shown to be closely related to *Relicina* (Crespo *et al.* 2010; Buaruang *et al.* 2015). Information on material studied, including GenBank Accession numbers, is reported in Table 1.

DNA extraction and PCR amplification

Total genomic DNA was extracted from small pieces of thallus devoid of any visible damage or contamination using the USB PrepEase Genomic DNA Isolation Kit (USB, Cleveland, OH, USA) in accordance with the manufacturer's instructions. We generated sequence data from nuclear ribosomal markers, the ITS region and a fragment of the nuLSU, in addition to a fragment of the mtSSU. Polymerase chain reaction (PCR) amplifications were performed using Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA) using dilutions of total DNA. Fungal ITS rDNA was amplified using primers ITS1F (Gardes & Bruns 1993), ITS4 and ITS4A (White *et al.* 1990; Larena *et al.* 1999); nuLSU rDNA was amplified using LR0R and LR5 (Vilgalys & Hester 1990); and mtSSU rDNA was amplified using the primers mrSSU1, mrSSU3R and mrSSU2R (Zoller *et al.* 1999). PCR 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, USA.

Sequence editing and alignment

New sequences were assembled and edited using Geneious v8.1.7 (Biomatters Ltd 2005–2015). Multiple sequence alignments for each locus were performed using the program MAFFT v7 (Katoh *et al.* 2005; Katoh & Toh 2008). For the ITS and nuLSU sequences we used the G-INS-i alignment algorithm and '20PAM/K = 2' scoring matrix with an offset value of 0·3 and the remaining parameters set to default values. We used the E-INS-i alignment algorithm and '20PAM/ K = 2' scoring matrix, with the remaining parameters set to default values, for the mtSSU sequences. The program Gblocks v0.91b (Talavera & Castresana 2007) was used 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 including "Allow smaller final blocks", "Allow gap positions within the final blocks" and "Allow less strict flanking positions" options.

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, thus relationships were estimated from a concatenated, three-locus (ITS, nuLSU, 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, with 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 multilocus data

Downloaded from https:/www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

TABLE 1. *Specimens used in this study together with location, reference collection detail and GenBank Accession numbers. Newly obtained sequences for this study are in bold and missing data are indicated with a dash (–).*

Taxon (DNA sample number)	Location and collection information	GenBank Accession numbers		
		ITS	nuLSU	mtSSU
Notoparmelia crambidiocarpa	New Zealand, *Knight* 60590 (OTA)	GU994571	KM657289	GU994665
N. cunninghamii	New Zealand, *Knight* 60608 (OTA)	GU994572	KM657290	GU994666
N. subtestacea	New Zealand, *Knight* 60609 (OTA)	GU994573	GU994573	GU994668
Pseudoparmelia cyphellata (8609)	Mexico, *Nash* 46672 (ASU)	KM657272	KM657291	KM657311
P. floridensis (KS3)	USA, *Scharnagl* KS3 (F)	KM657274	KM657293	KM657313
P. floridensis (KS11)	USA, *Scharnagl* KS11 (F)	KM657273	KM657292	KM657312
P. floridensis (KS30)	USA, *Scharnagl* KS30 (F)	KM657275	KM657294	KM657314
P. uleana (8706)	USA, *Seavey* 1386 (LSU)	KM657276	KM657295	KM657315
Relicina abstrusa (37426)	Australia, *Elix* 37426 (CANB)	GU994580	GU994580	–
R. abstrusa (1085)	Thailand, *Lumbsch* 19756g (F)	KM657278	KM657297	KM657317
R. abstrusa (1082)	Thailand, *Lumbsch* 19754f (F)	KM657277	KM657296	KM657316
R. abstrusa (3194)	Thailand, *Buarang et al.* 24368 (RAMK)	KM657279	KM657298	KM657318
R. abstrusa (3195)	Thailand, *Buarang et al.* 24369 (RAMK)	KM657280	KM657299	KM657319
R. abstrusa (4505)	Kenya, *Kirika* 4505 (EA, F, MAF)	**–**	**–**	**KX434467**
R. abstrusa 9603)	Kenya, *Kirika* 4506 (EA, F, MAF)	**KX434464**	**KX434472**	**–**
R. abstrusa (9608)	Kenya, *Kirika* 4541 (EA, F, MAF)	**–**	**KX434473**	**–**
R. abstrusa (9619)	Kenya, *Kirika & Lumbsch* 4032 (EA, F, MAF)	**KX434465**	**KX434474**	**KX434469**
R. echinocarpa (9317)	Kenya, *Kirika & Mugambi* 3567 (EA, F)	**–**	**KX434471**	**KX434468**
R. echinocarpa (9623)	Kenya, *Kirika* 4432 (F, MAF)	**–**	**KX434476**	**KX434470**
R. filsonii	Australia, *Elix* 37267 (CANB)	KM657281	–	–
R. subabstrusa (3193)	Thailand, *Buarang et al.* 24370 (RAMK)	KM657282	KM657300	KM657320
R. subnigra	Australia, *Louwhoff et al.* (MAF-Lich 10184)	AY785274	AY785267	AY785281
R. sydneyensis	Australia, *Lumbsch & Mangold* 19179a (F)	GU994581	GU994630	GU994675
Relicinopsis intertexta (1083)	Thailand, *Lumbsch* 19756g (F)	KM657283	KM657301	KM657323
R. intertexta (3177)	Thailand, *Buarang et al.* 24372 (RAMK)	–	KM657302	KM657324
R. malaccensis (9621)	Kenya, *Kirika* 4499 (EA, F, MAF)	**KX434466**	**KX434475**	**–**
R. malaccensis (9635)	Kenya, *Kirika* 4508 (EA, F, MAF)	**–**	**KX434477**	**–**
R. malaccensis (628)	Australia, *Elix* 36972 (hb. Elix)	**–**	GU994631	GU994677
R. malaccensis (1084)	Thailand, *Lumbsch* 19752a (F)	KM657284	KM657303	KM657325
R. malaccensis (3172)	Thailand, *Buarang et al.* 24373 (RAMK)	–	KM657304	KM657326
R. malaccensis (3173)	Thailand, *Buarang et al.* 24374 (RAMK)	–	KM657305	KM657327
R. malaccensis (3174)	Thailand, *Buarang et al.* 24375 (RAMK)	–	KM657306	KM657328
R. rahengensis (3169)	Thailand, *Buarang et al.* 24376 (RAMK)	KM657285	KM657307	–
R. rahengensis (3170)	Thailand, *Buarang et al.* 24377 (RAMK)	KM657286	KM657308	KM657329
R. rahengensis (3171)	Thailand, *Buarang et al.* 24378 (RAMK)	KM657287	KM657309	KM657330
R. stevensiae (1073)	Australia, *Elix* 37835 (CANB)	KM657288	KM657310	–

Downloaded from https://www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

matrix under BI using the program BEAST v1.8.2 (Drummond & Rambaut 2007). We ran two independent Markov chain Monte Carlo (MCMC) chains for 20 million generations, implementing a relaxed lognormal clock, a birth-death speciation process prior. The most appropriate model of DNA sequence evolution was selected for each marker using the program PartitionFinder v1.1.1 (Lanfear *et al.* 2012) treating the ITS1, 5.8S, ITS2, nuLSU, and mtSSU as separate partitions. The first 2 million generations were discarded as burn-in. Chain mixing and convergence were evaluated in Tracer v1.5 (Rambaut & Drummond 2009) considering effective sample size (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.

Alternative hypothesis testing

Since the results of the phylogenetic analyses did not support the monophyly of *Relicina* as currently circumscribed, we tested whether our data were sufficient to reject the monophyly of that genus. For the hypothesis testing two different methods were employed: 1) Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa 1999) and 2) expected likelihood weight (ELW) test (Strimmer & Rambaut 2002). The SH and ELW tests were performed using TREE-PUZZLE 5.2 (Schmidt *et al.* 2002) with the combined data set on a sample of the best trees agreeing with the null hypotheses and the unconstrained ML tree. These trees were inferred in TREE-PUZZLE employing the GTR+I+G nucleotide substitution model.

Morphological and chemical studies

Morphological characters, including lobe shape, size and width, and cilia and rhizines were studied using a Leica Wild M8 dissecting microscope. Key morphological and chemical features used to segregate *Relicina* and *Relicinopsis* are listed in Table 2.

Chemical constituents were identified by high performance thin-layer chromatography using standard methods (Arup *et al.* 1993; Lumbsch 2001) with a Camag horizontal developing chamber (Oleico Laboratory, Stockholm) using solvent system A.

Results and Discussion

Molecular phylogeny and phenotypic features

The aligned matrix contained 455 unambiguously aligned nucleotide positions in the ITS, 808 in the nuLSU and 735 in the mtSSU rDNA data sets. The final alignment of the concatenated data set was 1999 positions in length, with 548 variable characters. The ITS PCR product obtained ranged between 600–800 bp. Differences in size were due to the presence or absence of a group I intron of *c.* 200 bp at the 3' end of the 18S rDNA (Gutierrez *et al.* 2007). Introns from the ribosomal gene (18S) were removed from the analysis. GTR+I+G for ITS1, K80+I+G for 5.8S rDNA, TrN+G for ITS2, TrN+I+G for nuLSU rDNA and GTR+G for mtSSU rDNA were estimated as best fit models of evolution for each partition. All the newly generated sequences for this study were deposited in GenBank under Accession numbers KX434464–KX434477 (Table 1).

Tests for topological incongruence showed no supported conflicts (results not shown). The partitioned ML analysis of the concatenated data matrix yielded an optimal tree with ln likelihood value = − 8048·95 (Fig. 1). In the Bayesian analysis, ESS values of all estimated parameters were well above 200 indicating that convergence among parallel runs was reached. ML and Bayesian topologies were largely similar and did not show well-supported conflict (e.g. PP ≥ 0·95 and ML bootstrap ≥70%) and thus the ML tree topology is shown here with the Bayesian posterior probabilities added (Fig. 1).

TABLE 2. *Main morphological and chemical features used to distinguish* Relicina *and* Relicinopsis.

Features	*Relicina*	*Relicinopsis*
Ascospores (μm)	Ellipsoid (6–8 × 3–5) to bicornute (10–12 × 3)	Ellipsoid (5–8 × 3–5)
Conidia (μm)	Bifusiform (6–10 × 1)	Fusiform or cylindrical (5–7 × 1)
Marginal cilia	Bulbate	Simple (without swollen base)
Rhizines	Simple, furcate or agglutinate	Simple or agglutinate
Habitat	Tropical-subtropical to temperate	Tropical

Downloaded from https:/www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

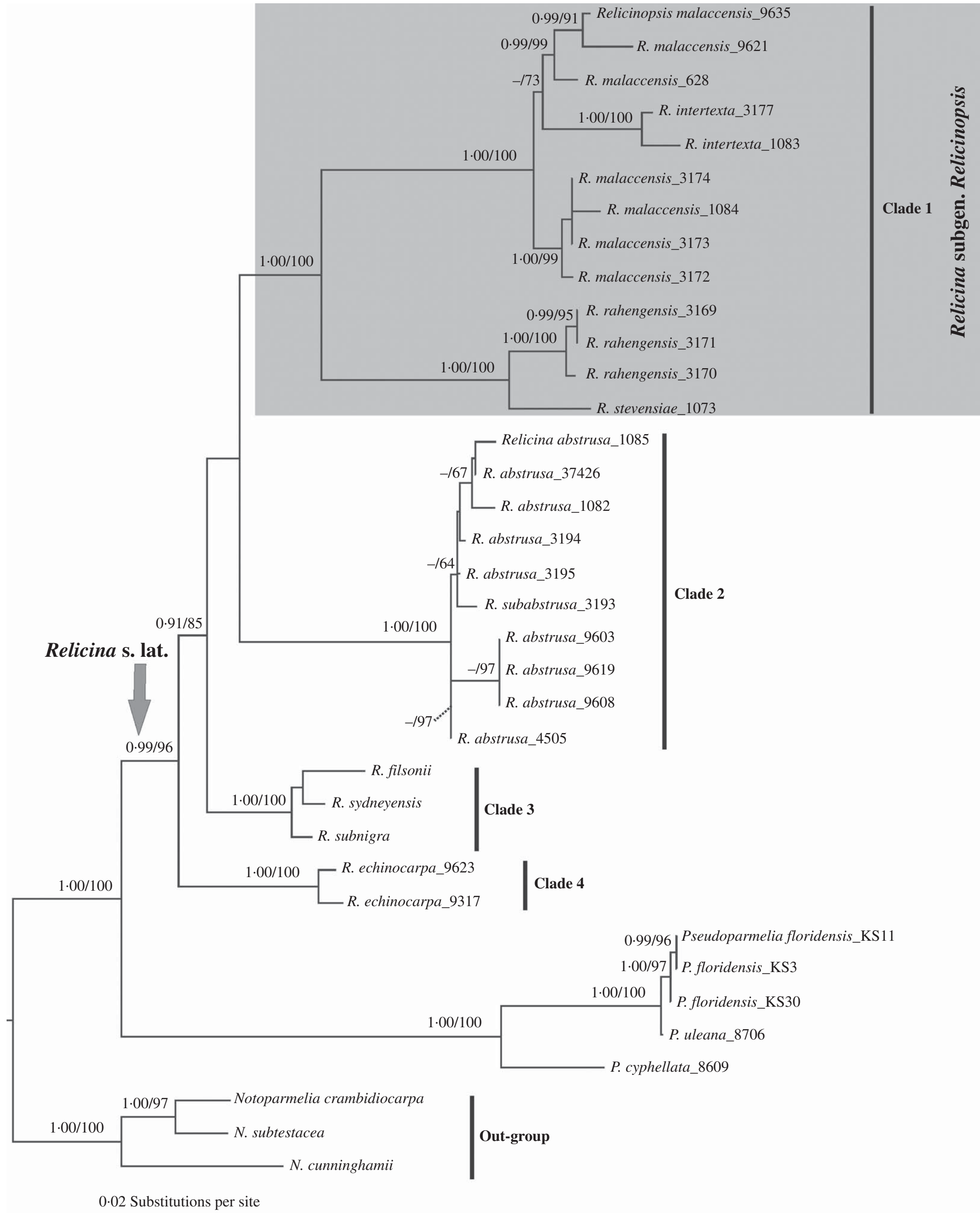

FIG. 1. Phylogenetic relationships of the genera *Relicina* and *Relicinopsis* based on maximum likelihood (ML) and Bayesian analyses of a concatenated three locus data set (ITS, nuLSU & mtSSU rDNA.) The ML tree obtained with RAxML is shown here. Posterior probabilities ≥0·95 from the Bayesian analysis (before the slash) and ML bootstrap values ≥70% (after the slash) are given above branches. Three species of *Notoparmelia* (*N. crambidiocarpa*, *N. cunninghamii* and *N. subtestacea*) were used as the outgroup.

Downloaded from https://www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

Results of the multilocus phylogeny showed that species of the genus *Relicinopsis* did not cluster with *Pseudoparmelia* in which they had previously been classified based on morphology (Hale 1975; Swinscow & Krog 1988). In agreement with previous molecular studies (Buaruang *et al.* 2015; Divakar *et al.* 2015), however, they grouped with *Relicina* species. Morphological similarities between *Relicinopsis* and *Relicina* species have been discussed previously (Elix *et al.* 1986; Elix 1993; Divakar & Upreti 2005). In this study, *Relicina* was recovered as paraphyletic with *Relicinopsis* nested within it (Fig. 1). Both the SH and ELW tests significantly rejected monophyly of *Relicina* as currently circumscribed ($P \leq 0{\cdot}005$). These data clearly indicate that the current phenotype-based generic circumscription (Hale 1975) does not reflect evolutionary relationships. Genus-level paraphyly has been found in other groups of parmelioid lichens, including *Hypotrachyna* (Vain.) Hale (Divakar *et al.* 2013) and *Bulbothrix* Hale (Divakar *et al.* 2010), and similar patterns have been found in other groups of lichen-forming fungi (reviewed in Lumbsch 2007; Printzen 2010).

All species of *Relicinopsis* were recovered in a well-supported (PP = 1·00 and ML bootstrap = 100%) monophyletic clade (clade 1), nested within *Relicina* (Fig. 1). Clade 1 of *Relicinopsis* included four of the five species currently known in this genus, including the type species *R. intertexta* (Mont. & Bosch.) Elix & Verdon. Species of *Relicina* were grouped in three well-supported monophyletic clades (clades 2, 3 and 4). However, the sister-group relationship of clade 2 and clade 1 recovered in the ML tree lacked support (Fig 1). Furthermore, in the Bayesian tree, clade 2 formed a well-supported (PP = 0·95) sister-group relationship with clade 3 (data not shown). Clade 2 included samples of *Relicina abstrusa* (Vain.) Hale and *R. subabstrusa* (Gyeln.) Hale from Australia, Kenya and Thailand, whereas clade 3 consisted of three species, *viz. R. filsonii* Elix & J. Johnston, *R. sydneyensis* (Gyeln.) Hale and *R. subnigra* Elix & J. Johnston occurring in Australasia and South-East Asia. Clade 4 included two samples of *R. echinocarpa* (Kurok.) Hale from Kenya. This relationship was strongly supported in the ML analysis (ML bootstrap = 85%) but received weak support in the Bayesian tree reconstruction (PP = 0·91). Our results showed that the relationships among these clades remain unresolved suggesting that additional sampling is necessary to better understand the evolutionary relationships among the clades within the *Relicina-Relicinopsis* clade. In fact, although all but one of the described *Relicinopsis* species were studied, only 15 samples collected from Africa, Australia and South-East Asia, representing only six of 54 described *Relicina* species, were sampled here.

Relicina was initially thought to be closely related to *Bulbothrix* (Hale 1975, 1976; Elix 1993) since both genera are characterized by the presence of bulbate cilia. However, molecular data showed that the two genera were only distantly related with *Bulbothrix* belonging to the *Parmelina* clade and *Relicina* belonging to the *Parmelia* clade, closely related to *Relicinopsis* (Crespo *et al.* 2010; Divakar *et al.* 2015). The key phenotypic features used to delineate the genera *Relicina* and *Relicinopsis* are summarized in Table 2. Both genera differ in the morphology of the marginal cilia (simple in *Relicinopsis* vs. bulbate in *Relicina*) and the type of conidia (fusiform or cylindrical in *Relicinopsis* vs. bifusiform in *Relicina*). Other characters, such as ascospore form and size, and rhizine morphology overlap (Table 2). Different types of conidia can be found in a number of currently accepted genera in *Parmeliaceae* such as *Hypotrachyna*, *Melanelixia* O. Blanco *et al.*, *Melanohalea* O. Blanco *et al.*, *Myelochroa* (Asahina) Elix & Hale, *Parmotrema* A. Massal., *Punctelia* Krog and *Xanthoparmelia* (Vain.) Hale (Crespo *et al.* 2010); thus this feature can be variable within genera in this family. Consequently, *Relicinopsis* is reduced here to synonymy with *Relicina*. However, given that *Relicinopsis* species formed a well-supported monophyletic clade (clade 1) and are distinguished by conidium morphology, we propose recognising the clade at subgeneric rank. Consequently, the subgenus *Relicina* is paraphyletic with *Relicinopsis* nested within it. However, following

Downloaded from https://www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

Divakar *et al.* (2013) we consider recognition of the monophyletic clade at the subgeneric level preferable since no paraphyletic taxa at generic level are produced (Hörandl & Stuessy 2010).

While most of the traditionally circumscribed species in *Relicina* s. lat. sampled for this study were recovered in monophyletic clusters, a few species did not form monophyletic groups, such as *Relicinopsis malaccensis* (clade 1) and *Relicina abstrusa* (clade 2). Additional studies are necessary to evaluate species boundaries in these nominal taxa.

Taxonomic Treatment

Relicina subgen. Relicinopsis (Elix & Verdon) Kirika, Divakar & Lumbsch comb. et stat. nov.

MycoBank No.: MB 817621

Relicinopsis Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 281 (1986); type species: *Relicina intertexta* (Mont. & Bosch) Kirika, Divakar & Lumbsch, *Lichenologist* XX: XX (2016).

Parmelia intertexta Mont. & Bosch, in Miquel, *Pl. Jungh*. **4:** 445 (1855).— *Pseudoparmelia intertexta* (Mont. & Bosch) Hale, *Phytologia* **29:** 190 (1974).— *Relicinopsis intertexta* (Mont. & Bosch) Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 281 (1986).

A subgenus in the genus *Relicina*, corresponding to clade 1 in Fig. 1, including all species currently placed in *Relicinopsis* (Elix *et al.* 1986; Elix 1993).

New Combinations

Relicina dahlii (Hale) Kirika, Divakar & Lumbsch comb. nov.

MycoBank No.: MB 817622

Pseudoparmelia dahlii Hale, *Smithson. Contr. Bot.* **31:** 28 (1976).—*Relicinopsis dahlii* (Hale) Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 281 (1986).

Relicina intertexta (Mont. & Bosch) Kirika, Divakar & Lumbsch comb. nov.

MycoBank No.: MB 817624

Parmelia intertexta Mont. & Bosch, in Miquel, *Pl. Jungh*. **4:** 445 (1855).— *Pseudoparmelia intertexta* (Mont. & Bosch) Hale, *Phytologia* **29:** 190 (1974).— *Relicinopsis intertexta* (Mont. & Bosch) Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 281 (1986).

Relicina malaccensis (Nyl.) Kirika, Divakar & Lumbsch comb. nov.

MycoBank No.: MB 817623

Parmelia malaccensis Nyl., *J. Linn. Soc., Bot.* **20:** 52 (1883).—*Pseudoparmelia malaccensis* (Nyl.) Hale, *Phytologia* **29:** 190 (1974).—*Relicinopsis malaccensis* (Nyl.) Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 282 (1986).

Relicina rahengensis (Vain.) Kirika, Divakar & Lumbsch comb. nov.

MycoBank No.: MB 817625

Parmelia rahengensis Vain., *Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo* **1:** 39 (1923).—*Pseudoparmelia rahengensis* (Vain.) Hale, *Phytologia* **29:** 191 (1974).—*Relicinopsis rahengensis* (Vain.) Elix & Verdon, in Elix *et al.*, *Mycotaxon* **27:** 282 (1986).

Relicina stevensiae (Elix & J. Johnst.) Kirika, Divakar & Lumbsch comb. nov.

MycoBank No.: MB 817626

Relicinopsis stevensiae Elix & J. Johnst., *Mycotaxon* **31:** 504 (1988).

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

References

Arup, U., Ekman, S., Lindblom, L. & Mattsson, J. E. (1993) High performance thin layer chromatography (HPTLC), an improved technique for screening lichen substances. *Lichenologist* **25:** 61–71.

Biomatters Ltd (2005–2015) *Geneious r8*. Available at: http://www.geneious.com/previous-versions

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

Crespo, A., Kauff, F., Divakar, P. K., Amo, G., Arguello, A., Blanco, O., Roca-Valiente, B., Núñez-Zapata, J., Cubas, P., Argüello, A. *et. al.* (2010) Phylogenetic generic classification of

Downloaded from https:/www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

parmelioid lichens (*Parmeliaceae*, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* **59:** 1735–1753.

Crespo, A., Divakar, P. K. & Hawksworth, D. L. (2011) Generic concepts in parmelioid lichens, and the phylogenetic value of characters used in their circumscription. *Lichenologist* **43:** 511–535.

Divakar, P. K. & Crespo, A. (2015) Molecular phylogenetic and phylogenomic approaches in studies of lichen systematics and evolution. In *Recent Advances in Lichenology: Modern Methods and Approaches in Lichen Systematics and Culture Techniques, Volume 2* (D. K. Upreti, P. K. Divakar, V. Shukla & R. Bajpai, eds): 45–60. New Delhi: Springer India.

Divakar, P. K. & Upreti, D. K. (2005) *Parmelioid Lichens in India (A Revisionary Study)*. Dehra Dun: Bishen Singh Mahendra Pal Singh.

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

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

Divakar, P. K., Crespo, A., Wedin, M., Leavitt, S. D., Hawksworth, D. L., Myllys, L., McCune, B., Randlane, T., Bjerke, J. W., Ohmura, Y. *et al.* (2015) Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist* **208:** 1217–1226.

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

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

Elix, J. A. (1993) Progress in the generic delimitation of *Parmelia* sensu lato lichens (Ascomycotina: *Parmeliaceae*) and a synoptic key to the *Parmeliaceae*. *Bryologist* **96:** 359–383.

Elix, J. A. (1994) *Parmeliaceae*. *Flora of Australia* **55:** 1–360.

Elix, J. A., Johnston, J. & Verdon, D. (1986) *Canoparmelia*, *Paraparmelia* and *Relicinopsis*, three new genera in the *Parmeliaceae* (lichenized Ascomycotina). *Mycotaxon* **27:** 271–282.

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

Gutierrez, G., Blanco, O., Divakar, P. K., Lumbsch, H. T. & Crespo, A. (2007) Patterns of group I intron presence in nuclear SSU rDNA of the lichen family *Parmeliaceae*. *Journal of Molecular Evolution* **64:** 181–195.

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

Hale, M. E. (1975) A monograph of the lichen genus *Relicina* (*Parmeliaceae*). *Smithsonian Contributions to Botany* **26:** 1–32.

Hale, M. E. (1976) A monograph of the lichen genus *Pseudoparmelia* Lynge (*Parmeliaceae*). *Smithsonian Contributions to Botany* **31:** 1–62.

Hörandl, E. & Stuessy, T. F. (2010) Paraphyletic groups as natural units of biological classification. *Taxon* **59:** 1641–1653.

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

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

Lanfear, R., Calcott, B., Ho, S. Y. & Guindon, S. (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

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

Lumbsch, H. T. (2001) Analysis of phenolic products in lichens for identification and taxonomy. In *Protocols in Lichenology: Culturing, Biochemistry, Ecophysiology and Use in Biomonitoring* (I. Kranner, R. Beckett & A. Varma, eds): 281–295. Berlin: Springer.

Lumbsch, H. T. (2007) Recent trends in phylogeny and classification of lichen-forming ascomycetes. In *Fungi: Multifaceted Microbes* (B. N. Ganguli & S. K. Deshmukh, eds): 153–168. Delhi: Anamaya.

Printzen, C. (2010) Lichen systematics: the role of morphological and molecular data to reconstruct phylogenetic relationships. *Progress in Botany* **71:** 233–279.

Rambaut, A. & Drummond, A. J. (2009) *Tracer version 1.5*. Available at: http://beast.bio.ed.ac.uk.

Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. (2002) TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18:** 502–504.

Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16:** 1114–1116.

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

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology* **57:** 758–771.

Strimmer, K. & Rambaut, A. (2002) Inferring confidence sets of possibly misspecified gene trees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269:** 137–142.

Downloaded from https:/www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https:/www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

Swinscow, T. D. V. & Krog, H. (1988) *Macrolichens of East Africa*. London: British Museum (Natural History).

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

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

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.

White, T. J., Bruns, T. D., Lee, S. & Taylor, J. (eds) (1990) *Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics*. San Diego: Academic Press.

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

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

Downloaded from https://www.cambridge.org/core. University of Chicago, on 31 May 2017 at 21:36:10, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S0024282916000748

View publication stats