Phytotaxa 289 (1): 036–048
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.289.1.2

Polyphyly of the genus *Canoparmelia*—uncovering incongruences between phenotype-based classification and molecular phylogeny within lichenized Ascomycota (Parmeliaceae)

PAUL M. KIRIKA[1, 2], PRADEEP K. DIVAKAR[3], ANA CRESPO[3] STEVEN D. LEAVITT[4], GEORGE MUGAMBI[5], GRACE W. GATHERI[1] & H. THORSTEN LUMBSCH[6]

[1]*Department of Plant Sciences, Kenyatta University, P. O Box 43844-00100, Nairobi, Kenya; email: pmkirika@gmail.com, ggatheri@yahoo.com*
[2]*Botany Department, National Museums of Kenya, P.O. Box 40658-00100, Nairobi, Kenya: email: pkirika@museums.or.ke*
[3]*Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid 28040 Spain; pdivakar@farm.ucm.es; email: acrespo@farm.ucm.es*
[4]*Monte L. Bean Life Science Museum, Brigham Young University, 193 MLBM, Provo, UT 84602, USA; email: steve_leavitt@byu.edu*
[5]*Department of Biological Sciences, School of Pure and Applied Sciences, Meru University of Science and Technology, P. O. Box 972-60200, Meru, Kenya; email: gkmugambi@gmail.com*
[6]*Science & Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, U.S.A.; email: sleavitt@fieldmuseum.org, tlumbsch@fieldmuseum.org*

Abstract

Many phenotypical features traditionally used to classify genera in Parmeliaceae and in lichens in general have evolved several times independently, potentially limiting their taxonomic utility. Here, we aim to elucidate evolutionary relationships of *Canoparmelia* s. lat. among other parmotremoid taxa. A multilocus dataset (ITS, nuLSU and mtSSU rDNA sequences) was gathered and analyzed within a phylogenetic framework. *Canoparmelia* s. lat. was recovered as highly polyphyletic within the parmelioid clade, and three divergent lineages representing *Canoparmelia* s. lat. were identified in addition to the previously segregated *Crespoa* group. Of these, two formed a sister relationship with *Parmotrema*. However, no apparent diagnostic morphological features were found distinguishing the distinct *Canoparmelia* s. lat. clades reconstructed in the phylogenetic analyses. As a consequence, we propose to restrict the circumscription of *Canoparmelia* to clade 1 (i.e. the *C. texana* group) and to include clades 2 and 3 in *Parmotrema*. We propose to recognize these well-supported monophyletic clades at subgeneric level. Consequently, the new subgeneric name *Parmotrema* subgen. *Africanae* is proposed for clade 3 recovered in this study. Since clade 4, which clusters with the genera *Nesolechia* and *Punctelia,* is only represented by a single sequenced specimen, we refrain from proposing any taxonomic changes. The new combinations *Parmotrema epileucum*, and *P. zimbabwense* are proposed.

Key words: Africa, classification, integrative taxonomy, molecular systematics, parmotremoid lichens

Introduction

The family Parmeliaceae comprises approximately 2800 species distributed worldwide, including Antarctica (Thell *et al.* 2012). The generic circumscription in Parmeliaceae and in lichen forming fungi in general are continually being revised as a consequence of new understandings and advent of new technologies—from light microscopes to DNA sequences, and through extrolites and molecular phylogenetic analyses (reviewed in Lumbsch 1998; Nimis 1998; Grube & Winka 2002; Lumbsch 2007; Printzen 2010; Crespo *et al.* 2011; Thell *et al.* 2012; Divakar & Crespo 2015).

While phenotypical and chemical features have traditionally been used for generic segregation in Parmeliaceae, in recent years, a number of taxonomic re-evaluations were based primarily on molecular phylogenies. For example, nine genera were synonymized within *Xanthoparmelia*, four within *Parmotrema*, three within *Hypotrachyna*, and more recently, *Bulborrhizina* within *Bulbothrix* (reviewed by Crespo *et al.* 2011; Thell *et al.* 2012; and Divakar *et al.* 2013a; Kirika *et al.* 2015). At the same time, molecular phylogenies have helped to uncover previously unrecognized genus-level lineages such as: *Melanelixia* Blanco *et al.* (2004: 881), *Melanohalea* Blanco *et al.* (2004: 882) and *Montanelia*

 Accepted by Mohammad Sohrabi: 4 Nov. 2016; published: 20 Dec. 2016

Divakar *et al.* (2012: 2022), each segregated from *Melanelia* Esslinger (1978: 46) s. lat. (Blanco *et al.* 2004; Divakar *et al.* 2012). Other examples include: *Austroparmelina* Crespo *et al.* (2010a: 209) which was segregated from *Parmelina* Hale (1974a: 481); *Remototrachyna* Divakar *et al.* (2010: 584) segregated from *Hypotrachyna* Hale (1974b: 340) s. lat. (Divakar *et al.* 2010); and more recently *Notoparmelia* Crespo *et al.* (2014: 59) was segregated from *Parmelia* Acharius (1803: xxxiii) (Ferencova *et al.* 2014). Based on the most recently available data, approximately 80 genera are currently accepted in the family Parmeliaceae (Divakar *et al.* 2015).

Within Parmeliaceae, the genus *Canoparmelia* (ca. 35 species) belongs to the parmelioid group, specifically clustering within the '*Parmotrema* clade' (Crespo *et al.* 2010b; Divakar *et al.* 2015). *Canoparmelia* species are characterized by relatively narrow, subirregular lobes with rotund or subrotund eciliate margins, pored epicortex, presence of isolichenan in the cell walls, bifusiform conidia and simple rhizines (Elix 1993; Crespo *et al.* 2010b). Species are widely distributed with centers of distribution in the Americas and Africa. Previous molecular studies have shown that *Canoparmelia*, as originally circumscribed (Elix *et al.*, 1986), is highly polyphyletic (see Crespo *et al.* 2010a,b). Consequently, some species were placed in the genus *Austroparmelina* (Crespo *et al.* 2010a), *Canoparmelia norsticticata* was transferred to *Parmotrema* (Crespo *et al.* 2010b) and a few species of *C. crozalsiana* group were accommodated in *Parmotrema* subgenus *Crespoa* D. Hawksw. (2011: 647). The latter was raised to generic level as *Crespoa* (D. Hawksw.) Lendemer & Hodkinson (2012: 3) more recently.

Phylogenetic relationships of *Canoparmelia* species have been partially explored recently using molecular and morphological data, although congeners from African populations have not been well studied (Crespo *et al.* 2010a, b). Furthermore, previous studies have shown that a number of morphological and chemical characters traditionally used for generic segregation in parmelioid lichens have evolved several times independently during the evolutionary history of this group (see e.g. Divakar *et al.* 2013b). Therefore, including a molecular phylogenetic approach, in addition to other types of data, is arguably prerequisite for robust generic circumscription in the parmelioid core. The present work constitutes an effort to clarify the phylogenetic relationships among species currently placed in *Canoparmelia*, and their position relative to other parmelioid genera. Specifically, we ask the following questions: (1) How many clades are included in *Canoparmelia* as currently recognized? (2) What are the relationships among species of *Canoparmelia* to other genera of parmotremoid clade? (3) Do phylogenetic relationships support major biogeographic patterns in *Canoparmelia*, particularly supporting the African species as a distinct lineage?

Material and Methods

Taxon sampling:—Data matrices of 65 specimens comprising 51 species from 9 genera of parmelioid lichens were assembled and analyzed. The DNA data matrix comprised nu LSU, ITS and mitochondrial SSU rDNA. GenBank accession numbers and information of studied materials are shown in Table 1. The data sets include 153 sequences from previous study (Divakar *et al.* 2015), and 30 newly generated sequences for this study. Two specimens of *Melanohalea* were used as an out-group since the genus is known to be closely related to the parmotremoid clade (Crespo *et al.* 2010).

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

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Austroparmelina endoleuca	Australia: Australian Capital Territory	Elix 38802	Herb Elix, MAF-Lich	GU183185	GU183192	GU183178
Austroparmelina macrospora	Australia: Western Australia	Elix 32408	Herb Elix, MAF-Lich	GU183187	GU183194	GU183180
Austroparmelina pruinata	Australia: Western Australia	E. McCrum s.n.	MAF-Lich 14270	EF042905	EF025481	EF042914

...Continued on next page

TABLE 1. (Continued)

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Austroparmelina pseudorelicina	Australia: New South Wales	Amo de Paz 1159	MAF-Lich 16115	GU183188	GU183195	GU183181
Canoparmelia caroliniana	USA: North Carolina	Perlmutter 1000	NCU	GU994542	AY584613	GU994584
*Canoparmelia caroliniana*_9304	Kenya: Western Province	Kirika, 3419	EA, F	**KX369243**	-	**KX369261**
Canoparmelia caroliniana 9309	Kenya: Western Province	Kirika, 3389	EA, F	**KX369244**	**KX369256**	**KX369262**
Canoparmelia cf. *zimbabwensis*_9290	Kenya: Eastern Province	Kirika & Lumbsch, 3828	EA, F, MAF	**KX369245**	-	**KX369263**
Canoparmelia ecaperata 9293	Kenya: Eastern Province	Kirika, Malombe & Matheka, 3692	EA, F	**KX369246**	-	**KX369264**
Canoparmelia eruptens 9388	Kenya: Coast Province	Kirika, Mugambi & Lumbsch, 2405	EA, F	**KX369247**	-	-
Canoparmelia eruptens 9630	Kenya: Coast Province	Kirika, 4483	EA, F, MAF	**KX369248**	**KX369257**	**KX369265**
Canoparmelia zimbabwensis 9390	Kenya: Coast Province	Kirika, Mugambi & Lumbsch, 2292	EA, F	**KX369249**	-	**KX369266**
Canoparmelia concrescens	Kenya: Western Province	Divakar, Mangold & Lumbsch 19538f	MAF-Lich 15547	GU994543	KR995317	GU994585
Canoparmelia epileuca 9292	Kenya: Eastern Province	Kirika & Lumbsch, 3871	EA, MAF, F	**KX369250**	-	**KX369267**
Canoparmelia epileuca 9508	Kenya: Eastern Province	Kirika & Lumbsch, 3866	EA, MAF, F	**KX369251**	**KX369258**	**KX369268**
Canoparmelia nairobiensis	Kenya: Western Province	Divakar, Mangold & Lumbsch 19538g	MAF-Lich 15544	GU994545	KR995318	GU994587
Canoparmelia nairobiensis 9682	Kenya: Central Province	Kirika, 4423	EA, MAF, F	**KX369252**	**KX369259**	**KX369269**
Canoparmelia schelpei 3248	Mozambique	s.n	MAF	**KX369255**	-	**KX369270**
Canoparmelia aff. *nairobiensis* 9288	Kenya: Western Province	Kirika, 3424	EA, F	**KX369253**	-	**KX369271**
Canoparmelia sp.	South Africa: Eastern Cape	Crespo *et al.* 49h	MAF-Lich 15508	KR995273	KR995319	KR995387
Canoparmelia texana	India: Uttaranchal	Divakar GPGC 02-000637	MAF-Lich 14272	EF042906	-	EF042915
Cetrelia cetrarioides	Spain: Asturias	Divakar s.n.	MAF-Lich 15552	JN943844	GU994636	GU994591
Cetrelia olivetorum	China:Yunnan	Crespo et al s.n.	MAF-Lich 15507	JN943843	KR995321	GU994593

...Continued on next page

TABLE 1. (Continued)

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Cetrelia pseudolivetorum	China: Yunnan	Crespo *et al.* s.n.	MAF-Lich 15506	GU994548	GU994639	GU994594
Crespoa carneopruinata	Costa Rica: Sarchi	Lücking 15171a	F	EF042904	EF025480	EF042913
Crespoa crozalsiana	Spain: Cádiz	Crespo *et al.* s.n.	MAF-Lich 7658	AY586571	AY586594	AY584831
Crespoa crozalsiana 9589	Kenya: Coast Province	Kirika & Lumbsch, 3964	EA, MAF, F	**KX369254**	**KX369260**	**KX369272**
Crespoa inhaminensis	Kenya: Western Province	Divakar, Lumbsch & Mangold 19529l	MAF-Lich 15545	GU994544	GU994633	GU994586
Crespoa schelpei	Kenya: Nairobi	Crespo, Divakar & Lumbsch 19501j	MAF-Lich 15546	GU994546	GU994634	GU994588
Flavoparmelia baltimorensis	USA: Maryland	Molina s.n.	MAF-Lich 7660, 10174	AY586559	AY586583	AY584832
Flavoparmelia caperata	China: Yunnan	Crespo *et al.* s.n.	MAF-Lich 10175	AY586561	AY586585	AY584834
Flavoparmelia citrinescens	Argentina: Bariloche	Messuti s.n.	MAF-Lich 15521	GU994550	GU994641	GU994596
Flavoparmelia marchantii	Australia: Western Australia	Elix s.n.	MAF-Lich 10492	DQ299905	GU994642	GU994598
Flavoparmelia soredians	Spain: Cáceres	Crespo *et al.* s.n.	MAF-Lich 10176	AY586562	AY586586	AY584835
Flavoparmelia springtonensis	Australia: South Australia	Elix 31200	MAF-Lich 14271	EF042907	EF025483	EF042916
Flavoparmelia subambigua	Argentina: National Park of Calilegua	Amo de Paz s.n.	MAF-Lich 15520	GU994551	GU994643	GU994599
Flavopunctelia flaventior	Spain: Teruel	Crespo *et al.* s.n.	MAF-Lich 6046	AY581060	AF351164	AY578923
Flavopunctelia soredica	USA: Minnesota	Cole 11220	MAF-Lich 17771	KR995280	KR995327	GU994600
Melanohalea elegantula	USA: California	Esslinger 18874	F	JN943705	JQ813114	JN939524
Melanohalea exasperata	The Netherlands; Gelderland	Aptroot 68148	F	JN943701	JQ813122	JN939535
Nesolechia oxyspora 1	Portugal: Azores	Ertz 16840	BR	KR995295	-	KR995417
Nesolechia oxyspora 2	Norway: Troms	Fröberg 10/08/2003	UPS	DQ980020	DQ923642	DQ923669
Parmotrema cetratum	Uruguay: Maldonado	Osorio 9424	MVM, MAF-Lich 7649	AY586576	AY586598	AY584847

...Continued on next page

TABLE 1. (Continued)

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Parmotrema crinitum	Portugal: Lisboa	Crespo s.n.	MAF-Lich 6061	AY586565	EU562699	AY584837
Parmotrema fistulatum	Uruguay: Maldonado	Geymonat, 9423	MVM, MAF-Lich 7655	AY581057	EU562700	AY578920
Parmotrema haitiense	Australia: Australian Capital Territory	Lowhoff *et al.* s.n.	MAF-Lich 7657	AY581055	AY582295	AY578918
Parmotrema hypoleucinum	Spain: Cádiz	Crespo *et al.*	MAF-Lich 7637	AY586567	AY586590	AY584839
Parmotrema norsticticatum	South Africa: Cape Province	Crespo *et al.* 49h	MAF-Lich 15510	GU994576	-	GU994622
Parmotrema perforatum	USA: North Carolina	Cole 7983	MAF-Lich 7651	AY586568	AY586591	AY584840
Parmotrema perlatum	Portugal: Sintra	Crespo *et al.* s.n.	MAF-Lich 6965	AY586566	AY586580	AY584838
Parmotrema pilosum	Uruguay: Maldonado	Sacarabino	MAF-Lich 7656	AY581056	EU562701	AY578919
Parmotrema reticulatum	Portugal: Lisboa	Crespo s.n.	MAF-Lich 6067	AY586579	AF351184	AY584850
Punctelia bolliana	USA: Minnesota	Cole 11219	MAF-Lich 17774	GU994579	GU994673	GU994628
Punctelia borreri	Portugal: Castello Vide	Crespo *et al.* s.n.	MAF-Lich 9919	AY581088	AY582324	AY578954
Punctelia jeckeri	Germany: Düsseldorf	Crespo s.n.	MAF-Lich 10251	AY613406	AY613426	GU994625
Punctelia pseudocoralloidea	Australia: New South Wales	Louwhoff *et al.* s.n.	MAF-Lich 6922	AY586572	AY586595	AY584843
Punctelia reddenda	Chile: Valdivia	Sancho s.n.	MAF-Lich 10247	AY613410	AY613430	GU994627
Punctelia rudecta	USA: Maryland	Molina s.n.	MAF-Lich 7661	AY586573	AY586596	AY584844
Punctelia subflava	Australia: Red rock	Elix 42705	MAF-Lich 7322	AY586575	EU562704	AY584846
Xanthoparmelia azaniensis	South Africa: Matroosberg	Crespo *et al.* s.n.	MAF-Lich 14269	EF042900	EF025478	EF042910
Xanthoparmelia chlorochroa	USA: North Dakota	Leavitt 55437	BRY-C	HM578887	KR995372	HM579298
Xanthoparmelia conspersa	Spain: Zamora	Blanco & Crespo s.n.	MAF-Lich 6793	AY581096	AF351186	AY578962
Xanthoparmelia exornata	South Africa: Cape Province	Crespo *et al.* s.n.	MAF-Lich 14266	EF042908	EF025485	EF108318
Xanthoparmelia hottentotta	South Africa: Cape Province	Crespo *et al.* s.n.	MAF-Lich 14267	EF042909	EF025486	EF042919

...Continued on next page

TABLE 1. (Continued)

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Xanthoparmelia mougeotii	Spain: La Rioja	Blanco & Crespo s.n.	MAF-Lich 9916	AY581100	AY582336	AY578967
Xanthoparmelia pokornyi	Spain: Zaragoza		MAF-Lich 9908	AY581075	EU562707	AY578939
Xanthoparmelia saxeti	Uruguay: Florida	s.n.	BRY-C	HM578888	KR995373	HM579299

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) 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 the dilutions of total DNA. Fungal ITS rDNA was amplified using ITS1F primers (Gardes and Bruns 1993), ITS4 and ITS4A (White *et al.* 1990; Larena *et al.* 1999); mtSSU rDNA was amplified using the primers mrSSU1, mrSSU3R and mrSSU2R (Zoller *et al.* 1999); nuLSU rDNA was amplified using LR0R and LR5 (Vilgalys and Hester 1990). 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, IL, 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 and 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 were set to default values. We used the E-INS-i alignment algorithm and '20PAM / K=2' scoring matrix, with the remaining parameters were set to default values for the mtSSU sequences. The program Gblocks v0.91b (Talavera and 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 the '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 multi-locus data matrix under BI using the program BEAST v1.8.2 (Drummond and 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 was selected 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 and 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.

Morphological and chemical studies:—Morphological characters, including lobe shape, size and width, cilia and rhizines were studied using a Leica Wild M 8 dissecting microscope. Key morphological and chemical features largely used to segregate genera in parmotremoid lichens were tabulated (Table 2).

TABLE 2. Key morphological and chemical features used to segregate genera in parmotremoid lichens.

Features	Clade 3 "*Canoparmelia*" p.p.	***Clade 2*** **Crespoa**	Clade 1 **Canoparmelia* s.str.	**Parmotrema**
Ascospore size (μm)	8–13 x 4–5	9–13 x 5–9	8–19 x 5–8	15–35 x 8–18 (rarely 10–14 × 5–7)
Conidia (μm)	Bifusiform 6–7 x 1	Filiform 12–15 x 1	Bifusiform 6–8 x 1	Sublageniform 5–8 x 1 or filiform 12–20 x 1
Cell wall polysaccharide	Isolichenan	Isolichenan	Isolichenan	Intermediate-type lichenan
Lobe morphology	Narrow, eciliate, sublinear, 1–2 mm wide	Narrow, eciliate, sublinear to subirregular, 1–6 mm wide	Narrow, eciliate, sublinear to subirregular, 1–8 mm wide	Broad, ciliate or eciliate, irregular to subirregular
Upper surface	Plane	Wrinkled and reticulately ridged to coarsely foveolate	Plane to rigulose	Plane to rigulose, reticulate
Chemistry	Atranorin, protocetraric acid	Atranorin, stictic acid, protocetraric acid	Atranorin, usnic acid, perlatolic acid, divaricatic acid, protolichesterinic acid	Varied
Distribution	From sea level to 300 m elevation. Africa	100 to 2000 m elevation. Wide, Pantropical	From sea level to 3000 m elevation. Cosmopolitan	From sea level to 4500 m elevation. Cosmopolitan

*Only species included in the phylogenetic tree were evaluated.

Chemical constituents were identified by thin layer chromatography using standard methods (Orange *et al.* 2010). Extraction of secondary metabolites for TLC analysis was done by placing small pieces of the thallus in Eppendorf tubes and then adding a few drops of acetone in the tube. The resulting extract was then spotted on glass plates coated with Silica gel using capillary tubes. Plates were developed in Camag horizontal developing chamber (Oleico Lab Stockholm) using solvent system A (Toluene:Dioxane:acetic acid, 45:15:2), plates were then air dried, sprayed with 10% sulphuric acid and then heated in an oven at 110°C to visualize the spots. Spots were identified by comparisons with controls (Orange *et al.* 2010).

Results and Discussion

DNA sequence data and phylogenetic reconstructions:—In this study, we generated a total of 30 new sequences, these comprise 13 nuclear ITS, 12 nuLSU and 5 mitochondrial SSU rDNA from thirteen samples of *Canoparmelia* s. lato from Eastern Africa (Table 1). These were deposited in Genbank under accession numbers KX369243–KX369272. The aligned data matrix contained 471 unambiguously nucleotide position characters in ITS, 846 in nuLSU and 780 in mt SSU. The final alignment of three-locus concatenated data set was 2098 positions in length, with 670 variable characters. The ITS PCR product obtained ranged between 600 to 800 base pairs (bp). Differences in size were due to the presence or absence of insertions of about 200 bp identified as group I introns (Gutierrez *et al.* 2007) at the 3 ′ end of the SSU rDNA. We excluded group I introns and a 160 bp of the mtSSU 56 bp of the ITS1, and 35 bp of the ITS2 alignments from the analysis using GBlocks. SYM+I+G, TrN+I+G and HKY+I+G were resulted as best fit model of evolution for ITS, nu LSU and mt SSU, respectively.

Topologies of single-locus analyses did not show supported conflicts (results not shown) and hence the concatenated three-locus data matrix (ITS, nuLSU and mtSSU) was used for all subsequent phylogenetic analyses. The partitioned ML analysis of the concatenated data matrix yielded the optimal tree with ln likelihood value = –15032.46. The

effective sample sizes (ESS) of all estimated parameters were well above 200 in Bayesian analysis, indicating that convergence among parallel runs was reached. The maximum likelihood (ML) and Bayesian topologies were largely similar and did not show any supported conflict (e.g., PP ≥ 0.95 and ML bootstrap ≥ 70%), and therefore the ML tree topology was used as a working hypothesis of phylogenetic relationships (Fig. 1).

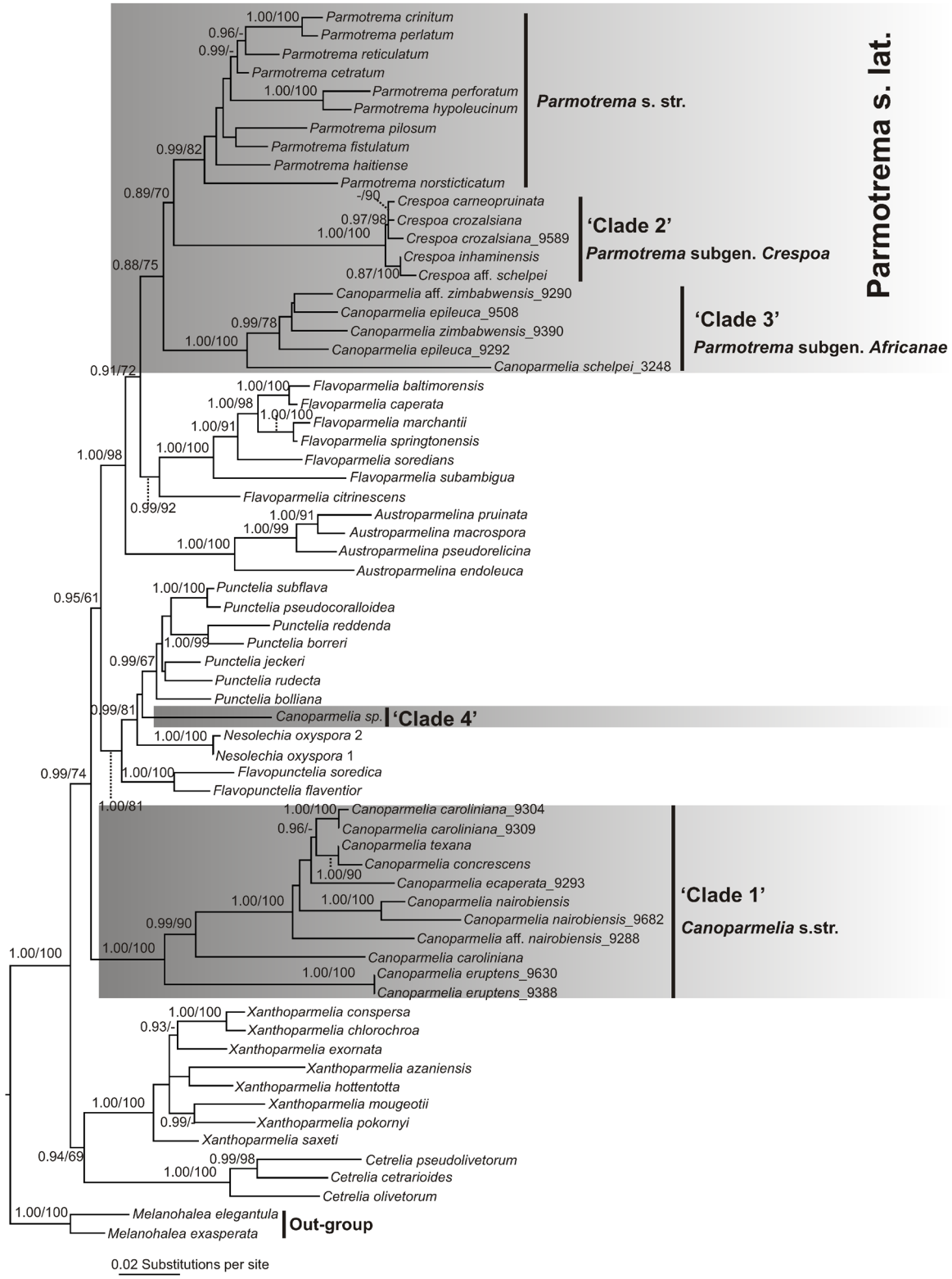

FIGURE 1. Phylogenetic relationships of *Canoparmelia* s. lat. among parmotremoid taxa based on a maximum-likelihood (ML) analysis of a concatenated, three locus dataset (ITS, nuLSU & mtSSU rDNA.) Since the ML and Bayesian inference topologies were identical, only the ML topology is shown here. Posterior probabilities ≥ 0.95/ ML bootstrap values ≥ 70% are given above the branches. Two species of *Melanohalea* (*M. elegantula* and *M. exasperata*) were used as out-group.

Our results showed that species in the genus *Canoparmelia* sensu lato were not recovered within a single, monophyletic group, in agreement with previous studies indicating the non-monophyly of *Canoparmelia* (Crespo *et al.* 2010a, b). In this study, *Canoparmelia* specimens were recovered in four distinct clades-'clade 1', 'clade 2', 'clade 3' and 'clade 4' (Fig. 1). This pattern is inconsistent with a generic circumscription based on phenotypical features (Elix *et al.* 1986; Elix 1993; Brodo *et al.* 2001; Divakar & Upreti 2005). Genus-level polyphyly is not an unusual phenomenon in Parmeliaceae and such patterns have been found in many other groups of lichen forming fungi as well (reviewed in Lumbsch 2007; Printzen 2010; Crespo *et al.* 2011; Thell *et al.* 2012; Divakar & Crespo 2015).

With our extended taxon sampling, species of *Canoparmelia* clustered in four different clades within the parmotremoid clade (Crespo *et al.* 2010b). 'Clade 1' formed a sister relationship to rest of the genera included in parmotremoid clade. The relationship was strongly supported in both analyses (pp = 0.99, bs = 74%). This clade included species distributed in wide geographic regions and habitats ranging from sea level to about 3000 m elevation (Table 2). Moreover, the type species of the genus *Canoparmelia* (*C. texana*) clustered within this clade and hence clade 1 is here considered as *Canoparmelia* s.str. 'Clade 2' consisted of species recently accommodated in *Crespoa* either at generic or subgeneric rank (Hawksworth 2011, Lendemer & Hodkinson 2012), which was recovered as sister to the genus *Parmotrema* s.str. This relation is consistent with a previous study (Crespo *et al.* 2010b). Initially, species clustered in this clade were segregated as *Parmotrema* subgenus *Crespoa* based on its monophyly in phylogenetic reconstructions and in having wrinkled and reticulately ridged to coarsely foveolate upper surface (Hawksworth 2011). Subsequently, a generic rank as *Crespoa* was proposed for this group by Lendemer & Hodkinson (2012). Species within this clade have been characterized by narrow eciliate, sublinear to subirregular, 1–6 mm wide lobes, wrinkled and reticulately ridged to coarsely foveolate upper surface, filiform conidia, and stictic, constictic and protocetraric acids medullary extrolites (Table2). They are widely distributed in pantropical regions from ca. 100 to 2000 m elevation. Other species with similar morphology (except foveolate upper surface) and chemistry can be found in 'clade 1' (*Canoparmelia* s.str.) and 'clade 3'; and filiform conidia are common in *Parmotrema* s.str. (Table 2). Upper surface morphology is a widely variable feature in the genus *Parmotrema* and wrinkled upper surface and stictic and constictic acids can be found in several species in this genus (Hale 1965).

'Clade 3' formed a supported sister group relation to *Parmotrema* s. str. + 'clade 2'. 'Clade 3' included species distributed in coastal areas from sea level to 300 m elevation in Africa. Species included in this clade can be characterized by sublinear narrow lobes upto 2 mm wide, protocetraric acid medullary extrolites and their restricted distribution to coastal areas in Africa. However, species with similar chemistry can be found in 'clade 2' and *Parmotrema* s.str. (Table 2). 'Clade 4' included a single undescribed species, endemic to South Africa that was recovered as sister to *Punctelia* with low statistical support (Fig. 1). This has already been shown in a previous study (Divakar *et al.* 2015).

Phenotypic features such as lobe morphology, marginal cilia, and chemistry have evolved several times independently within the parmelioid core, indicating that that they have an adaptive value in certain habitats (Divakar *et al.* 2013b). Further, morphological and chemical features have also been shown to be highly plastic in other groups of lichenized fungi (e.g. Caliciales, Prieto *et al.* 2013; Cladoniaceae, Parnmen *et al.* 2010; Collemataceae, Otálora *et al.* 2013; Graphidaceae, Rivas Plata & Lumbsch 2011; Roccellaceae, Tehler & Irestedt 2007). Therefore, it is not surprising that the monophyly of *Canoparmelia* based on phenotypic feature was not recovered in our molecular phylogenetic analyses.

Some species in the genus *Canoparmelia* s.str. ('clade 1') and 'clade 3', such as *C. caroliniana, C. epileuca*, *C. nairobiensis*, *C. schelpei* and *C. zimbabwensis*, were not found to be monophyletic (Fig. 1). Additionally, a specimen representing *C. schelpei* from Kenya clustered in 'clade 2' (subgen. *Crespoa*) and a sample from Mozambique was recovered in the newly uncovered 'clade 3'. Since the type material of this species is described from Mozambique, the sample clustered in 'clade 3' most likely belongs to *C. schelpei* s.str., and the sample from Kenya recovered in 'clade 2' may belong to an undescribed species. Additional studies are necessary to clarify the current species delimitations in this group, which is largely based on macromorphological and chemical characters. A detailed investigation evaluating the cryptic diversity in this group is under progress and will be discussed in a forthcoming paper.

Taxonomic conclusions:—Based on our molecular phylogenetic analyses and morphological re-evaluation, we propose to transfer *Canoparmelia* species clustered in 'clade 3' to *Parmotrema* and accept *Crespoa* at a subgeneric rank within *Parmotrema* as proposed earlier (Hawksworth 2011). We also propose to recognize the species clustered in 'clade 3' as *Parmotrema* subgen. *Africanae* and leave the remaining unstudied species unclassified within the genus *Canoparmelia* ('clade 1'). 'Clade 4' included only a single sample. A detailed study of this clade is under progress and results will be discussed in a subsequent paper. The description of the new subgenus and new combinations are proposed below.

New subgenus

Parmotrema* subgen. *Africanae Kirika, Divakar & Lumbsch, *subgen. nov.* MycoBank No.: MB 817400

Type species:—*Parmotrema epileucum* (Hale) Kirika, Divakar & Lumbsch (2016: XXX); *Canoparmelia epileuca* (Hale) (Hale) Elix & Hale, in Elix *et al.* (1986: 278); *Pseudoparmelia epileuca* (Hale) Hale (1974: 190). *Parmelia epileuca* Hale (1972: 343).

A new subgenus in the genus *Parmotrema*, corresponding to 'clade 3' in Fig. 1. This new subgenus is characterized by having sublinear, very narrow lobes up to 2.0 mm wide and the presence of atranorin and protocetraric acid. All species included are endemic to Africa and distributed in coastal areas from sea level to 300 m elevation.

New combinations

Parmotrema epileucum (Hale) Kirika, Divakar & Lumbsch, *comb. nov.* MycoBank No.: 817401

Canoparmelia epileuca (Hale) (Hale) Elix & Hale, in Elix *et al.* (1986: 278); *Pseudoparmelia epileuca* (Hale) Hale (1974: 190); *Parmelia epileuca* Hale (1972: 343).

Parmotrema zimbabwense (Hale) Kirika, Divakar & Lumbsch, *comb. nov.* MycoBank No.: MB 817402

Canoparmelia zimbabwensis (Hale) Elix & Hale, in Elix *et al.* (1986: 279); *Pseudoparnielia zimbabwensis* (Hale) Hale (1974: 191); *Parmelia zimbabwensis* Hale (1972: 346).

Note: In our cirumscription of subgenera in *Parmotrema, P. schelpei* (Hale) D. Hawksw. (2011: 648), is classified in *Parmotrema* subgen. *Africanae* rather than *Parmotrema* subgen. *Crespoa***.**

Acknowledgements

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 of the IDP/The Field Museum Africa Training Fund and the Spanish Ministerio de Ciencia e Innovación (CGL2013-42498-P).

References

Acharius, E. (1803) *Methodus qua Omnes Detectos Lichenes*. Stockholm: F. D. D. Ulrich.

Biomatters Ltd. (2005–2015) GENEIOUSr8. Available from: http://www.geneious.com/previous-versions (accessed 19 December 2016)

Blanco, O., Crespo, A., Divakar, P.K., Esslinger, T.L., Hawksworth, D.L. & Lumbsch, H.T. (2004) *Melanelixia* and *Melanohalea*, two new genera segregated from Melanelia (Parmeliaceae) based on molecular and morphological data. *Mycological Research* 108: 873–884.
https:/doi.org/10.1017/S0953756204000723

Brodo, I.M., Sharnoff, S.D. & Sharnoff, S. (2001) *Lichens of North America*. Yale University Press, New Haven & London, 795 pp.

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.
https:/doi.org/10.1017/S0024282911000570

Crespo, A., Ferencova, Z., Pérez-Ortega, S., Argüello, A., Elix, J.A. & Divakar, P.K. (2010a) *Austroparmelina*, a new Australasian lineage in parmelioid lichens (Parmeliaceae, Ascomycota): a multigene and morphological approach. *Systematics and Biodiversity* 8: 209–221.
https:/doi.org/10.1080/14772001003738320

Crespo, A., Kauff, F., Divakar, P.K., Amo, G., Arguello, A., Blanco, O., Roca-Valiente, B., Núñez-Zapata, J., Cubas, P., Argüello, A., Elix, J.A., Esslinger, T.L., Hawksworth, D.L., Millanes, A., Molina, M.C., Wedin, M. Ahti, T., Aptroot, A., Barreno, E., Bungartz,

B., Calvelo, S., Candan, M., Cole, M., Ertz, D., Goffinet, B., Lindblom, L., Lücking, R., Lutzoni, F., Mattsson, J.-E., Messuti, M.I., Miadlikowska, J., Piercey-Normore, M., Rico, V.J., Sipman, H.J.M., Schmitt, I., Spribille, T., Thell, A., Thor, G., Upreti, D.K. & Lumbsch, H.T. (2010b) Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* 59: 1735–1753.

Divakar, P.K. & Crespo, A. (2015) Molecular phylogenetic and phylogenomic approaches in studies of lichen systematics and evolution. *In*: Upreti, D.K., Divakar, P.K., Shukla, V. & Bajpai, R. (Eds.) *Recent Advances in Lichenology*: *Modern methods and approaches in Lichen systematics and culture techniques*, volume 2. Springer India, pp. 45–60.

Divakar, P.K., Crespo, A., Núñez-Zapata, J., Flakus, A., Sipman, H.J.M., Elix, J.A. & Lumbsch, H.T. (2013a) A molecular perspective on generic concepts in the *Hypotrachyna* clade (Parmeliaceae, Ascomycota). *Phytotaxa* 132: 21–38.
https:/doi.org/10.11646/phytotaxa.132.1.2

Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D., Hawksworth, D.L., Myllys, L., McCune, B., Randlane, T., Bjerke, J.W., Ohmura, Y., Schmitt, I., Boluda, C.G., Alors, D., Roca-Valiente, B., Del-Prado, R., Ruibal, C., Buaruang, K., Núñez-Zapata, J., Amo de Paz, G., Rico, V.J., Molina, M.C., Elix, J.A., Esslinger, T.L., Tronstad, I.K.K., Lindgren, H., Ertz, D., Gueidan, C., Saag, L., Mark, K., Singh, G., Dal Grande, F., Parnmen, S., Beck, A., Benatti, M.N., Blanchon, D., Candan, M., Clerc, P., Goward, T., Grube, M., Hodkinson, B.P., Hur, J.-S., Kantvilas, G., Kirika, P.M., Lendemer, J., Mattsson, J.-E., Messuti, M.I., Miadlikowska, J., Nelsen, M., Ohlson, J.I., Pérez-Ortega, S., Saag, A., Sipman, H.J.M., Sohrabi, M., Thell, A., Thor, G., Truong, C., Yahr, R., Upreti, D.K., Cubas, P. & Lumbsch, H.T. (2015) Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist* 208: 1217–1226.

Divakar, P.K., Del Prado, R., Lumbsch, H.T., Wedin, M., Esslinger, T.L., Leavitt, S.D. & Crespo, A. (2012) Diversification of the newly recognized lichen forming fungal lineage *Montanelia* (Parmeliaceae, Ascomycota) and its relation to key geological and climatic events. *American Journal of Botany* 99: 2014–2026.
http://dx.doi.org/10.3732/ajb.1200258

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.
http://dx.doi.org/10.3732/ajb.0900140

Divakar, P.K, Kauff, F., Crespo, A, Leavitt, S.D. & Lumbsch, H.T. (2013b) Understanding Phenotypical Character Evolution in Parmelioid Lichenized Fungi (Parmeliaceae, Ascomycota). *PLoS ONE* 8 (11): e83115.
https:/doi.org/10.1371/journal.pone.0083115

Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D., Hawksworth, D.L., Myllys, L., McCune, B., Randlane, T., Bjerke, J.W., Ohmura, Y., Schmitt, I., Boluda, C.G., Alors, D., Roca-Valiente, B., Del-Prado, R., Ruibal, C., Buaruang, K., Núñez-Zapata, J., Amo de Paz, G., Rico, V.J., Molina, M.C., Elix, J.A., Esslinger, T.L., Tronstad, I.K.K., Lindgren, H., Ertz, D., Gueidan, C., Saag, L., Mark, K., Singh, G., Dal Grande, F., Parnmen, S., Beck, A., Benatti, M.N., Blanchon, D., Candan, M., Clerc, P., Goward, T., Grube, M., Hodkinson, B.P., Hur, J.-S., Kantvilas, G., Kirika, P.M., Lendemer, J., Mattsson, J.-E., Messuti, M.I., Miadlikowska, J., Nelsen, M., Ohlson, J.I., Pérez-Ortega, S., Saag, A., Sipman, H.J.M., Sohrabi, M., Thell, A., Thor, G., Truong, C., Yahr, R., Upreti, D.K., Cubas, P. & Lumbsch, H.T. (2015) Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist* 208: 1217–1226.
https:/doi.org/10.1111/nph.13553

Divakar, P.K. & Crespo, A. (2015) Molecular phylogenetic and phylogenomic approaches in studies of lichen systematics and evolution. *In*: Upreti, D.K., Divakar, P.K., Shukla, V. & Bajpai, R. (Eds.) *Recent Advances in Lichenology*: *Modern methods and approaches in Lichen systematics and culture techniques*, *volume 2*. Springer India. pp. 45–60.
https:/doi.org/10.1007/978-81-322-2235-4_3

Divakar, P.K. & Upreti, D.K. (2005) *Parmelioid lichens in India (A revisionary study)*. Bishen Singh Mahendra Pal Singh, Dehra Dun. 488 pp.

Divakar, P.K., Del Prado, R., Lumbsch, H.T., Wedin, M., Esslinger, T.L., Leavitt, S.D. & Crespo, A. (2012) Diversification of the newly recognized lichen forming fungal lineage *Montanelia* (Parmeliaceae, Ascomycota) and its relation to key geological and climatic events. *American Journal of Botany* 99: 2014–2026.
https:/doi.org/10.3732/ajb.1200258

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.
https:/doi.org/10.3732/ajb.0900140

Drummond, A. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.
https:/doi.org/10.1186/1471-2148-7-214

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.

https:/doi.org/10.1093/molbev/mss075
Elix, J.A., Johnston, J. & Verdon, D. (1986) *Canoparmelia, Paraparmelia* and *Relicinopsis*, three new genera in the Parmeliaceae (lichenized Ascomycotina). *Mycotaxon* 27: 271–282.
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.
https:/doi.org/10.2307/3243867
Ferencova, Z., Cubas, P., Divakar, P.K., Molina, M.C. & Crespo, A. (2014) *Notoparmelia*, a new genus of Parmeliaceae (Ascomycota) based on overlooked reproductive anatomical features, phylogeny and distribution pattern. *Lichenologist* 46: 51–67.
https:/doi.org/10.1017/S0024282913000649
Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* 2: 113–118.
Grube, M. & Winka, K. (2002) Progress in understanding the evolution and classification of lichenized ascomycetes. *Mycologist* 16: 67–76.
https:/doi.org/10.1017/S0269915X02002069
Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* 2: 113–118.
https:/doi.org/10.1111/j.1365-294X.1993.tb00005.x
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.
https:/doi.org/10.1007/s00239-005-0313-y
Hale, M.E. & Kurokawa, S. (1964) Studies on *Parmelia* subgenus *Parmelia*. *Contributions from the United States National Herbarium* 36: 121–191.
Hale, M.E. (1965) A monograph of *Parmelia* subgenus *Amphigymnia*. *Contributions from the United States National Herbarium* 36: 193–358.
Hale, M.E. (1972). New Species of *Parmelia* Section *Cyclocheila* in Southern Africa. *Bryologist* 75:342–348.
https:/doi.org/10.2307/3241472
Hale, M.E. (1974) New combinations in the lichen genus *Pseudoparmelia* Lynge. *Phytologia* 29: 188–191.
Hale, M.E. & Kurokawa, S. (1964) Studies on *Parmelia* subgenus *Parmelia*. *Contributions from the United States National Herbarium* 36: 121–191.
Hawksworth, D.L. (2011) *Parmotrema* subgen. Crespoa subgen. nov. for the *Canoparmelia crozalsiana* clade. *Lichenologist* 43: 647–648.
https:/doi.org/10.1017/S0024282911000399
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.
https:/doi.org/10.1093/nar/gki198
Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
https:/doi.org/10.1093/bib/bbn013
Kirika, P.M., Leavitt, S.D., Divakar, P.K., Crespo, A., Gatheri, G.W., Mugambi, G. & Lumbsch, H.T. (2015) The monotypic genus *Bulborrhizina* belongs to *Bulbothrix* sensu lato (Parmeliaceae, Ascomycota). *Bryologist* 118: 164–169.
https:/doi.org/10.1639/0007-2745-118.2.164
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.
https:/doi.org/10.1093/molbev/mss020
Lendemer, J.C. & Hodkinson, B.P. (2012) Recognition of the *Parmelia crozalsiana* group as the genus *Crespoa*. *North American Fungi* 7 (2): 1–5.
https:/doi.org/10.2509/naf2012.007.002
Lumbsch, H.T. (1998) Taxonomic use of metabolic data in lichen-forming fungi. *In*: Frisvad, J.C., Bridge, P.D. & Arora, D.K. (Eds.) *Chemical fungal taxonomy*. Marcel Dekker, New York, pp. 345–387.
Lumbsch, H.T. (2007) Recent trends in phylogeny and classification of lichen-forming ascomycetes. *In*: Ganguli, B.N. & Deshmukh, S.K. (Eds.) *Fungi: multifaceted microbes*. Anamaya, Delhi, pp 153–168.
Nimis, P.L. (1998) A critical appraisal of modern generic concepts in lichenology. *Lichenologist* 30: 427–438.
Otálora, M.A., Aragón, G., Martínez, I. & Wedin, M. (2013) Cardinal characters on a slippery slope—a re-evaluation of phylogeny, character evolution, and evolutionary rates in the jelly lichens (Collemataceae s. str). *Molecular Phylogenetics and Evolution* 68:

185–198.
https:/doi.org/10.1016/j.ympev.2013.04.004

Parnmen, S., Rangsiruji, A., Mongkolsuk, P., Boonpragob, K., Elix, J.A. & Lumbsch, H.T. (2010) Morphological disparity in Cladoniaceae: The foliose genus *Heterodea* evolved from fruticose *Cladia* species (Lecanorales, lichenized Ascomycota). *Taxon* 59: 841–849.

Prieto, M., Baloch, E., Tehler, A. & Wedin, M. (2013) Mazaedium evolution in the Ascomycota (fungi) and the classification of mazaediate groups of formerly unclear relationship. *Cladistics* 29:296–308.
https:/doi.org/10.1111/j.1096-0031.2012.00429.x

Printzen, C. (2010) Lichen systematics: the role of morphological and molecular data to reconstruct phylogenetic relationships. *In*: Lüttge, U., Beysch, W., Büdel, B. & Francis, D. (Eds.) *Progress in botany*, vol 71, pp. 233–279.
https:/doi.org/10.1007/978-3-642-02167-1_10

Rambaut, A. & Drummond, A.J. (2009) Tracer version 1.5. Available from: http://tree.bio.ed.ac.uk/software/tracer/ (accessed 19 December 2016)

Rivas Plata, E. & Lumbsch, H.T. (2011) Parallel evolution and phenotypic disparity in lichenized fungi: a case study in the lichen-forming fungal family Graphidaceae (Ascomycota: Lecanoromycetes: Ostropales). *Molecular Phylogenetics and Evolution* 61: 45–63.
https:/doi.org/10.1016/j.ympev.2011.04.025

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
https:/doi.org/10.1093/bioinformatics/btl446

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology* 57: 758–771.
https:/doi.org/10.1080/10635150802429642

Talavera, G. & Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56: 564–577.
https:/doi.org/10.1080/10635150701472164

Tehler, A. & Irestedt, M. (2007) Parallel evolution of lichen growth forms in the family Roccellaceae (Arthoniales, Ascomycota). *Cladistics* 23: 432–454.
https:/doi.org/10.1111/j.1096-0031.2007.00156.x

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.
https:/doi.org/10.1111/j.1756-1051.2012.00008.x

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.
https:/doi.org/10.1128/jb.172.8.4238-4246.1990

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, CA: Academic Press.

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.