DIVERSITY AND MOLECULAR PHYLOGENETIC ANALYSES OF PARMELIOID LICHENS (PARMELIACEAE, ASCOMYCOTA) IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or for any other award.

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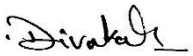

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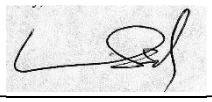

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DEDICATION

I dedicate this work to my family for their love and unwavering support; my wife Jennifer Muigai and our daughters Wambui, Wairimu and Mumbi. You have always been the source of my inspiration and have always given me the reason to push on.

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ABBREVIATIONS AND ACRONYMS

BEAST	Bayesian Evolutionary Analysis Sampling Trees (software)
BI	Bayesian Inference
BM	British Museum
B/MCMC	Bayesian / Markov Chain Monte Carlo
DNA	Deoxyribonucleic acid
EA	East African Herbarium
ELW	Expected Likelihood Weight
ESS	Effective Sample Size
F	The Field Museum of Natural History, Chicago, USA
HPTLC	High Performance Thin Layer Chromatography
ITS	Internal Transcribed Spacer
LSU	Large Subunit
MAF	Herbarium Complutense University of Madrid, Spain
MAFFT	Multiple Alignment using Fast Fourier Transform-for sequences alignment
MCC	Maximum Clade Credibility
MB	Mycobank
ML	Maximum Likelihood
mtDNA	Mitochondrial DNA
mtSSU	Mitochondrial Small Subunit DNA
nuLSU	nuclear Large Subunit
PCR	Polymerase Chain Reaction

PP	Posterior Probability
RAxML	Randomized Axelerated Maximum Likelihood- Programme
rDNA	Ribosomal DNA
SH	Shimodaira–Hasegawa
S.lat.	*Sensu lato*
S.str.	*Sensu stricto*
TLC	Thin Layer Chromatography

ABSTRACT

Lichens are symbiotic associations of fungi and algae or cyanobacteria.They are ecologically important as bioindicators for air pollution, forest age and health, and as sources of food, shelter and nesting materials for animals. Man has historically used them for dyes, medicines, poisons and in the manufacture of perfumes and antibiotics. Globally there are an estimated 28,000 species of lichens. Parmeliaceae is the largest family with over 2800 species in about 80 genera. Parmelioid clade is the largest group comprising 75% of the total number of species in the family. About 180 species of parmelioid lichens distributed in 19 genera are known for Kenya. The advent of molecular tools has shown that, the morphological and chemical characters traditionally used to delimit taxa in lichens underestimate diversity. Application of molecular data has therefore become a prerequisite while making taxonomic evaluations in this group. Molecular DNA sequence data were used to evaluate the phylogenetic relationships and to re-asses traditional phenotype-based taxon delimitation in *Bulborrhizina*, *Bulbothrix, Canoparmelia, Hypotrachyna, Parmelinella, Relicina* and *Relicinopsis*. Seventy seven samples were collected from 5 floral regions in Kenya and duplicates deposited in EA, F and MAF herbaria. Genomic DNA were extracted from newly collected samples and the ITS, nuLSU and mtSSU regions of rDNA sequenced using Sanger sequencing approach. To infer phylogenetic relationships, 6 datasets of concatenated ITS, nuLSU and mtSSU comprising 746 DNA sequences were analyzed using ML and BI methods. Three species, *Bulbothrix kenyana, Hypotrachyna himalayana* and *Parmelinella schimperiana* were described as new to science, and taxonomic re-evaluation of 22 taxa carried out. Eight new combinations were proposed: *Bulbothrix sublaevigatoides, Parmotrema epileucum, P. zimbabwense, Relicina dahlii, R. intertexta, R. malaccensis, R. rahengensis* and *R. stevensiae.* The monotypic genus *Bulborrhizina* had not been studied previously using molecular data and its phylogenetic position hitherto unknown. DNA sequences of *Bulborrhizina africana* were analyzed with 95 other samples of parmelioid lichens. In the resultant phylogenetic tree, *B. africana* clustered with *Bulbothrix*, in the *Parmelina* clade. Species boundaries in *Bulbothrix isidiza* and *B. tabacina*, two pantropical and asexually reproducing species were re-examined using mutilocus dataset of *Bulbothrix* specimens from E. Africa, Asia and S. America. Five species-level lineages in *Bulbothrix isidiza s.lat.* and three in *B. tabacina s.lat* were recovered. Alternative hypothesis testing using SH and ELW tests significantly rejected monophyly of *B. isidiza* and *B. tabacina,* respectively. In the phylogenetic analysis of *Canoparmelia s.lat*. the genus was recovered as polyphyletic with three divergent lineages, two formed a sister group relationship with *Parmotrema.* Consequently they were included in *Parmotrema* and recognized at subgeneric level as *Parmotrema* subgenus *Africanae* and *Crespoa,* the former described as new. Genetic diversity of the pantropical sorediate species *Hypotrachyna sorocheila* was assessed with the resultant phylogeny forming two distinct species-level lineages.The pantropical species *Parmelinella wallichiana s.lat*. was assessed, samples of *P. wallichiana* were recovered in four well-supported clades. Evolutionary relationships of *Relicina* and *Relicinopsis* were elucidated; *Relicina* was recovered nested with *Relicinopsis*. However, based on differences in conidia, *Relicinopsis* is accepted at a subgenus rank as *Relicina* subgen. *Relicinopsis*. The use of DNA sequence data in understanding true diversity and biogeography in Parmeliaceae is underscored. A wider taxon sampling is recommended for the lineages that remain undescribed in this study.

CHAPTER 1

INTRODUCTION

1.1 Background Information

Fungi are heterotrophic eukaryotic organisms, usually filamentous and devoid of chlorophyll, they possess chitinous cell walls and produce spores. Fungi are able to enter into symbiotic relationships with other organisms (Nash, 2008). Lichens are symbiotic phenotypes of lichen forming-fungi (mycobiont) and algae or cyanobacteria (photobiont). In the lichen symbiosis, green algae in the genus *Trebouxia* Puymaly are the most common photobionts and less often species of *Trentepohlia* Martius (Lumbsch, 2007), while the most common cyanobacteria in these associations is *Nostoc* Vaucher ex Bornet and Flahault (Rikkinen *et al*., 2013). The lichen association is thought to be mutualistic with the photobiont, which contains chlorophyll and thus provides energy for growth and development of the lichen while the fungus protects the photosynthetic partner from high insolation in addition to possibly aiding in water and mineral uptake. Lichens therefore exist as autotrophic organisms similar to green plants (Will-Wolf *et al*., 2004). Lichens are poikilohydric, lacking any water storage system, consequently their water status is determined by the prevailing environmental conditions, thus are able to survive long periods of dry conditions in a dormant state (Nash, 2008). Lichens are therefore unique in nature and highly sensitive to climatic conditions making them ideal bioindicators for atmospheric pollution and climate change (Crespo *et al*., 2004; Root *et al*., 2014)

Most lichen fungi are species-specific in their choice of the photosynthetic partner (algae or cyanobacteria) (Brodo *et al*., 2001). However some exceptions exists where; (i) the same lichen in different geographical locations may contain different species of algae; (ii)

some lichen species may contain different algal species during different stages of their life cycles; (iii) another fungus growing on a lichen may share the alga of its host and later swap it for another species; (iv) some lichen thalli may contain both algae and cyanobacteria leading to the existence of the same lichen in different forms (photomorphs) depending on the photobiont it contains (Purvis, 2000).

The most common lichens are those with flat leaf-like thalli (foliose lichens). Their thallus has a dorsiventral organization and often partially attached to the substrate by hyphae of the lower cortex or by rhizines. The thallus is often irregularly lobed, and variable in their size and diversity. Traditionally, the shape of the lobes, their length and width and their configuration are important characters in distinguishing lichen species.

Lichens reproduce sexually through production of ascospores by the mycobiont, however for the successful establishment of lichen symbiosis; the germinating spores must meet with the proper photosynthetic partner (algae or cyanobacteria) (Nash, 2008). Lichens also reproduce vegetatively (asexually) through the production of special structures containing both the fungus and the photobiont, most commonly being soredia and isidia. These structures form important characters in lichen classification (Brodo *et al*., 2001).

Lichens produce an array of unique and diverse chemical extrolites, which are produced by the fungal component and deposited on the surface of the hyphae (Brodo *et al*., 2001). Each lichen species usually has a constant chemical makeup, however in some cases, species that are identical in appearance may have different chemistry ("chemical species") and can only be distinguished by chemical tests (Hale, 1979). Thus the presence

or absence of specific extrolites provides a useful and practical means of identifying lichens (Hale, 1979; Lumbsch, 1998b).

The total number of fungi worldwide has been estimated at 1.5 million species (Hawksworth, 1991, 2001), whilst the number of currently described species is 100,000 (Kirk *et al*., 2009). Globally there are an estimated 28,000 species of lichens occurring in all terrestrial habitats and in all continents (Thell *et al*., 2012) of these about 16,000 species has been described to date (Lücking *et al*., 2009), consequently a large number of lichens species are yet to be documented (Lumbsch and Leavitt, 2011). So far about 800 species are reported for Kenya (Swinscow and Krog, 1988; Kirika and Lumbsh, 2011; Kirika *et al*., 2012). Majority of the undescribed species are thought to be largely found in the poorly studied areas, especially in the tropical regions of the world (Sipman and Aptroot, 2001; Hawksworth, 2012). In addition there is a growing body of evidence particularly in lichens, suggesting that the current phenotype-based method of species recognition actually underestimates the number of species (Lumbsch and Leavitt, 2011).

The vast majority of lichenized fungi are ascomycetes (98%) and only 2% are basidiomycetes (Purvis, 2000). Ascomycetes are distinguished by the production of reproductive meiospores (ascospores) contained in small sac-like structures called asci (Taylor *et al*., 2000). Lichens are classified in about 60 orders, but the largest order of lichen-forming fungi is Lecanorales, comprising about 20 families. Within this order Parmeliaceae is indeed the largest family of lichen-forming fungi with over 2800 species in about 80 genera (Thell *et al*., 2012). The family has a worldwide distribution and includes mostly macrolichens. The family is megadiverse in the tropical ecosystems and includes many common and well-known species frequently used as bioindicators for

atmospheric pollution (Crespo *et al*., 2004). Parmelioid lichens are the largest group within Parmeliaceae (75%) with over 1800 species in 27 genera, with their centers of distribution in the Southern Hemisphere (Crespo *et al.*, 2010b).

Parmelioid lichens are phenotypically characterized by having foliose thalli with rhizines on the lower surface, cup-shaped apothecia on the thallus upper surface and *Lecanora*-type asci with hyaline ascospores. The most speciose genera within the group include *Hypotrachyna* (Vainio) Hale, *Parmotrema* Massal. and *Xanthoparmelia* (Vainio) Hale. In Kenya, parmelioid lichens are the most speciose group comprising about 180 species distributed in 19 genera (Swinscow and Krog, 1988).

Traditionally, the classification of lichens is based on morphological, anatomical and/or chemical characters and new taxa are usually described based on these features (Purvis *et al*. 1992; Divakar and Upreti, 2005). However the advent of the polymerase chain reaction (PCR) and the subsequent ease of obtaining DNA sequences have greatly enhanced our knowledge and understanding of lichen forming-fungi (Lumbsch, 2007). Studies using DNA sequence data have in some cases shown incongruence between the conventional phenotype-based species circumscription and molecular phylogenetic reconstruction (Lumbsch and Leavitt, 2011). Often morphological or chemical differences in lichens have been interpreted as intraspecific variability, however, molecular phylogenetic reconstruction studies have often recovered numerous distinct lineages hidden under a single species name as traditionally described using morphology (see e.g. Grube and Kroken, 2000; Molina *et al*., 2004; Arguello *et al*., 2007; Baloch and Grube, 2009). Further re-examination of morphology with the background of a molecular phylogenetic estimate often revealed, subtle, morphological and/or chemical characters

supporting the distinction of these lineages at species level (Molina *et al*., 2004; Divakar *et al*., 2005a, b; Arguello *et al*., 2007). Further, cryptic species in which no morphological characters could be used to distinguish distinct lineages can now be separated using molecular data (Wirtz *et al*., 2008; Crespo and Lumbsch, 2010; Leavitt *et al*., 2016).

Lichens are important bioindicators for air pollution, forest age and health, and soil quality (Hawksworth, 1971; Nash and Gries, 1991; Richardson, 1991; Nash and Gries, 2002). Indeed lichens are among the most widely used biomonitors in the terrestrial environment (Nimis *et al.*, 2002). Thus, today, there is an increasing interest in the documentation of lichen diversity to further understand their role in the ecosystems and because of their pharmacological potential or use as biocontrol agents (Huneck and Yoshimura, 1996; Gómez-Serranillos *et al*., 2014). This study is therefore aimed at increasing our current knowledge of parmelioid lichens in Kenya by reassessing their diversity as currently circumscribed using morphology and chemistry with the background of DNA molecular data.

1.2.1 Statement of the Problem

In contrast to many groups of lichen-forming fungal species in temperate regions, which are relatively well studied (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011), tropical species are generally less explored (Parnmen *et al*., 2012; Kraichak *et al*., 2015). Indeed, several tropical regions (e.g. Africa) are biodiversity hotspots and therefore likely to harbor a large number of lichen forming fungal species yet they are least studied (Hawksworth, 2012). While undescribed species of lichenized fungi are expected to be found in unexplored regions, there is also a growing body of

evidence through molecular phylogenetic studies indicating that numerous distinct species-level lineages may also be hidden under a single nominal taxon (reviewed in Leavitt *et al*., 2015b). DNA sequence data coupled with empirical species delimitation methods have advanced our knowledge of species boundaries in lichen forming fungi (reviewed in Leavitt *et al*., 2015b). In a number of cases, phenotype-based approaches to species recognition have been shown to underestimate the number of species in lichenized fungi. For instance, in the family Parmeliaceae alone, Crespo and Lumbsch (2010) have estimated at least 80 cryptic lineages hidden under widely distributed or disjunct species. Based on this new perspective, species delimitation studies and taxonomic revisions now commonly incorporate molecular sequence data (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011; Leavitt *et al*., 2015b).

1.2.2 Justification

Lichens are ecologically important, providing food for animals, shelter and nesting materials, while humans have historically used them as food, clothing, dyes, perfume additives, medicines, poisons and tannins. Lichens are highly sensitive to climatic conditions due to their poikilohydric nature making them ideal organisms for biomonitoring of air pollution and provide effective monitoring system for climate change studies (Ellis *et al*., 2007). Accurate knowledge of lichen diversity and species delimitations in this group is therefore critical.

The advent of DNA sequence technologies and advances in molecular phylogenetic methods have revolutionized our understanding on species delimitation and systematics in lichens and fungi in general (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011; Divakar and Crespo, 2015; Leavitt *et al*., 2015b).

In a number of taxonomic re-evaluations based on molecular phylogenies generated from DNA sequence data have led to the discovery of new phylogenetic species (Crespo *et al*., 2002; Molina *et al*., 2004: Divakar *et al*., 2005a, b, 2010a). Molecular tools have also become vital in the evaluations of the taxonomic significance of morphological characters (Blanco *et al*., 2006; Divakar *et al*., 2006, 2013b; Lumbsch, 2007; Crespo *et al*., 2007). The use of molecular data has therefore emerged as an important additional character for accurate species assessment.

Further, in fungi and lichens, biogeographical patterns have largely been neglected, with researchers accepting wide distribution ranges for species (Lumbsch and Leavitt, 2011). However this may be as a result of inappropriate methods of species recognition (Weisse and Rammer, 2006; Chantangsi *et al*., 2007; Lumbsch and Leavitt, 2011) as revealed by a number of studies (Burt *et al*., 1996; Geiser *et al*., 1998; Taylor *et al*., 2000; Papke *et al*., 2003; Whitaker *et al*., 2003) based on molecular DNA sequence data.

This study is therefore an attempt to better understand the diversity, distribution and phylogenetic relationships of parmelioid lichens with congeners from African populations using molecular DNA sequence data coupled with analytical tools.

1.3 Hypotheses

i. The diversity and phylogenetic relationships of parmelioid lichens in the tropics is currently not well known.
ii. Traditional methods of species recognition using morphology and chemistry greately underestimate the true species diversity in Parmeliaceae.

iii. In Parmeliaceae, vegetatively reproducing species with pantropical or disjunct biogeographic distribution may actually consist of different independent species in different geographic regions.

iv. The vegetative characters traditionally used to circumscribe taxa in Parmeliaceae are homoplacious and therefore phylogenetically invaluable.

1.4 Research questions

i. What is the phylogenetic relationship of parmelioid lichens in Kenya?

ii. Does the traditional method of species delimitation using morphology and chemistry represent the true diversity in Parmeliaceae?

iii. Do isidiate and sorediate species with a pantropical and/or disjunct geographic patterns of distribution represent monophyletic groups?

iv. Are the vegetative characters used in delimitation of taxa in Parmeliaceae phylogenetically valuable?

1.5 Objectives

1. 5.1 General Objective

This study aimed to evaluate the evolutionary relationships, species delimitations, biogeographic patterns and to reassess the taxonomic importance of some morphological and chemical features traditionally used in species delimitation within parmelioid lichens using molecular DNA sequence data.

1.5.2 Specific Objectives

i. To infer the evolutionary relationships of the Kenyan populations of Parmelioid lichens using molecular DNA sequence data.

ii. To re-asses species boundaries of morphologically delimited species in Parmeliaceae, using DNA sequence data.

iii. To review the biogeographic distribution of some vegetatively reproducing species with pantropical or disjunct distribution patterns.

iv. To re-examine the taxonomic importance of morphological features traditionally used to characterize genera and species of Parmelioid lichens using molecular data.

1.6 Significance and Expected Outputs

This study will enhance our knowledge of the diversity of lichen mycobiota in the tropics. In addition to providing important baseline information and data that may be useful for better management of biodiversity in tropical ecosystems and for the future detection and monitoring of environmental changes using lichens.

This study will provide a better understanding of the evolutionally relationships in parmelioid lichens and enhance our knowledge of species diversity and biogeographic distribution of taxa in Parmeliaceae. Collected specimens will greatly enrich the lichen research collection with additional herbarium voucher specimens. Further, new DNA sequences will be generated and deposited in public resporitory databases e.g. NCBI Genbank. This will provide a baseline data for DNA barcoding of tropical taxa in addition to providing correctly identified sequences for further phylogenetic analyses. It is also expected that novel taxa will be discovered and described.

CHAPTER 2

LITERATURE REVIEW

2.1 Molecular Phylogenetic Relationships of Parmeliaceae

Traditionally, systematics in lichens is based on morphological, anatomical and/or chemical characters and new taxa are usually described based on these characters (Purvis *et al*., 1992; Divakar and Upreti, 2005). However molecular sequence data are today largely used to evaluate traditional, phenotype-based species delimitations in lichen-forming fungi. The use of DNA data in re-assessing species circumscriptions have often helped reveal distinct independent lineages in widely distributed morphological species (Molina *et al*., 2004; Divakar *et al*., 2005a and b; Muggia *et al*., 2008; Wirtz *et al*., 2008; Wedin *et al*., 2009; Crespo and Lumbsch, 2010). Todate, the use of molecular data has therefore emerged as an important additional character for accurate species assessment.

Parmeliaceae is the largest family among the lichen-forming ascomycetes. Within the family, six strongly supported monophyletic groups are distinguished, namely; alectorioid, cetrarioid, hypogymnioid, letharioid, parmelioid and psiloparmelioid (Amo de Paz *et al*., 2011; Crespo *et al*., 2007; Divakar *et al*., 2015). Parmelioid lichens are the largest group and constitute a monophyletic clade (Fig. 2.1). They include species that are mainly foliose, mostly rhizinate, with laminal lecanorine-type of apothecia and simple hyaline ascospores (Crespo *et al*., 2007). They have a worldwide distribution with the center of diversity in oceanic temperate, tropical, and subtropical ecosystems (Blanco *et al*., 2005). Phenotypical characters used in the taxonomy of Parmeliaceae include: the type of the upper cortex, pseudocyphellae, cell-wall polysaccharides, apothecial and spore characters, pycnidial and conidial traits, secondary thalline characters, medullary

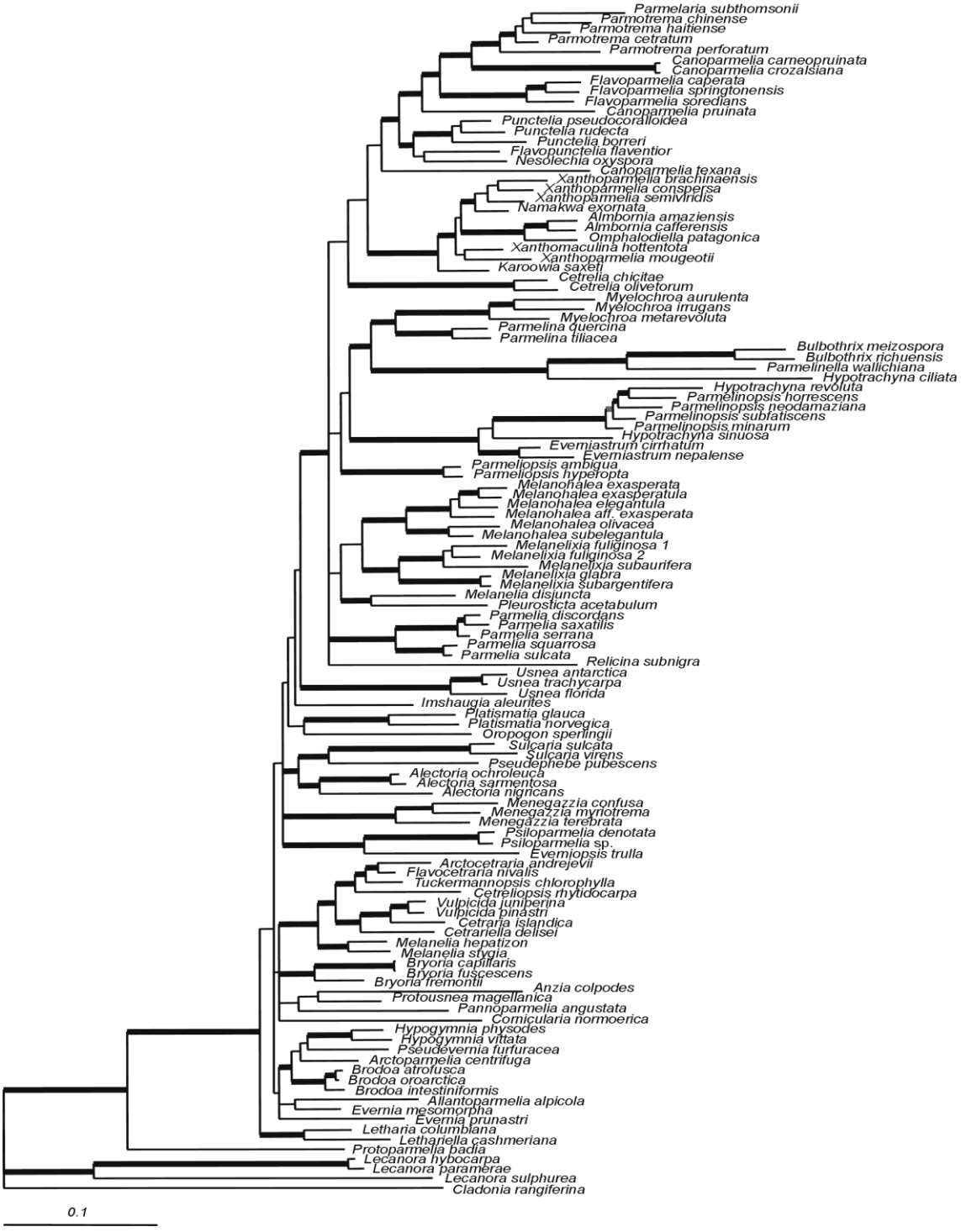

Figure 2.1. Phylogenetic relationships of Parmeliaceae inferred from a combined analysis of nuclear ITS, LSU, mitochondrial SSU rDNA, and nuclear RPB1 sequences. Majority-rule consensus tree of 38,000 trees sampled using a Bayesian MC/MCMC analysis. Branches with posterior probabilities above 0.94 and also bootstrap support under parsimony equal or above 70% are indicated in bold (Adopted from Crespo *et al*., 2007).

chemistry, geographical distribution and ecology (Brodo *et al.*, 2001; Crespo *et al.*, 1999; Elix, 1993).

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*., 2010b; 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 and Triebel, 1999). Studies have shown that a core group of parmelioid lichens is monophyletic (Blanco *et al*., 2006; Crespo *et al*., 2007) within which, nine monophyletic clades are distinguished namely; *Cetrelia* W. Culb. & C. Culb., *Hypotrachyna* (Vain.) Hale, *Melanohalea* O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, *Nipponoparmelia* (Kurok.) K.H. Moon *et al*., *Parmelia* Ach., *Parmelina* Hale, *Parmeliopsis* (Stizenb.) Nyl., *Parmotrema* A. Massal. and *Xanthoparmelia* (Vain.) Hale clades (Crespo *et al*., 2010b; Thell *et al*., 2012).

The generic circumscription in Parmeliaceae and in other groups of 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, 1998a, b; Nimis, 1998; Grube and Winka, 2002; Lumbsch, 2007; Printzen, 2010; Crespo *et al*., 2011; Thell *et al*., 2012; Divakar and Crespo, 2015). In a number of studies the 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 and Crespo, 2015). 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; 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, Crespo, Divakar, Essl., Hawksw. & Lumbsch (Blanco *et al*., 2004), *Melanohalea* (Blanco *et al*., 2004) and *Montanelia* Divakar, A. Crespo, Wedin & Essl. (Divakar *et al*., 2012), each segregated from *Melanelia* Essl. (Esslinger, 1978) *s.lat*. (Blanco *et al*. 2004; Divakar *et al*., 2012). Other examples include: *Austroparmelina* (Crespo *et al*., 2010a), which was segregated from *Parmelina* Hale (Hale, 1976d) *Remototrachyna* (Divakar *et al*., 2010a) segregated from *Hypotrachyna* Hale (1974b: 340) *s.lat.* (Divakar *et al*., 2010a); and more recently *Notoparmelia* (Crespo *et al*., 2014) was segregated from *Parmelia* (Acharius, 1803; 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). 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.

Further, molecular sequence data are increasingly being used to test traditional, phenotype-based species delimitations in lichen-forming fungi (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011; Leavitt, *et al*., 2015). While molecular data support phenotype-based species circumscriptions in numerous cases (Tehler and Källersjö, 2001; Pino Bodas *et al*., 2010; Sliwa *et al*., 2012; Kanz *et al*., 2015; Lendemer

et al., 2015), in other instances these data have challenged traditional delimitations (Alors *et al*., 2015; Cornejo and Scheidegger, 2015; Kraichak *et al*., 2015, Singh *et al*., 2015; Pérez-Ortega *et al*., 2016). Subsequent studies based on phylogenetic reconstructions may reveal previously unrecognized phenotypical differences that supported the independence of the distinct species-level clades (Arguello *et al*., 2007; Divakar *et al*., 2010a; Spribille *et al*., 2011; Orange, 2012; Pino-Bodas *et al*., 2012; Schneider *et al*., 2016). Other diverse examples of widely distributed nominal taxa masking multiple, species-level diversity include: *Cladia aggregata* (Parnmen *et al*., 2012), *Melanelixia glabra* (Divakar *et al*., 2010b), *Melanelixia fuliginosa, M. glabratula* (Leavitt *et al*., 2012), *Melanohalea elegantula, M. exasperata* (Leavitt *et al*., 2013a), *Montanelia tominii* (Leavitt *et al.,* 2015a), *Parmelia saxatilis* (Molina *et al.,* 2011a), *P. sulcata* (Divakar *et al*., 2005b; Molina *et al*., 2011b), *Parmelina quercina* (Argüello *et al*., 2007), *P. tiliacea* (Nuñez-Zapata *et al*., 2011), *Parmotrema reticulatum* (Del-Prado *et al*., 2011), *Protoparmelia badia* (Singh *et al*., 2015), *Physconia distorta* (Divakar *et al.,* 2007), *Rhizoplaca melanophthalma* (Leavitt *et al*., 2013b), and *Sphaerophorus globosus* (Högnabba and Wedin, 2003). These studies demonstrate that some supposedly cosmopolitan taxa may include previously unrecognized diversity. Thus re-evaluation of widely distributed species applying molecular sequence data is prerequisite to understanding biogeographic patterns in broadly distributed taxa. Furthermore, inaccurate species assessment may have negative consequences for conservation purposes or understanding of diversification patterns.

Further, despite the progress made in understanding the 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*., 2010b). Further congeners from the African populations have largely not been included in these analyses.

2.2.1 *Bulborrhizina* Kurok.

Among the genera of parmelioid lichens that have not yet been studied using molecular markers include, *Bulborrhizina* Kurok. and *Parmotremopsis* Elix & Hale. The genus *Pseudoparmelia* Lynge had not been included in molecular phylogenetic studies; however, recently *Pseudoparmelia* Lynge was found to be a distinct genus related to *Relicina* (Hale & Kurok.) Hale and *Relicinopsis* Elix & Verdon (Buaruang, *et al*., 2015).

Bulborrhizina africana Kurok. 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.,* 2013a), since both genera have linear elongate lobes. *Bulborrhizina* was said to differ in having 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 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.*, 2010b). 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; Divakar *et al.*, 2010a).

On a recent field trip to south-eastern Kenya, fresh material of the monotypic genus *Bulborrhizina* were collected, representing a new record of the species for Kenya and the second only known population of *B. africana*. With the fresh material of *Bulborrhizina* available, DNA sequence data were generated 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, A. Crespo, Sipman, Elix & Lumbsch or species with bulbate cilia as found in the two *Bulbothrix* clades or the genus *Relicina.*

2.2.2 *Bulbothrix* Hale

Bulbothrix Hale, is one of the tropical lichen forming fungal genera belonging to the parmelioid core of Parmeliaceae (Crespo *et al*., 2010b; Divakar *et al*., 2015). The genus includes ca. 60 species, widespread in tropical regions reaching its highest diversity in semi-arid woodlands and secondary forests in the Neotropics (Hale, 1976a; Swinscow and Krog, 1988; Elix, 1994; Benatti, 2010, 2012a). Species in *Bulbothrix* are characterized by a small foliose thallus, corticate above and below, laciniate and usually adnate to loosely attached to their substrate. The species have bulbate marginal cilia, an upper cortex consisting of a palisade plectenchyma with a pored epicortex and with

isolichenan in the cell walls, and a whitish to brownish mineral gray upper cortex. The cilia and rhizinae are simple to branched. The apothecia are smooth to coronate imperforate, containing hyaline unicellular ellipsoid to bicornute ascospores, 5.0−21.0 × 4.0−12.0 µm. The conidia are bacilliform to bifusiform, 5.0−10.0× 0.5− 1.0 µm (Hale, 1976a; Elix, 1993; Elix, 1994; Divakar and Upreti, 2005; Benatti, 2010). *Bulbothrix* was initially segregated from *Parmelia* Ach., on the basis of black bulbate marginal cilia (Hale, 1974).

Bulbothrix is morphologically similar to *Relicina* (Hale & Kurok.) Hale, chiefly differing in cortical chemistry with the former having atranorin and the latter usnic acid (Hale, 1974). However, 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.,* 2010b). The taxonomic history of these taxa underscores many of the challenges of circumscribing natural groups and inferring evolutionary relationships based on morphological characters alone.

The genus *Bulbothrix* as currently circumscribed does not form a monophyletic lineage, but is paraphyletic with *Parmelinella* Elix & Hale, nested within (Divakar *et al*., 2006). Two distinct lineages are currently identified: a predominantly neotropical clade and a predominantly paleotropical clade, the latter being sister to *Parmelinella* (Divakar *et al*., 2010a). Additionally, studies have shown that some species grouped in these clades are not monophyletic (Divakar *et al*., 2010a; Kirika *et al*., 2015). However, such studies have been limited by sparse taxonomic and specimen sampling, and since the phylogenetic position of the type species of *Bulbothrix* is currently unknown, no taxonomic conclusions have yet been drawn.

Bulbothrix isidiza (Nyl.) Hale and *B. tabacina* (Mont. & Bosch) Hale, belong to the predominantly paleotropical clade of this genus (Divakar *et al*., 2010a). Both species are commonly found occurring in seasonally wet and secondary forests in Africa and throughout the tropics (Hale, 1976a; Benatti, 2013). They grow either on tree bark or on siliceous rocks and are characterized by the presence of asexual reproductive structures, isidia which are cylindrical and pale. They also have subirregular lobes, and the thallus has simple rhizines on the lower surface and contains atranorin and salazinic acid. *B. isidiza* differs from *B. tabacina*, mainly in having a pale brown lower surface with concolorous rhizines (Hale, 1976a; Benatti, 2013).

This study aims to re-examine the species boundaries of these two widespread isidiate species occurring in tropical regions – *Bulbothrix isidiza* and *B. tabacina*. Specifically, aiming to answer the following questions; i) do the samples of *B. isidiza* and *B. tabacina*, each form monophyletic groups? and ii) do populations distributed in disjunct geographic and/or ecological regions correspond to distinct lineages?

2.2.3 *Canoparmelia* Elix & Hale

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, and 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). More recently the latter was raised to generic level as *Crespoa* (D. Hawksw.) Lendemer and Hodkinson (2012: 3).

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, aiming to answer 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?

2.2.4. *Hypotrachyna* subgenus *Everniastrum* (Hale ex Sipman) Divakar *et al.*

Lichens that reproduce by means of vegetative propagules, e.g., isidia and soredia, are generally thought to have broad geographic distributions. However, some widely distributed nominal lichenized fungal species may consist of several distinct evolutionary lineages, inferred using information from DNA sequence data. Furthermore, species separated based on the presence or absence of soredia have commonly been shown to be conspecific (Buschbom and Mueller, 2006; Divakar *et al*., 2007; Wirtz *et al.*, 2012; Truong *et al*., 2013), although other studies support the independence of otherwise morphologically similar phenotypes with differing reproductive strategies as distinct species (Lücking *et al*., 2008; Cornejo *et al*., 2009). These studies imply that careful, case-by-case consideration may be required to accurately circumscribe species boundaries in well-known lichens with broad distributions.

The genus *Everniastrum* Hale ex Sipman was described by Hale (1976c) to include species formerly classified in *Parmelia* subgenus *Everniiformis.* In a recent molecular phylogenetic analysis of *Hypotrachyna s.lat*., the genus *Everniastrum* was found to be nested within the Hypotrachyna clade, subsequently reduced to synonymy with *Hypotrachyna*, and classified as a subgenus within *Hypotrachyna* (Divakar *et al*., 2013a). Recognition at the subgeneric level had the advantage that monophyletic lineages clustered within a paraphyletic *Hypotrachyna s.lat*. can be recognized without producing paraphyletic taxa (Hörandl and Stuessy, 2010).

Hypotrachyna subgenus *Everniastrum* (Hale ex Sipman) Divakar *et al*. comprises about 33 species (Divakar *et al.,* 2013a). Species in this subgenus are characterized by long, linear thalli with subcanaliculate to involute lobes with or without long marginal cilia and

rhizines. They have isolichenan in their cell walls, a pored epicortex and a palisade like upper cortex (Hale, 1973; Crespo *et al*., 2007). In addition, all species of this genus have atranorin in the cortex and most contain salazinic acid in the medulla, often accompanied by protolichesterinic acid. Other secondary extrolites, including alectoronic, constictic, fumarprotocetraric, gyrophoric, norstictic, and protocetraric acids, have also been reported (Hale and Wirth, 1971; Sipman, 1980; Culberson and Culberson, 1981; Sipman, 1986).

In *Hypotrachyna* subgen. *Everniastrum* five of the 33 described species form soredia (Sipman, 1986); and *Hypotrachyna sorocheila* is the only widely distributed sorediate species in the subgenus. This taxon is known from the tropics and warm temperate regions of both the Old and New Worlds. Other sorediate species in the group include: *Hypotrachyna catawbiensis, H. columbiensis*, *H. plana* and *H. subplana*. *Hypotrachyna catawbiensis* occurs in South America, eastern North America, Papua New Guinea and East Africa; whereas *H. columbiensis*, *H. plana* and *H. subplana* are only known from South America. *Hypotrachyna plana* has been considered a synonym of *H. catawbiensis* based on morphological variation in lobe morphology, marginal rhizines size and position of soralia on lobe tips (Culberson and Culberson, 1981). However, this has not been accepted by other authors (e.g. Sipman, 1986).

This study aims to elucidate evolutionary relationships in the widely distributed sorediate taxon *H. sorocheila* and to better understand the overall diversity of sorediate species in subgenus *Everniastrum*.

2.2.5. *Parmelinella* Elix & Hale

Parmelinella is a small genus (ca. 10 species) and belongs to the parmelioid clade in the family Parmeliaceae (Divakar *et al*., 2015). The species included in this genus are characterized by a pored epicortex, isolichenan in the cell walls, subirregular lobes, cylindrical or bifusiform conidia, simple cilia and rhizines, and a yellow-grey upper cortex - containing secalonic acid derivatives and atranorin (Elix, 1993; Crespo *et al*., 2010b). Species in the genus are mainly distributed in subtropical to tropical regions of Africa, Asia, Australasia and South America. *Parmelinella chozoubae, P. manipurensis* and *P. nimandairana* are restricted to Asia; *P. salacinifera*, is reported from Southeast USA, central and south America, and Thailand; *P. simplicior* occurs in Asia and East Africa; and *P. cinerascens, P. lindmanii, P. mutata* and *P. versiformis* are endemic to South America (Elisaro *et al*., 2010; Benatti, 2014). For a long time only four additional *Parmelinella* species were known from India (Divakar and Upreti, 2005), but recent studies added six species to the genus, most of which had previously been known to occur only in South America (Elisaro *et al*., 2010; Benatti, 2014). Of the ten species, only two, *P. simplicior* and *P. wallichiana*, have previously been reported from East Africa (Swinscow and Krog, 1988; Alstrup *et al*., 2010).

Parmelinella wallichiana is the only widely distributed species in this genus and is known from Africa, Asia, Australia and South America. While it is widespread in East Africa and Asia, the species is known from a few localities in Australia and South America. *Parmelinella wallichiana* normally reproduces asexually by isidia and grows in wide range of ecological environments. The species is most frequently epiphytic but also found rarely on rocks. Studies have demonstrated broad, intercontinental distributions of

a number of lichen-forming fungi that reproduce via asexual propagules (see e.g. Divakar *et al*., 2005b; Molina *et al*., 2011a and b; Leavitt *et al*., 2013a; Roca-Valiente *et al*., 2013; Divakar *et al*., 2016)

This study aims to assess biogeographic patterns in the widely distributed, isidiate, lichen-forming fungal species *Parmelinella wallichiana* and re-evaluate the phenotypical features in the light of relationships inferred from molecular phylogenetic reconstructions.

2.2.6. *Relicina* (Hale & Kurok.) and *Relicinopsis* Elix & Verdon

Currently in Parmeliaceae about 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*., 2010b; Divakar *et al*., 2015). The evolutionary relationships of those 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 cortical extrolite (Elix *et al.*, 1986). This genus includes a total of five species, which are widely distributed in Southeast Asia and Australasia (Hale, 1976b; Elix, 1993, 1994; Divakar and Upreti, 2005). The genus *Relicina* was segregated from *Parmelia* Ach. *s.lat.* (Hale, 1974) based on having bulbate marginal cilia, bifusiform conidia, and containing usnic acid in the upper cortex. This genus includes ca. 54 species (Thell *et al*., 2012), with a centre of distribution in Southeast 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*. (2010b).

In this study, an extended taxon sampling is used to: i) examine the monophyly of *Relicina* and *Relicinopsis*, and ii) evaluate the taxonomic significance of phenotypic features in these two genera.

CHAPTER 3

MATERIALS AND METHODS

3.1 Specimens and DNA sequences sampled.

Kenya has diverse and unique ecoregions, on one hand, it has afro-montane ecoregions, which are generally cooler and more humid than the surrounding lowlands, and on the other hand it has tropical moist forest regions in the western part and along the east coast. Specimens for this study were collected randomly from different ecological regions in Kenya, including the montane and afro-montane regions of the Aberdares and Mt. Kenya in Nyeri and Meru Counties, Mt. Elgon in Trans Nzioa County and in Bomet, Nakuru, Baringo and Kajiado Counties in the Rift Valley, the inselbergs in south eastern Kenya in Kitui, Makueni and Taita Taveta Counties and in the Coastal forests of Kwale and Kilifi Counties (Plate. 3.1). Seventy seven voucher specimens were collected in triplicates using standard methods (Brodo *et al*., 2001) and copies of each voucher specimen were deposited in the East African Herbarium (EA) while duplicates were sent to the Field Museum, Chicago USA (F) and Complutense University of Madrid (MAF), Spain. Further, addition samples of parmelioid lichens from different tropical regions in Africa, South America and Asia were borrowed and later analyzed. Collected samples were identified at species level following the standard identification keys and monographs.

DNA sequences from previous studies were downloaded from the GenBank database website, the National Centre for Biotechnology Information (NCBI) at; https://blast.ncbi.nlm.nih.gov/ Blast.cgi - 2015

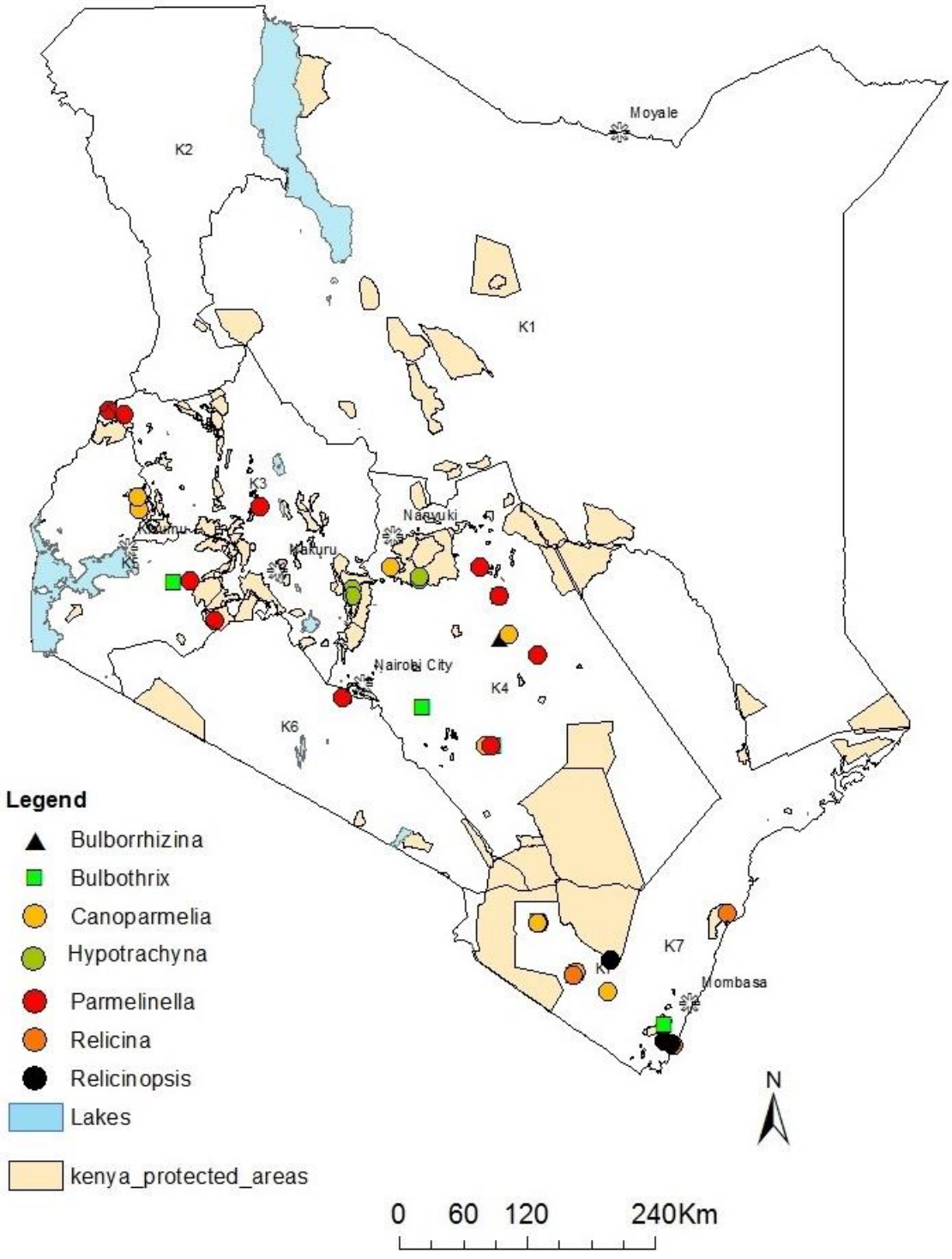

Plate 3.1. Map of Kenya Showing the Floral Regions where Specimens of the Six genera Studied were Collected.

3.2 Morphological and Chemical Studies

Morphological characters, including lobe shape, size and width, cilia and rhizines, observation of maculae were done using a dissecting microscope. Observations and measurements of ascospores were made in water, at ×40 (objective) and ×10 (eye piece) magnification with a Leica DM RB microscope. Initial morphological studies were done at the EA herbarium and further investigations at the Field Museum, Chicago (USA) and at the Department of Biology II, University of Madrid (Spain).

Chemical extrolites were identified by thin layer chromatography using standard methods (Arup *et al*., 1993; Orange *et al.,* 2010). Extraction of secondary metabolites for TLC analysis was done by placing small pieces of the thallus in 1.5 ml Eppendorf tubes. A few drops of acetone were then added in the tube and then left for about 10 minutes. The resulting extract was then spotted on glass plates coated with silica gel using capillary tubes. Plates were developed in TLC tanks or in Camag horizontal developing chamber (Oleico Lab Stockholm) using solvent system A (Toluene:Dioxane:acetic acid, 45:15:2) and B (Toluene-acetic acid, 170:30), plates were then air dried, sprayed with 10% sulphuric acid and then heated in an oven at 110 degrees Celsius to visualize the spots. Identification of the spots was done by comparing the spots with controls (Arup *et al*., 1993; Orange *et al*., 2010).

3.3 DNA Extraction and PCR Amplification

DNA was extracted from 78 samples, 50 from specimens collected in Kenyan and 28 were borrowed from other tropical regions. 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. DNA extraction was done at at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, in USA and at the SYSTEMOL Laboratory, Complutense University of Madrid in Spain.

For the 170 new sequences generated for this study, polymerase-chain-reaction (PCR) amplifications were performed using Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA). Fungal ITS rDNA was amplified using primers ITS1F (Gardes and Bruns, 1993), ITS4 and ITS4A (White *et al*., 1990; Larena *et al.*, 1999); nuLSU rDNA was amplified using LR0R and LR5 (Vilgalys and Hester, 1990); and mtSSU rDNA was amplified using the primers mrSSU1 with either mrSSU3R or mrSSU2R (Zoller *et al*., 1999). The amplifications for ITS and LSU rDNA were carried out in an automatic thermocycler and performed using the following programs: initial denaturation at 94°C for 5 min, and 30 cycles of: 94°C for 1 min, 55–60°C (ITS rDNA) and 60°C (LSU rDNA) for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min. The PCR amplification for mitochondrial rDNA was performed using the following program: initial denaturation at 94°C for 5 min and 35 cycles of: 94°C for 1 min, 57–58°C for 1 min, and 72°C for 1.5 min, and a final extension at 72°C for 5 min.

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.

3.4 Samples used in the phylogenetic analysis of genera

3.4.1 Samples used in the phylogenetic analysis of *Bulborrhizina.*

Freshly collected specimens of *Bulborrhizina africana* were sampled from a robust population recently found in south-eastern Kenya at Yambyu dam area, Mwingi sub-county, Kitui County, 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.

In the phylogenetic analysis of *Bulborrhizina*, a total of 96 specimens representing all major groups in the *Hypotrachyna* and *Parmelina* clades (Crespo *et al.*, 2010b) (Appendix 1) were included. Representatives of the following genera were incorporated: *Bulbothrix s.lat.* (Divakar *et al*., 2006), *Hypotrachyna* – including representatives for each subgenus (Divakar *et al.*, 2013a), *Myelochroa*, *Parmelina*, *Parmelinopsis*, and *Remototrachyna* (Divakar *et al*., 2010a).

3.4.2. Samples used in the phylogenetic analysis of *Bulbothrix.*

Thirty-seven samples representing nine species of *Bulbothrix*, including newly collected samples from East Africa, Asia and South America, were compiled for this study (Appendix 2). A multilocus DNA data matrix was assembled, comprising of the nuclear ribosomal internal transcribed spacer region (ITS) and a fragment of the nuclear ribosomal large subunit (nuLSU), in addition to a fragment of the mitochondrial ribosomal small subunit (mtSSU). The dataset included a total of 85 sequences, 34 from previous studies (Divakar *et al*., 2010a; Divakar *et al.*, 2015; Buaruang *et al*., 2015) and 51 new sequences generated for this study. Three samples of *Parmelinella wallichiana*

were used as an out-group since the genus has been shown to be closely related to *Bulbothrix* (Divakar *et al*., 2006, Divakar *et al*., 2010a; Kirika *et al*., 2015).

3.4.3 Samples used in the phylogenetic analysis of *Canopamelia*.

Data matrices of 65 specimens comprising 51 species from 9 genera of parmelioid lichens were assembled and analyzed. The DNA data matrix comprised nuLSU, ITS and mitochondria SSU rDNA. GenBank accession numbers and information of studied materials are shown in Appendix 3. 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*., 2010b).

3.4.4. Samples used in the phylogenetic analysis of *Hypotrachyna*.

A data matrix comprised of 30 samples, representing 11 species of *Hypotrachyna* subgen. *Everniastrum* (Divakar *et al*., 2013a) was assembled using sequences of nuclear ITS, nuLSU and mitochondrial SSU rDNA. The dataset included numerous samples of *H. sorocheila* from different geographic regions, including Africa, Asia, Australasia, and South America (Appendix 4). GenBank accession numbers and information of studied materials are shown in Appendix 4. The data sets include 30 sequences from previous studies (Divakar *et al*., 2006; Divakar *et al*., 2010a; Divakar *et al*., 2013a), and 43 were newly generated for this study. Two species of *Hypotrachyna*, *H. kaernefeltii* and *H. dubitans* were used as the out-group since they are outside subgenus *Everniastrum.*

3.4.5 Samples used in the phylogenetic analysis of *Parmelinella*.

A DNA data matrix was assembled using sequences of nuclear ITS, nuLSU and mitochondrial SSU rDNA of 21 samples, representing 18 specimens of *P. wallichiana*

s.lat. from Africa, Asia and South America assembled together with DNA sequences of *P.* aff. *wallichiana* and *P. lindmanii* (Elisaro *et al*., 2010) downloaded from the GenBank. GenBank accession numbers and information of studied materials are shown in Appendix 5. The data sets include 12 sequences from previous publications (Blanco *et al.*, 2004; Divakar *et al*., 2006; Divakar *et al*., 2010b: Eliasaro *et al.*, 2010: Kirika *et al*., 2015), and 25 were newly generated for this study. Three specimens of *Bulbothrix isidiza* were used as an out-group since it has been shown to belong to a sister group in a previous study (Kirika *et. al.*, 2015).

3.4.6 Samples used in the phylogenetic analysis of *Relicina* and *Relicinopsis*.

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 those genera collected from East Africa. A multilocus DNA data matrix was assembled, comprised of nuLSU, ITS and mtSSU rDNA to infer evolutionary relationships. The multilocus data set included 7 sequences from a previous study (Buaruang *et al*., 2015), and 14 sequences generated for this study. Three species of *Notoparmelia* were used as the out-group since the genus has been shown to be closely related to *Relicina* (Crespo *et al*., 2010b; Buaruang *et al*., 2015). Information of studied materials, including GenBank accession numbers, is reported in Appendix 6.

3.5 Sequence Editing and Alignment

New sequences were assembled and edited using Geneious v8.1.7 (http://www.geneious.com, 2015; Kearse *et al*., 2012). Multiple sequence alignments for each locus were performed using the program MAFFT v7 (Katoh *et al*., 2005; Katoh and Toh, 2008) and manually adjusted. For the ITS and nuLSU sequences, G-INS-i alignment

algorithm and ‘20PAM / K=2’ scoring matrix were used, with an offset value of 0.3, and the remaining parameters were set to default values. For the mtSSU sequences, E-INS-i alignment algorithm and ‘20PAM / K=2’ scoring matrix were used, with the remaining parameters set to default values. 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), 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.

3.6 Phyogenetic Analysis

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 conflicts, thus relationships were estimated from a concatenated, three-locus (ITS, nuLSU, mtSSU) data matrix using a total-evidence approach (Wiens, 1998). The program RAxML v8.1.11 (Stamatakis, 2014) was used to reconstruct the single and concatenated ML gene-tree using the CIPRES Science Gateway server (http://www.phylo.org/portal2/). The ‘GTRGAMMA’ model was implemented with locus-specific model partitions for concatenated dataset treating all loci as separate partitions, and evaluated nodal support using 1000 bootstrap pseudoreplicates. Exploratory analyses using alternative partitioning (e.g. ITS1, 5.8S, ITS2, nuLSU and mtSSU) schemes resulted in identical topologies and highly similar bootstrap support values. Phylogenetic relationships were

reconstructed from the concatenated multi-locus data matrix under BI using the program BEAST v1.8.2 (Drummond and Rambaut, 2007). A run of two independent Markov Chain Monte Carlo (MCMC) chains for 20 million generations were performed, implementing a relaxed lognormal clock and 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 ITS, 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 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.

3.7 Alternative hypothesis testing

Two different methods were employed for the hypothesis testing: 1). Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and 2), expected likelihood weight (ELW) test (Strimmer and 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.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Phylogenetic reconstruction in *Bulborrhizina*

New DNA sequences of the mtSSU, nuLSU, and ITS of *Bulborrhizina africana* were generated for this study (Appendix1). The matrix of the combined data set included 2079 unambiguously aligned nucleotide position characters (784 mtSSU, 849 nuLSU, 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 of ITS, nuLSU and mtSSU were analyzed. Since the ML and BI analyses were identical in their topology and therefore only the ML tree with support values of both analyses is shown (Fig. 4.1).

In the resultant phylogenetic tree, *Bulborrhizina africana* clusters within *Bulbothrix* 'clade II' (Fig. 4.1), which includes the predominantly paleotropical species of *Bulbothrix s.lat*. (Divakar *et al*., 2006; Divakar *et al*., 2010a). However, the relationships within this clade remain uncertain, since the topology is unsupported in this part of the tree.

These results confirm the polyphyly of *Bulbothrix* as currently circumscribed with a predominantly Neotropical clade I and a predominantly palaeotropical clade II 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; 1976a) 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.*

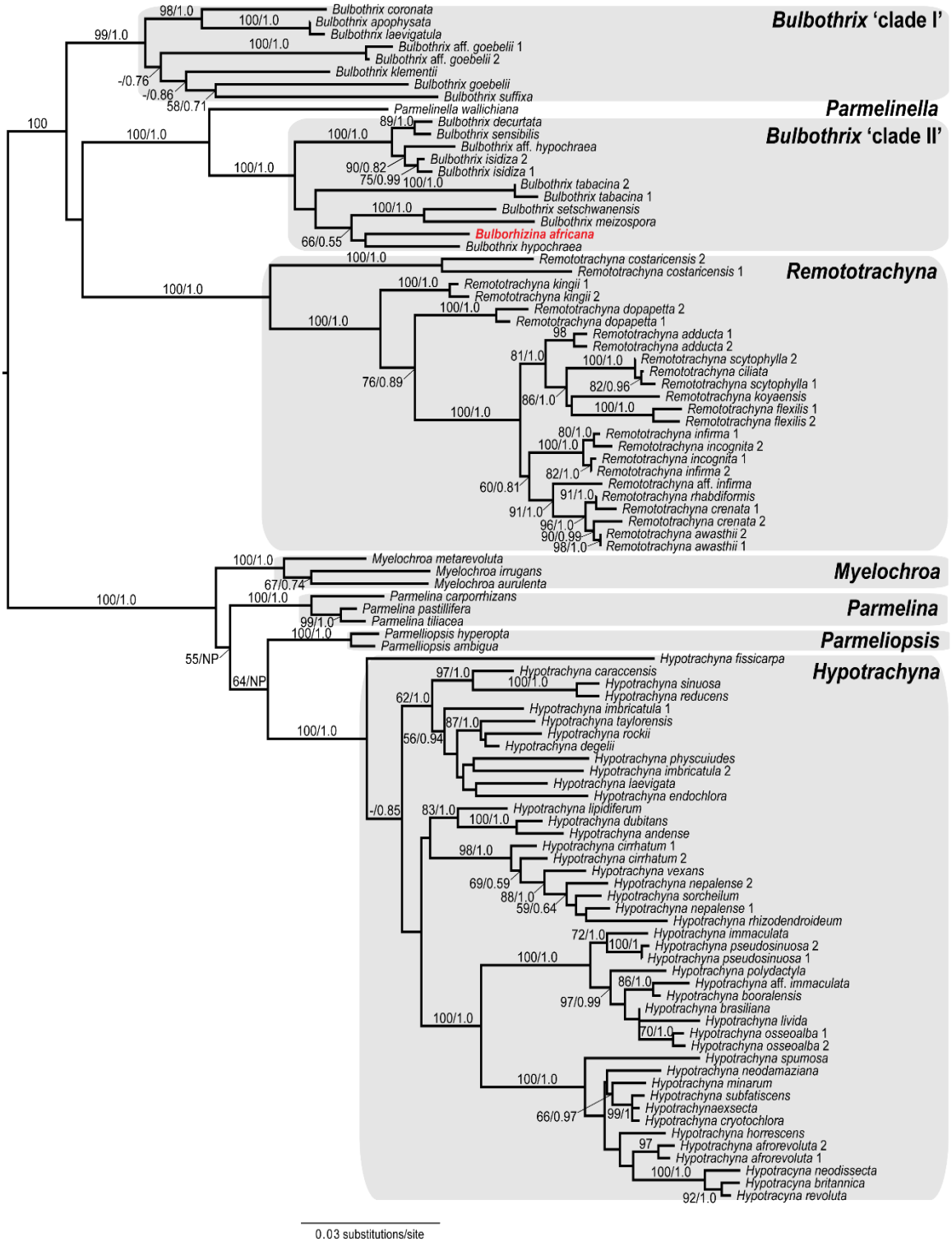

Figure 4.1. Phylogenetic placement of *Bulborrhizina africana* based on a maximum-likelihood (ML) analysis of a concatenated, three-marker dataset (mtSSU, nuLSU, ITS). ML and Bayesian inference topologies were identical, and only the ML topology is reported. Values at each node indicate non-parametric bootstrap support (BS)/ posterior probability values, with support indices > 50 BS/0.50 PP are indicated.

Alternatively, all species of clade II could be transferred to an emended genus *Parmelinella.* Taxonomic changes are withheld until additional samples of the 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, this 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, A. Crespo, Sipman, Elix & Lumbsch 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 separately 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 and Crespo, 2002; Thell *et al*., 2006). Morphological 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 that species support the placement of the species in that 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 on 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). ‘Clade1’ and ‘clade 2’ correspond to previously recognized clades in this polyphyletic genus (Divakar *et al*., 2006).

4.2 Phylogenetic reconstruction in *Bulbothrix*

In the phylogenetic analysis of *Bulbothrix* a total of 51 new sequences, including 16 nuclear ITS, 21 nuLSU and 14 mtSSU rDNA from 37 samples from Asia, Eastern Africa and South America were generated in this study and deposited in Genbank under accession numbers KX539173-KX539223 (Appendix 2). The aligned ITS data matrix contained 481 unambiguously aligned nucleotide position, the nuLSU included 842 and the mtSSU 854 characters. In the ITS alignment, 187 characters were variable and of those 147 parsimony informative; in the nuLSU 119 were variable and 77 parsimony informative; and in the mtSSU, 114 positions were variable and 56 parsimony informative 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. Group I introns were excluded and a 74 bp of the mtSSU, a 26 bp region of the ITS1, and 24 bp of the ITS2 alignments from the analysis using Gblocks. SYM+G, TrNef+I+G, and HKY+I+G resulted as best fitting models of evolution for ITS, nuLSU and mtSSU, respectively.

Testing for topological incongruence showed no supported conflicts and hence the concatenated three-locus data matrix (ITS, nuLSU and mtSSU) was analyzed. The partitioned ML analysis of the concatenated data matrix yielded an optimal tree with a ln

likelihood value = -7676.92 (Fig. 4.2). 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 any supported conflict (e.g., PP ≥ 0.95 and ML bootstrap ≥ 70%), and thus the ML tree topology is depicted here with the Bayesian posterior probabilities added (Fig. 4.2).

Results of the phylogenetic analysis of *Bulbothrix* indicate that both *Bulbothrix isidiza* and *B. tabacina* do not form monophyletic groups as currently circumscribed, although a number of distinct, well-supported clades were recovered (Fig. 4.2). Samples representing *Bulbothrix isidiza* were recovered in five well-supported, independent clades, hereafter named as 'clade I1', 'clade I2', 'clade I3', 'clade I4' and 'clade I5' (Fig. 4.2). Similar to *B. isidiza s.lat.*, specimens collected from pantropical populations of *B. tabacina* were not recovered in a single monophyletic clade. Rather, these specimens fell into three independent, well-supported monophyletic clades, here after named as 'cladeT1', 'cladeT2' and 'cladeT3' (Fig. 4.2). Both the SH and ELW tests significantly rejected monophyly of *B. isidiza* and *B. tabacina,* respectively ($p \leq 0.001$).

Bulbothrix isidiza and *B. tabacina* have traditionally been circumscribed based on isidia morphology, color of the thallus on the lower surface, and extrolite compositions (Hale, 1976a). This traditional classification is not corroborated by phylogenetic reconstructions. Species level polyphyly is a common phenomenon in Parmeliaceae and in lichenized fungi in general. Moreover, cryptic diversity masked under widespread and or disjunct phenotypical species have repeatedly been shown in diverse groups of lichen-forming fungi (Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011;

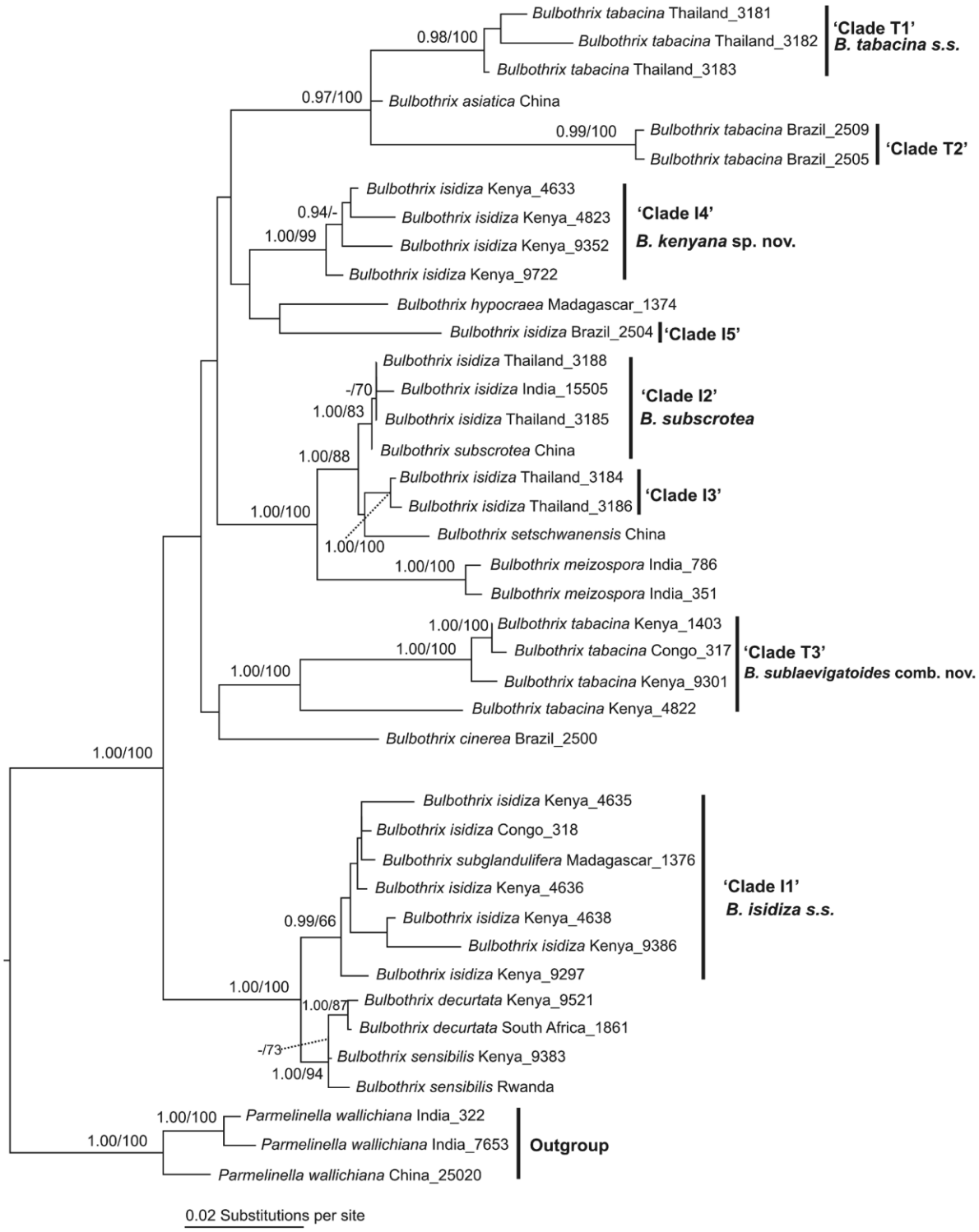

Figure 4.2. Phylogenetic relationships of *Bulbothrix* species based on a maximum-likelihood (ML) analysis of a concatenated, three-locus dataset (ITS, nuLSU & mtSSU rDNA.). ML bootstrap values ≥ 70% and posterior probabilities ≥ 0.95 from the Bayesian analysis are given above branches. *Parmelinella wallichiana* was used as out-group.

Leavitt *et al*., 2015a; Alors *et al*., 2016; Kirika *et al.*, 2016a, b); and the occurrence of cryptic species is common in other groups of fungi as well (see e.g. Hibbett, 2016).

Bulbothrix isidiza, which was thought to have a pantropical distribution (Hale, 1976a), probably has a more restricted distributional range, probably endemic to Africa, in a predominantly African phylogenetic lineage (Fig. 4.2). Samples collected from montane regions of Congo, Kenya and Madagascar were recovered in 'clade I1'. Since the type of *B. isidiza* is described from Serra Chella, a mountain range in central Angola (Nylander, 1884), and 'clade I1' is widely distributed in montane regions of sub-Saharan Africa, we here interpret this clade as *B. isidiza s.str*. Samples collected in dry woodlands and dry forest in Kenya formed 'clade I4', in contrast to specimens from montane regions, which belong to *B. isidiza s.str.* 'clade I1'. *Bulbothrix isidiza s.str*. forms a well-supported (BS = 100, PP = 0.99) sister group relationship with a clade including samples of *B. decurtata* (Kurok.) Hale and *B. sensibilis* (J.Steiner & Zahlbr.) Hale. *Bulbothrix decurtata* is an obligate isidiate species endemic to Africa and *B. sensibilis* is an obligate apotheciate species commonly occurring in Africa (Hale, 1976a; Swinscow and Krog, 1988). The saxicolous *B. decurtata* differs from *B. isidiza s.str.* ('Clade I1') in having a black lower thallus surface and black-tipped isidia. Further, it differs from *B. sensibilis* in having obligate asexual reproduction, an isidiate upper surface, emaculate upper cortex and bifusiform conidia.

Bulbothrix subscortea (Asahina) Benatti was recently resurrected from *B. isidiza s.lat*. based solely on morphological features (Benatti, 2012b). Benatti (2012b) characterized *B. subscortea* by having a saxicolous habitat, emaculate upper thallus surface, simple to slightly ramified isidia on the upper surface, longer cilia and rhizines with basal bulbs. In

this analyses, ‘clade I2’ grouped with samples of *B. isidiza s.lat.* collected in India and Thailand with one sample of *B. subscortea* from China. Since the type specimen of *B. subscortea* is described from Taiwan (Asahina, 1957), here the sample from east Asian in ‘clade I2’ is considerered as *B. subscortea s.str.* Samples clustered in ‘clade I2’ were collected from epiphytic and saxicolous habitats, indicating the substrate is not useful in distinguishing *B. subscortea* from *B. isidiza s.str*. Therefore, the only phenotypic feature that currently distinguishes both species (including samples clustered in ‘clade I2’) is the presence or absence of maculae on the upper surface (Benatti, 2012b; Zhang *et al.*, 2014). This study indicates that caution should be taken when making nomenclatural changes based solely on morphological features in this group of lichen-forming fungi. Moreover, *B. isidiza s.str*. is only known from Africa, whereas *B. subscortea* is only known from Asia.

Not all Asian samples clustered in the *B. subscortea s.str.* clade; two samples from Thailand grouped in ‘clade I3’ (Fig. 4.2). This clade formed an unsupported sister group relationship with the apotheciate species *B. setschwanensis* (Zahlbr.) Hale, endemic to the Himalayas (Divakar and Upreti, 2005). Since only one sample representing *B. setschwanensis* was included in this study and phylogenetic relationships remain unresolved, additional studies are necessary to clarify the phylogenetic position of samples grouped in ‘clade I3’.

Samples of *B. isidiza s.lat.* clustered in ‘clade I4’ (Fig. 4.2) are characterized by a combination of features: emaculate upper surface, papillate lower marginal zone, bulbate cilia reduced to nodules, apices lacking and ascospores of 7.5–15 × 5–7.5 μm. *Bulbothrix isidiza s.str*. (‘clade I1’) differs in having a maculate upper surface, bulbate cilia with

long apices up to 1 mm, simple to furcate, and relatively larger ascospores 10.0– 16.0 (–17.5) × 5.0–9.0 µm (Benatti, 2013). Further, our studies indicate that the species found in dry thickets, woodlands/dry forests vs. those growing in montane localities in Africa are not closely related. Alternative topology tests significantly rejected the monophyly of the African 'clade I1' and 'clade I4' ($P \leq 0.05$). However, the phylogenetic position of 'clade I4' remained unresolved. Since no name is available for this clade, a new species is describe to accommodate the African samples growing in dry, low-elevation forests clustered in it.

Finally, 'clade I5' included a single sample from Brazil, the phylogenetic placement of which remains unresolved. Given the limited number of samples, the formal description of 'clade I5' must await additional studies, especially increased sampling of specimens from Neotropical populations.

Although the resurrection of *B. subscortea* based on phenotypic features by Benatti (2012b) was in part supported by our molecular phylogenetic tree, the resurrection of *B. subglandulifera* (Hue) Hale (Hale, 1974; Benatti, 2013) was not supported. The type specimen of *B. subglandulifera* was from Madagascar (Hue, 1899). The single specimen from Madagascar included in this study, morphologically similar to *B. subglandulifera*, clustered in the *B. isidiza s.str.* clade ('clade I1', Fig. 4.2). Thus, results suggest that *B. subglandulifera* should be included in *B. isidiza s.str.*, although the formal synonymization must await the study of additional material of *B. subglandulifera* for confirmation. This species was traditionally separated from *B. isidiza* by the presence of narrow lobes and granular isidia sometimes dissolving into soredia. However, in the type material, young and poorly developed true isidia and even the largest isidia always

remain corticated (they never become sorediate; see Benatti, 2013). In fact, these phenotypic features are plastic in *B. isidiza s.str*. the *B. isidiza* group and isidiate species in parmelioid lichens in general (reviewed in, e.g., Crespo and Lumbsch, 2010; Divakar and Hawksworth, 2011) and are thus not reliable for taxon differentiation. *Bulbothrix isidiza s.str*. (‘clade I1’) is distinguished by a combination of features, having an isidiate upper surface, maculate upper cortex, bulbate cilia with long apices up to 1 mm, simple to furcate, and relatively larger ascospores 10.0–16.0 (–17.5) × 5.0–9.0 μm (Benatti, 2013), and is restricted in its distribution to montane regions of Africa. Indeed, vegetative phenotypic features have repeatedly been shown to be homoplasious in Parmeliaceae and in lichen-forming fungi in general (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011; Divakar *et al.*, 2013b; Parnmen *et al.*, 2012; Prieto *et al.*, 2013; Kraichak *et al.*, 2015; Leavitt *et al.*, 2015a) and caution must be taken in this group of fungi when making any nomenclatural changes based solely on vegetative phenotypic features.

Bulbothix tabacina is another asexually reproducing, isidiate species with a supposedly pantropical distribution (Hale, 1976a). Specimens collected from pantropical populations of *B. tabacina* were not recovered in a single clade, but fell into three independent, well supported clades, namely ‘clade T1, T2 and T3’ (Fig. 4.2). ‘Clade T1’ included samples from Thailand. Since the type of *B. tabacina* was from east Asia (Montagne, 1856), this clade is considerered to represent *B. tabacina s.str*. This clade appeared closely related to *B. asiatica* Y.Y.Zhang & Li S.Wang. The latter is a recently described species from Cambodia and China, distinguished from *B. tabacina* in having an emaculate thallus upper surface and coralloid isidia and has been shown to be distinct using ITS sequences

alone (Zhang *et al.*, 2014). However, in the multi-locus phylogenetic analysis, the placement of *B. asiatica* appeared unresolved, indicating that additional studies are required.

Two samples of *B. tabacina s.lat.* from Brazil clustered in ‘clade T2’ (Fig. 4.2) and may belong to an undescribed species or these samples could belong to *B. tabacina* in a broader concept including *B. asiatica*. However, since only two samples were included here, a formal taxonomic conclusion must await additional sampling. Lastly, ‘clade T3’ included four samples from *B. tabacina s.lat*. populations collected in East Africa. This clade had an unsupported relationship to another isidiate species, *B. cinerea* Marcelli & Kalb. For the East African populations (‘clade T3’), the name *Parmelia sublaevigatoides* Dodge (1959) described from Uganda is available. Based on morphological features, *P. sublaevigatoides* has been considered as a synonym of *B. tabacina.* Consequently, we use this name to accommodate the samples clustered in ‘clade T3’, resurrecting it below and making the necessary combination into *Bulbothrix*. Unfortunately, we were unable to find any distinctive feature to characterize ‘clade T3’. Nonetheless, the samples clustered in ‘clade T3’ are currently only known from East Africa, whereas *B. tabacina s.str*. does not occur in East Africa.

East African material of *B. decurtata* has previously been treated under *B. tabacina* (Swinscow and Krog, 1988). Saxicolous *B. decurtata* differs from *B. tabacina* in minor morphological features, such as black-tipped isidia, and therefore the distinction of the two species has not been generally accepted (see Benatti, 2013). However, in our phylogenetic tree Kenyan samples representing *B. decurtata* grouped with South African material of that species and were not closely related to *B. tabacina.* Instead, specimens

representing *B. decurtata* formed a well-supported sister relationship to the apotheciate *B. sensibilis* (Fig. 4.2), supporting *B. decurtata* as a valid, independent species. Although the relationship between the two samples of *B. sensibilis* was unresolved in the ML analysis (Fig. 4.2), these formed a monophyletic group in the Bayesian analysis. This is consistent with previous findings (Divakar *et al.*, 2010a). *Bulbothrix decurtata* is distinguished from *B. sensibilis* in having an isidiate upper surface, lacking apothecia, bifusiform conidia and emaculate upper cortex.

In agreement with other species complexes in parmelioid lichens (Crespo *et al.*, 2002; Molina *et al.*, 2011b; Leavitt *et al.*, 2012; Alors *et al.*, 2016), the results presented here highlight that isidiate species generally thought to have wide distributional ranges may show striking phylogeographical structure. In both phenotypically circumscribed species, *B. isidiza s.lat*. and *B. tabacina s.lat*. morphologically similar populations occurring in different continents or ecological habitats correspond to distinct, independent lineages and probably represent distinct species that had previously been overlooked.

This study highlights and unmasks the presence of a higher species diversity in the isidiate *Bulbothrix* spp. than previously assumed. In this group, five species are found in Kenya, including one new species, *B. kenyana* sp. nov. In the light of these results, other species reported from East Africa, namely *B. bulbochaeta* (Hale) Hale, *B. coronata* (Fée) Hale, *B. goebelii* (Zenker) Hale, *B. hypocraea* (Vain.) Hale, *B. meizospora* (Nyl.) Hale, *B. pustulata* (Hale) Hale, *B. suffixa* (Stirt.) Hale and *B. ventricosa* (Hale & Kurok.) Hale (Swinscow and Krog, 1988), need to be revised to evaluate potentially hidden diversity like that detected here. Further, other ecological regions, especially woodland/dry forest areas and the coastal province of Kenya and surrounding countries in East Africa, need to

be sampled to examine the species diversity in this group of lichen forming fungi critically.

4.2.1. Taxonomic treatment

4.2.2. New species

Bulbothrix kenyana Kirika, Divakar & Lumbsch, **sp. nov**. **(Plate. 4.1**)

MycoBank No.: 817700

Diagnosis

Morphologically similar to *B. isidiza* but differs in having emaculate upper surface, papillate marginal zone, cilia lacking apices and molecular phylogenetic position ('Clade I4'; Fig. 4.2).

Plate 4.1. ***Bulbothrix kenyana***, habit (holotype *Kirika* & *Lumbsch*, 4823 [EA])

Type specimen

KENYA. Kitui County, Mwingi, Nuu Hill inselberg, 880-980m 1˚01'S, 38˚19'E, on bark, 25th January 2015, *P. Kirika* 4823 & *H.T. Lumbsch* (holotype: EA, isotypes: F, MAF).

*Genbank accession number***:** ITS KX539174 and nuLSU KX539204.

Etymology

The taxon name is based on its occurrence in Kenya.

Description

Thallus foliose, adnate, 4–6 cm across. Lobes broad, irregularly to subirregularly branched, 2–5 mm wide, rounded crenate, with rotund apices, margins bulbate. Cilia lacking apices, only bulb in the crenate and axils. Upper surface grey, smooth, emaculate, isidiate. Isidia laminal, cylindrical, mostly simple or rarely branched 0.2-0.4 mm high, concolorous with the upper surface and with pale brown tips. Medulla white. Lower surface pale brown, richly rhizinate, papillate margins. Rhizines pale brown, evenly distributed, simple, 0.2–1 mm long. Apothecia laminal, adnate to sessile, 1-6 mm in diameter, amphithecium isidiate. Disc concave, brown, imperforate. Asci 8-spored. Ascospores oval to ellipsoid, 7.5-15 x 5-7.5 µm (M = 6-7 × 10.3-11.75 µm, ±SD = 1.3-4 × 1.8-1.7 µm, n = 40). Pycnidia absent.

Secondary chemistry: Cortex K+ yellow, UV–; medulla K+ yellow turning red, C–, KC–, P+ orange-red, UV–; upper cortex with atranorin, medulla with salazinic acid.

Remarks

Bulbothrix kenyana can easily be confused with *B. isidiza* in the field, but the former differs in having emaculate upper surface, papillate to naked marginal zone and cilia lacking apices. Ascospore size in both species largely overlaps and they contain atranorin

and salazinic acid. In spite of morphological similarities, *B. kenyana* do not form sister relation with *B. isidiza* in our multilocus molecular phylogenetic reconstruction, but fell far apart. The new species is also morphologically highly similar to *B. subscortea*, which is endemic to Asia and has relatively larger ascospores (16.0 × 9.0µm) and cilia with usually longer apices, up to 0.8 mm. In the molecular phylogenetic reconstruction *B. subscortea* form sister group relationship with an apotheciate species *B. setschwanensis*, endemic to Asia. *Bulbothrix kenyana* occurs corticolous on *Acacia* and *Commiphora* spp. and saxicolous at lower elevation ranging from 800 to 1850m in dry thickets, woodlands/dry forests areas of Kenya. At present it is only known from six localities in Kitui, Makueni, and Bomet Counties in Kenya.

Additional specimens examined

KENYA. Makueni County, Makueni Wote, Ngutwa village, Matooi hill Dry woodland, 1400m, 01˚49'S, 37˚56E, on rocks, 12th December 2013, *P. Kirika* 3695, *I. Malombe* & *K. Matheka* (EA, F, MAF); Kitui County, Mwingi, Nuu Hill, inselberg with dry woodland dominated by *Terminalia*, *Combretum* and *Acacia* spp. c. 1000m, 01˚02'S, 38˚20'E, on bark, 12th March 2014, *P. Kirika 3857* & *H.T. Lumbsch* (EA, F, MAF), 4633 (EA, F, MAF); Makueni County, Sultan Hamud, Emali Hill, degraded *Acacia-Commiphora* woodland, 1352m, 02˚04'S, 37˚24'E ,on bark, 22 September 2014, *P. Kirika* 4586 (EA, F, MAF); Makueni County, Utu, Chyulu Hills National Reserve, dry rocky woodland, 1121m, 02˚42'S, 37˚58'E, on bark, 23 September 2014, *P. Kirika* 4620 (EA, MAF); Nakuru County, Mt. Suswa Conservancy, rocky wooded bushland, 1846m, 01˚07'S, 36˚24'E, on bark, 22 June 2015, *P.Kirika* 4888 (EA, F, MAF); 4889 (EA, F, MAF); Ngoina, Kipsonoi river, Unilever Estate along Ikonge-Ngoina road, degraded dry

riparian forest on *Eucalyptus*, 1633m, 00˚30'S, 35˚04'E, on bark, 23 August 2015, *P. Kirika* 4937 (EA, F).

4.2.3 New combination

Bulbothrix sublaevigatoides (Dodge) Kirika, Divakar & Lumbsch, **comb. nov.**

MycoBank No.: MB 817715

Basionym*: Parmelia sublaevigatoides* Dodge, *Annals of the Missouri Botanical Garden* 46: 88. (1959).

Type: UGANDA. Mount Elgon (BM, lectotype).

4.3. Phylogenetic reconstruction in *Canoparmelia*

In this study, a total of 30 new sequences were generated, these comprised 13 nuclear ITS, 12 nuLSU and 5 mitochondrial SSU rDNA from thirteen samples of *Canoparmelia s.lat.* from Eastern Africa (Appendix 3). 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 mtSSU. 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 and 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. Group I introns were excluded and 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, nuLSU and mtSSU, respectively.

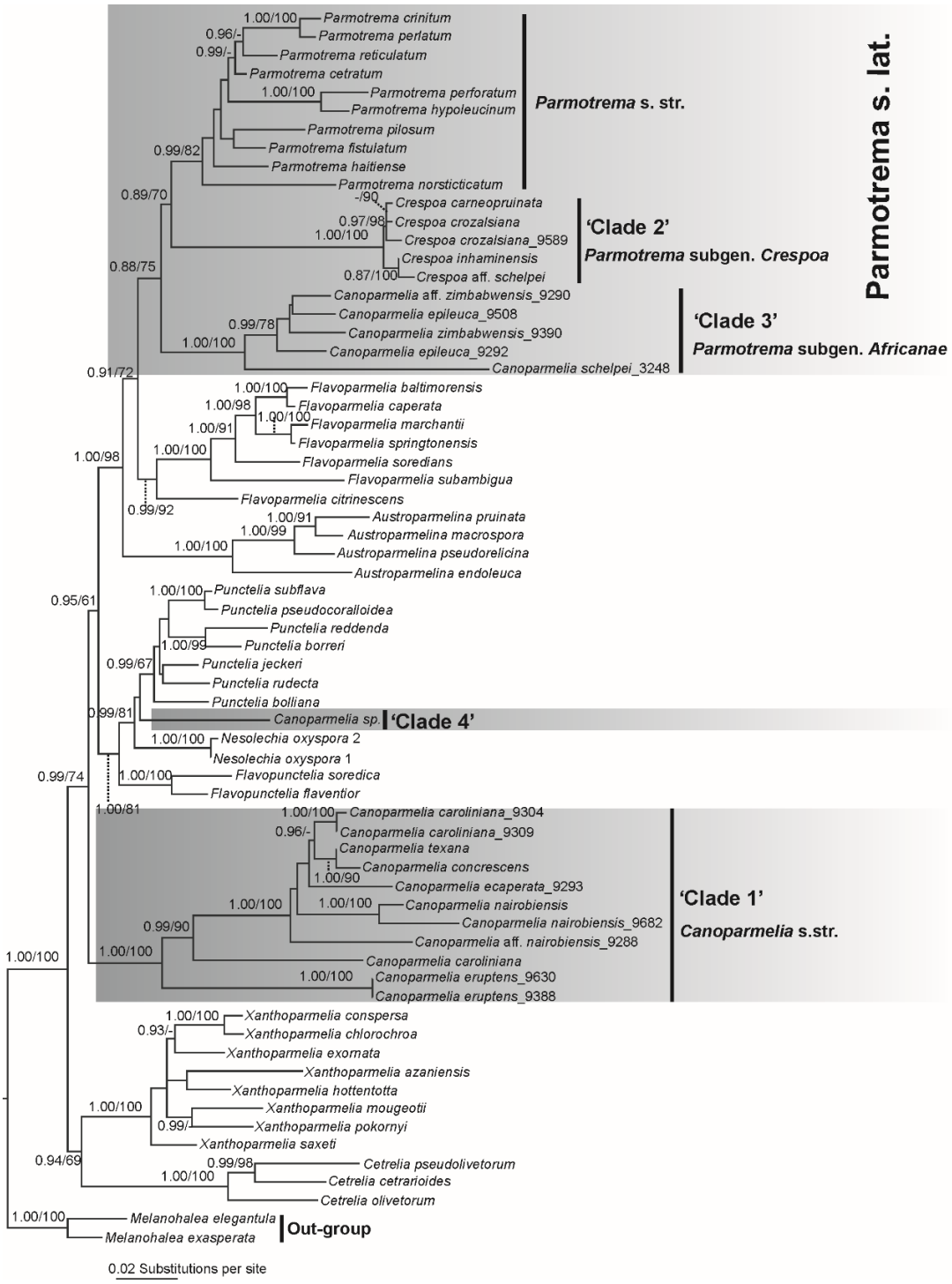

Figure 4.3. Phylogenetic relationships of *Canoparmelia s.lat.* among parmotremoid taxa based on a maximum-likelihood (ML) analysis of a concatenated, three locus dataset (ITS, nuLSU & mtSSU rDNA.) Since the ML and Bayesian inference topologies were identical, only the ML topology is shown here. Posterior 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.

Topologies of single-locus analyses did not show supported conflicts and hence the concatenated three-locus data matrix of the 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 $\geq$ 0.95 and ML bootstrap $\geq$ 70%), and therefore the ML tree topology was used as a working hypothesis of phylogenetic relationships (Fig.4.3).

The resultant phylogenetic tree showed that species in the genus *Canoparmelia s.lat*. 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, specimens of *Canoparmelia* were recovered in four distinct clades - 'clade1', 'clade 2', 'clade 3' and 'clade 4' (Fig. 4.3). This pattern is inconsistent with a generic circumscription based on phenotypical features (Elix *et al*., 1986; Elix, 1993; Brodo *et al*., 2001; Divakar and 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 and Crespo, 2015).

With an extended taxon sampling, species of *Canoparmelia* clustered in four different clades within parmotremoid clade (Crespo *et al*., 2010b). 'Clade 1' formed a sister

relationship to the 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 3000m elevation (Appendix 3). 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 and 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 rise to generic rank as *Crespoa* was proposed for this group by Lendemer and 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 (Table 4.1). 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 4.1). 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.

Table 4.1. 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	Sublagenifor m 5-8 x 1 or filiform 12-20 x 1
Cellwall 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

Species included in this clade can be characterized by sublinear narrow lobes up to 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 4.1). ‘Clade 4’ included a single undescribed species, endemic to South Africa that was recovered as sister to *Punctelia* with low statistical support (Fig. 4.3). 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 and Lumbsch, 2011; Roccellaceae, Tehler and Irestedt, 2007). Therefore, it is not surprising that the monophyly of *Canoparmelia* based on phenotypic feature was not recovered in the 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. 4.3). 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.

4.3.1 Taxonomic treatment

Based on the molecular phylogenetic analyses and morphological re-evaluation, *Canoparmelia* species clustered in ‘clade 3’ are transferred to *Parmotrema* and *Crespoa* is accepted at a subgeneric rank within *Parmotrema* as proposed earlier (Hawksworth, 2011). Thus, species clustered in ‘clade 3’ are recognized as *Parmotrema* subgen. *Africanae*.The remaining unstudied species are left unclassified within the genus *Canoparmelia* (‘clade 1’). ‘Clade 4’ included only a single sample. A detailed study of this clade is in progress and results will be discussed later. The description of the new subgenus and new combinations are proposed below.

4.3.2 New subgenus

Parmotrema* subgen. *Africanae Kirika, Divakar & Lumbsch, subgen. nov.

MycoBank No.: MB 817400

Type species

Parmotrema epileucum (Hale) Kirika, Divakar & Lumbsch (2016: 45); *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. 4.3. 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.

4.3.3 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); *Pseudoparmelia zimbabwensis* (Hale) Hale (1974: 191); *Parmelia zimbabwensis* Hale (1972: 346).

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

4.4 Phylogenetic reconstruction in *Hypotrachyna* subgen *Everniastrum*

For this analysis 43 new DNA sequences were generated, including ITS, nuLSU and mtSSU of the following *Hypotrachyna* subgen. *Everniastrum* species: *H. catawbiensis, H. columbiensis, H. sorocheila,* and *H. vexans*. The matrix of the concatenated data set included 2160 unambiguously aligned nucleotide position characters, 500 unambiguously aligned nucleotide position characters in ITS, 835 in nuLSU and 783 in mtSSU. The ITS PCR product obtained ranged between 600 to 800 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, which were excluded from the final analyses. 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. 4.4). Alignments and trees associated with this study are available in Treebase (https://treebase.org/treebase-web/home.html).

In the inferred multilocus topology, all the samples of *Hypotrachyna* subgen. *Everniastrum* formed a strongly supported monophyletic group as in a previous study (Divakar *et al*., 2013a). While some species in this subgenus formed monophyletic groups, others did not, including *H. cirrhata, H. sorocheila* and *H. vexans* (Fig. 4.4). The polyphyly of *H. cirrhata* has been shown in previous studies (Divakar *et al*., 2006, 2013a), whereas non-monophyly of *H. sorocheila* and *H. vexans* is shown here for the first time. An Asian sample of the latter species formed a strongly supported monophyletic group with samples of other species collected in Asia, and the Kenyan sample of *H. vexans* formed a sister relation to this Asian clade, resulting in paraphyly of *H. vexans* as currently circumscribed. *Hypotrachyna vexans* is an isidiate species described from Taiwan (Hale, 1976c; Culberson and Culberson, 1981; Divakar and Upreti, 2005) and the specimen from Kenya may belong to an undescribed species. However, additional sampling is necessary in order to better understand the delimitation of this species.

Samples of *H. sorocheila* were recovered in two distinct, well-supported clades. Clade '1' included samples from Colombia, Kenya, Madeira, and New Zealand, whereas clade '2' included samples from China and India. Clade '2' clustered in the Asian clade (Fig. 4.4). Clade '1' formed a sister-group relationship with two South American samples of an apotheciate species *H. cirrhata* and clade '2' formed a sister-group relationship with the

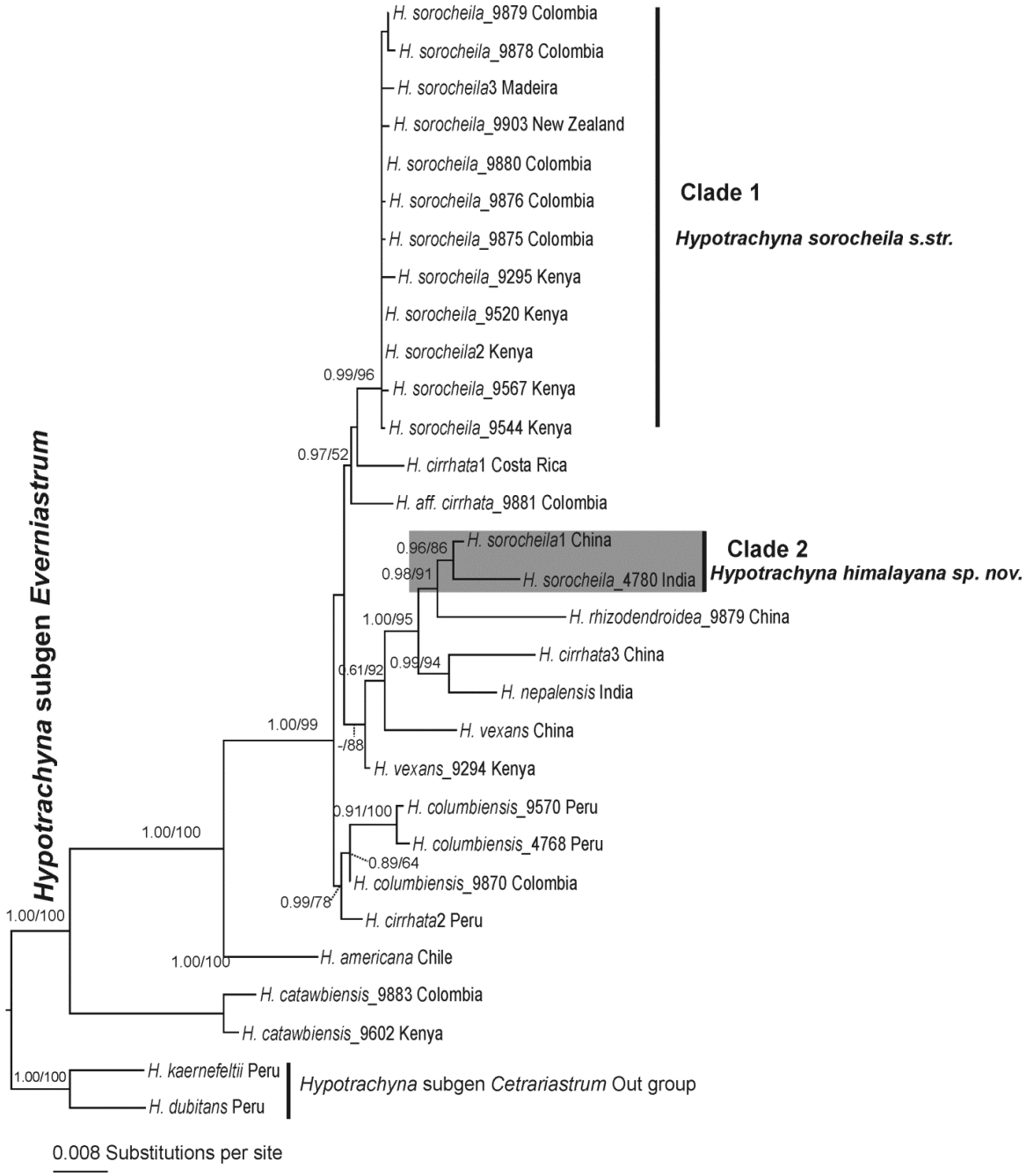

Figure 4.4. Phylogenetic relation among *Hypotrachyna* subgen *Everniastrum* taxa based on a maximum-likelihood (ML) analysis of a concatenated, three locus dataset, ITS, nuLSU & mtSSU rDNA. Since ML and Bayesian inference topologies were identical, only the ML topology is reported here. Posterior probabilities ≥ 0.95/ ML bootstrap values ≥ 70% are given above the branches.

Asian apotheciate species *H. rhizodendroidea*. Interestingly, both sorediate lineages formed sister-group relationships with apotheciate taxa.

While *H. sorocheila* is common in South America and East Africa, it is rarely collected in Asia (Culberson and Culberson, 1981; Sipman, 1980, 1986). In India the species is known from two localities in West and East Himalayas (Divakar and Upreti, 2005). *Hypotrachyna sorocheila* is here reported for the first time from Madeira. It is a sorediate species that was originally described from Colombia. Since all Colombian material belongs to clade '1', this clade is therefore considered as *H. sorocheila s.str.* Consequently, a new species is described to accommodate samples from Asia (clade '2'). Furthermore, these results demonstrate that samples from neo- and paleo-tropics belong to a single lineage, supporting the pantropical distribution of *H. sorocheila s.str*.

Hypotrachyna catawbiensis and *H. columbiensis* are the only other sorediate species in this subgenus. These two species clustered in separate lineages. The Neotropical *H. columbiensis* formed a well-supported monophyletic group sister to a *H. cirrhata* sample from Peru. *Hypotrachyna catawbiensis* had a sister-group relationship to all other species in the subgenus. The species is known from the New World and East Africa. The samples from Kenya and Colombia clustered together indicating that this species is comprised of geographically disjunct populations. While the disjunct and wide distribution pattern of sorediate taxa in this subgenus has been discussed previously (Culberson and Culberson, 1981; Sipman, 1980, 1986), this has not been tested before, using molecular data.

These results suggest that sorediate morphs in *Hypotrachyna* subgen. *Everniastrum* belong to independent lineages distinct from esorediate taxa. Further confirming that

some sorediate species-level lineages in this subgenus can be widely distributed (e.g., *H. catawbiensis* and *H. sorocheila*). Studies have shown that species distributed in different continents may belong to distinct lineages (e.g., Argüello *et al*., 2007; Divakar *et al*., 2010b; Alors *et al*., 2016; Leavitt *et al*., 2015a). In some cases, sorediate morphs in other genera have been shown to be conspecific with esorediate counterparts (Buschbom and Mueller, 2006; Divakar *et al*., 2007; Wirtz, *et al*., 2012; Truong *et al*., 2013). In other instances, sorediate taxa have been shown to represent lineages distinct from esorediate counterparts that are otherwise morphologically similar (Lücking *et al*., 2008; Cornejo *et al*., 2009), as observed in *Hypotrachyna* subgen. *Everniastrum* in this study. Hence, the taxonomic significance of a reproductive trait varies among lineages of lichen-forming fungi (Tehler *et al*., 2009; Leavitt *et al*., 2015a).

Here more evidence is added supporting the distinction of a number of sorediate species in *Hypotrachyna* subgen. *Everniastrum*. These results show different distribution patterns in these sorediate taxa, with some being widely distributed (e.g., *H. catawbiensis* and *H. sorocheila*) and others with more restricted distributions in tropical regions (e.g., *H. columbiensis* and *H. himalayana* sp. nov.).

4.4.1. Taxonomic treatment

4.4.2. New species

Hypotrachyna himalayana Divakar & Kirika, **sp. nov**. (Plate 4.2)

MycoBank No. MB 817198

Type specimen

China, Yunnan, Jianchuan Co., ridge on trail to Lao Sueri Shan, *Rhododendron* forest – scrub with scattered *Abies*, on tree trunk, 26.37N, 99.43E, alt. 3980m, 19th Oct. 2002, *A.*

Crespo, O. Blanco & *A. Arguello* (MAF-Lich 10375, holotype). Genbank accession number: ITS DQ279490, nu LSU EU562677 and mtSSU DQ287798.

Plate 4.2. ***Hypotrachyna himalayana*** habit (MAF-Lich 10375)

Etymology

The taxon name is based on its occurrence in the Himalayan region.

Diagnosis

The species is morphologically similar to *H. sorocheila* but differs in geographic distribution restricted to Asia, molecular phylogenetic tree topology and consists of samples grouped in clade '2', within the Asian clade (Fig. 4.4).

Description

Thallus loosely attached to the substratum, suberect, ca. 3.0 cm across, dichotomously lacinate lobate. Lobes sub-linear, elongate, tapering at apices, 1-2.5 mm wide. Margin flat to involute especially near apices, ciliate. Cilia sparse, simple, ca. 0.8 mm long. Upper surface grey, smooth, sorediate. Soralia granular, subterminal, sorediate apices involute. Medulla white. Lower surface black with narrow brown to dark brown colored zone near tip of lobes, canaliculated, smooth or sometimes transversely wrinkled in lower parts, sparsely rhizinate near margins. Rhizines absent or a few, simple to branched, black, to 1 mm long. Apothecia and pycnidia not seen in the specimen examined.

Chemistry: Cortex K+ yellow; medulla K+ yellow turning red, C-, KC-, P+ orange-red; atranorin and salazinic acid.

Remarks

This taxon is morphologically most similar to *H. sorocheila*, however, in the current phylogenetic reconstruction it appears to be more closely related to the apotheciate species *H. rhizodendroidea* known from Asia (Fig. 4.4, Appendix 4). It is worth emphasizing that no morphological differences have yet been found distinguishing *H. sorocheila* and the new segregate, *H. himalayana*, however, the latter lacked galbinic acid and protolichesterinic acid (Culberson and Culberson, 1981). *Hypotrachyna himalayana* is the only sorediate species known so far from Asia in the *Hypotrachyna* subgen. *Everniastrum*. It occurs corticolous in *Rhododendro*n forest and scattered *Abies* and other tree trunks at higher elevation ranging from 3000 to 4000 m in Himalayan regions of China and India. At the moment it is only known from three localities of Himalayan region.

Hypotrachyna sorocheila (Vain.) Divakar *et al*. (2013a)

Type: Colombia: "prope Bogota", 8500' s.m., J. Weir 5 pr.p. (BM, holotype)

Note

A full description of *H. sorocheila* is found elsewhere (Sipman, 1980; Culberson and Culberson, 1981). Morphologically, it is similar to *H. himalayana* but in the phylogenetic tree it appears close to two samples of *H. cirrhata* from South America. The species grows on trees and mossy shrubs in South America, Australasia, Africa and Madeira. So far *H. sorocheila* is not known from Asia.

4.5 Phylogenetic reconstruction in *Parmelinella*

For the phylogenetic reconstruction in *Parmelinella*, a total of 28 new DNA sequences of *Parmelinella wallichiana* were generated for this study (Appendix 5). These were deposited in GenBank under accession numbers KX341978-KX342008. The dataset included samples from wide geographic regions as Asia, East Africa and South America. The final alignment of the combined data set was 2174 positions in length and was comprised of 458 unambiguously aligned nucleotide position characters in ITS, 844 in the nuLSU, and 872 in the mtSSU. As the topologies of the single locus phylogenies did not show any conflicts they were analyzed in a concatenated data matrix of the three loci (ITS, nuLSU and mtSSU). The ML and BI analyses were identical in their topology and hence only the ML tree with support values of both analyses is depicted in Fig. 4.5.

Specimens representing *Parmelinella wallichiana* did not form a monophyletic lineage (Fig. 4.5). This is inconsistent with currently hypothesized species boundaries based on phenotypical features (Divakar and Upreti, 2005; Benatti, 2014). Species-level

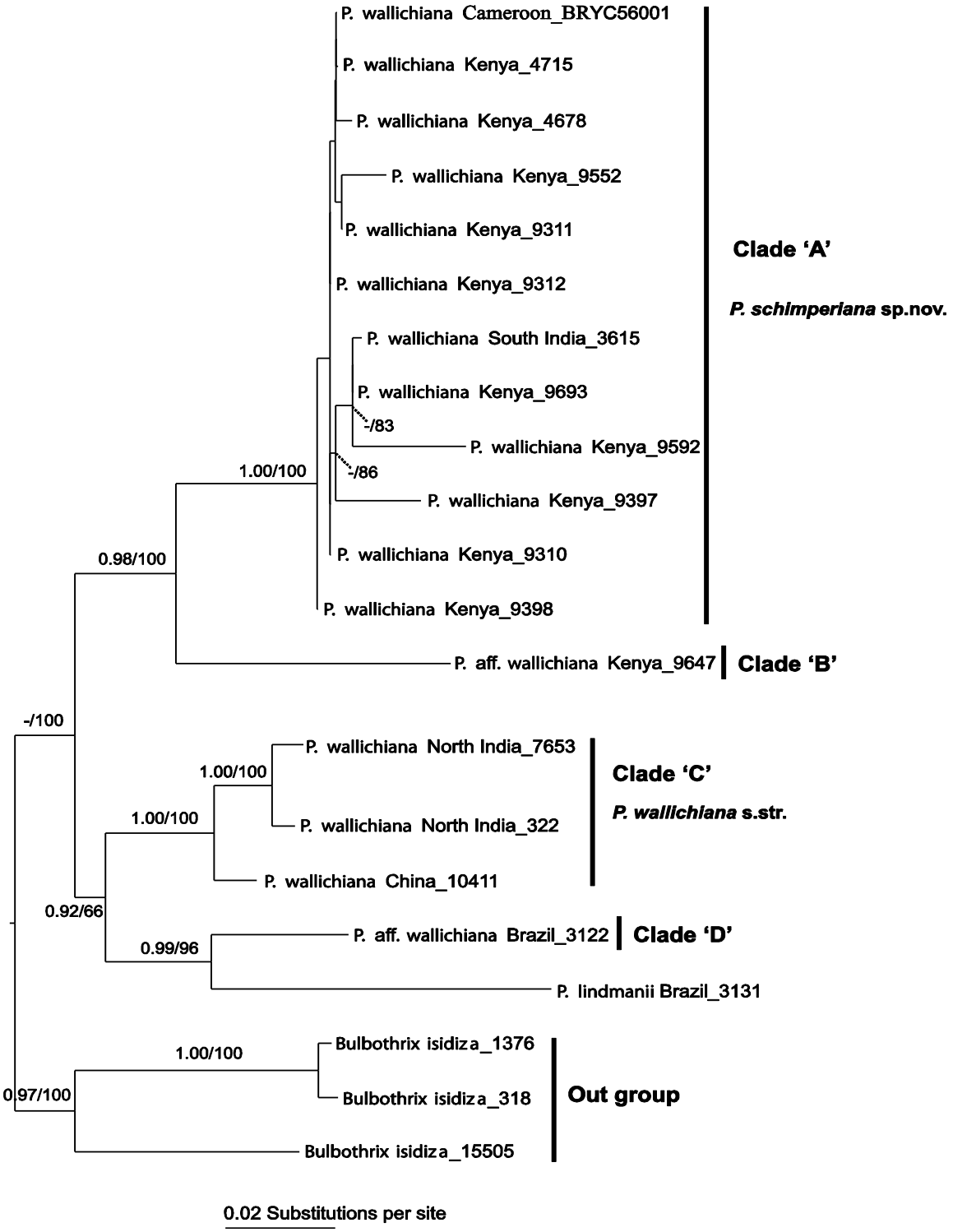

Figure 4.5. Phylogenetic relationships among *Parmelinella* taxa based on a maximum-likelihood (ML) analysis of a concatenated, three locus dataset (ITS, nuLSU & mtSSU rDNA.) Since the ML and Bayesian inference topologies were identical, only the ML topology is show is shown here. Posterior probabilities ≥ 0.95/ ML bootstrap values ≥ 70% are given above the branches.

polyphylies are commonly found in Parmeliaceae and other groups of lichen-forming fungi (see reviews by Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011). Specimens representing *P. wallichiana s.lat*. fell into four distinct well-supported clades. Clade 'A' included samples from Kenya, Cameroon, and a single sample from South India. Clade 'B' included a single sample from coastal region (Kwale County) of Kenya. Clade 'C' included most samples from Asia; and clade 'D' was represented by a single sample from South America (Brazil). Specimens in clade 'A 'are characterized in having smaller ascospores (5-10 x 5-7.5µm), whereas they are larger (15-20 x 9-14 µm) in clade 'C'. Further, the same strongly supported monophyletic clades – 'A' and 'C' – were recovered in reciprocally monophyletic clades in the independent gene trees (Hudson and Coyne, 2002). Presence of the same clades in different single-locus genealogies can be taken as strong evidence that the clades are reproductively and evolutionarily isolated lineages representing distinct species-level lineages (Dettman *et al*., 2003; Pringle *et al*., 2005; De Quieroz, 2007). The relationships among the clades were well supported (Fig. 4.5). Clade 'B' formed sister-group relationship with clade 'A', whereas clade 'D' was sister to *P. lindmanii*, and clade 'C' sister to a clade including clade 'D' and *P. lindmanii*. The type material of *Parmelinella wallichiana* is from Nepal in the Himalayas (Hale, 1976d; Divakar and Upreti, 2005). Since all samples sequenced from the Himalayan regions (China and India) clustered in clade 'C', this clade is considered as *P. wallichiana s.str*.

For clade 'A' there are a few potential names available that were studied. For example, *Parmelia junodi* was described from the Cape Province in South Africa (Steiner, 1907) and *Parmelia tiliacea* var. *eximia* has been described from Tanzania (Steiner, 1888).

These taxa have previously been considered synonyms of *P. wallichiana* (Hale, 1976d). However, according to a recent study by Benatti (2014), *Parmelia tiliacea* var. *eximia* is a synonym of *Parmelinella cinerascens* and the type material of *Parmelia junodi* contained mixture of different species, such as *Parmelinopsis minarum* or *P. horrescens* and a fragment belonged to *Parmelinella cinerascens*. Thus these names are considered as synonyms of *Parmelinella cinerascens*. The latter is a rare species occurring in South America and until recently was classified in the genus *Canoparmelia* (Elix *et al*., 1986). Recently, based on morphological data, *Canoparmelia cinerascens* was transferred to the genus *Parmelinella* (see Benatti, 2014). Unfortunately, this species has not been sequenced and hence the phylogenetic position of *C. cinerascens* cannot be confirmed. Samples clustered in clade 'A' collected from Africa and South India are morphologically similar to *Parmelinella wallichiana s.lat.* Since there is no name available for this clade, a new species is described below to accommodate samples from Africa and South India (clade 'A'). Further, the segregation of this new taxon from *P. wallichiana s.str*. is corroborated by morphological data, discussed below. The new species has a disjunct distribution occurring in Africa and South India. There are abundant examples of this disjunct distribution pattern in flowering plants (see e.g. Mani, 1974; Kadereit, 2004).

Clades 'B' and 'D' were each represented by a single specimen from Kenya and Brazil, respectively. The sample from the coastal region of Kenya (clade 'B') has a deviating morphology, i.e. very narrow, sublinear and dichotomous lobes, although the specimen from coastal Brazil (clade 'D') was more similar to *P. wallichiana s.lat*. In both cases, study of additional samples will be required before a formal description of these putative

species is made.

These results add a further example to a growing body of evidence of the existence of distinct lineages hidden under currently circumscribed species (reviewed in Bickford *et al*., 2007; Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011). Whereas, some studies found no obvious phenological differences and interpreted the discovered additional species diversity as cryptic (reviewed in Crespo and Lumbsch, 2010; Lumbsch and Leavitt, 2011; Hibbett, 2016), re-examination of material falling into different clades uncovered previously unrecognized morphological differences. This has been shown in other cases as well (see e.g. *Parmelia barrenoae*, Divakar *et al*. (2005b); *Physconia thorstenii*, Divakar *et al*. (2007); *Caloplaca citrina* group Vondrák *et al*. (2009); *Melanelixia californica*, Divakar *et al*. (2010b); *Parmelia mayi*, Molina *et al.* (2011a); *Cladia aggregata* group, Parnmen *et al*. (2012) and demonstrates the importance of careful re-analysis of morphological and chemical characters in order to phenotypically circumscribe species. Further, the species-level lineages uncovered in this widely distributed isidiate taxon showed biogeographic structure in what was previously believed to be a pantropical species. Although geographical structure of species detection using molecular data has recently been shown to be a common phenomenon in lichenized fungi (Argüello *et al*., 2007; Divakar *et al*., 2010a; Otalora *et al*., 2010; Amo de Paz *et al*., 2012; Parnmen *et al*., 2012; Moncada *et al*., 2014; Leavitt *et al*., 2015a; Alors *et al*., 2016); caution must be taken to generalizing for all isidiate lichen taxa (Leavitt *et al*., 2013a; Roca-Valiente *et al*., 2013; Divakar *et al*., 2016).

4.5.1 Taxonomic treatment

4.5.2 New species

Parmelinella schimperiana Kirika & Divakar, **sp. nov**. Plate. 4.3

MycoBank No. MB 817294

Plate 4.3. Morphology of the new species; ***Parmelinella schimperiana*** (holotype [EA])

Type specimen

KENYA, Makueni County, Wote, Ngutwa village, Matooi hill, dry woodland, 01º49'S, 37º66'E, 1400m, on bark, 12th December 2013, *P. Kirika, I. Malombe* & *K. Matheka*, 3703 (holotype: EA, isotype: F). **Genbank accession numbe**r**.** ITS KX341985, nuLSU KX342003.

Diagnosis

Morphologically similar to *P. wallichiana* but differs in having smaller ascospores (5-10 x 5-7.5µm), being restricted in distribution to Africa and South India, and molecular phylogenetic position (Clade 'A'; Fig. 4.5).

Etymology

The taxon name is in the honor of W.G. Schimper, the first botanist to collect lichens in East Africa.

Description

Thallus foliose, adnate to loosely adnate, 3–7 cm across. Lobes broad, irregularly to subirregularly branched, 3–8 mm wide, rounded crenate, with rotund apices, margins ciliate. Cilia simple, frequent in the lobe axils, 0.1–0.6 mm long. Upper surface grey, grey-green smooth, emaculate, usually pruinose, thallus irregularly cracked towards the center on older parts, isidiate. Isidia laminal, cylindrical, mostly simple or branched 0.1-0.5 mm high, concolorous with the upper surface. Medulla white. Lower surface black with more than 2 mm broad, brown papillate margins, and rhizinate. Rhizines black, evenly distributed, simple, 0.2–1 mm long. Apothecia laminal, adnate to sessile, 1-5 mm in diameter. Disc concave, brown, imperforate. Asci 8-spored. Ascospores ellipsoid to subglobose, 5-10 x 5-7.5µm (M= 5.5–6.4 x 7.6–8.5 µm, ±SD=0.7–1.0 x 1.0–2.3 µm, n=100). Pycnidia absent.

Secondary chemistry – Cortex K+ yellow, UV–; medulla K+ yellow turning red, C–, KC–, P+ orange-red, UV–; upper cortex with secalonic acid A and atranorin, medulla with salazinic acid.

Distribution and ecology

At present the new species is known from Kenya, Cameroon and South India. It occurs in montane regions and in dry woodland areas. It is predominantly corticolous and sometimes saxicolous rarely terricolous, found corticolous on *Mangifera indica*, *Juniperus procera*, *Podocarpus* spp., *Lannaea* spp. and on *Eucalyptus* in artificial habitats.

Additional specimens examined

KENYA. Marsabit County, Marsabit National Park, Lake Paradise, disturbed forest on ridge, 2º16'N 37º56'E, 1434m, on bark, *P. Kirika* 4678 & *H.T. Lumbsch* (EA, F, MAF): Marsabit National Park at roadside between Marsabit Lodge and Lake Paradise, forest on slope, 2º18'N 37º57'E, 1513m, on bark, *P. Kirika* 4715 & *H.T. Lumbsch* (EA, F, MAF); Tharaka Nithi County, Chiakariga, Kijege Hill, *Acacia-Commiphora* woodland, 00º16'S, 37º50'E, 1160m, on bark, *P. Kirika* 3432 (EA, F); Kitui County, Mumoni Hill, *Eucalyptus* plantation, 00º31'S, 38º00'E, 1620-1695m, on bark, *P. Kirika* 3487 & *G. Mugambi* (EA, F); Tharaka Nithi County, Chiakariga, Kijege Hill, *Acacia-Commiphora-Encephalartos* woodland, 00º16'S, 37º50'E, 1160m, *P. Kirika* 3436 (EA, F); Baringo County, Eldama Ravine, Lembus forest off Eldama Ravine-Eldoret Road, remnant montane forest, 0º13'N, 35º69'E, 2275m, on bark, *P. Kirika* 2870, *G. Mugambi* & *H.T. Lumbsch* (EA, F); 0º16'N, 35º75'E, 2137m, on rock, *P. Kirika* 2815, *G. Mugambi* & *H.T. Lumbsch* (EA, F); Kericho County, Kericho, James Finlay Tea Estate, Chomogondy, secondary forest, 00º23'S, 35º18'E, 2056m, on bark, *P. Kirika* 3145 (EA, F); Kericho County, small disturbed remnant forest in tea plantation, 0º44'S, 35º31'E, 2049m, on bark, *P. Kirika* 2974 *G. Mugambi* & *H.T. Lumbsch* (EA, F); Bomet County, Koiwa, Unilever riparian forest, 00º35S, 35º17'E, 2030m, on bark, *P.Kirika* 4900 (EA); Kajiado

County, Ngong Hills, upland grassland with rocky outcrops, 01˚24’S, 36˚38’E, 2430m, on Soil, *P. Kirika* 3334 (EA, F); CAMEROON. E of Mount Cameroon, vic. of Ekona, *E.A. Orock* 56009 (BRY-C); INDIA. S India, Tamil Nadu, Vellore distr., Yellagiri hills, 13º30’N, 79º05’E, 1393m, on *Mangifera indica* tree trunk, hill side with teak and *Eucalyptus* vegetation, *H.T., Lumbsch, P.K. Divakar, D.K. Upreti* & *J. Tandon* 19705a (MAF).

Remarks

Parmelinella schimperiana is morphologically most similar to *P. wallichiana*, but differs in having smaller ascospores (5-10 x 5-7.5µm), whereas the ascospore size in *P wallichiana* is: 15-20 x 9-14 µm.

4.6 Phylogenetic reconstruction in *Relicina* and *Relicinopsis*

Aligned DNA data matrix for this analysis, contained 455 unambiguously aligned nucleotide positions in the ITS, 808 in the nuLSU and 735 in the mtSSU rDNA datasets. The final alignment of concatenated dataset was 1999 positions in length, with 548 variable characters. The ITS PCR product obtained ranged between 600 to 800 bp. Differences in size were due to the presence or absence of a group I intron of about 200 bp at the 3’ end of the 18S rDNA (Gutierrez *et al.,* 2007). Introns from 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 model of evolution for each partition. All the newly generated sequences for this analysis were deposited in GenBank under accession numbers KX434464-KX434477 (Appendix 6).

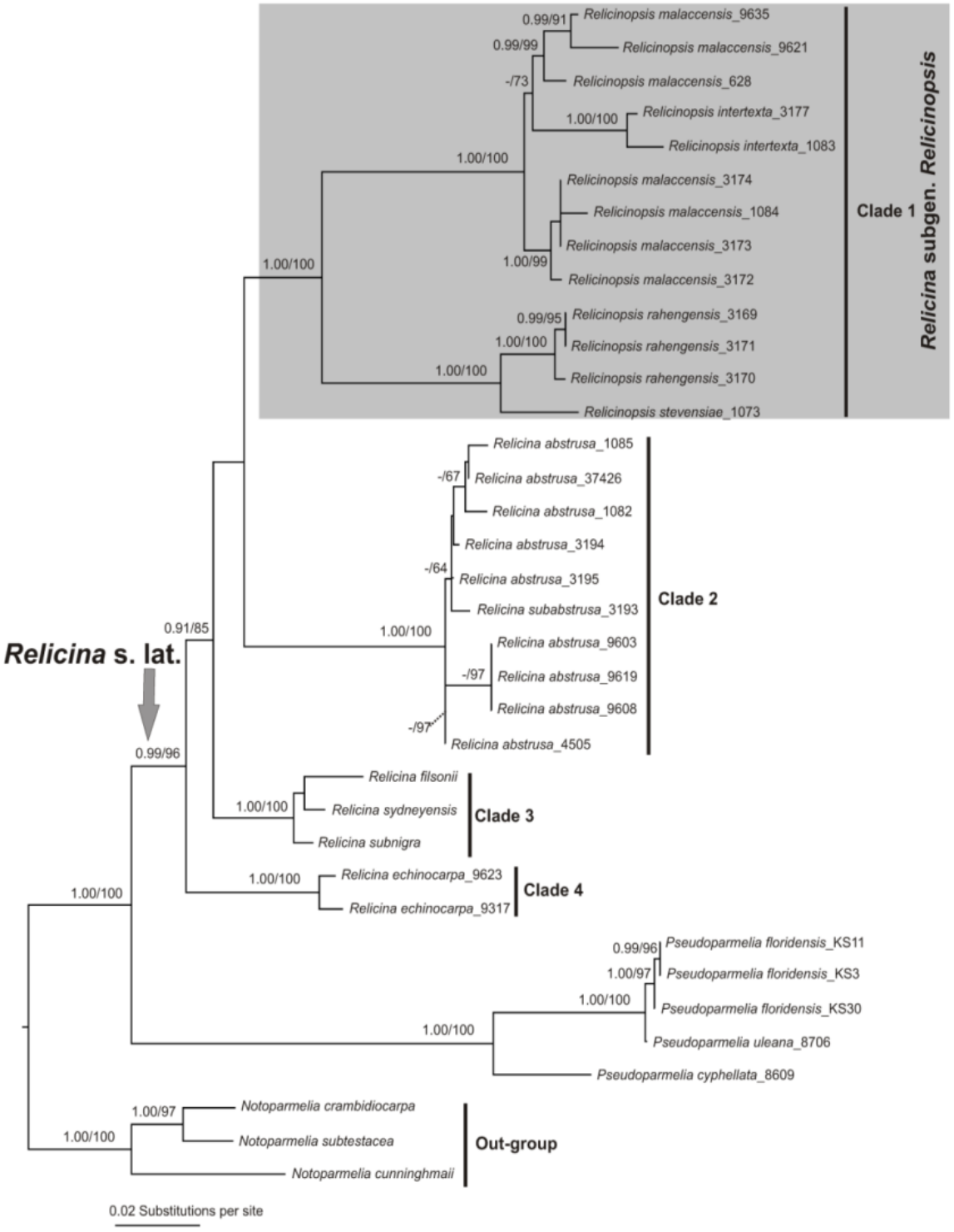

Figure 4.6. Phylogenetic relationships of the genera *Relicina* and *Relicinopsis* based on a maximum-likelihood (ML) and Bayesian analyses of a concatenated, three locus dataset (ITS, nuLSU & mtSSU rDNA.) The ML tree obtained with RAxML is shown here. Posterior probabilities ≥ 0.95 from the Bayesian analysis and ML bootstrap values ≥ 70% are given above branches. Three species of *Notoparmelia* (*N. crambidiocarpa*, *N. cunninghamii* and *N. subtestacea)* were used as the out-group.

Tests for topological incongruence showed no supported conflicts. The partitioned ML analysis of the concatenated data matrix yielded an optimal tree with ln likelihood value = -8048.95 (Fig. 4.6). In 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. 4.6).

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 and Krog, 1988). This is in agreement with previous molecular studies (Buaruang *et al*., 2015; Divakar *et al*., 2015), however, they grouped with *Relicina* spp. Morphological similarities of *Relicinopsis* and *Relicina* species have been discussed previously (Elix *et al*., 1986; Elix, 1993; Divakar and Upreti, 2005). In this study, *Relicina* was recovered as paraphyletic with *Relicinopsis* nested within (Fig. 4.6). Both the SH and ELW tests significantly rejected monophyly of *Relicina* as currently circumscribed ($p \leq 0.005$). 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*., 2013a) and *Bulbothrix* Hale (Divakar *et al*., 2010a) 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. 4.6).

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 4.6). Further, in the Bayesian tree, clade 2 formed a well-supported (PP = 0.95) sister-group relationship with clade 3. Clade 2 included samples of *Relicina abstrusa* and *R. subabstrusa* 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 & Johnston, occurring in Australasia and Southeast Asia. Clade 4 included two samples of *R. echinocarpa* (Kurok.) Hale from Kenya. This relationship was strongly supported (ML bootstrap = 85%) in the ML analysis, but received weak support (PP = 0.91) in the Bayesian tree reconstruction. These 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 fifteen samples collected from Africa, Australia and Southeast 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, whereas *Relicina* belongs to the *Parmelia* clade, closely related to *Relicinopsis* (Crespo *et al*., 2010b; Divakar *et al*., 2015). The key

phenotypic features used to delineate the genera *Relicina* and *Relicinopsis* are summarized in Table 4.2. 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 are overlapping (Table 4.2).

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.,* 2010b), thus this feature can be

Table 4.2. Main morphological and chemical features used to distinguished *Relicina* and *Relicinopsis*.

Features	***Relicina***	***Relicinopsis***
Ascospores (µm)	Ellipsoid (6-8 x 3-5) to bicornute (10-12 x 3)	Ellipsoid (5-8 x 3-5)
Conidia (µm)	Bifusiform (6-10 x 1)	Fusiform or cylindrical (5-7 x 1)
Marginal cilia	Bulbate	Simple (without swollen base)
Lobe morphology	Sublinear, subdichotomously to dichotomously branched	Sublinear, subdichotomously to dichotomously branched
Rhizines	Simple, furcated-branched or agglutinate	Simple or rhizoids agglutinated
Cell walls polysaccharide	Isolichenan	Isolichenan
Epicortex	Pored	Pored
Cortical extrolites	Usnic acid	Usnic acid
Distribution	Africa, Asia, Australasia, Central and South America	Southeast Asia, Australia, East Africa, India
Habitat	Tropical-subtropical to temperate	Tropical

variable within genera in this family. Consequently, *Relicinopsis* is here reduced to synonymy with *Relicina.* However, given that *Relicinopsis* species formed a well-supported monophyletic clade (clade 1) and is distinguished by distinct conidia morphology, the clade is recognized at the subgeneric rank. Consequently, the subgenus *Relicina* is paraphyletic with *Relicinopsis* nested within. However, following Divakar *et al.* (2013a), recognition of the monophyletic clade at the subgeneric level is preferable here, since no paraphyletic taxa at generic level are produced (Hörandl and 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 samples are necessary to evaluate species boundaries in these nominal taxa.

4.6.1 Taxonomic treatment

4.6.2. New subgenus

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

MycoBank No.: 817621

Relicinopsis Elix & Verdon, in Elix *et al*., *Mycotaxon* **27**: 281 (1986).

Type species: *Relicina intertexta* (Mont. & Bosch) Kirika & Lumbsch, *Lichenologist* 49: 189-197 (2017)

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 the clade 1 in Fig. 4.6, including all species currently placed in *Relicinopsis* (Elix *et al.* 1986; Elix 1993).

4.6.3 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) (as "*stevensii*").

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The re-assesment of species boundaries using molecular data undertaken in this study show that phenotypic features traditionally used to delimit taxa in lichens underestimate true diversity, more species are described in Parmeliaceae. Further, the use of molecular sequence data has helped recover previously unknown lineages, thus enabling a better understanding of species diversity, their evolutionary relationships and geographic distributions. Cryptic species are also uncovered while taxa previously thought to be wide spread are shown to have restricted distributional ranges.

- The phylogenetic position of *Bulborrhizina* was elucidated and contrary to previous thinking *Bulborrhizina africana* is neither a phylogenetically isolated species nor related to the morphologically similar species of *Hypotrachyna* subgen. *Cetrariastrum* and subgen *Everniastrum* but belongs to the paleotropical *Bulbothrix* clade. However the bulbate appendages found in that species support the placement of the species in that clade and suggest that the distinction of cilia and rhizines as used by Kurokawa (1994) resulted in a misinterpretation of phylogenetic relationships.
- Three new species namely; *Bulbothrix kenyana*, *Hypotrachyna himalayana* and *Parmelinella schimperiana* were described as new to science from some of the independent species level lineages recovered. Two new subgenera were proposed; *Parmotrema* subgen. *Africana* and *Relicina* subgen. *Relicinopsis* and one new combination made *Bulbothrix sublaegtoides.* Three new records were made for

Kenya, namely; *Bulbothrix sublaegtoides*, *Relicina echinocarpa* and *Bulborrhizina africana* which becomes the second known population of *B. Africana.*

- Eight independent species level lineages were recovered in *Bulbothrix*, four in *Parmelinella,* four in *Canoparmelia* and two in *Hypotrachyna* subgen. *Everniastrum*. While the genus *Relicina* was also recovered as paraphyletic with three well supported clades. Showing that the traditional methods of taxa circumscription using morphology and chemistry underestimates the actual number of species in Parmeliaceae. Suggesting that some of the macromorphological features used in the circumscription of genera and species in Parmeliaceae are homoplasious and therefore phylogenetically uninformative.
- In the re-assessment of species boundaries in two asexually reproducing isidiate species; *Bulbothrix isidiza* and *B. tabacina*. *Bulbothrix isidiza,* which was previously thought to have a pantropical distribution (Hale, 1976a), is found to have a restricted distributional range, probably endemic to Africa. In *B. isidiza s.lat.* and *B. tabacina s.lat*. morphologically similar populations occurring in different continents or ecological habitats correspond to independent lineages and represent distinct species that had previously been overlooked. This study suggests that *Bulbothrix tabacina s.str*. does not occur in East Africa contrary to previous knowledge. While *B. decurtata* is supported as an independent species contrary to the previous opinion that it does not significantly differ from *B. tabacina* (Swinscow and Krog, 1988).

- Some sorediate species are shown to have a wide distributional range, *Hypotrachyna sorocheila s.str*. was shown to have a pantropical distribution including populations in African, Europe, Asia and South America while *H. catawbiensis* has a disjunct distribution in Africa and S. America. Other sorediate species in this group showed a restricted distribution such as *H. columbiensis* occurring only in South America and *H. himalayana* found in Asia. Consequently highlighting the fact that the taxonomic significance of reproductive traits may vary among lineages of lichen-forming fungi and the need for careful case-by-case studies.
- An isidiate species *Parmelinella wallichiana* which was thought to be widely distributed is probably restricted to Asia or Australasia. Based on this limited sampling, this study suggest that *P. wallichiana s.str*. does not likely occur in Africa contrary to previous knowledge.
- A total of 170 DNA sequences comprising of 60 ITS, 66 nuLSU and 44 mtSSU were newly generated mainly from the Kenyan samples and were deposited with the NCBI genbank.

5.2 Recommendations

- A DNA sequence based sample identification is necessary in order to access the actual biological diversity in tropical parmelioid lichens.
- Three species level lineages in *Bulbothrix* two in *Parmelinella* and two in Canoparmelia remain undescribed due to the limited number of specimens sampled; further studies with a wider sampling are therefore required in order to better understand and delimit these lineages.

- In the light of this study revealing hidden diversity in the genus *Bulbothrix*, all species of this genus occurring in East Africa namely; *B. bulbochaeta, B. coronata, B. goebelii, B. hypocraea, B. meizospora, B. pustulata, B. suffixa* and *B. ventricosa* need to be revised to evaluate potentially hidden diversity. More studies are proposed in the *Bulbothrix subscortea* group to reassess the species boundaries.
- To fully understand the biogeographic distribution of *Parmelinella*, specimens from Australia need to be examined in order to elucidate their relationship to other *P. wallichiana* lineages recovered in this study.
- The polyphyly of *Hypotrachyna cirhatta* and *H. vexans* imply there could be more species masked under these nominal taxa. More studies with wider sampling are therefore necessary.
- Additional studies are proposed to clarify the current species delimitations in the African congeners of *Canoparmelia*, especially in the polyphyletic species recovered namely; *Canoparmelia caroliniana, C. epileuca, C. nairobiensis, C. schelpei* and *C. zimbabwensis* and the specimen representing *C. aff. schelpei* from Kenya clustering in the subgen. *Crespoa* that may belong to an undescribed species.
- The genus *Relicina* was recovered as paraphyletic and its sister relationship with *Relicina* subgen. *Relicinopsis* was unsupported, however only six out of 54 described species of *Relicina* were sampled, more studies including wider sampling to include more species is therefore recommended in order to understand taxa delimitation in this group. Further, *Relicinopsis malaccensis* and

Relicina abstrusa species did not form monophyletic groups, additional studies are necessary to evaluate species boundaries in these taxa.

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APPENDICES

Appendix 1. Specimens used in the study of *Bulborrhizina*, including collection details (locality and voucher information), and GenBank accession numbers.

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Bulborrhizina africana	**Kenya: Kitui County**	***Kirika* 4819 & *Lumbsch* (F)**	**KR445661**	**KR445662**	**KR445663**
Bulbothrix aff. goebelii 1	Fiji: Taveuni Island	*Lumbsch* 19817e (F)	GQ919260	GQ919235	GQ919208
Bulbothrix aff. goebelii 2	Fiji: Taveuni Island	*Lumbsch* 19817g (F)	GQ919261	GQ919236	GQ919209
Bulbothrix aff. hypochraea	Madagascar: Col de Tapia	*Ertz* 12888 (BR)	n/a	GQ919240	GQ919213
Bulbothrix apophysata	Costa Rica: San Jose	*Lücking* 16650b (F)	DQ279481	EU562670	DQ287788
Bulbothrix coronata	South Africa: W Cape	*Crespo et al.* s/n (MAF-Lich 13987)	DQ279482	EU562671	DQ287789
Bulbothrix decurtata	South Africa: W Cape	*Crespo et al*. s/n (MAF-Lich 13988)	DQ279483	EU562672	DQ287790
Bulbothrix goebelii	South Africa: W Cape	*Lumbsch* s/n (MAF-Lich 13985)	DQ279484	EU562673	DQ287791
Bulbothrix hypochraea	Madagascar: Col de Tapia	*Ertz* 12876 (BR)	n/a	GQ919239	GQ919212
Bulbothrix isidiza 1	Congo: Kahuri-Biega National Park	*Mamush* s/n (MAF-Lich 15511)	GQ919262	GQ919237	GQ919210
Bulbothrix isidiza 2	Madagascar: Col de Tapia N Ambositra	*Ertz* 12878 (BR)	GQ919263	GQ919238	GQ919211
Bulbothrix klementii	Costa Rica: Puntarenas	*Lücking* 15170a (F)	DQ279485	n/a	DQ287792
Bulbothrix laevigatula	Costa Rica: Puntarenas	*Lücking* 15045b (F)	GQ919264	n/a	GQ919214
Bulbothrix meizospora	India: Uttaranchal	*Divakar* s/n (GPGC 02-000786)	AY611068	AY607780	AY611127
Bulbothrix sensibilis	Rwanda: W. Province	*Ertz* 11025 (BR)	GQ919265	GQ919241	n/a
Bulbothrix setschwanensis	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10212)	AY611069	AY607781	n/a
Bulbothrix suffixa	Madagascar: Col de Tapia	*Ertz* 12889 (BR)	GQ919266	GQ919242	GQ919215
Bulbothrix tabacina 1	Congo: Kahuri-Biega National Park	*Mamush* s/n (MAF-Lich 16111)	GQ919267	GQ919243	n/a

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Bulbothrix tabacina 2	Kenya: Kakamega County	*Crespo, Lumbsch* & *Divakar* s/n (MAF-Lich16112)	GQ919268	GQ919244	GQ919216
Hypotrachyna aff. immaculata	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10413)	DQ279505	EU562680	DQ287814
Hypotrachyna afrorevoluta 1	Australia: New South Wales	*Elix* 28562 (MAF-Lich 15619)	GQ919286	GQ919259	GQ919233
Hypotrachyna afrorevoluta 2	Canary Islands: Tenerife	*Crespo* s/n (MAF-Lich 10409)	DQ279529	EU562681	DQ287839
Hypotrachyna andese	Peru: Ancash	*Lumbsch, Wirtz* & *Ramirez* 19334 F (MAF-Lich 15620)	GQ919269	GQ919245	GQ919217
Hypotrachyna booralensis	Australia: Queensland	*Lumbsch* s/n (MAF-Lich 13969)	DQ279493	EU562682	DQ287801
Hypotrachyna brasiliana	Brazil: Municipio de Piraquara	*Sanders* 99802.4 (MAF-Lich)	n/a	n/a	DQ287802
Hypotrachyna britannica	Ireland: Kerry	*Crespo* & *Gavilan* s/n (MAF-Lich 15415)	GQ919273	GQ919249	GQ919221
Hypotrachyna caraccensis	—	—	n/a	DQ912336	DQ912280
Hypotrachyna cirrhata 1	Costa Rica: San Jose	*Trest* 149 (MAF-Lich 7465)	AY611070	AY607782	AY611128
Hypotrachyna cirrhata 2	Peru: QuebradaParon	*Lumbsch* 19342r (MAF-Lich 13976)	DQ279487	EU562674	DQ287795
Hypotrachyna cryotochlora	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10398)	DQ279535	EU562695	DQ287845
Hypotrachyna degelii	—	—	n/a	DQ912337	DQ912281
Hypotrachyna dubitans	Peru: Ancash	*Lumbsch, Wirtz* & *Ramirez* 19366 (F, MAF-Lich 15621)	GQ919270	GQ919246	GQ919217
Hypotrachyna endochlora	Great Britain: Scotland	*Coppins* s/n (MAF-Lich 10178)	AY 611072	AY607784	AY611130
Hypotrachyna exsecta	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10380)	DQ279498	EU562684	DQ287807
Hypotrachyna fissicarpa	South Africa: W Cape	*Crespo* & al. s/n (MAF-Lich 13991)	DQ279501	n/a	DQ287810
Hypotrachyna horrescens	Spain: La Coruña	*Carvallal* s/n (MAF-Lich 9913)	AY581085	AY578951	AY582321

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Hypotrachyna imbricatula 1	Costa Rica: Manzanillo	*Molina* s/n (MAF-Lich 10382)	DQ279502	GQ919253	DQ287811
Hypotrachyna imbricatula 2	South Africa: W Cape	*Crespo* & *al*. s/n (MAF-Lich 13990)	DQ279503	EU562686	DQ287812
Hypotrachyna immaculata	Australia: Queensland	*Louwhoff, Molina* & *Elix* s/n (MAF-Lich 7462)	AY611073	AY607785	AY611131
Hypotrachyna laevigata	Great Britain: Scotland	*Coppins* s/n (MAF-Lich 10177)	AY611074	AY607786	AY611132
Hypotrachyna lipidifera	Peru: QuebradaCojup	*Lumbsch* 19309b (MAF-Lich 13966)	DQ279488	EU562675	DQ287796
Hypotrachyna livida	Argentina: Salta	*Argüello* s/n (MAF-Lich 15519)	GQ919282	n/a	GQ919230
Hypotrachyna minarum	Spain: Cádiz	*Crespo* & *al.* s/n (MAF-Lich 7639)	AY581086	AY579852	AY582322
Hypotrachyna neodamaziana	Australia: Queensland	*Louwhoff, Molina* & *Elix* s/n (MAF-Lich 10182)	AY611107	AY607820	AY611166
Hypotrachyna neodissecta	South Africa: W Cape	*Crespo* & *al.* s/n (MAF-Lich 13986)	DQ279510	EU562689	DQ287820
Hypotrachyna nepalense 1	India: Uttaranchal	*Divakar* s/n (GPGC 02-000924)	AY611071	AY607783	AY611129
Hypotrachyna nepalense 2	—	—	DQ383642	JN939605	n/a
Hypotrachyna osseoalba 1	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10390)	DQ279512	EU562690	DQ287822
Hypotrachyna osseoalba 2	South Africa: W. Cape	*Crespo et al.* s/n (MAF-Lich 15608)	GQ919279	n/a	GQ919227
Hypotrachyna physcioides	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10391)	DQ279513	EU562691	DQ287823
Hypotrachyna polydactyla	Kenya: Western	*Divakar* &. *Lumbsch* s/n (MAF-Lich 15518)	GQ919283	GQ919258	GQ919231
Hypotrachyna pseudosinuosa 1	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10392)	DQ279516	EU562692	DQ287826
Hypotrachyna pseudosinuosa 2	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10393)	DQ279517	GQ919257	DQ287827
Hypotrachyna reducens	Costa Rica: Nat. Park Irazu	*Lücking* 15450 (F)	DQ279520	n/a	DQ287830

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Hypotrachyna revoluta	Spain: Puerto Urkiola, Vizcaya	*Noya* & *Olea* s/n (MAF-Lich 6047)	AY611075	AY607787	AF351166
Hypotrachyna rhizocendroideum	China: Yunnan	*Aptroot* 55665 (ABL)	DQ279489	EU562676	DQ287797
Hypotrachyna rockii	Peru: QuebradaParon	*Lumbsch* 19342l (MAF-Lich 13965)	DQ279524	EU562693	DQ287834
Hypotrachyna sinuosa	Great Britain: Scotland	*Coppins* s/n (MAF-Lich 10179)	AY611076	AY607788	AY611133
Hypotrachyna sorcheilia	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10375)	DQ279490	EU562677	DQ287798
Hypotrachyna spumosa	USA: Pennsylvania	*Lendemer* & *Macklin* s/n (HB. *Lendemer* 2386 (MAF-Lich 15618))	GQ919287	n/a	GQ919234
Hypotrachyna subfatiscens	Australia: Queensland	*Louwhoff, Molina* & *Elix* s/n (MAF-Lich 6878)	AY611108	AY607821	AF351174
Hypotrachyna taylorensis	Great Britain: Scotland	*Hawksworth* s/n (MAF -Lich 9921)	AY581061	AY578924	AY582298
Hypotrachyna vexans	China: Yunnan	*Aptroot* 56597 (ABL)	DQ279491	EU562678	DQ287799
Myelochroa aurulenta	India: North Sikkim	*Divakar* s/n (MAF -Lich 13992)	DQ279530	EF042917	EF025484
Myelochroa irrugans	China: Yunnan	*Crespo* & *al.* s/n. (MAF-Lich 10207)	AY611103	AY607815	AY611160
Myelochroa metarevoluta	China: Yunnan	*Crespo et al*. s/n (MAF-Lich 10208)	AY611102	AY607814	AY611159
Parmelina carporrhizans	Spain: Madrid	*Crespo* s/n (MAF-Lich 6057)	AY611105	AY607818	AY611164
Parmelina pastillifera	Spain: Cádiz	*Crespo* s/n (MAF-Lich 6058)	AY611104	AY607817	EU562697
Parmelina tiliacea	Spain: Teruel	*Crespo et al.* s/n (MAF-Lich 6056)	AY581084	AY578950	AF351173
Parmelinella wallichiana	India: Sikkim	*Chatterjee* & *Divakar* s/n (MAF-Lich 7653)	AY611106	AY607819	AY611165
Parmeliopsis ambigua	—	—	AF410829	AY607822	EU562698
Parmeliopsis hyperopta	Spain: Madrid	*Blanco* s/n (MAF -Lich 10181)	AY611109	AY607823	AY611167
Remototrachyna adducta 1	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10378)	DQ279492	n/a	DQ287800

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Remototrachyna adducta 2	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10206)	AY785270	AY785263	AY785277
Remototrachyna aff. crenata	India: Karnatka	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15616)	GQ919275	GQ919250	GQ919223
Remototrachyna aff. infirma	India: Karnataka	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15611)	GQ919278	GQ919254	GQ919226
Remototrachyna awasthii 1	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15614)	GQ919271	GQ919247	GQ919219
Remototrachyna awasthii 2	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15615)	GQ919272	GQ919248	GQ919220
Remototrachyna ciliata	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10185)	AY785273	AY785266	AY785280
Remototrachyna costaricensis 1	Costa Rica: VolcanArenal	*Molina* s/n (MAF-Lich 10211)	AY785269	AY785262	AY785276
Remototrachyna costaricensis 2	Cuba: Granma	*Pérez Ortega* s/n (MAF-Lich 15607)	GQ919274	n/a	GQ919222
Remototrachyna crenata	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10377)	DQ279495	EU562683	DQ287804
Remototrachyna dopapetta 1	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15612)	GQ919276	GQ919251	GQ919224
Remototrachyna dopapetta 2	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15613)	GQ919277	GQ919252	GQ919225
Remototrachyna flexilis 1	India: North Sikkim	*Divakar* s/n (MAF-Lich 13974)	DQ279499	n/a	DQ287808
Remototrachyna flexilis 2	India: North Sikkim	*Divakar* s/n (MAF-Lich 13975)	DQ279500	EU562685	DQ287809
Remototrachyna incognita 1	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10385)	DQ279506	EU562687	DQ287815
Remototrachyna incognita 2	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10384)	DQ279507	n/a	DQ287816
Remototrachyna infirma 1	China: Yunnan	*Crespo, Blanco* &*Arguello* s/n (MAF-Lich 10386)	DQ279508	n/a	DQ287817

Taxon	Location	Voucher	ITS	nrLSU	mtSSU
Remototrachyna infirma 2	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10210)	AY785271	AY785264	AY785278
Remototrachyna kingii 1	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15610)	GQ919280	GQ919255	GQ919228
Remototrachyna kingii 2	India: Tamil Nadu	*Divakar, Lumbsch* & *Upreti* s/n (MAF-Lich 15609)	GQ919281	GQ919256	GQ919229
Remototrachyna koyensis	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10388)	DQ279509	EU562688	DQ287819
Remototrachyna rhabdiformis	India: North Sikkim	*Divakar* s/n (MAF-Lich 15617)	GQ919284	n/a	n/a
Remototrachyna scytophylla 1	China: Yunnan	*Crespo, Blanco* & *Arguello* s/n (MAF-Lich 10410)	DQ279525	EU562694	DQ287835
Remototrachyna scytophylla 2	India: Uttaranchal	*Divakar* s/n (MAF-Lich 15606)	GQ919285	n/a	GQ919232

Appendix 2. Specimens used in the study of *Bulbothrix*, with location, reference collection details and GenBank accession numbers. Newly obtained sequences are in bold face and missing data are indicated with a dash (—).

Taxon label	**Locality**	**Collector(s)**	**voucher specimen**	**Genbank accession number**		
				ITS	**mtSSU**	**nuLSU**
Bulbothrix asiatica China	China: Yunnan	*Li Song Wang et al.*	14-44427	KM285403	—	—
***Bulbothrix cinerea* Brazil_2500**	**Brazil**	**s.n**	**MNB3071**	**—**	**KX539200**	**KX539219**
***Bulbothrix decurtata* Kenya_9521**	**Kenya: Taita-Taveta County, Ngangao**	***Kirika* 4489**	**EA, MAF**	**KX539182**	**—**	**KX539211**
Bulbothrix decurtata South Africa_1861	South Africa: W Cape	*Crespo et al.* s/n	MAF-Lich 13988	DQ279483	DQ287790	EU562672
Bulbothrix hypochraea Madagascar_1374	Madagascar: Col de Tapia	*Ertz* 12876	BR	—	GQ919212	GQ919239
***Bulbothrix isidiza* Brazil_2504**	**Brazil**	**s.n**	**MNB3125**	**—**	**KX539199**	**KX539218**
Bulbothrix isidiza Congo_318	Congo	*Mamush* s/n	MAF-Lich 15511	GQ919262	GQ919210	GQ919237
Bulbothrix isidiza India_15505	India: Sikkim	Divakar	MAF	KX341979	—	KX341998
***Bulbothrix isidiza* Kenya_4633**	**Kenya: Kitui County, Nuu Hill**	***Kirika* & *Lumbsch* 3869**	**EA, F, MAF**	**KX539173**	**KX539189**	**KX539203**
***Bulbothrix isidiza* Kenya_4635**	**Kenya: Baringo County, Eldama Ravine**	***Kirika, Mugambi* & *Lumbsch* 2829**	**EA, F**	**KX539177**	**KX539191**	**KX539206**
***Bulbothrix isidiza* Kenya_4636**	**Kenya: Nyeri County, Mt. Kenya**	***Kirika* 4363B**	**EA, F, MAF**	**KX539178**	**KX539192**	**KX539207**
***Bulbothrix isidiza* Kenya_4638**	**Kenya: Nyeri County, Mt. Kenya**	***Kirika* 4364**	**EA, F, MAF**	**KX539179**	**KX539193**	**KX539208**
***Bulbothrix isidiza* Kenya_4823**	**Kenya: Kitui County, Nuu Hill**	***Kirika* & *Lumbsch* 4823**	**EA, F, MAF**	**KX539174**	**—**	**KX539204**

Taxon label	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Bulbothrix isidiza **Kenya_9297**	**Kenya: Nyeri County, Mt. Kenya**	***Kirika* 4363C**	**MAF**	**KX539180**	**KX539194**	**KX539209**
Bulbothrix isidiza **Kenya_9352**	**Kenya: Makueni County, Wote**	***Kirika, Malombe* & *Matheka* 3695**	**EA, F**	**KX539175**	**KX539190**	**KX539205**
Bulbothrix isidiza **Kenya_9386**	**Kenya: Kericho County**	**Kirika 3214**	**EA, F**	**KX539181**	**KX539195**	**KX539210**
Bulbothrix isidiza **Kenya_9722**	**Kenya: Kitui County, Nuu Hill**	***Kirika* & *Lumbsch* 3857**	**EA, F, MAF**	**KX539176**	**—**	—
Bulbothrix isidiza Madagascar_1376	Madagascar: Col de Tapia N Ambositra	*Ertz* 12878	Ertz 12878 (BR)	GQ919263	GQ919238	GQ919211
Bulbothrix isidiza **Thailand_3184**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27987**	—	—	**KX539215**
Bulbothrix isidiza **Thailand_3185**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27986**	—	—	**KX539216**
Bulbothrix isidiza **Thailand_3186**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27988**	—	—	**KX539217**
Bulbothrix isidiza **Thailand_3188**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27989**	**—**	**—**	**KX539223**
Bulbothrix meizospora India_351	India	*Divakar*	(MAF-Lich 17013)	JN943846	KR995316	JN939599
Bulbothrix meizospora India_786	India: Uttaranchal	*Divakar*	GPGC 02-000786	AY611068	AY611127	AY607780
Bulbothrix sensibilis **Kenya_9383**	**Kenya: Taita Taveta County, Ngangao**	***Kirika, Mugambi* & *Lumbsch* 2427**	**EA, F**	**—**	**KX539198**	**KX539214**

Taxon label	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Bulbothrix sensibilis Rwanda_3	Rwanda: W. Province	*Ertz* 11025	BR	GQ919265	—	GQ919241
Bulbothrix setschwanensis China_10212	China: Yunnan	*Crespo, Blanco & Arguello*	MAF 10212	AY611069	—	AY607781
Bulbothrix subscortea China	China: Yunnan Prov., Nanjian Co.	*Li Song Wang et. al.*	12-37673	KM249907	—	—
***Bulbothrix tabacina* Brazil_2505**	**Brazil**	**s.n**	**MNB3177**	**KX539185**	—	—
***Bulbothrix tabacina* Brazil_2509**	**Brazil**	**s.n**	**MNB3188**	**KX539186**	—	—
Bulbothrix tabacina Congo_317	Congo: Kahuri-Biega National	*Mamush* s/n	MAF-Lich 16111	GQ919267	—	GQ919243
Bulbothrix tabacina Kenya_1403_	Kenya: Kakamega County	*Crespo, Lumbsch & Divakar* s/n	MAF-Lich16112	GQ919268	GQ919216	GQ919244
***Bulbothrix tabacina* Kenya_4822**	**Kenya: Kitui County, Nuu Hill**	***Kirika* & *Lumbsch* 4822**	**EA, F, MAF**	**KX539183**	**KX539197**	**KX539212**
***Bulbothrix tabacina* Kenya_9301**	**Kenya: Makueni County, Wote**	***Kirika, Malombe* & *Matheka* 3704**	**EA, F**	**KX539184**	**—**	**KX539213**
***Bulbothrix tabacina* Thailand_3181**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27991**	**KX539187**	**KX539201**	**KX539220**
***Bulbothrix tabacina* Thailand_3182**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27990**	**KX539188**	**—**	**KX539221**
***Bulbothrix tabacina* Thailand_3183**	**Thailand: Khao Yai National Park**	***Buaruang***	**RAMK27992**	**—**	**KX539202**	**KX539222**
Parmelinella wallichiana China_250204	China: Yunnan	*Crespo, Blanco & Arguello*	MAF-10411	DQ279532	DQ287842	**KX341978**
Parmelinella Wallichiana India_322	India: Uttaranchal	*Divakar*	MAF	KX341980	KX341990	KX341999

Taxon label	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
Parmelinella Wallichiana India_7653	India: Sikkim	*Chatterjee* & *Divakar*	MAF-7653	AY611106	AY611165	AY607819

Appendix 3. Specimens used in the study of *Canoparmelia*, with location, reference collection details 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
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: Kakamega County**	***Kirika* 3419**	**EA, F**	**KX369243**	**—**	**KX369261**
***Canoparmelia caroliniana* 9309**	**Kenya: Kakamega County**	***Kirika* 3389**	**EA, F**	**KX369244**	**KX369256**	**KX369262**
***Canoparmelia* cf. *zimbabwensis*_9290**	**Kenya: Kitui County**	***Kirika* & *Lumbsch* 3828**	**EA, F, MAF**	**KX369245**	**—**	**KX369263**
***Canoparmelia ecaperata* 9293**	**Kenya: Makueni County**	***Kirika, Malombe* & *Matheka* 3692**	**EA, F**	**KX369246**	**—**	**KX369264**
***Canoparmelia eruptens* 9388**	**Kenya: Taita Taveta County**	***Kirika, Mugambi* & *Lumbsch* 2405**	**EA, F**	**KX369247**	**—**	**—**
***Canoparmelia eruptens* 9630**	**Kenya: Taita Taveta County**	***Kirika* 4483**	**EA, F, MAF**	**KX369248**	**KX369257**	**KX369265**

Species	**Locality**	**Collector (s)**	**voucher specimen**	**Genbank accession number**		
				ITS	**mtSSU**	**nuLSU**
***Canoparmelia zimbabwensis* 9390**	**Kenya: Taita Taveta County**	***Kirika, Mugambi* & *Lumbsch* 2292**	**EA, F**	**KX369249**	**—**	**KX369266**
Canoparmelia concrescens	Kenya: Western	*Divakar, Mangold* & *Lumbsch* 19538f	MAF-Lich 15547	GU994543	KR995317	GU994585
***Canoparmelia epileuca* 9292**	**Kenya: Kitui County**	***Kirika* & *Lumbsch* 3871**	**EA, MAF, F**	**KX369250**	**—**	**KX369267**
***Canoparmelia epileuca* 9508**	**Kenya: Kitui County**	***Kirika* & *Lumbsch*, 3866**	**EA, MAF, F**	**KX369251**	**KX369258**	**KX369268**
Canoparmelia nairobiensis	Kenya: Kakamega County	*Divakar, Mangold* & *Lumbsch* 19538g	MAF-Lich 15544	GU994545	KR995318	GU994587
***Canoparmelia nairobiensis* 9682**	**Kenya: Nyeri County**	***Kirika* 4423**	**EA, MAF, F**	**KX369252**	**KX369259**	**KX369269**
***Canoparmelia schelpei* 3248**	**Mozambique**	**s.n**	**MAF**	**KX369255**	**—**	**KX369270**
***Canoparmelia* aff. *nairobiensis* 9288**	**Kenya: Kakamega County**	***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
Cetrelia pseudolivetorum	China: Yunan	*Crespo et al*. s.n.	MAF-Lich 15506	GU994548	GU994639	GU994594

Species	Locality	Collector(s)	voucher specimen	Genbank accession number		
				ITS	mtSSU	nuLSU
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: Taita Taveta County**	***Kirika & Lumbsch,* 3964**	**EA, MAF, F**	**KX369254**	**KX369260**	**KX369272**
Crespoa inhaminensis	Kenya: Kakamega County	*Divakar, Lumbsch* & *Mangold* 19529l	MAF-Lich 15545	GU994544	GU994633	GU994586
Crespoa schelpei	Kenya: Nairobi County	*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: Flinders Ranges	*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

Species	Locality	Collector(s)	Voucher specimen	GenBank accession number		
				ITS	mtSSU	nuLSU
Flavopunctelia flaventior	Spain: Teruel	*Crespo et al.* s.n.	MAF-Lich 6046	AY581060	AF351164	AY578923
Flavopunctelia soredica	USA: Minesota	*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
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

Species	Locality	Collector(s)	Voucher specimen	GenBank accession number		
				ITS	mtSSU	nuLSU
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: Mayrland	*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

Species	Locality	Collector(s)	Voucher specimen	GenBank accession number		
				ITS	mtSSU	nuLSU
Xanthoparmelia hottentotta	South Africa: Cape Province	*Crespo et al*. s.n.	MAF-Lich 14267	EF042909	EF025486	EF042919
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	*Leavitt* 55567	BRY-C	HM578888	KR995373	HM579299

Appendix 4. Specimens used in the study of *Hypotrachyna* subgen. *Everniastrum*, with location, reference collection details and GenBank accession numbers. Newly obtained sequences are in bold face.

Taxa	**Seq/DNA code**	**Locality**	**Collector(s)**	**Voucher**	**ITS**	**mtSSU**	**nuLSU**
Hypotrachyna (=*Cetrariastrum*) *dubitans*	CEDU 200	Peru: Ancash	*Lumbsch, Wirtz & Ramirez,* 19366	F (MAF-Lich 15621)	GQ919270	GQ919217	GQ919246
Hypotrachyna (=*Cetrariastrum*) *kaernefeltii*	CEAN 199	Peru: Ancash	*Lumbsch, Wirtz & Ramirez,* 19334	MAF-Lich15620	GQ919269	GQ919217	GQ919245
Hypotrachyna* (=*Everniastrum*) aff_*cirrhata	**9881**	**Colombia; Tolima**	***Silano* JS14_042 37745**	**F**	**KX254119**	**KX254134**	**KX254146**
Hypotrachyna (=*Everniastrum*) *americana*	EVAM	Chile	*Feuerer* s.n	DNA1223(HBG, LD)	AY251418	—	—
Hypotrachyna* (=*Everniastrum*) *catawbiensis	**9602**	**Kenya; Central**	***Kirika,* 4325A**	**EA, F, MAF**	**KX254120**	**KX254135**	**KX254147**
Hypotrachyna* (=*Everniastrum*) *catawbiensis	**9883**	**Colombia; Cuaca**	***Salinas* 32b**	**F**	**KX254121**	—	—
Hypotrachyna (=*Everniastrum*) *cirrhata*1	OECIRRHA	Costa Rica: San José	*Trest* 149	MAF-Lich 7465	AY611070	AY611128	AY607782
Hypotrachyna (=*Everniastrum*) cirrhata2	EVCI 1894	Peru	*Lumbsch* 19342r	MAF 13976	DQ279487	DQ287795	EU562674
Hypotrachyna (=*Everniastrum*) *cirrhata*3	ECIRRHAT	China	*Crespo et al.*	MAF 10374	DQ279486	DQ287794	—
Hypotrachyna* (=*Everniastrum*) *columbiensis	**4768**	**Peru**		**MAF**	**KX254122**	—	—
Hypotrachyna* (=*Everniastrum*) *columbiensis	**9570**	**Peru**		**MAF(PY01)**	**KX254123**	**KX254136**	**KX254148**
Hypotrachyna (=*Everniastrum*) *nepalensis*	ENEPAL	India: Uttarakhand	*Divakar* s/n	GPGC02-000924	AY611071	AY611129	AY607783

Taxa	Seq/DNA code	Locality	Collector(s)	Voucher	ITS	mtSSU	nuLSU
Hypotrachyna (=*Everniastrum*) *rhizodendroidea*	ERHYZODE	China: Yunnan	*Aptroot* 55665	ABL	DQ279489	DQ287797	EU562676
Hypotrachyna* (=*Everniastrum*) *sorocheila	**4780**	**India**		**MAF**	**KX254124**	—	—
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9295**	**Kenya: Kirinyaga County**	***Kirika* 3653**	**EA, F**	—	**KX254137**	**KX254149**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9520**	**Kenya: Western**	***Divakar et al.* 19566P**	**MAF**	**KX254125**	**KX254138**	**KX254150**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9544**	**Kenya; Nyeri County**	***Kirika* 4325B**	**EA, F, MAF**	**KX254126**	**KX254139**	**KX254151**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9567**	**Kenya; Nyeri County**	***Kirika* 4349**	**EA, F, MAF**	**KX254127**	**KX254140**	**KX254152**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9870**	**Colombia: Cundinamarca**	***Lomana* 299**	**F**	—	**KX254141**	**KX254153**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9875**	**Colombia; Narino**	***Moncada* 7577**	**F**	**KX254128**	**KX254142**	**KX254154**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9876**	**Colombia; Cundinamarca**	***Avella,* 351a**	**F**	**KX254129**	**KX254143**	**KX254155**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9878**	**Colombia; Cundinamarca**	***L. Castro* 27**	**F**	**KX254130**	—	**KX254156**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9879**	**Colombia; Cundinamarca**	***Uribe* 24**	**F**	**KX254131**	—	**KX254157**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9880**	**Colombia; Cundinamarca**	***Uribe* 40**	**F**	**KX254132**	—	**KX254158**
Hypotrachyna* (=*Everniastrum*) *sorocheila	**9903**	**New Zealand**		**F**	**KX254133**	**KX254144**	**KX254159**

Taxa	**Seq/DNA code**	**Locality**	**Collector(s)**	**Voucher**	**ITS**	**mtSSU**	**nuLSU**
Hypotrachyna (=*Everniastrum*) *sorocheila*1	ESORECHI	China	*Crespo, Blanco* & *Arguello*	MAF-10375	DQ279490	DQ287798	EU562677
Hypotrachyna (=*Everniastrum*) *sorocheila*2	EVSO501	Kenya: Western	*Divakar, Mangold* & *Lumbsch* 19566e	F, MAF-Lich	JN943841	KR995337	JN939606
***Hypotrachyna* (=*Everniastrum*) *sorocheila*3**	**3514**	**Portugal: Madeira**	***P.K. Divakar* & M. *Talavera*, 6974P**	**MAF**	**KX341977**	—	—
Hypotrachyna* (=*Everniastrum*) *vexans	**9294**	**Kenya: Kirinyaga County**	**Kirika 3662**	**EA, F**	—	**KX254145**	**KX254160**
Hypotrachyna (=*Everniastrum*) *vexans*	EVEXANS	China: Yunnan	*Aptroot* 56597	ABL	DQ279491	DQ287799	EU562678

Appendix 5. Specimens used in the study of *Parmelinella*, with the location, reference collection details and GenBank accession numbers. Newly obtained sequences are in bold face.

Species	**Seq/DNA code**	**Locality**	**Collector(s)**	**Voucher specimen**	**GenBank accession numbers**		
					ITS	**mtSSU**	**nuLSU**
Bulbothrix isidiza	**15505**	**India: Sikkim**	***Divakar***	**—**	**KX341979**	—	**KX341998**
Bulbothrix isidiza1	BUIS 318it	Congo	*Mamush* s/n	MAF 15511	GQ919262	GQ919210	GQ919237
Bulbothrix isidiza2	BUIS 1376I	Madagascar: Col de Tapia N Ambositra	*Ertz* 12878 (BR)		GQ919263	GQ919238	GQ919211
Parmelinella aff. wallichiana	3122	Brazil: Curitiba	*S. Eliasaro*	UPCB	GQ267691	—	—
Parmelinella sp aff wallichiana	BRYC56001	Cameroon: Ekona	*Orock*	BRYC 56001	JQ673451	—	—
Parmelinella wallichiana1	7653	India: Sikkim	*Chatterjee & Divakar*	MAF-7653	AY611106	AY611165	AY607819
Parmelinella wallichiana	**322**	**India: Uttaranchal**	***Divakar***	**—**	**KX341980**	**KX341990**	**KX341999**
Parmelinella wallichiana	**3615**	**India: South India**	***Lumbsch et al.***	**—**	**KX341981**	—	—
Parmelinella wallichiana	**4678**	**Kenya: Marsabit County**	***Kirika* & *Lumbsch* 4678**	**EA, F, MAF**	**KX341982**	**KX341991**	—
Parmelinella wallichiana	**4715**	**Kenya: Marsabit County**	***Kirika* & *Lumbsch* 4715**	**EA, F, MAF**	**KX341983**	**KX341992**	KX342000
Parmelinella wallichiana	**9310**	**Kenya: Tharaka Nithi County**	***Kirika* 3432**	**EA, F**	—	**KX341993**	**KX342001**
Parmelinella wallichiana	**9311**	**Kenya: Kitui County**	***Kirika* 3487**	**EA, F**	**KX341984**	—	**KX342002**
Parmelinella wallichiana	**9312**	**Kenya: Makueni County**	***Kirika, Malombe & Matheka 3703***	**EA, F**	**KX341985**	—	**KX342003**
Parmelinella wallichiana	**9398**	**Kenya: Kericho County**	***Kirika 3145***	**EA, F**	**—**	—	**KX342005**

Species	**Seq/DNA code**	**Locality**	**Collector(s)**	**Voucher specimen**	**GenBank accession numbers**		
					ITS	**mtSSU**	**nuLSU**
Parmelinella wallichiana	**9552**	**Kenya: Eldama Ravine**	***Kirika, Mugambi & Lumbsch* 2815**	**EA, F**	—	**KX341995**	**KX342006**
Parmelinella wallichiana	**9592**	**Kenya: Kericho County**	***Kirika Mugambi & Lumbsch* 2974**	**EA, F**	**KX341987**	—	—
Parmelinella cf. wallichiana	**9647**	**Kenya: Kwale County**	***Kirika* & *Lumbsch* 4033**	**F,EA, MAF**	**KX341988**	**KX341996**	**KX342007**
Parmelinella wallichiana	**9693**	**Kenya: Trans Nzoia County**	***Kirika* 4280**	**F,EA, MAF**	**KX341989**	**KX341997**	**KX342008**
Parmelinella wallichiana	250204	China: Yunnan	*Crespo, Blanco & Arguello*	MAF-10411	DQ279532	DQ287842	—
Parmelinella lindmanii	3131	Brazil: Curitiba	*S. Eliasaro*	UPCB	GQ267190	—	—

Appendix 6. Specimens used in the phylogenetic analysis of *Relicina* and *Relicinopsis*, with location, reference collection details and GenBank accession numbers. Newly obtained sequences for this study are in bold face and missing data are indicated with a dash (—).

Taxon label	Collection details	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, *Nayarit Nash* 46672 (ASU)	KM657272	KM657291	KM657311
*P. floridensis*_KS3	USA, Florida, *Scharnagl* KS3 (F)	KM657274	KM657293	KM657313
*P. floridensis*_ KS11	USA, Florida, *Scharnagl* KS11 (F)	KM657273	KM657292	KM657312
*P. floridensis*_ KS30	USA, Florida, *Scharnagl* KS30 (F)	KM657275	KM657294	KM657314
*P. uleana*_8706	USA, Florida, *Seavey* 1386 (LSU)	KM657276	KM657295	KM657315
*Relicina abstrusa*_37426	Australia, *Elix* 37426 (CANB)	GU994580	GU994580	-
*R. abstrusa*_1085	Thailand, *Lumbsch* 19756g	KM657278	KM657297	KM657317
*R. abstrusa*_1082	Thailand, Khao Kew, *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, Kwale County, *Kirika* 4505 (EA, F, MAF)**	-	-	**KX434467**
***R. abstrusa*_9603**	**Kenya, Kwale County, *Kirika* 4506 (EA, F, MAF)**	**KX434464**	**KX434472**	-
***R. abstrusa*_9608**	**Kenya, Kilifi County, *Kirika* 4541 (EA, F, MAF)**	-	**KX434473**	-
***R. abstrusa*_9619**	**Kenya, Kwale County, *Kirika* & *Lumbsch* 4032 (EA, F, MAF)**	**KX434465**	**KX434474**	**KX434469**
***R. echinocarpa*_9317**	**Kenya, Taita Taveta County, *Kirika* & *Mugambi* 3567 (EA, F)**	-	**KX434471**	**KX434468**
***R. echinocarpa*_9623**	**Kenya, Taita Taveta County, *Kirika* 4432 (F, MAF)**	-	**KX434476**	**KX434470**

Taxon label	Collection details	ITS	nuLSU	mtSSU
R. filsonii	Australia, New South Wales *Elix* 37267 (CANB)	KM657281	-	-
*R. subabstrusa*_3193	Thailand, *Buarang et al.* 24370 (RAMK)	KM657282	KM657300	KM657320
R. subnigra	Australia, Molonglo Gorge Reserve, *Louwhoff et al*. (MAF-Lich 10184)	AY785274	AY785267	AY785281
R. sydneyensis	Australia, *Lumbsch* & Mangold 19179a (F)	GU994581	GU994630	GU994675
*Relicinopsis intertexta*_1083	Thailand, Khao Khew, *Lumbsch* 19756g (F)	KM657283	KM657301	KM657323
*R. intertexta*_3177	Thailand, *Buarang et al*. 24372 (RAMK)	-	KM657302	KM657324
***R. malaccensis*_9621**	**Kenya, Kwale County, *Kirika* 4499 (EA, F, MAF)**	**KX434466**	**KX434475**	-
***R. malaccensis*_9635**	**Kenya, Kwale County, *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, Northern Territory, *Elix* 37835 (CANB)	KM657288	KM657310	-

Appendix 7. Papers Published from this Research Work

1. Kirika, P.M., Leavitt, S.D., Divakar, P.K., Crespo, A., Gatheri, G.W., Mugambi, G. and Lumbsch, H.T. (2015). The monotypic genus *Bulborrhizina* belongs to *Bulbothrix* sensu lato (Parmeliaceae, Ascomycota). *Bryologist*, 118(2):164-169.
2. Kirika, P.M., Divakar, P.K., Crespo, A., Leavitt, S.D., Mugambi, G., Gatheri, G.W. and Lumbsch, H.T. (2016). Polyphyly of the genus *Canoparmelia* - uncovering incongruences between phenotype-based classification and molecular phylogeny within lichenized Ascomycota (Parmeliaceae). *Phytotaxa* 289 (1): 036–048.
3. Kirika, P.M., Divakar, P.K., Crespo, A., Gatheri, G.W., Mugambi, G., Leavitt, S.D., Moncada, B. and Lumbsch, H.T. (2016). Molecular data show that *Hypotrachyna sorocheila* (Parmeliaceae) is not monophyletic. *Bryologist* 119: 172-180.
4. Kirika, P.M., Divakar, P.K., Crespo, A., Mugambi, G., Orock, E.A., Leavitt, S.D., Gatheri G.W. and Lumbsch, H.T. (2016). Phylogenetic studies uncover a predominantly African lineage in a widely distributed lichen-forming fungal species. *Mycokeys* 14: 1-16.
5. Kirika, P.M., Divakar, P.K., Leavitt, S.D, Buruang, K., Crespo, A., Mugambi, G., Gatheri, G.W. and Lumbsch, H.T. (2017). The genus *Relicinopsis* is nested within *Relicina* (Parmeliaceae, Ascomycota). *The Lichenologist* 49(03):189-197
6. Kirika, P.M., Divakar, P.K., Buaruang, K., Leavitt, S.D., Crespo, A., Gatheri, G.W, Mugambi, G., Benatti, M. and Lumbsch, H.T. (2017) Molecular phylogenetic studies unmask overlooked diversity in the tropical lichenized fungal genus *Bulbothrix* s.l. (Parmeliaceae, Ascomycota). *Botanical Journal of the Linnean Society* 184:387–399

Appendix 8. Research Authorization Documents

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date:

1st November, 2013

NACOSTI/12B/013/66

Paul Muigai Kirika
Kenyatta University
P.O.Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "***Diversity and molecular systematics of parmelioid lichens (parmeliaceae, Ascomycota) in Kenya,***" I am pleased to inform you that you have been authorized to undertake research in **selected Counties** for a period ending **31st December, 2014.**

You are advised to report to **the County Commissioners and the County Directors of Education of the selected Counties** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. M. K. RUGUTT, PhD, HSC.
DEPUTY COMMISSION SECRETARY
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Copy to:

The County Commissioners
The County Directors of Education
Selected Counties.

PAGE 2

THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Paul Muigai Kirika
of (Address) Kenyatta University
P.O.Box 43844-00100, Nairobi.
has been permitted to conduct research in
Location
District
Counties
Selected
On the topic: Diversity and molecular systematics of parmelioid lichens (parmeliaceae, Ascomycota).
for a period ending: 31st December, 2014.

Applicant's Signature

PAGE 3

Research Permit No. *NACOSTI/RCD/12B/013/66*
Date of issue *1st November, 2013*
Fee received *KSHS. 2000*

For: Secretary
National Commission for Science
Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 555

CONDITIONS: see back page

Designed & Printed by:
Jaw Enterprises

nema
mazingira yetu | uhai wetu | wajibu wetu

NATIONAL ENVIRONMENT MANAGEMENT AUTHORITY

Tel: (254-020)-6005522 / 3 / 6 / 7, 6001945, 6008767
Mobile line: 0724 253 398, 0723 363 010, 0735 013 046, 0735 010 237
Telkom Wireless: 020-2101370
Fax: (254-020)-6008997
Hotline: 020-8077233, 020-6006041

P. O. Box 67839 - 00200
Popo Road, Nairobi, Kenya
E-mail: dgnema@nema.go.ke
website: www.nema.go.ke

Ref: NEMA/AGR/39/2013

22nd October, 2013

Paul M. Kirika
National Museums of Kenya,
Botany Department
P.O Box 40568-00100
NAIROBI

RE: ACCESS PERMIT APPLICATION TO ACCESS LICHENS (PARMELIACEAE) IN VARIOUS LOCATIONS IN KENYA

The National Environment Management Authority (NEMA) has reviewed your application for an access permit to access lichens (Parmeliaceae) in Mt. Kenya, Aberdares, Mt. Elgon, Chyulu hills, Shimba Hills, Esegeri Hills Mattews Ranges and Marsabit and the following issues need to be addressed to help the Authority to make informed decisions:

I. Provide Prior Informed Consent with Mutually Agreed Terms from Kenya Forest Service (as it is noted that the resources will be sourced from forests) and also from communities surrounding these forests.

II. Provide valid research clearance from National Council for Science and Technology

Kindly provide the above information.

VERONICAH KIMUTAI
For: DIRECTOR GENERAL

NATIONAL ENVIRONMENT MANAGEMENT AUTHORITY

Tel: (254-020)-6005522 / 3 / 6 / 7, 6001945, 6008767
Mobile line: 0724 253 398, 0723 363 010, 0735 013 046, 0735 010 237
Telkom Wireless: 020-2101370
Fax: (254-020)-6008997
Hotline: 020-8077233, 020-6006041

P. O. Box 67839 - 00200
Popo Road, Nairobi, Kenya
E-mail: dgnema@nema.go.ke
website: www.nema.go.ke

NEMA/ AGR/39/2013

22nd January, 2014

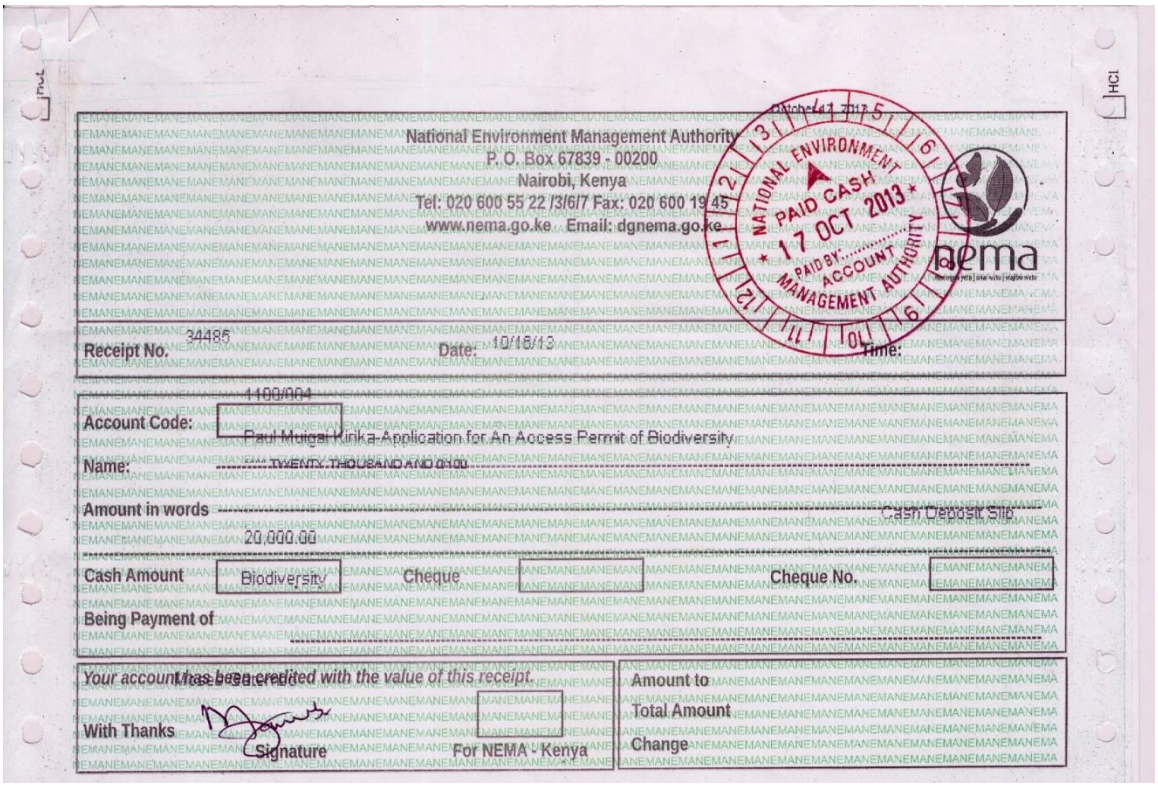
Paul M. Kirika
National Museums of Kenya
Botany Department
P.O. BOX 40568-00100
NAIROBI, KENYA.

Re: Approval Conditions to Access Lichen Thalli of Parmelioid Lichens (*Parmeliaceae*)

The National Environment Management Authority (NEMA) has reviewed your application for an access permit to access lichen thalli of Parmelioid (*Parmeliaceae*) Lichens from Mt.Kenya, Aberdares, Mt. Elgon, Chyulu Hills, Shimba Hills, Esageri, Matthews Range and Marsabit.

In light of the provisions of Environmental Management and Coordination (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing) Regulations, 2006, the Authority has approved the application subject to the following conditions:

1. This approval shall be valid for **12 months** from the date of issuance and shall not be transferable.
2. This approval is for accessing Lichen Thalli from Parmelioid (*Parmeliaceae*) Lichens.
3. The access of the genetic resources and its derivatives shall be strictly for understanding Biodiversity and Molecular Systematics of Parmelioid (*Parmeliaceae*) Lichens in Kenya.
4. The genetic resource and its derivatives shall not be transferred to a third party without the consent from the genetic resource providers, NEMA, Kenya Forest Service, Kenya Wildlife Service and National Museums of Kenya.
5. The genetic resource and the derivative shall not be used for commercial or profit purpose without understanding from NEMA, Kenya Forest Service, Kenya Wildlife Service and National Museums of Kenya.



National Environment Management Authority
P. O. Box 67839 - 00200
Nairobi, Kenya
Tel: 020 600 55 22 /3/6/7 Fax: 020 600 19 45
www.nema.go.ke Email: dgnema.go.ke

NATIONAL ENVIRONMENT MANAGEMENT AUTHORITY
PAID CASH
17 OCT 2013
PAID BY.....
ACCOUNT

Receipt No. 34485 Date: 10/16/13 Time:

Account Code: 1400/004
Name: Paul Muigai Kirika-Application for An Access Permit of Biodiversity
Amount in words TWENTY THOUSAND AND 00/00 Cash Deposit Slip
20,000.00
Cash Amount Biodiversity Cheque Cheque No.
Being Payment of

Your account has been credited with the value of this receipt.

With Thanks Signature For NEMA - Kenya

Amount to
Total Amount
Change

(r. 1

Application Reference No: NEMA/AGR/39/2013

Registration:.....0032.....

Form of Access Permit

This Permit is hereby granted to M/s .....PAUL MUIGAI KIRIKA, NATIONAL MUSEUMS OF KENYA, BOTANY DEPARTMENT P.O. BOX 40568 – 00100 NAIROBI.....

..... (*insert name, contact address and description of applicant)* in accordance with Regulation 11 of the Environmental Management and Co-ordination (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing) Regulations, 2006 for the collection of the following genetic resources:

....LICHEN THALLI OF PARMELIOID LICHENS.....

.....

.....

(insert description of the genetic resource, its derivatives, product(s) or intangible component(s) as stated in the Material Transfer Agreement) located at .....

.....MT. KENYA, ABERDARES, MT. ELGON, CHYULU HILLS, ESEGERI HILLS, SHIMBA HILLS, MATTHEWS RANGE, MARSABIT FOREST.....

..... (*insert geographical description of the location of the genetic resources*).

This Permit is issued subject to the Regulations and all agreements concluded pursuant to its grant, and may be suspended, cancelled or revoked should the holder breach any of those agreements and the conditions of issue and those contained in the Regulations.

M/s .....PAUL MUIGAI KIRIKA..... (*insert name of applicant*) being the holder of this Permit, including his agents and assignees, undertake to abide by the conditions of this Permit and to promptly report to the National Environment Management Authority any matter that may prejudice the interests of Kenya and other parties concluded pursuant to the grant of this Permit.

Signed:..... Date: .....28TH JANUARY, 2014

For *Director General*
The National Environmental Management Authority *(Seal)*

PERMIT CONDITIONS:

1: .....

2: .....

3: .....

PERMIT CONDITIONS-NEMA/AGR/35/2013

1. This approval shall be valid for **12 months** from the date of issuance and shall not be transferable.

2. This approval is for accessing Lichen Thalli from Parmelioid (*Parmeliaceae*) Lichens.

3. The access of the genetic resources and its derivatives shall be strictly for understanding Biodiversity and Molecular Systematics of Parmelioid (*Parmeliaceae*) Lichens in Kenya.

4. The genetic resource and its derivatives shall not be transferred to a third party without the consent from the genetic resource providers, NEMA, Kenya Forest Service, Kenya Wildlife Service and National Museums of Kenya.

5. The genetic resource and the derivative shall not be used for commercial or profit purpose without understanding from NEMA, Kenya Forest Service, Kenya Wildlife Service and National Museums of Kenya.

6. You shall deposit and keep records of all duplicates and holotypes of the genetic resources with the National Museums of Kenya and provide evidence of deposition.

7. You shall ensure that there is reasonable access by all Kenyan citizens to all genetic resources collected whether such genetic resources and intangible components are held locally or abroad.

8. Ensure that all agreements entered into with respect to access of genetic resources shall be strictly for the purposes for which they were entered into.

9. You shall execute the Material Transfer Agreement with National Museums of Kenya enjoined with Kenya Wildlife Service and Kenya Forests Service and a copy deposited with NEMA.

10. You shall furnish quarterly reports to NEMA on the status of research, including all discoveries from research involving genetic resources and/or intangible components thereof and a final report and peered reviewed publication.

11. You shall comply with the Improvement Orders issued by NEMA's environmental inspectors throughout the project cycle.

12. You shall abide by the laws of Kenya.

13. NEMA reserves the right to suspend, cancel, revoke or vary this permit in the event of a breach of the conditions stated here in or any contravention to the Environmental Management and Coordination Act, 1999 and regulations thereunder.

14. Upon the completion of the intended studies, you shall return the remaining genetic resource (if any specimen is exported) to Kenya and deposit with National Museums of Kenya and notify NEMA of the return

15. Publications arising from knowledge generated from the accessed genetic material should provide the site of sample collection, include local collaborators in the authorship and should have the following acknowledgement: "Genetic resources utilized in this study were provided by the Government and people of Kenya''.

KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

Winner: COYA 2010 Awards in Corporate Citizenship & Environment, and Human Resource Management.

KWS/BRM/5001

10 September 2013

Dr. M.P.H. Gathaara
Department of Plant and
Microbial Sciences
Kenyatta University
P.O.Box 43844
NAIROBI
e-mail: chairman-pms@ku.ac.ke ; pmkirika@gmail.com

Dear Dr. Gathaara,

CONSENT TO OBTAIN AN ACCESS PERMIT TO GENETIC RESOURCES FROM THE NATIONAL ENVIRONMENT MANAGEMENT AUTHORITY (NEMA) TO COLLECT LICHENS FROM PROTECTED AREAS

We acknowledge receipt of your letter dated 3 September 2013 requesting for permission for Mr. Paul M. Kirika to conduct research on a project titled: '*Diversity and Molecular Systematics of Parmeloid Lichens (Parmeliaceae) in Kenya*'. Consent is hereby given to Mr. Kirika to initiate the process of acquiring an Access Permit to Genetic Resources from NEMA.

This is in compliance with the NEMA Legal Notice No. 160 of 1999 and revised version of 2006, where researchers are required to first obtain an access permit to genetic resources from NEMA to collect samples from the field and thereafter seek permission from respective resource management authorities to access the required specimens for research purposes.

KWS will consider issuing to Mr. Kirika permission to conduct the research in protected areas after submission of his application and the access permit to genetic resources from NEMA.

Yours sincerely,

SAMUEL M. KASIKI, PhD, OGW
DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:
- Director-General NEMA
- Paul M. Kirika

KENYA WILDLIFE SERVICE

ISO 9001:2008 Certified

KWS/BRM/5001

10 March 2014

Mr. Paul Muigai Kirika
Kenyatta University
P.O.Box 40658-00100
NAIROBI
e-mail: pkirika@gmail.com
mobile: 0722568191

Dear Paul

PERMISSION TO CONDUCT RESEARCH ON LICHENS IN AND OUTSIDE PROTECTED AREAS

We acknowledge receipt of your letter dated 3rd September 2013 requesting for permission to conduct research on a project titled: '*Diversity and Molecular Systematics in Parmelioid Lichens (Parmeliaceae) in Kenya*'. The study will generate data and information that will help in the understanding of the spatial and temporal distribution of lichens in high altitude montane ecosystems.

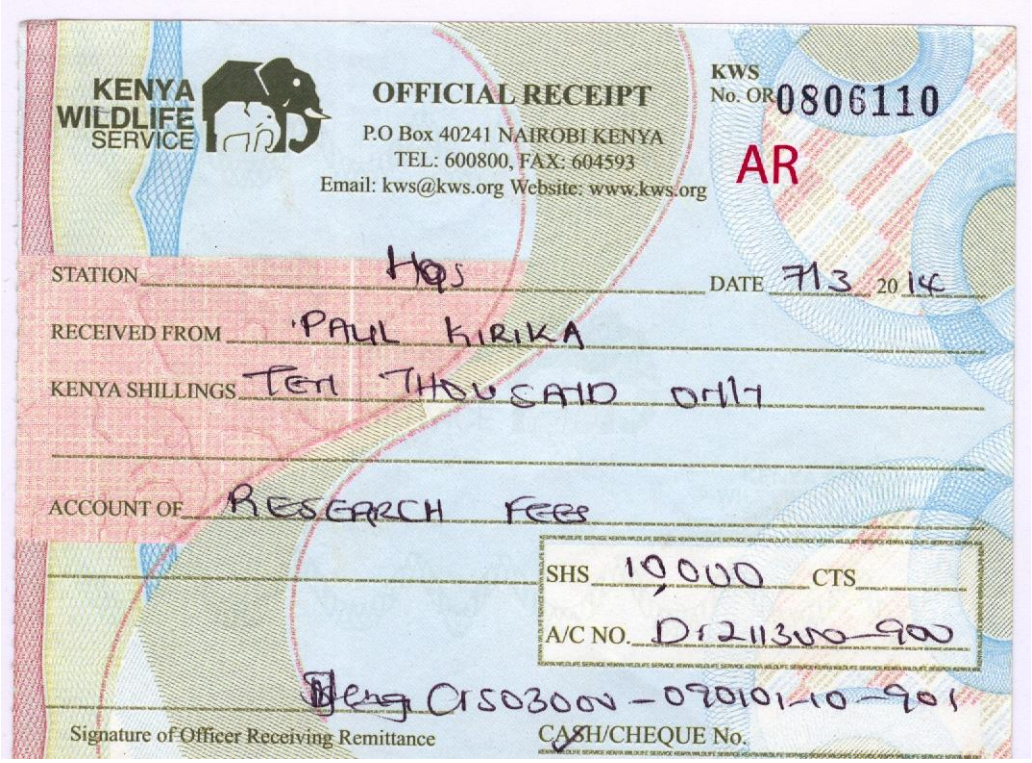
You have been granted permission to conduct the study from **March 2014 t0 March 2017** upon payment to KWS of academic research fees of **Ksh. 10000**. However, you will abide by the set KWS regulations and guidelines regarding the conduct of research in and outside protected areas. You will also be required to work closely with our Senior Scientist in-charge of Mountain Conservation Area (MCA), Western Conservation Area (WCA), Tsavo Conservation Area (TCA), Coast Conservation Area (CCA) and Northern Conservation Area (NCA), whom you will give a copy of the research proposal and regular progress reports on the study.

You will submit a copy of your PhD thesis to the KWS Deputy Director, Biodiversity Research and Monitoring on completion of the study.

Yours Sincerely

DR. ERUSTUS KANGA
FOR: DEPUTY DIRECTOR
BIODIVERSITY RESEARCH AND MONITORING

Copy to:
- SAD-Parks & Reserves
- Senior Scientist, MCA, WCA, TCA, CCA, NCA
- Senior Warden, Aberdare N. Park, Mt. Kenya N. Park, Tsavo West N. Park, Mt. Elgon N. Park, Marsabit N. Park



KENYA WILDLIFE SERVICE

OFFICIAL RECEIPT

P.O Box 40241 NAIROBI KENYA
TEL: 600800, FAX: 604593
Email: kws@kws.org Website: www.kws.org

KWS No. OR 0806110

AR

STATION HQS DATE 7/3/2014

RECEIVED FROM PAUL KIRIKA

KENYA SHILLINGS TEN THOUSAND ONLY

ACCOUNT OF RESEARCH FEES

SHS 10,000 CTS

A/C NO. D:211300-900

Eng C150300V-090101-10-901

Signature of Officer Receiving Remittance

CASH/CHEQUE No.

KENYA
Forest Service

Kenya Forest Service
Karura, Off Kiambu Rd
P.O. Box 30513-00100
Nairobi, Kenya

Ref: No. RESEA/1/KFS/51

Date: 10th September 2013

The Chairman
Department of Plant and Microbial Sciences
P.O. Box 43844
NAIROBI

RE: PERMISSION TO UNDERTAKE RESEARCH FOR A PhD THESIS FOR MR. PAUL .K. KIRIKA REG. NO. 184/23317/2012

I refer to your letter of 3rd September, 2013 on the above subject. Kenya Forest Service appreciates the role played by research in generating knowledge that contributes to informed decision making in management.

Permission is hereby granted to access Endau, Nuu, Taita Hills and Loita Hills, with the following conditions;

i. The requirements of the Legal Notice No. 160, "The Environment Management and Coordination Act, . Regulation 2006 (Conservation of Biological Diversity and Resources, Access to Genetic Resources and Benefit Sharing)," Section 9, are complied with.
ii. A copy of the summary of the findings is submitted to Kenya Forest Service.

By a copy if this letter, the Ecosystem Conservators of Kitui, Taita Taveta, Kajiado, Laikipia, Kilifi, Mombasa, Lamu, and Kwale are instructed to facilitate access.

D.K. Mbugua
Director

Copy to:- Ecosystem Conservators: – Kitui, Taita Taveta, Kajiado, Laikipia, Kilifi, Mombasa, Lamu, and Kwale.

JM/ca

Trees for better lives

Tel: (254) 020-3754904/5/6, (254) 020-2014663, (254) 020-2020285, Fax: (254) 020-2385374
Email: info@kenyaforestservice.org, Website: www. kenyaforestservice.org