The Lichenologist **50**(3): 299–312 (2018) doi:10.1017/S0024282918000233

Assessing phylogeny and historical biogeography of the largest genus of lichen-forming fungi, *Xanthoparmelia* (*Parmeliaceae*, Ascomycota)

Steven D. LEAVITT, Paul M. KIRIKA, Guillermo AMO DE PAZ, Jen-Pan HUANG, Jae-Seoun HUR, John A. ELIX, Felix GREWE, Pradeep K. DIVAKAR and H. Thorsten LUMBSCH

Abstract: Species richness is not evenly distributed across the tree of life and a limited number of lineages comprise an extraordinarily large number of species. In lichen-forming fungi, only two genera are known to be 'ultradiverse' (>500 species), with the most diverse genus, *Xanthoparmelia*, consisting of *c.* 820 species. While Australia and South Africa are known as current centres of diversity for *Xanthoparmelia*, it is not well known when and where this massive diversity arose. To better understand the geographical and temporal context of diversification in this diverse genus, we sampled 191 *Xanthoparmelia* specimens representing *c.* 124 species/species-level lineages from populations worldwide. From these specimens, we generated a multi-locus sequence data set using Sanger and high-throughput sequencing to reconstruct evolutionary relationships in *Xanthoparmelia*, estimate divergence times and reconstruct biogeographical histories in a maximum likelihood and Bayesian framework. This study corroborated the phylogenetic placement of several morphologically or chemically diverse taxa within *Xanthoparmelia*, such as *Almbornia*, *Chondropsis*, *Karoowia*, *Namakwa*, *Neofuscelia*, *Omphalodiella*, *Paraparmelia*, *Placoparmelia* and *Xanthomaculina*, in addition to improved phylogenetic resolution and reconstruction of previously unsampled lineages within *Xanthoparmelia*. Our data indicate that *Xanthoparmelia* most likely originated in Africa during the early Miocene, coinciding with global aridification and development of open habitats. Reconstructed biogeographical histories of *Xanthoparmelia* reveal diversification restricted to continents with infrequent intercontinental exchange by long-distance dispersal. While likely mechanisms by which *Xanthoparmelia* obtained strikingly high levels of species richness in Australia and South Africa remain uncertain, this study provides a framework for ongoing research into diverse lineages of lichen-forming fungi. Finally, our study highlights a novel approach for generating locus-specific molecular sequence data sets from high throughput metagenomic reads.

Key words: Africa, arid regions, Australia, BioGeoBEARS, diversification, hyperdiversity, ultradiverse

Accepted for publication 12 *February* 2018

Introduction

Species richness is not evenly distributed across the tree of life and evolutionary forces have resulted in some lineages comprising an extraordinarily large number of species (Büdel *et al.* 2014). Understanding how these exceptionally species-rich lineages arose is a central pursuit of evolutionary biology (Rabosky *et al.* 2007). Elucidating potential

S. D. Leavitt: Department of Biology & M. L. Bean Life Science Museum, Brigham Young University, Provo, Utah, USA. Email: steve_leavitt@byu.edu
P. M. Kirika: National Museums of Kenya, Nairobi, Kenya.
G. Amo de Paz: Departamento de Física de la Materia Condensada, Facultad de Ciencias, Universidad Autónoma de Madrid, Spain.
J.-P. Huang, F. Grewe and H. T. Lumbsch: Science & Education, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA.
J.-S. Hur: Korean Lichen Research Institute, Sunchon National University, Suncheon 57922, Republic of Korea.
J. A. Elix: Research School of Chemistry, Building 137, Australian National University, Canberra, ACT 2601, Australia.
P. K. Divakar: Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid 28040, Spain.

causes of the vast disparities in species numbers among clades has broad-ranging implications, from evolutionary theory to biodiversity conservation and to systematics.

Clade age and differences in per-lineage rates of speciation have generally been invoked to explain disparities in the number of species among groups (Benton & Emerson 2007; McPeek & Brown 2007). These observations are based, in part, on studies investigating variation in diversification rates across deep phylogenetic and taxonomic scales, such as angiosperms (Magallón & Castillo 2009), amphibians (Roelants *et al*. 2007) or passerine birds (Jetz *et al*. 2012). However, other factors, in addition to clade age and speciation rates, are likely contribute to the creation of hyperdiverse radiations. These may include climatic shifts (Tolley *et al*. 2008), species interactions (Susoy & Herrmann 2014) and dispersal capacity (Claramunt *et al*. 2012). How these forces interact to create extremely diverse lineages and their relative importance can be investigated by focusing on radiations at lower taxonomic scales, and by investigating a broad range of factors potentially influencing discrepancies in species richness across lineages (Papadopoulou & Knowles 2016).

The current perspective of factors driving variation of species richness across extant clades is generally biased towards arctic and temperate/tropical forest regions, with much less focus on arid habitats (Byrne *et al*. 2008). However, arid and semi-arid regions occupy approximately one third of the Earth's total terrestrial environments, incorporating diverse habitats across the world. Many of these regions, including arid and semi-arid habitats in Australia and the Cape Region of South Africa, harbour unique and diverse biological communities (Crisp *et al*. 2004) and are particularly vulnerable to environmental disturbances (Bowker *et al*. 2008).

In lichen-forming fungi, only a limited number of genera are known to be ultra-diverse (>500 species; two genera), hyper-diverse (201–500 species; 17 genera) or megadiverse (genera with 101–200 species) (Lücking *et al*. 2016). These can provide singular examples to identify factors that generate disproportionately high levels of diversity. With more than 800 described species (Thell *et al*. 2012), *Xanthoparmelia* is the most diverse genus of lichen-forming fungi. Congeners occur in exposed environments worldwide and are particularly abundant in arid regions (Hale 1990; Elix 1994). Radiations within this genus are hypothesized to be associated with a shift to a drier habitat (Lumbsch *et al*. 2008), a switch to rocks and soils (Divakar *et al*. 2013), and the emergence of novel arid habitats in the Southern Hemisphere after the splitting of modern Australia from South America (Amo de Paz *et al*. 2011). Recent research is consistent with these hypotheses, which show a correlation between the increased speciation rate in *Xanthoparmelia* and the expansion of drylands during the Oligocene-Miocene transition (Kraichak *et al*. 2015).

Xanthoparmelia has two main centres of diversity, Australia and the Cape Region of South Africa. Each region harbours distinct assemblages of largely endemic species and more than 300 species are known to occur in each region. However, biogeographical patterns of regional endemism in both Australia and Africa cannot be explained by vicariance since these landmasses separated much earlier than the origin of *Xanthoparmelia* (Amo de Paz *et al*. 2011). Therefore, both long-distance dispersal and regional radiations appear to have played important roles in shaping *Xanthoparmelia* diversity. For example, the '*Xanthoparmelia pulla* group' originated in South Africa and diversified during the Miocene, with different lineages migrating to multiple distinct geographical regions via long-distance dispersal and subsequent radiations (Amo de Paz *et al*. 2012). Whether the high levels of diversity in Australia and southern Africa are associated with continuously high rates of speciation since its origin or more recent radiations tied to increasing aridification during the late Pliocene remains untested.

In contrast to the high diversity in Australia and the Cape Region, North and South America each harbour only *c*. 100 species, with even fewer species distributed across Asia and Europe (Hale 1990). Current evidence

indicates that the diversity of most North American *Xanthoparmelia* species originated during the Pliocene and Pleistocene (Leavitt *et al*. 2013), long after the initial diversification of the genus during the late Oligocene or early Miocene (Divakar *et al*. 2015). However, it is not known whether the lower diversity in Asia, North and South America, and Europe is a result of more recent evolutionary histories than the diversifications in Africa and Australia. Alternatively, speciation rates may be accelerated in the two centres of *Xanthoparmelia* diversity (Lumbsch *et al*. 2008), thus reflecting differences in diversification dynamics rather than clade age.

To provide an insight into factors that have generated ultradiversity in *Xanthoparmelia*, we sampled 191 *Xanthoparmelia* specimens collected from populations throughout the world, with an emphasis on Australia, East Africa, the Cape Region of South Africa and North America. Our overall objective was to address diversification patterns and the historical biogeography in this species-rich genus. Specifically, our aim was to elucidate the geographical origin of *Xanthoparmelia* and better understand the temporal and geographical context for diversification in this lineage. To meet these aims, we used multilocus sequence data, including a novel approach to generate locus-specific molecular sequence data from high throughput metagenomic reads, to: 1) reconstruct evolutionary relationships in *Xanthoparmelia*, providing the most comprehensive phylogeny to date for this group; 2) estimate divergence times for major lineages; 3) reconstruct biogeographical histories.

Materials and Methods

Specimen sampling

Data from a total of 200 specimens were included in this study, including sequences from previous studies and newly generated sequences from 105 freshly collected specimens (see Supplementary Material Table S1, available online). The focal group, *Xanthoparmelia*, included 191 specimens representing 124 species/species-level lineages collected from worldwide populations. Our sampling emphasized species occurring in Africa (both South and eastern Africa), Australia and North America, in addition to a more limited sampling in Asia, Europe and South America. Specimens collected for this study were inspected under an Olympus SZH stereomicroscope and extrolites were identified using thin-layer chromatography (TLC) in solvent system A or G (Orange *et al*. 2001). Specimen identifications followed Hale (1990), Esslinger (1977) and more recent taxonomic treatments (Nash *et al*. 1995; Elix 2001, 2003; Nash 2016). Representatives from other genera closely related to *Xanthoparmelia* were also included in the molecular phylogenetic analyses: *Austroparmelina pruinata*, *Canoparmelia crozalsiana*, *C. texana*, *Canoparmelia* sp., *Cetrelia cetrarioides*, *Flavoparmelia marchantii*, *Parmotrema crinitum* and *Punctelia* aff. *bolliana*. Outgroup taxa (*Melanelixia glabroides* and *Melanohalea clairi*) were selected based on previous studies (Crespo *et al*. 2010; Divakar *et al*. 2015).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from specimens collected for this study using either the USB PrepEase Genomic DNA Kit (Affymetrix, Santa Clara, CA, USA; product discontinued) or the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research). For this study we generated molecular sequence data for a total of nine markers: two nuclear ribosomal loci including a gene encoding the large-subunit (nuLSU) and the internal transcribed spacer region (ITS); a fragment of the gene encoding the mitochondrial small subunit (mtSSU); fragments from six nuclear protein-coding loci, including β-tubulin, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), mini-chromosome maintenance complex component 7 (*Mcm7*), RNA polymerase II subunit 1 (*RPB1*), RNA polymerase II subunit 2 (*RPB2*), and the pre-rRNA processing Trypanosoma serine–arginine 1 (*Tsr1*). Sequences were generated using either Sanger sequencing or assembling consensus sequences from metagenomic reads from Illumina's NextSeq and MiSeq platforms (Illumina, San Diego, CA, USA). Conditions for polymerase chain reaction (PCR) amplifications for all loci follow previous studies (Blanco *et al*. 2004; Schmitt *et al*. 2009; Leavitt *et al*. 2013). PCR amplifications were performed using Ready-To-Go PCR Beads (GE Healthcare, Pittsburgh, PA, USA) and cycling parameters followed a 55–50 °C touchdown reaction (Lindblom & Ekman 2006). PCR products were visualized on 1% agarose gel and cleaned using ExoSAP-IT (USB, Cleveland, OH, USA), following the manufacturer's recommendations. We sequenced complementary strands with the same primers used for PCR amplifications and sequencing reactions were performed using BigDye 3.1 (Applied Biosystems, Foster City, CA, USA). Products were run on an ABI 3730 automated sequencer (Applied Biosystems) at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, Chicago, USA.

Metagenomic reads were generated from a total of 47 *Xanthoparmelia* specimens. Libraries were constructed from total DNA extractions (from intact *Xanthoparmelia* thalli) using either the TruSeq DNA PCR-Free Library Preparation Kit or the Nextera XT DNA Library

Preparation Kit (Illumina, San Diego, CA), following the manufacturers' recommendations. TruSeq libraries were sequenced on Illumina's MiSeq platform at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, Chicago, USA. Nextera libraries were sequenced on the NextSeq platform at the Core Genomics Facility at the University of Illinois at Chicago, USA.

Sequencing assembly and alignments

Sanger reads were assembled and edited using the program Sequencher 4.10 (Gene Codes Corporation, Ann Arbor, MI). Sequences were assembled from Illumina metagenomic reads for the nine sampled loci (ITS, nuLSU, mtSSU, β-tubulin, *GAPDH*, *Mcm7*, *RPB1*, *RPB2* and *Tsr1*). Reads from each specimen were mapped to reference sequences of each of the nine markers (ITS, GenBank Accession no. AY581063; nuLSU, HM125760; mtSSU, KR995373; β-tubulin, HM577782; *GAPDH*, AY249628; *Mcm7*, HM579689; *RPB1*, EF092115; *RPB2*, MG695956 and *Tsr1*, MG696003) using the 'Map to Reference' feature in the program Geneious v.6.1.8. We used the 'Medium-Low Sensitivity' option, with fine tuning set to 'Iterate up to 5 times'. The iterative read mapping extended the loci beyond the reference sequences and we exported consensus sequences from the extended mappings from each locus for subsequent analyses.

Sequences were aligned using the program MAFFT v.7 (Katoh *et al*. 2005; Katoh & Toh 2008). We implemented the G-INS-i alignment algorithm and '1PAM/K = 2' scoring matrix, with an offset value of 0·9, and the remaining parameters were set to default values for the protein-coding (β-tubulin, *GAPDH*, *Mcm7*, *RPB1*, *RPB2* and *Tsr1*) and nuLSU markers. For the ribosomal ITS and mtSSU, we used the same parameters, with the exception of an offset value set to 0·1 rather than 0·9 and 'unalignlevel' set to 0·6. Furthermore, ambiguously aligned regions were delimited and removed from the mtSSU and *GAPDH* alignments using the Gblocks webserver (Talavera & Castresana 2007; http://molevol.cmima.csic.es/castresana/Gblocks_server.html), implementing the options for a less stringent selection including 'Allow smaller final blocks', 'Allow gap positions within the final blocks' and 'Allow less strict flanking positions'.

Phylogenetic inference

Phylogenetic relationships among the sampled fungal lineages were inferred using both maximum likelihood (ML) and Bayesian inference (BI) methods. Exploratory phylogenetic analyses of individual gene topologies showed no evidence of well-supported topological conflict (conflicting nodes with ≥70% bootstrap values; data not shown). Hence, we chose to infer relationships using a concatenated gene-tree approach based on the complete, nine-locus data matrix ($n = 200$) which included *c.* 45% missing data (see Supplementary Material Table S1, available online). An ML topology was reconstructed from the nine-locus matrix using the program RAxML v.8.2.1 (Stamatakis 2006; Stamatakis *et al*. 2008) in the CIPRES Science Gateway server (http://www.phylo.org/portal2/), treating each locus as a separate partition and implementing the 'GTRGAMMA' model. Nodal support was evaluated using 1000 bootstrap pseudoreplicates. Exploratory analyses of alternative partition strategies provided highly similar topologies and nodal support values.

Divergence estimates

We attempted to place the diversification history of *Xanthoparmelia* taxa in a temporal context using the program BEAST v.1.8.3 (Drummond & Rambaut 2007; Heled & Drummond 2010) and based on secondary calibrations. We used a node-calibrated approach based on recent divergence estimates for *Parmeliaceae* (Divakar *et al*. 2015). Specifically, we set the age of the most recent common ancestor (MRCA) of *Austroparmelina*, *Canoparmelia* s. lat., *Cetrelia*, *Flavoparmelia*, *Flavopunctelia*, *Parmotrema*, *Punctelia* and *Xanthoparmelia* to 60·55 Mya (node '8' in Divakar *et al*. (2015)), with a second node calibration set to 52 Mya for the MRCA of *Austroparmelina*, *Flavoparmelia* and *Parmotrema* (Divakar *et al*. 2015). A normal prior distribution was set to calibrate both nodes, with standard deviations set to '5' and '4' for each node, respectively, to approximate the 95% confidence intervals inferred previously (Divakar *et al*. 2015). The BI analyses were performed under a Yule speciation process prior and with the data matrix partitioned by individual gene regions. Substitution models for each locus were estimated using jModelTest v.2.1.10 (Darriba *et al*. 2012). Divergence times were estimated under both a strict molecular clock and an uncorrelated relaxed lognormal molecular clock (Drummond *et al*. 2006). For both strict and relaxed lognormal estimates, two independent MCMC runs of 50 million generations were performed, sampling every 1500 steps. Chain mixing and convergence were inspected using the program Tracer v.1.6 (Rambaut & Drummond 2003), considering ESS (effective sample size) values >200 as good indicators. After excluding the first 25% of sampled trees as burn-in, trees from the two independent runs were combined using the program LogCombiner v.1.8.3 (Rambaut & Drummond 2013), and the final MCC tree was estimated from the combined posterior distribution of trees using TreeAnnotator v.1.8.3 (Rambaut & Drummond 2009).

Ancestral range reconstructions

The biogeographical history of *Xanthoparmelia* was reconstructed using the dispersal, extinction and cladogenesis model DEC (Ree & Smith 2008) and the DEC + J (founder event speciation) model (Matzke 2014), with all analyses performed using the R package BioGeoBEARS (Matzke 2014). As an input topology for BioGeoBEARS, we used the BEAST chronogram inferred under a strict clock after excluding the outgroup specimens and all but one specimen in each species-level lineage in the concatenated BEAST tree using the 'drop.tip' function in the 'ape' R package (Paradis *et al*. 2004).

Each tip was assigned to continent-wide geographical states where the lineage occurs, for example Africa, Asia, Australia, Europe, North America and South America (see Supplementary Material Table S1, available online). Species occurring across broad, intercontinental distributions were assigned to multiple states in the BioGeoBEARS analyses corresponding to their distribution. Since exploratory analyses revealed that *Xanthoparmelia* species in South Africa and East Africa (Kenya) were recovered in different lineages distributed across the phylogeny, specimens from Africa were divided into two geographical regions, 'East Africa' and 'South Africa', which included a single specimen from Namibia. Species/lineage distributions were generally obtained from selected literature (Hale 1990; Amo de Paz *et al.* 2010*a*, 2012; Leavitt *et al.* 2011*a*, *b*, 2013). In cases where a nominal taxon was found to have a well-supported phylogeographical substructure, we treated each clade comprised of specimens from a distinct geographical region as a separate species-level lineage. Similarly, lineages in nominal taxa that were not recovered as monophyletic or were recovered with a deep phylogenetic substructure were also treated as separate, putative lineages. Geographical distributions of each sampled species/species-level lineage can be found in Supplementary Table S1 (available online). Subsequently, we used the likelihood ratio test and the Akaike Information Criterion (AIC) for comparing different biogeographical history reconstructions.

TABLE 1. *List of loci included in the study, including alignment length (in number of base pairs = bp) and the nucleotide substitution model used in the analysis, selected by the Akaike Information Criterion (AIC) in jModelTest.*

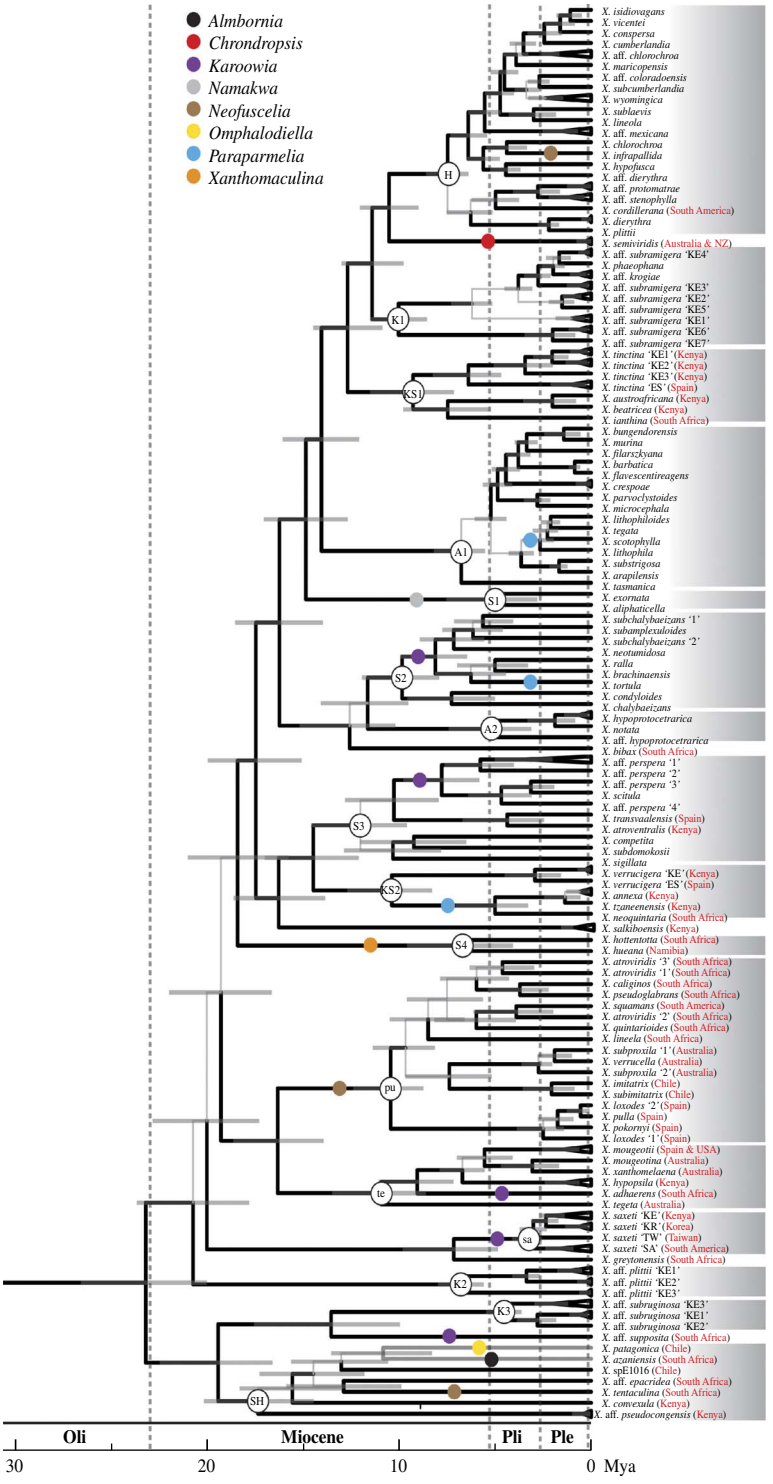
Locus	Alignment length (bp)	Model
ITS	654	TrNef+I+G
nuLSU	852	TrN+I+G
β-tubulin	1689	TIM1+G
GAPDH	1835	TrNef+G
Mcm7	1489	TIM3ef+I+G
RPB1	807	TrNef+I+G
RPB2	1871	TrN+I+G
Tsr1	1441	TrN+I+G
mtSSU	795	TPM1uf+I+G

Results

New sequences generated in association with this study have been deposited in GenBank under Accession numbers MG695404–MG696048 and MG825903–MG825905. The complete data matrix ($n = 200$ specimens; Supplementary Material Table S1) included 11475 aligned nucleotide position characters across the nine selected loci and was submitted to TreeBASE (submission no. 20980). Additional alignment information, including the inferred substitution models, is reported in Table 1.

Both the BEAST (Fig. 1; Supplementary Material Fig. S1) and ML (Supplementary Material Fig. S2) reconstructions resulted in similar branching patterns and nodal support values across the topology, although a limited number of well-supported clades in the BEAST topology (posterior probabilities (PP) >0·95) were recovered with only moderate to weak statistical support in the ML topology (bootstrap (BS) < 70%). In all analyses, *Xanthoparmelia* was recovered as monophyletic with strong statistical support. Relationships among clades within *Xanthoparmelia* were generally recovered with strong statistical support, although a small number of backbone relationships remained unsupported (Fig. 1). A number of well-supported, infrageneric lineages were recovered within *Xanthoparmelia*, many of which consisted of species from distinct geographical regions, and multiple East African, South African and Australian clades were distributed across the phylogeny (Fig. 1). Other well-supported clades included species that corresponded to members of genera that were found to be nested within *Xanthoparmelia* in previous studies, such as *Almbornia*, *Chrondropsis*, *Karoowia*, *Namakwa*, *Neofuselia*, *Omphalodiella*, *Paraparmelia* and *Xanthomaculina* (Fig. 1).

Nodal support values were very similar across different BEAST analyses. Divergence estimates for *Xanthoparmelia* were also largely similar for inferences under both a relaxed and strict clock, although dates inferred under a relaxed clock tended to be slightly older (data not shown). However, low ESS values (<50) were obtained for many parameters in the analyses implementing a relaxed molecular clock. Therefore, the relationships and divergence dates estimated under a relaxed clock were not considered further. In contrast, ESS values for nearly all parameters were >200 for the analyses using a strict clock; therefore, we used a maximum clade credibility chronogram inferred from the combined posterior sampling of the two independent runs as



our best estimate of divergence times for *Xanthoparmelia* (Fig. 1). Consistent with previous estimates, the MRCA for *Xanthoparmelia* was estimated near the Oligocene-Miocene boundary 23 million years ago (Mya) (Highest Posterior Density (HPD) = 20·0–26·6 Mya; Fig. 1). Our results suggested a Miocene-dominated diversification history for *Xanthoparmelia*, with more limited diversification during the Pliocene and into the Pleistocene (Fig. 1).

Based on current sampling, Asian, European and North American *Xanthoparmelia* species generally comprised a single well-supported clade, 'Holarctic clade' (node 'H', Fig. 1), which also included a single South American representative, *X. cordillerana* (Gyeln.) Hale. Species recovered in this clade were estimated to share an MRCA *c.* 7·2 Mya (HPD = 6·2–8·3 Mya), with diversification continuing into the Pliocene and Pleistocene (Fig. 1). A number of notable exceptions where Asian, European and/or North American species were recovered outside of the 'Holarctic' clade included: the widespread taxon *X. mougeotii* (Schaer.) Hale ('*tegeta* clade'), European and Central Asian populations of *X. tinctina* (Maheu & A. Gillet) Hale ('Kenya/South Africa 1' clade), Asian populations of *X. saxeti* (Stizenb.) Amo de Paz *et al.* ('*saxeti* group'), *X. transvaalensis* Hale *et al.* ('South Africa 3' clade), and some members of the *Xanthoparmelia pulla* group, such as *X. loxodes* (Nyl.) O. Blanco *et al.* and *X. pulla* (Ach.) Blanco *et al.* ('*pulla* group' clade) (Fig. 1).

Australian species were recovered in three well-supported clades including 'Australia 1', 'Australia 2' and intermixed with species from other locations in the '*pulla*' clade (node 'pu', Fig. 1). The majority of Australian species sampled were recovered within the 'Australia 1' clade (node 'A1', Fig. 1) and comprised a number of closely related species sharing an MRCA at *c.* 6·9 Mya (HPD = 5·5–8·7 Mya), with a number of closely related species estimated to have diverged during the Pleistocene (Fig. 1). Clade 'Australia 2' (node 'A2', Fig. 1) comprised three specimens, two of which were identified as *X. hypoprotocetrarica* but shared an MRCA at *c.* 5 Mya. Brown Australian *Xanthoparmelia* species in the '*pulla*' group (formerly recognized as *Neofuscelia*) were recovered as monophyletic within the '*pulla* group' clade.

African species were recovered in nine well-supported clades (three East African, four South African and two East/South African lineages), widely distributed across the phylogeny (nodes 'K1', 'K2', 'K3', 'S1', 'S2', 'S3', 'S4', 'KS1' and 'KS2'; Fig. 1). *Xanthoparmelia* species from East and South Africa were consistently recovered in multiple divergent clades, suggesting a deep evolutionary independence between East and South African *Xanthoparmelia* species.

Distinct phylogeographical patterns were observed for a number of nominal *Xanthoparmelia* species, including *X. saxeti*, *X. tinctina* and *X. verrucigera*. *Xanthoparmelia saxeti* specimens were recovered in four distinct clades corresponding to distinct geographical regions: East Africa, East Asia, Taiwan and South America (node 'sa', Fig. 1). The '*saxeti* group' shares an MRCA near the end of the Pliocene, *c.* 3·3 Mya. Specimens representing *X. tinctina* were recovered in two separated clades within the more broadly circumscribed 'Kenya/South Africa 1' clade (node 'KS1', Fig. 1), one with specimens from East Africa and the other comprising specimens from

FIG. 1. Time-calibrated chronogram for *Xanthoparmelia* inferred from a nine-locus, concatenated data matrix (only the *Xanthoparmelia* clade is shown; complete BEAST topology is reported in Supplementary Fig. S1, available online). Provisionally named clades are indicated to the right of the chronogram and shortened, abbreviated codes are found at corresponding nodes. In cases where the geographical origin of the specimen does not coincide with a clade's provisional name, or the provisional name is geographically ambiguous, the geographical origin of the species is shown in red. Clades representing genera that have previously been synonymized with *Xanthoparmelia* are indicated with coloured dots along branches. Note that *X. neotumidosa* in the 'South Africa 2' clade was not previously circumscribed within *Karoowia*. Posterior probabilities ≥0·95 from the Bayesian analysis are indicated by thickened branches and nodal support <0·95 is represented by thinner, grey branches. The Oligocene ('Oli'), Miocene, Pliocene ('Pli') and Pleistocene ('Ple') epochs and the scale of branch lengths (in Mya) are indicated above and below the chronogram, respectively.

Europe, with an estimated MRCA at *c.* 6·4 Mya. A similar pattern was observed for *X. verrucigera*, within the more broadly circumscribed 'Kenya/South Africa 2' clade (node 'KS2', Fig. 1), which consisted of two clades, one with specimens from East Africa and the other from Europe. Other nominal taxa were recovered with substantial phylogenetic substructure at regional scales: *X.* aff. *plittii* (Kenyan populations; 'Kenya 2' clade), *X. subramigera* ('Kenya 1' clade) and *X. perspera* ('South Africa 3' clade) (Fig. 1). The *X. subramigera* group, clade 'Kenya 1', was estimated to share an MRCA at *c.* 10·1 Mya.

Our ancestral area reconstructions supported an African origin for *Xanthoparmelia*, with the most likely geographical origin for most species reconstructed as South Africa (Fig. 2). These data support multiple, independent dispersal events from Africa into other continental landmasses where independent radiations occurred (Fig. 2). Specific models and parameters in the ancestral area reconstruction analysis are reported in Table 2.

Discussion

The genus *Xanthoparmelia* is the most species-rich genus of lichen-forming fungi (Jaklitsch *et al.* 2016; Lücking *et al.* 2016), despite its relatively recent origin. In this study, we demonstrated that *Xanthoparmelia* most likely originated in Africa near the Oligocene-Miocene boundary (23·3 Mya; Fig. 2), with a general pattern of subsequent diversification coinciding with global aridification and development of open habitats (Flowers & Kennett 1994; Sepulchre *et al.* 2006). Other genera in *Parmeliaceae* that have a similar age of origin, such as *Canoparmelia* (26 Mya), *Montanelia* (23 Mya) or *Parmelia* (23 Mya), have considerably lower species-level diversity (Divakar *et al.* 2012, 2015; Molina *et al.* 2017).

Xanthoparmelia is known for its broad, intercontinental geographical distribution (Hale 1990). Phylogenetic relationships inferred in this study revealed more biogeographical structure within the genus than previously recognized, with a number of well-supported lineages restricted to distinct regions, especially Australia and East or South Africa (Fig. 1). For example, the general pattern of phylogenetic separation of East African vs. South African species of *Xanthoparmelia* was not expected. In addition, most species occurring in Australia, a centre of diversity for *Xanthoparmelia*, were recovered in clades exclusively comprised of Australian species (e.g. 'Australia 1', 'Australia 2' and the Australian clade within the '*pulla* group' clade; Fig. 1). However, in other lineages, such as the 'Southern Hemisphere' and '*tegeta* clade' (Fig. 1), biogeographical patterns are less apparent and additional sampling may help elucidate a more nuanced perspective.

Given that in the early Miocene the continents had already separated, these results suggest that the evolution of *Xanthoparmelia* was driven to a large degree by diversification restricted to continents with infrequent intercontinental exchange by long-distance dispersal (see also Amo de Paz *et al.* 2012). This is consistent with other groups of lichen-forming fungi in which infrequent long-distance dispersal was found (Otálora *et al.* 2010; Sérusiaux *et al.* 2011; Amo de Paz *et al.* 2012; Del-Prado *et al.* 2013). Recent studies highlight the promise of incorporating fossil evidence into divergence dating of lichen-forming fungi (Divakar *et al.* 2015; Kaasalainen *et al.* 2015); however, we caution against over-interpreting our divergence estimates and only include them as a hypothesis-generating approach for considering the temporal context of diversification in this group.

Our study further suggests that the main diversification of the genus took place in Australia and Africa, as indicated by the radiation of early diverging clades within the genus being largely restricted to these continents, with at least two migrations into the Holarctic in the late Miocene or Pliocene. Holarctic samples fell into two clades: the derived 'Holarctic clade' and the '*pulla* group' (Fig. 1). Specimens collected in the Holarctic were also recovered in the '*tegata*' clade (*X. mougeotii*), 'South Africa 3' clade (*X. transvaalensis*), 'Kenya/South Africa 1'

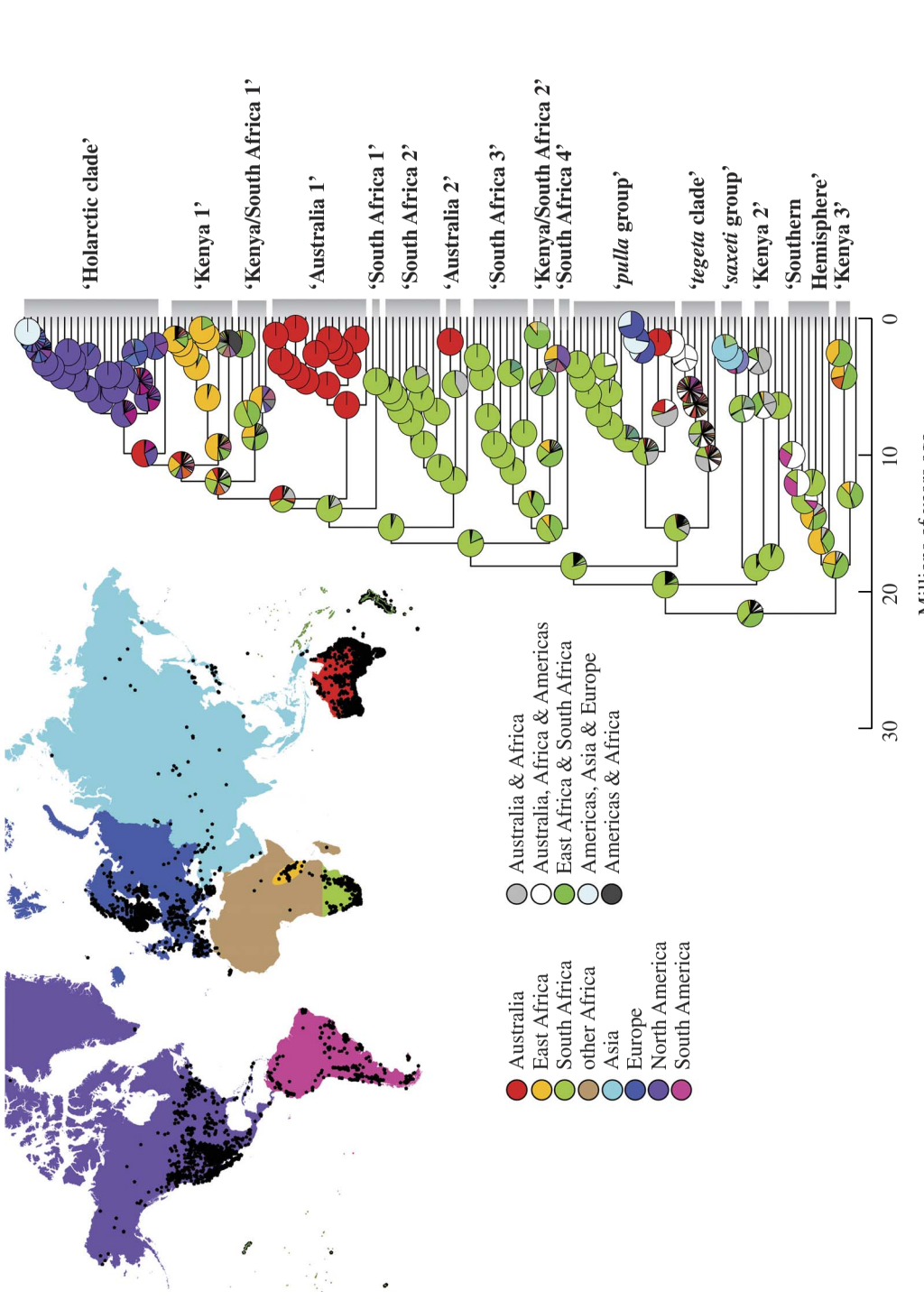


FIG. 2. Maximum likelihood reconstructions of geographical range evolution in *Xanthoparmelia* according to the DEC (dispersal, extinction and cladogenesis) model using a BioGeoBEARS analysis (only the *Xanthoparmelia* clade is shown; complete BEAST topology is reported in Supplementary Fig. S1). Pie charts at the nodes represent the relative probabilities of possible geographical ranges, including reconstructions supporting broad, intercontinental ancestral areas. The map represents the geographical distribution of *Xanthoparmelia* populations and the delimited geographical regions used in ancestral area reconstructions.

TABLE 2. *Parameters of biogeographical models used in the ancestral area reconstructions analysis, including the DEC (dispersal, extinction, and cladogenesis) model and the DEC + J (founder event speciation) model.*

			Parameter estimates			
Model	LnL	Number of parameters	*d*	*e*	*j*	AIC
DEC	−391·1326	2	0·009079	3·313e-03	0·0	786·2652
DEC+J	−386·7134	3	0·007892	1·0 e-12	0·011631	779·4268

Parameters: the rate of range expansion ("dispersal"), parameter *d*; range contraction ("extinction"), parameter *e*; weight of each jump dispersal event in the cladogenesis matrix, parameter *j*. Akaike Information Criterion, AIC.

clade (*X. tinctina*) and the 'Kenya/South Africa 2' clade (*X. verrucigera*) (Fig. 1).

In addition to improved phylogenetic resolution, especially at the backbone of the phylogeny which lacked support in previous studies, and phylogenetic placement of previously unsampled lineages, especially from African and Australian lineages within *Xanthoparmelia* (Fig. 1), our extended taxon sampling also confirmed the phylogenetic placement of morphologically or chemically diverse taxa which were recently grouped together with *Xanthoparmelia* (i.e. *Almbornia*, *Chondropsis*, *Karoowia*, *Namakwa*, *Neofuscelia*, *Paraparmelia*, *Omphalodiella*, *Placoparmelia*, *Xanthomaculina*) (Blanco *et al.* 2004; Thell *et al.* 2006; Amo de Paz *et al.* 2010*a*, *b*, 2011; Crespo *et al.* 2010; Divakar *et al.* 2015). In some cases, such as the subcrustose *Xanthoparmelia* spp. (previously recognized in *Karoowia*), our phylogenetic estimates suggest that these phenotypes evolved several times independently within the genus (Fig. 1), although this should be tested further. In another lineage, the 'Southern Hemisphere' clade (Fig. 1), members of three former genera (*Almbornia*, *Neofuscelia* and *Omphalodiella*) were recovered along with species traditionally recognized as *Xanthoparmelia*. This phenotypic diversity of growth forms in a single clade within *Xanthoparmelia* is remarkable given that our estimates suggest that the genus has a relatively recent origin during the early Miocene (Fig. 2). In comparison, fossils of another genus in the *Parmeliaceae*, *Anzia*, are dated from the Eocene (*c.* 40 Mya; Kaasalainen *et al.* 2017), and this genus represents markedly less diversity in growth forms than *Xanthoparmelia*.

In addition, our study indicates issues with species delimitation in *Xanthoparmelia*, highlighting the fact that in spite of the high levels of species diversity in this genus, traditional taxonomic approaches have failed to adequately characterize this diversity (Leavitt *et al.* 2011*a*, *b*). While the present study was not designed for dense, intraspecific sampling, multiple representatives of nominal taxa were recovered in separate, well-supported clades in a number of cases. This is consistent with results found in other groups of *Parmeliaceae*, and lichen-forming fungi in general, further supporting the suggestion that phenotypic delimitation of species in these symbiotic fungi might misrepresent true diversity (Crespo & Pérez-Ortega 2009; Crespo & Lumbsch 2010; Lumbsch & Leavitt 2011; Leavitt *et al.* 2016*a*). In contrast to the high morphological variability in *Xanthoparmelia*, in a number of cases morphologically similar specimens, such as nominal *X. plittii* ('Kenya 2' and 'Holarctic' clades, Fig. 1) and *X. tasmanica* (Hodkinson & Lendemer 2011), were found in distinct, deeply divergent clades. A number of specimens from Kenya were identified as *X.* aff. *plittii* ('Kenya 2' clade, Fig. 1) but were only distantly related to North American and European populations of *X. plittii* ('Holarctic clade', Fig. 1), which was originally described from eastern North America (Gyelnik 1931). In fact, the clade comprising all *X.* aff. *plittii* lineages from Kenya was recovered as one of the earlier diverging clades in *Xanthoparmelia* and shared an estimated MRCA with *X. plittii* populations in North America ('Holarctic clade') at *c.* 19 Mya (Fig. 1).

In some species, including *X. saxeti* and *X. tinctina*, populations from distinct geographical regions formed monophyletic groups separated for several million years (Fig. 1). These results revealed that some nominal taxa in *Xanthoparmelia* have a phylogeographical substructure that potentially corresponds to evolutionarily independent species-level lineages occurring on different continents. A similar pattern was found in the *Cladia aggregata* complex, where geographically distinct lineages were often interpreted as different species (Parnmen *et al.* 2012, 2013).

Relative to other genera in the *Parmeliaceae*, *Xanthoparmelia* has been shown to associate with a much broader range of algal partners (Leavitt *et al.* 2015). How this diversity varies across regions and how patterns in symbiont interactions relate to patterns in *Xanthoparmelia* diversity have not yet been tested. Specifically, whether certain symbiotic associations or the switch between partners might pertain to a higher speciation rate remains untested in lichen-forming fungi. Evaluating the effect of symbiotic relationships, as well as trait-dependent and evolutionary time-dependent factors, on speciation dynamics will provide an insight into how megadiverse lineages arise in symbiotic fungi.

While diversification dynamics associated with increased aridity have been studied for a number of plant (Crisp *et al.* 2004; Griffin & Hoffmann 2014) and animal groups (Rabosky *et al.* 2014), the diversification dynamics of symbiotic fungal radiations remain poorly characterized. Our study of the ultradiverse lichen-forming fungal genus *Xanthoparmelia* provides a valuable framework for continued research. However, the exact mechanisms by which *Xanthoparmelia* became extremely successful in Australia and South Africa are still not clear. Here we show that *Xanthoparmelia* likely originated in Africa and relatively high levels of species diversity may reflect, in part, clade age. Based on our sampling, *Xanthoparmelia* in Australia appear to have a more recent origin than African lineages (Fig. 1) but with similar levels of species diversity compared to the African Cape Region. In fact, it appears that diversification in Australian lineages generally coincides with *Xanthoparmelia* diversification in the Holarctic ('Australia 1' and 'Australia 2' clades, Fig. 1) but resulted in approximately three times the number of species (Hale 1990). *Xanthoparmelia* has recently been shown to have increased rates of speciation relative to other genera in the *Parmeliaceae* (Kraichak *et al.* 2015), and the present study suggests there are differences in speciation and/or extinction rates among lineages within *Xanthoparmelia*. Future studies identifying clades with increased rates of speciation will assist in more precisely identifying factors that drive diversification in this group of symbiotic fungi.

As metagenomic reads from lichen samples become increasingly available, utilizing these data for phylogenetic studies remains largely untested (but see Grewe *et al.* 2017). This study highlights a novel approach for generating multilocus datasets from high-throughput metagenomic sequencing reads. While we generated data for only nine loci here, hundreds to thousands of homologous genomic markers could be recovered from metagenomic data using appropriate genomic references, such as the core eukaryotic genes (Leavitt *et al.* 2016*b*) or for universal single-copy orthologs in fungi (Simão *et al.* 2015), and used to infer robust phylogenomic datasets.

We dedicate this publication to our friend and colleague, Ana Crespo de las Casas, on the occasion of her 70th birthday. Ana has been our mentor and collaborator and contributed enormously to our knowledge of lichenized fungal evolution, especially in the family *Parmeliaceae*. We appreciate the thoughtful comments and perspective from Associate Editor Mats Wedin and constructive feedback from anonymous reviewers. We thank Drs. Trevor Goward, Matthew Nelsen, Roger Rosentreter and Larry St. Clair for invaluable conceptual help and insightful comments. We are also indebted to various colleagues for providing material and field assistance, notably Trevor Goward, Larry St. Clair, Prof. Mark Seaward, Roger Rosentreter and the Leavitt family. We thank Dr Kevin Feldheim for his invaluable help with sequencing. The authors thank the Negaunee Foundation and the Field Museum for financial support, and the Lauer family for the generous gift of the MiSeq sequencer to the Pritzker Laboratory for Molecular Systematics at the Field Museum. Additional support was received from the USDA Forest Service and the College of Life Sciences, Brigham Young University.

Supplementary Material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S0024282918000233

References

Amo de Paz, G., Lumbsch, H. T., Cubas, P., Elix, J. A. & Crespo, A. (2010*a*) The genus *Karoowia* (*Parmeliaceae*, Ascomycota) includes unrelated clades nested within *Xanthoparmelia*. *Australian Systematic Botany* **23:** 173–184.

Amo de Paz, G., Lumbsch, H. T., Cubas, P., Elix, J. A. & Crespo, A. (2010*b*) The morphologically deviating genera *Omphalodiella* and *Placoparmelia* belong to *Xanthoparmelia* (*Parmeliaceae*). *Bryologist* **113:** 376–386.

Amo de Paz, G., Cubas, P., Divakar, P. K., Lumbsch, H. T. & Crespo, A. (2011) Origin and diversification of major clades in parmelioid lichens (*Parmeliaceae*, Ascomycota) during the Paleogene inferred by Bayesian analysis. *PLoS ONE* **6:** e28161.

Amo de Paz, G., Cubas, P., Crespo, A., Elix, J. A. & Lumbsch, H. T. (2012) Transoceanic dispersal and subsequent diversification on separate continents shaped diversity of the *Xanthoparmelia pulla* group (Ascomycota). *PLoS ONE* **7:** e39683.

Benton, M. J. & Emerson, B. C. (2007) How did life become so diverse? The dynamics of diversification according to the fossil record and molecular phylogenetics. *Palaeontology* **50:** 23–40.

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

Bowker, M. A., Miller, M. E., Belnap, J., Sisk, T. D. & Johnson, N. C. (2008) Prioritizing conservation effort through the use of biological soil crusts as ecosystem function indicators in an arid region. *Conservation Biology* **22:** 1533–1543.

Büdel, B., Colesie, C., Green, T. G. A., Grube, M., Lázaro Suau, R., Loewen-Schneider, K., Maier, S., Peer, T., Pintado, A., Raggio, J., *et al.* (2014) Improved appreciation of the functioning and importance of biological soil crusts in Europe: the Soil Crust International Project (SCIN). *Biodiversity and Conservation* **23:** 1639–1658.

Byrne, M., Yeates, D. K., Joseph, L., Kearney, M., Bowler, J., Williams, M. A. J., Cooper, S., Donnellan, S. C., Keogh, J. S., Leys, R., *et al.* (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* **17:** 4398–4417.

Claramunt, S., Derryberry, E. P., Remsen, J. & Brumfield, R. T. (2012) High dispersal ability inhibits speciation in a continental radiation of passerine birds. *Proceedings of the Royal Society of London B: Biological Sciences* **279:** 1567–1574.

Crespo, A. & Lumbsch, H. T. (2010) Cryptic species in lichen-forming fungi. *IMA Fungus* **1:** 167–170.

Crespo, A. & Pérez-Ortega, S. (2009) Cryptic species and species pairs in lichens: a discussion on the relationship between molecular phylogenies and morphological characters. *Anales del Jardin Botanico de Madrid* **66:** 71–81.

Crespo, A., Kauff, F., Divakar, P. K., del Prado, R., Pérez-Ortega, S, Amo de Paz, G., Ferencova, Z., Blanco, O., Roca-Valiente, B., Núñez-Zapata, J., *et al.* (2010) Phylogenetic generic classification of parmelioid lichens (*Parmeliaceae*, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* **59:** 1735–1753.

Crisp, M., Cook, L. & Steane, D. (2004) Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **359:** 1551–1571.

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

Del-Prado, R., Blanco, O., Lumbsch, H. T., Divakar, P. K., Elix, J. A., Molina, M. C. & Crespo, A. (2013) Molecular phylogeny and historical biogeography of the lichen-forming fungal genus *Flavoparmelia* (Ascomycota: *Parmeliaceae*). *Taxon* **62:** 928–939.

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

Divakar, P. K., Kauff, F., Crespo, A., Leavitt, S. D. & Lumbsch, H. T. (2013) Understanding phenotypical character evolution in parmelioid lichenized fungi (*Parmeliaceae*, Ascomycota). *PLoS ONE* **8:** e83115.

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

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

Drummond, A., Ho, S., Phillips, M. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4:** e88.

Elix, J. A. (1994) *Xanthoparmelia*. In *Flora of Australia. Volume 55 Lichens*-Lecanorales 2, Parmeliaceae (C. Grgurinovic, ed.): 201–308. Canberra: Australian Biological Resources Study.

Elix, J. A. (2001) A revision of the lichen genus *Paraparmelia* Elix & J. Johnst. *Bibliotheca Lichenologica* **80:** 1–224.

Elix, J. A. (2003) The lichen genus *Paraparmelia*, a synonym of *Xanthoparmelia* (Ascomycota, *Parmeliaceae*). *Mycotaxon* **97:** 395–403.

Esslinger, T. L. (1977) A chemosystematic revision of the brown *Parmeliae*. *Journal of the Hattori Botanical Laboratory* **42:** 1–211.

Flowers, B. P. & Kennett, J. P. (1994) The middle Miocene transition: East Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology* **108:** 537–555.

Grewe, F., Huang, J. P., Leavitt, S. D. & Lumbsch, H. T. (2017) Reference-based RADseq resolves robust relationships among closely related species of lichen-forming fungi using metagenomic DNA. *Scientific Reports* **7:** 9884.

Griffin, P. C. & Hoffmann, A. A. (2014) Limited genetic divergence among Australian alpine *Poa* tussock grasses coupled with regional structuring points to ongoing gene flow and taxonomic challenges. *Annals of Botany* **113:** 953–965.

Gyelnik, V. (1931) Additamenta ad cognitionem Parmeliarum - II. *Feddes Repertorium Specierum Novarum Regni Vegetabilis* **29:** 273–291.

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

Heled, J. & Drummond, A. J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27:** 570–580.

Hodkinson, B. P. & Lendemer, J. C. (2011) Molecular analyses reveal semi-cryptic species in *Xanthoparmelia tasmanica*. *Bibliotheca Lichenologica* **106:** 108–119.

Jaklitsch, W., Baral, H-O, Lücking, R., Lumbsch, H. T. & Frey, W (2016) *Syllabus of Plant Families-Adolf Engler's Syllabus der Pflanzenfamilien*, Part 1/2. Stuttgart: Borntraeger Verlagsbuchhandlung.

Jetz, W., Thomas, G., Joy, J., Hartmann, K. & Mooers, A. (2012) The global diversity of birds in space and time. *Nature* **491:** 444–448.

Kaasalainen, U., Heinrichs, J., Krings, M., Myllys, L., Grabenhorst, H., Rikkinen, J. & Schmidt, A. R. (2015) Alectorioid morphologies in Paleogene lichens: new evidence and re-evaluation of the fossil *Alectoria succini* Mägdefrau. *PLoS ONE* **10:** e0129526.

Kaasalainen, U., Schmidt, A. R. & Rikkinen, J. (2017) Diversity and ecological adaptations in Palaeogene lichens. *Nature Plants* **3:** 17049.

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

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

Kraichak, E., Divakar, P. K., Crespo, A., Leavitt, S. D., Nelsen, M. P., Lücking, R. & Lumbsch, H. T. (2015) A tale of two hyper-diversities: diversification dynamics of the two largest families of lichenized fungi. *Scientific Reports* **5:** 10028.

Leavitt, S. D., Johnson, L. A. & St. Clair, L. L. (2011*a*) Species delimitation and evolution in morphologically and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (*Parmeliaceae*, Ascomycota) in western North America. *American Journal of Botany* **98:** 175–188.

Leavitt, S. D., Johnson, L. A., Goward, T. & St. Clair, L. L. (2011*b*) Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (*Parmeliaceae*) in North America. *Molecular Phylogenetics and Evolution* **60:** 317–332.

Leavitt, S. D., Lumbsch, H. T., Stenroos, S. & St. Clair, L. L. (2013) Pleistocene speciation in North American lichenized fungi and the impact of alternative species circumscriptions and rates of molecular evolution on divergence estimates. *PLoS ONE* **8:** e85240.

Leavitt, S. D., Kraichak, E., Nelsen, M. P., Altermann, S., Divakar, P. K., Alors, D., Esslinger, T. L., Crespo, A. & Lumbsch, H. T. (2015) Fungal specificity and selectivity for algae play a major role in determining lichen partnerships across diverse ecogeographic regions in the lichen-forming family *Parmeliaceae* (Ascomycota). *Molecular Ecology* **24:** 3779–3797.

Leavitt, S. D., Divakar, P. K., Crespo, A. & Lumbsch, H. T. (2016*a*) A matter of time – understanding the limits of the power of molecular data for delimiting species boundaries. *Herzogia* **29:** 479–492.

Leavitt, S. D., Grewe, F., Widhelm, T., Muggia, L., Wray, B. & Lumbsch, H. T. (2016*b*) Resolving evolutionary relationships in lichen-forming fungi using diverse phylogenomic datasets and analytical approaches. *Scientific Reports* **6:** 22262.

Lindblom, L. & Ekman, S. (2006) Genetic variation and population differentiation in the lichen-forming ascomycete *Xanthoria parietina* on the island Storfosna, central Norway. *Molecular Ecology* **15:** 545–1559.

Lücking, R., Hodkinson, B. P. & Leavitt, S. D. (2016) The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – approaching one thousand genera. *Bryologist* **119:** 361–416.

Lumbsch, H. T. & Leavitt, S. D. (2011) Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* **50:** 59–72.

Lumbsch, H. T., Hipp, A., Divakar, P. K., Blanco, O. & Crespo, A. (2008) Accelerated evolutionary rates in tropical and oceanic parmelioid lichens (Ascomycota). *BMC Evolutionary Biology* **8:** e257.

Magallón, S. & Castillo, A. (2009) Angiosperm diversification through time. *American Journal of Botany* **96:** 349–365.

Matzke, N. J. (2014) Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology* **63:** 951–970.

McPeek, M. A. & Brown, J. M. (2007) Clade age and not diversification rate explains species richness among animal taxa. *American Naturalist* **169:** E97–E106.

Molina, M. C., Divakar, P. K., Goward, T., Millanes, A. M., Lumbsch, H. T. & Crespo, A. (2017) Neogene diversification in the temperate lichen-forming fungal genus *Parmelia* (*Parmeliaceae*, Ascomycota). *Systematics and Biodiversity* **15:** 166–181.

Nash, T. H., III (2016) *Xanthoparmelia* in Mexico. *Bibliotheca Lichenologica* **110:** 621–692.

Nash, T. H., III, Gries, C. & Elix, J. A. (1995) A revision of the lichen genus *Xanthoparmelia* in South America. *Bibliotheca Lichenologica* **56:** 1–157.

Orange, A., James, P. W. & White, F. J. (2001) *Microchemical Methods for the Identification of Lichens*. London: British Lichen Society.

Otálora, M. A. G., Martínez, I., Aragón, G. & Molina, M. C. (2010) Phylogeography and divergence date estimates of a lichen species complex with a disjunct distribution pattern. *American Journal of Botany* **97:** 216–223.

Papadopoulou, A. & Knowles, L. L. (2016) Toward a paradigm shift in comparative phylogeography driven by trait-based hypotheses. *Proceedings of the National Academy of Sciences of the United States of America* **113:** 8018–8024.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20:** 289–290.

Parnmen, S., Rangsiruji, A., Mongkolsuk, P., Boonpragob, K., Nutakki, A. & Lumbsch, H. T. (2012) Using phylogenetic and coalescent methods to understand the species diversity in the *Cladia aggregata* complex (Ascomycota, *Lecanorales*). *PLoS ONE* **7:** e52245.

Parnmen, S., Leavitt, S. D., Rangsiruji, A. & Lumbsch, H. T. (2013) Identification of species in the *Cladia aggregata* group using DNA barcoding (Ascomycota: *Lecanorales*). *Phytotaxa* **115:** 1–14.

Rabosky, D. L., Donnellan, S. C., Talaba, A. L. & Lovette, I. J. (2007) Exceptional among-lineage variation in diversification rates during the radiation of Australia's most diverse vertebrate clade. *Proceedings of the Royal Society of London B: Biological Sciences* **274:** 2915–2923.

Rabosky, D. L., Donnellan, S. C., Grundler, M. & Lovette, I. J. (2014) Analysis and visualization of complex macroevolutionary dynamics: an example from Australian scincid lizards. *Systematic Biology* **63:** 610–627.

Rambaut, A. & Drummond, A. J. (2003) *Tracer v.1.6.0*. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Rambaut, A. & Drummond, A. J. (2009) *TreeAnnotator v1.6.1*. Available at: http://beastbioedacuk/TreeAnnotator.

Rambaut, A. & Drummond, A. J. (2013) *LogCombiner v.1.8.0*. Available at: http://beastbioedacuk/TreeAnnotator.

Ree, R. H. & Smith, S. A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Roelants, K., Gower, D. J., Wilkinson, M., Loader, S. P., Biju, S. D., Guillaume, K., Moriau, L. & Bossuyt, F. (2007) Global patterns of diversification in the history of modern amphibians. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 887–892.

Schmitt, I., Crespo, A., Divakar, P. K., Fankhauser, J. D., Herman-Sackett, E., Kalb, K., Nelsen, M. P., Rivas-Plata, E., Shimp, A. D., Widhelm, T., *et al.* (2009) New primers for promising single-copy genes in fungal phylogenies and systematics. *Persoonia* **23:** 35–40.

Sepulchre, P., Ramstein, G., Fluteau, F., Schuster, M., Tiercelin, J.-J. & Brunet, M. (2006) Tectonic uplift and Eastern Africa aridification. *Science* **313:** 1419–1423.

Sérusiaux, E., Villarreal, A., J. C., Wheeler, T. & Goffinet, B. (2011) Recent origin, active speciation and dispersal for the lichen genus *Nephroma* (*Peltigerales*) in Macaronesia. *Journal of Biogeography* **38:** 1138–1151.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. M. (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31:** 3210–3212.

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

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

Susoy, V. & Herrmann, M. (2014) Preferential host switching and codivergence shaped radiation of bark beetle symbionts, nematodes of *Micoletzkya* (Nematoda: *Diplogastridae*). *Journal of Evolutionary Biology* **27:** 889–898.

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

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

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

Tolley, K. A., Chase, B. M. & Forest, F. (2008) Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. *Journal of Biogeography* **35:** 1402–1414.