

SYSTEMATICS AND PHYLOGENY

Molecular phylogeny and historical biogeography of the lichen-forming fungal genus *Flavoparmelia* (Ascomycota: Parmeliaceae)

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Abstract The lichen-forming fungal genus *Flavoparmelia* includes species with distinct distribution patterns, including subcosmopolitan, restricted, and disjunct species. We used a dataset of nuclear ITS and LSU ribosomal DNA including 51 specimens to understand the influence of historical events on the current distribution patterns in the genus. We employed Bayesian, maximum likelihood and maximum parsimony approaches for phylogenetic analyses, a likelihood-based approach to ancestral area reconstruction, and a Bayesian approach to estimate divergence times of major lineages within the genus. We identified two major clades in the genus, one of them separating into two subclades and one of those into four groups. Several of the groups and clades have restricted geographical ranges in the Southern Hemisphere, but two groups include species with wider distribution areas. Our analyses suggest that the genus originated in southern South America during the Eocene–Oligocene transition and that the diversification of the Australasian groups occurred recently. The subcosmopolitan distribution of species is explained by long-distance dispersal, while vicariance probably played a major role in the origin of the genus. Several currently accepted species were found to be non-monophyletic, indicating that the species delimitation in the genus requires further studies.

Keywords ancestral areas; distribution; lichens; long-distance dispersal; parmelioid lichens; phylogeny; Southern Hemisphere; vicariance

Received: 11 Jan. 2013; revision received: 10 May 2013; accepted: 29 Aug. 2013. DOI: <http://dx.doi.org/10.12705/625.22>

■ INTRODUCTION

With the wide availability of DNA sequence data and advances in analytical methods of distribution data in a phylogenetic context (Ree & al., 2005; Ree & Smith, 2008; Ree & Sanmartin, 2009), biogeographical studies in lichenized fungi have become popular (Printzen & Lumbsch, 2000; Crespo & al., 2002; Högberg & al., 2002; Printzen & Ekman, 2002; Arnerup & al., 2004; Divakar & al., 2010; Lumbsch & al., 2010; Otálora & al., 2010, 2011; Sérusiaux & al., 2011). Lichenized fungi tend to have wide distribution ranges with numerous cosmopolitan, bipolar or pantropical species (Culberson, 1972; Galloway & Aptroot, 1995; Crespo & al., 2002; Lücking, 2003; Feuerer & Hawksworth, 2007; Wirtz & al., 2008), leading to a common belief among lichenologists that distribution of these organisms is primarily shaped by ecological conditions, with only few classical studies invoking historical factors explaining current distribution patterns (Poelt, 1963; Yoshimura, 1968; Culberson, 1972; Galloway, 1987, 1988). However, there is a growing body of evidence from recent biogeographical studies employing molecular data indicating that the current

distribution of lichenized fungi is a combination of different historical events, including vicariance and long- or mid-distance dispersal often coupled with subsequent diversification in isolated areas (Otálora & al., 2010; Amo de Paz & al., 2011, 2012; Leavitt & al., 2012b, 2013).

In order to assess the impact of dispersal mechanisms in shaping the current distribution of organisms, estimates of the diversification dates are needed. Due to the poor fossil record for fungi this has been difficult and consequently, very few studies have used dated phylogenies in earlier phylogenetic studies of lichens (Printzen & Lumbsch, 2000). However, recent progress in dating molecular phylogenies of ascomycetes, including lichen-forming fungi, now enables us to estimate timing of diversification events more reliably (Berbee & Taylor, 2001; Taylor & Berbee, 2006; Lücking & al., 2009; Amo de Paz & al., 2011, 2012; Leavitt & al., 2012a, b, c, 2013).

The genus *Flavoparmelia* Hale is an ideal subject for study of the impact of historical events on current distributional ranges, since it exhibits a large disparity of distributions despite being a relatively small genus among parmelioid lichens (Parmeliaceae) with 38 accepted species (Crespo & al., 2010b).

It includes yellow-green foliose lichens, characterized by having broad rounded lobes, non-ciliate margins, a pored epicortex, bifusiform or fusiform conidia, a cortex containing usnic acid, and cell-walls composed of isolichenan (Crespo & al., 2011). The genus has a worldwide distribution with centers of distribution in temperate and subtropical areas. Species in the genus show different patterns of distribution, such as wide, restricted and disjunct. Some species have a restricted distribution, including the Australasian *F. haywardiana* Elix & J. Johnst., *F. euplecta* (Stirt.) Hale and *F. haysomii* (C.W. Dodge) Hale, the South American *F. citrinescens* (Gyeln.) O. Blanco & al. and *F. subambigua* (Hale) O. Blanco & al., and the North American *F. baltimorensis* (Gyeln. & F6riss) Hale and *F. subcapitata* (Nyl. ex Hasse) Hale ex DePriest & B.W. Hale. Other species are widely distributed and occur in most continents, such as *F. caperata* (L.) Hale and *F. soredians* (Nyl.) Hale. *Flavoparmelia rutidota* (Hook. f. & Taylor) Hale has a disjunct distribution, occurring in Australia and America.

So far, molecular phylogenetic studies focusing on parmelioid lichens included only few *Flavoparmelia* species (Blanco & al., 2006; Crespo & al., 2007, 2010b). These studies have supported the monophyly and elucidated the phylogenetic position of this genus within parmelioid lichens. As yet, however, no study focusing on the phylogeny of *Flavoparmelia* species has been undertaken. Thus, little is known about how the species in the genus are related to each other and how the different distribution ranges in the genus can be explained. Therefore we have assembled data of two ribosomal loci from 51 specimens representing 21 species of *Flavoparmelia* to address the following issues: (1) resolve major phylogenetic relationships in the genus, (2) estimate the timing of diversification events of main lineages, and (3) assess the impact of vicariance and long-distance dispersal in shaping the current distribution of *Flavoparmelia* species.

■ MATERIALS AND METHODS

Taxon sampling. — Sequence data of the nuclear ITS and LSU rDNA were analyzed for 51 specimens of *Flavoparmelia*. Of the 38 described species within *Flavoparmelia*, 21 species are included in this study, collected from various geographic regions including Asia, Australia, Europe, North and South America, and East and South Africa. Eight species of *Austroparmelina* A. Crespo & al. were selected as outgroup because this genus has previously been shown to be closely related to *Flavoparmelia* (Crespo & al., 2010a, b). The ITS dataset included 43 sequences from previous publications by our group (Blanco & al., 2004, 2005; Gutierrez & al., 2007; Crespo & al., 2010b; Del-Prado & al., 2010) and 16 generated for this study. The nuLSU dataset included 16 sequences from our previous publications (see above) and 39 newly generated sequences.

Details of the studied material, including GenBank accession numbers are shown in Appendix 1.

Flavoparmelia lacks fossil records and hence we prepared a larger dataset (142 taxa) including main parmelioid lineages published elsewhere (Crespo & al., 2010b) to include

the calibration points and constrain the tree with fossil records in molecular dating analyses.

Molecular methods. — Total DNA was extracted from freshly collected materials, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer, with slight modifications described in Crespo & al. (2001). Fungal nuclear LSU and ITS rDNA were amplified using the following primers: (1) for nuLSU: AL1R (D6ring & al., 2000), and LR6 (Vilgalys & Hester, 1990), and (2) for nuITS: ITS1F (Gardes & Bruns, 1993), ITS4A (Larena & al., 1999), ITS1-LM (Myllys & al., 1999), and ITS2-KL (Lohtander & al., 1998). Amplifications were performed in a 25 μ l volume containing 2.5 μ l 10 \times DNA buffer containing 2 mM MgCl₂ (Biotools, Madrid, Spain), 0.5 μ l dNTPs (10 mM of each base), 1.25 μ l of each primer (10 μ M), 0.625 μ l DNA polymerase (1 U/ μ l), 13.875 μ l distilled water and 5 μ l of DNA template.

The amplifications for nuLSU and ITS rDNA were carried out in an automatic thermocycler (Techno Progene, JepsonBolton & Co., Walford, Herts, U.K.) using the following parameters: initial denaturation at 94 $^{\circ}$ C for 5 min followed by 35 cycles at 94 $^{\circ}$ C for 1 min, 54 $^{\circ}$ C (AL1R/LR6, and ITS1F/ITS4A) or 56 $^{\circ}$ C (ITS1LM/ITS2KL) for 1 min, and 72 $^{\circ}$ C for 1.5 min; and a final extension at 72 $^{\circ}$ C for 10 min. Amplification products were visualized on 1% agarose gels stained with SYBR Safe DNA (Life Technologies Corporations, Grand Island, New York, U.S.A.) gel stain (10,000 \times concentrated in DMSO), and subsequently purified using the enzyme exoSAP-IT (GE Healthcare, Chalfont St. Giles, U.K.) according to the manufacturers' instructions.

Fragments were sequenced using Big Dye Terminator reaction kit (ABI PRISM, Life Technologies Corporations). Cycle sequencing reactions were performed with the same sets of primers used for PCR amplifications, as described previously (Del-Prado & al., 2010). Sequence fragments obtained were assembled with SeqMan v.4.03 (DNASTar, www.dnastar.com) and manually edited.

Sequence alignment and phylogenetic analysis. — The two datasets were aligned separately. For the nuLSU dataset, the program Clustal W v.2 (Thompson & al., 1994) was used followed by a manual adjustment. For the ITS marker, sequences were aligned using the program MAFFT v.6 (Katoh & al., 2009) with the parameters set to default values. The alignment of the combined dataset was analyzed using maximum parsimony (MP), maximum likelihood (ML), and a Bayesian Markov chain Monte Carlo approach (B/MCMC).

The nucleotide substitution models were selected using jModelTest v.2 (Posada, 2008) using the Akaike information criterion (AIC). The following substitution models were selected: (1) for ITS, the general time reversible model (Rodriguez & al., 1990) with estimation of invariant sites and the assumption of a gamma distribution with six rate categories (GTR+I+G); and (2) for the nuLSU region GTR+G was selected.

We used an MP approach to detect potential conflicts between the two data partitions with a threshold of 70% bootstrap support (Lutzoni & al., 2004). If no conflict was evident, it was assumed that the two datasets were congruent and could be combined.

MP analyses were performed using the program PAUP* v.4.0b10 (Swofford, 1993). Heuristic searches with 1000 random taxon addition replicates were conducted with the tree-bisection-reconnection (TBR) branch-swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein, 1985) was based on 4000 pseudoreplicates with random sequence additions. To assess homoplasy levels, the consistency (CI) and retention indices (RI) were calculated in PAUP*.

The ML analysis was performed using an online version of the program RAxML v.7.0.4 (<http://phylobench.vital-it.ch/raxml-bb/>) (Stamatakis, 2006; Stamatakis & al., 2008), assuming a GTR+G model and a bootstrap analysis using 1000 pseudoreplicates.

MRBAYES v.3.1.2 (Huelsenbeck & Ronquist, 2001) was employed to sample trees using a MCMC procedure. The combined dataset was partitioned into the two parts (ITS, nuLSU), and each partition was allowed to have its own parameters (Nylander & al., 2004). No molecular clock was assumed. Two parallel runs of 2 million generations were done, starting with a random tree and employing 12 simultaneous chains each. Every 100th tree was saved into a file. The first 200,000 generations (i.e., 2000 trees) were deleted as the “burn-in” of the chains. We used the program AWTY (Nylander & al., 2007) to compare split frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining 36,000 trees (18,000 from each parallel run), a majority-rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes.

Only clades that received bootstrap support equal to or above 70% in MP and ML analyses and posterior probabilities equal to or above 0.95 were considered as strongly supported. Phylogenetic trees were drawn using the program TREEVIEW v.X (Page, 1996).

Alignments are available at TreeBase (<http://www.treebase.org>) under study accession number S14222, and matrix and phylogenetic tree accession numbers M17024 and Tr63498.

Ancestral area reconstructions. — We used the likelihood approach of ancestral area reconstruction (AAR) developed by Ree and colleagues (Ree & al., 2005; Ree & Smith, 2008) to estimate the ancestral area of *Flavoparmelia* and explore the ancestral range of the main clades within the genus. Analyses were conducted on an ultrametric tree estimated using the lognormal relaxed clock model (Drummond & al., 2006) implemented in an MCMC framework in the program BEAST v.1.6.1 (Drummond & Rambaut, 2007), using the ML tree as the start tree and a GTR+I+G model of nucleotide substitution, with a total run of 10 million generations. This tree was imported into LaGrange v.2.0 (<http://lagrange.googlecode.com>) using the LaGrange configuration module (<http://www.reelab.net/lagrange/configurator/index>). Presence in six different areas was coded for all species (Australasia [i.e., Australia, New Zealand, New Guinea], North America, Eurasia and North Africa, East Africa, South Africa, South America) with no restrictions on the number of allowed areas in which ancestral species may have been present (Sanmartin & Ronquist, 2004).

Molecular dating analysis. — The divergence time analyses were performed using BEAST v.1.6.1 (Drummond & Rambaut, 2007) with the ML tree as starting tree transformed to an ultrametric tree using nonparametric rate smoothing (NPRS) implemented in TreeEdit v.10a10 (Rambaut, 2007) and the divergence of parmelioid lichens set at 60.28 Ma (Amo de Paz & al., 2011). The dataset used for the molecular dating analysis consisted of a combined alignment of the parmelioid dataset used previously in Amo de Paz & al. (2011) and the *Flavoparmelia* dataset used here for the phylogenetic analysis as described above. The BEAST analysis was performed with unlinked substitutions models (GTR+I+G) across the loci, a birth-death process tree prior, and a relaxed clock model (uncorrelated lognormal) for each partition.

Two points of calibration were used for this study: (C1) was the divergence time of 49.81–73.55 Ma for the crown of parmelioid lichens (Amo de Paz & al., 2011); (C2) was *Parmelia ambra* Poinar & al., a fossil from the Dominican amber calibrated at 15–45 Ma (Poinar & al., 2000). Calibration points were defined as prior distributions: (C1) was calibrated with a uniform distribution; (C2) was considered as minimal age and calibrated with a lognormal distribution (Ho & Phillips, 2009) at lognormal mean = 2.77, offset = 14, lognormal standard deviation = 0.5.

The analysis was run for 10 million generations with parameter values sampled every 1000th generation. We checked for a stationary plateau with Tracer v.1.4.1 (Rambaut & Drummond, 2007). We discarded 10% of the initial trees as burn-in and the consensus tree was calculated using Tree Annotator v.1.6.1 (Drummond & Rambaut, 2007). The results were visualized with FigTree v.1.3.1 (Rambaut, 2009). Ages were estimated for all nodes with significant support in the phylogenetic analyses shown in Fig. 1.

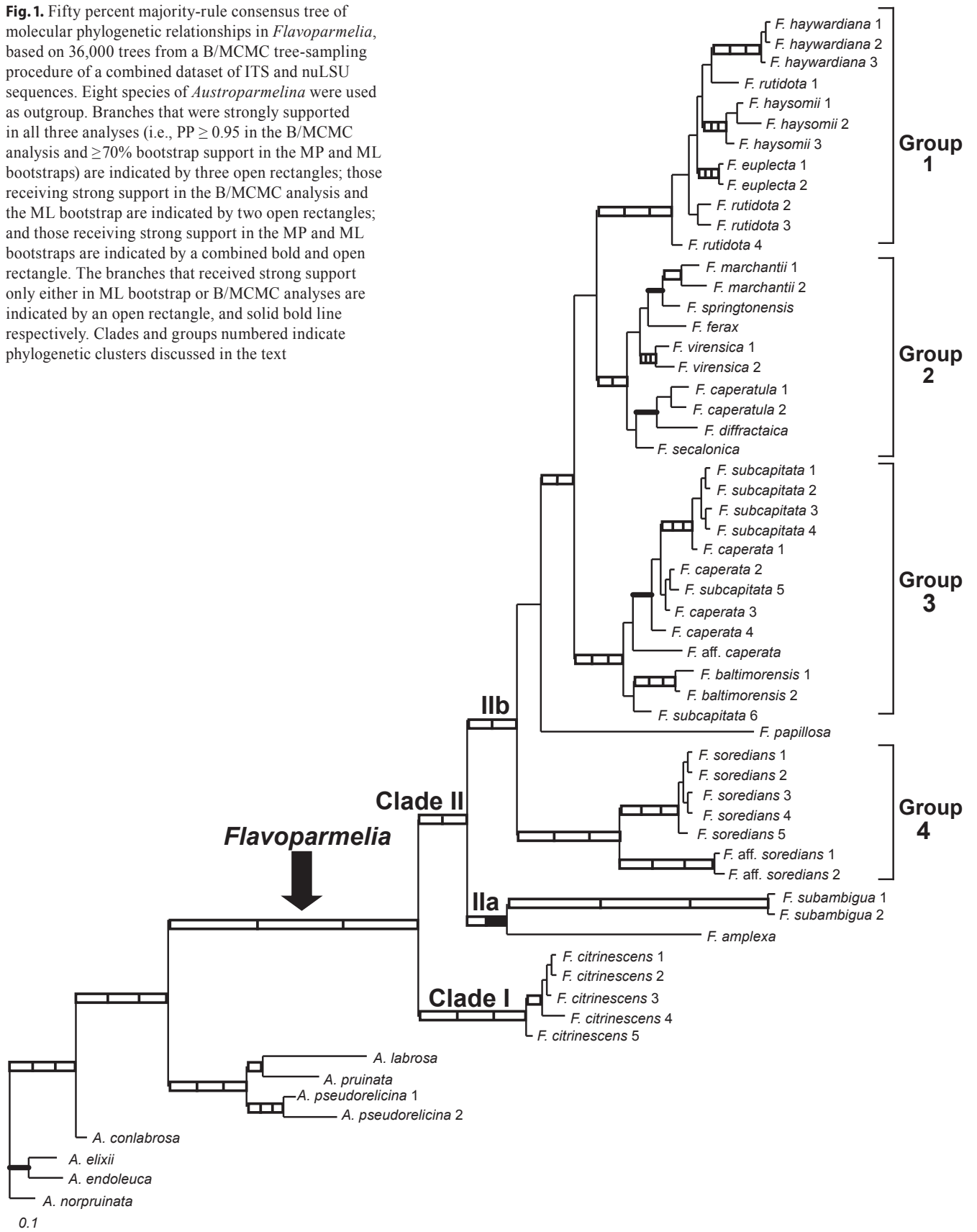
■ RESULTS

Phylogenetic analysis. — A total of 15 new nuclear ITS and 38 new nuLSU rDNA sequences were generated for this study (Appendix 1). These were aligned with 44 ITS and 17 nuLSU sequences that we previously generated (Appendix 1). Testing for topological incongruence showed no supported conflicts (results not shown); hence, the combined set was analyzed. A dataset including 51 *Flavoparmelia* samples and 8 *Austroparmelina* samples was analyzed. The aligned matrix included 478 unambiguously aligned nucleotide position characters in the ITS and 831 in the nuLSU. The concatenated alignment was 1309 positions long, with 266 variable characters.

The MP analysis of the combined data matrix resulted in 10,864 most parsimonious trees (tree length = 547 steps, CI = 0.625, RI = 0.879). In the combined matrix, 204 positions were parsimony-informative. The ML analysis of the concatenated data matrix yielded an optimal tree with likelihood value $\ln L = -4931.776$. For the Bayesian analysis the $\ln L$ value was -5104.696 with a standard deviation of ± 0.309 .

Since the topologies of the trees estimated from MP, ML and B/MCMC analyses did not show any supported conflict

Fig. 1. Fifty percent majority-rule consensus tree of molecular phylogenetic relationships in *Flavoparmelia*, based on 36,000 trees from a B/MCMC tree-sampling procedure of a combined dataset of ITS and nuLSU sequences. Eight species of *Austroparmelina* were used as outgroup. Branches that were strongly supported in all three analyses (i.e., PP ≥ 0.95 in the B/MCMC analysis and ≥70% bootstrap support in the MP and ML bootstraps) are indicated by three open rectangles; those receiving strong support in the B/MCMC analysis and the ML bootstrap are indicated by two open rectangles; and those receiving strong support in the MP and ML bootstraps are indicated by a combined bold and open rectangle. The branches that received strong support only either in ML bootstrap or B/MCMC analyses are indicated by an open rectangle, and solid bold line respectively. Clades and groups numbered indicate phylogenetic clusters discussed in the text



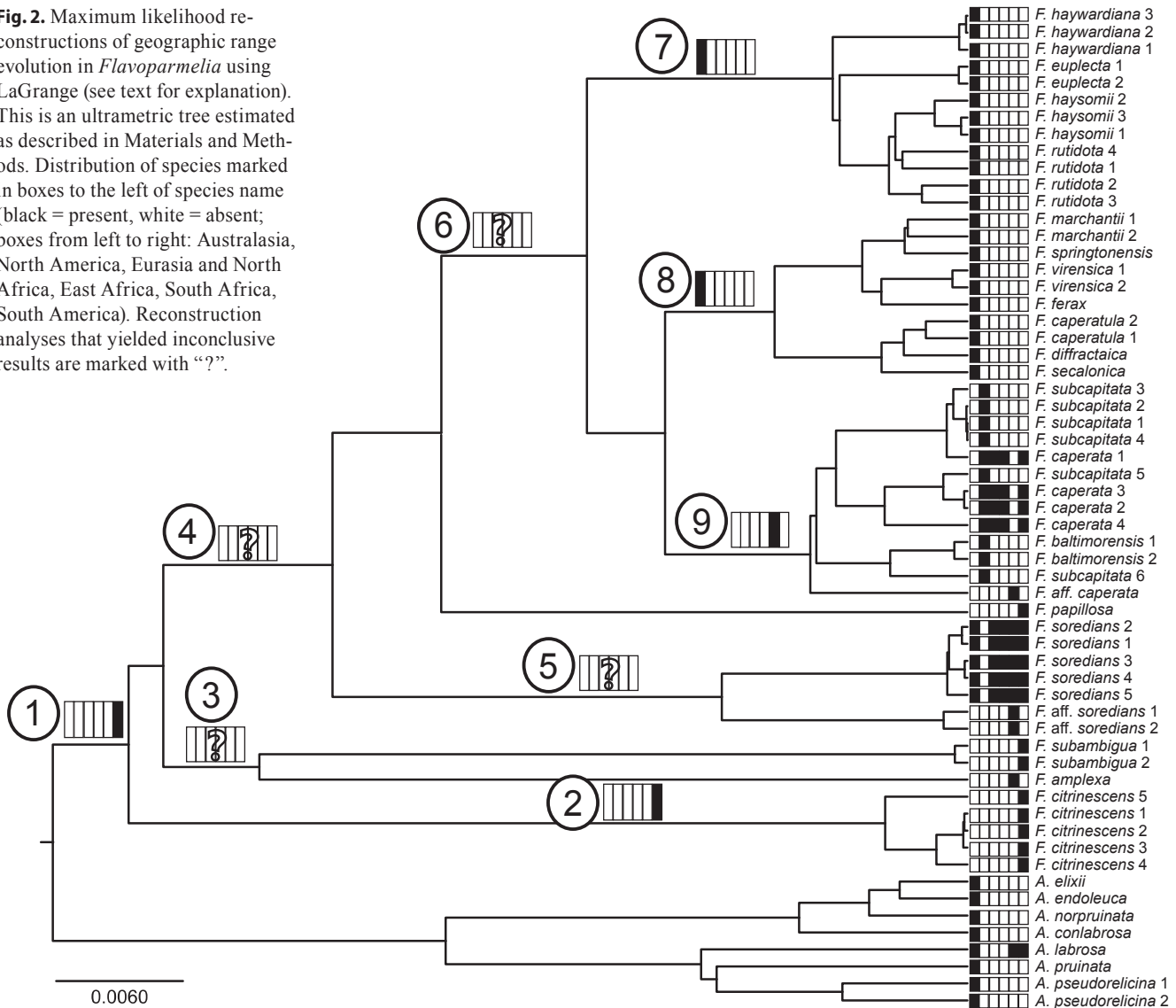
(i.e., PP ≥ 0.95 in B/MCMC analysis; MP and ML bootstrap ≥ 70%), only the 50% majority-rule consensus tree of the Bayesian tree sampling is shown here, with MP and ML bootstrap values plotted onto the Bayesian tree (Fig. 1).

In the phylogenetic tree (Fig. 1) *Flavoparmelia* forms a well-supported monophyletic group, which splits into two well-supported sister groups: (1) Clade I including samples of *F. citrinescens*, and clade II consisting of the remaining *Flavoparmelia* species. Within clade II, the samples of *F. subambigua* and *F. amplexa* (Stirt.) Hale (IIa) clustered together forming a sister-lineage to the remaining species (clade IIb). Within clade IIb, phylogenetic relationships among species are not completely resolved. However, four monophyletic groups can be identified. Group 1 includes specimens of *F. haywardiana*, *F. haysomii*, *F. euplecta*, and *F. rutidota*; (2) group 2 consists of samples of *F. marchantii* Elix & al., *F. springtonensis* (Elix) Hale, *F. ferax* (Müll. Arg.) Hale, *F. virensica* Elix & al., *F. caperatulula* (Nyl.) Elix & al., *F. diffractaica* Elix

& J. Johnst., and *F. secalonica* Elix & J. Johnst. All species in group 2 have an Australasian distribution. All the specimens belonging to *F. subcapitata*, *F. caperata* and *F. baltimorensis* formed a well-supported clade (group 3). Within this clade, phylogenetic relationships among species are ambiguous with *F. caperata* and *F. subcapitata* being non-monophyletic. All *F. soledians* specimens formed a well-supported clade (group 4) including two well-supported sister groups: one comprising specimens collected from various geographic regions, while the other includes two samples from South Africa, here identified as *F. aff. soledians*.

Ancestral area reconstructions. — The results of the ancestral range reconstructions are summarized in Fig. 2 and Table 1. The ancestral range reconstructions for five of the nodes revealed only a single most likely ancestral range within the confidence window of two log-likelihood units (Edwards, 1972). For nodes 1 (base of *Flavoparmelia*), and 2, South America was recovered as the most likely ancestral range. For

Fig. 2. Maximum likelihood reconstructions of geographic range evolution in *Flavoparmelia* using LaGrange (see text for explanation). This is an ultrametric tree estimated as described in Materials and Methods. Distribution of species marked in boxes to the left of species name (black = present, white = absent; boxes from left to right: Australasia, North America, Eurasia and North Africa, East Africa, South Africa, South America). Reconstruction analyses that yielded inconclusive results are marked with “?”.



nodes 7 and 8, the analyses strongly supported ancestral ranges in Australasia. At the base of clade 9 (*F. caperata* complex), the analysis supported an ancestral range in South Africa. For nodes 3 to 6 different ancestral ranges are statistically plausible, indicating localized uncertainty (Table 1).

Molecular dating analysis.— The calibrated chronogram of the *Flavoparmelia* dataset is depicted in Fig. 3. The mean node ages and divergence date ranges (95% highest posterior density intervals, HPD) of the clades are shown in Table 2. The

separation of *Flavoparmelia* from its closest relative *Parmotrema* A. Massal. was estimated at 33.2 Ma (23.70–44.67 Ma) (Fig. 3; Table 2). The basal radiation of *Flavoparmelia* took place around 28.49 Ma (19.88–38.98 Ma), between the late Eocene and the early Oligocene, when the main lineages of the genus originated.

Our analyses suggest five separate major divergence events that led to the evolution of the main lineages within *Flavoparmelia* (Fig. 3; Table 2). The earliest divergence is estimated around 28.49 Ma, when the basal southern South American lineage *F. citrinescens* (clade I) separated from the rest of *Flavoparmelia* (clade II). In subsequent divergence events between late Oligocene and early Miocene times, clade IIa split from the rest of *Flavoparmelia* (clade IIb) about 23.93 Ma (Fig. 3); *F. soredians* (group 4) differentiated from other species ca 19.33 Ma, and *F. amplexa* split from *F. subbambigua* about 19.22 Ma. During the late Miocene *F. soredians* separated from *F. aff. soredians* at 9.37 Ma.

The diversification of the *F. caperata* complex (group 3), and the Australasian clade (group 2), was estimated to be around 6.7 Ma and 6.5 Ma respectively. Within group 3, *F. baltimorensis* radiated around 1 Ma. Within group 2, *F. springtonensis* separated from *F. marchantii* ca. 4 Ma, and *F. diffractaica* from *F. caperatulula* ca. 4.1 Ma; *F. marchantii* radiated around 1.9 Ma and *F. virensica* around 2 Ma, and the most recent common ancestor (MRCA) of *F. ferax* and *F. secalonica* is calibrated around 6.5 Ma. In a second radiation event (around 5.4 Ma), the Australasian group 1 diversified, and *F. citrinescens* (clade I) was estimated to have radiated by the late Pliocene (2.5 Ma). Within group 1, the radiation of *F. haysomii* occurred at ca. 1.7 Ma, of *F. euplecta* ca. 0.3 Ma, and of *F. haywardiana* ca. 0.4 Ma; and the MRCA of the *F. rutidota* complex is estimated ca. 5.4 Ma.

Table 1. Inferences of ancestral area and range evolution parameters of *Flavoparmelia* at selected nodes as indicated in Fig. 2.

Node ^a	Area(s) inferred	–lnL	Rel. Prob.
1	F-F	154.7	0.356
	F-AF	156.3	0.074
	F-ACDEF	156.5	0.063
2	F-F	153.7	0.982
3	F-F	154.7	0.360
	E-F	155.0	0.268
	E-E	155.6	0.146
	A-A	156.4	0.067
4	F-F	155.2	0.225
	A-A	156.5	0.063
	F-ACDEF	156.8	0.043
	F-E	157.0	0.038
5	ACDEF-E	154.6	0.392
	ADEF-E	156.1	0.093
	E-E	156.3	0.075
	AEF-E	156.4	0.065
	AE-E	156.6	0.056
6	AF-A	155.5	0.172
	A-A	156.0	0.099
	ABF-A	156.1	0.088
	ABCEF-A	156.2	0.078
	AEF-A	156.4	0.064
	ABEF-A	156.6	0.056
	ABDEF-A	156.7	0.051
7	A-A	153.7	0.994
8	A-A	153.7	0.995
9	E-BCDF	154.5	0.450
	E-BCF	155.3	0.200
	E-BF	155.7	0.139
	E-BCD	156.3	0.071

Only inferences inside the confidence window of two log-likelihood units (Edwards, 1972) are listed. Relative probability (Rel. Prob.) of the global likelihood for the optimal optimization is given (in bold) and compared with the alternative(s). The first of the two distributions for each node leads to the upper daughter branch and the second to the lower daughter branch in Fig. 2. Global ML at root node: –lnL = 153.7 (dispersal = 28.06, extinction = 5.17). Ancestral range patterns were inferred using the following geographic areas: A, Australasia; B, North America; C, Eurasia and North Africa; D, East Africa; E, South Africa; F, South America.

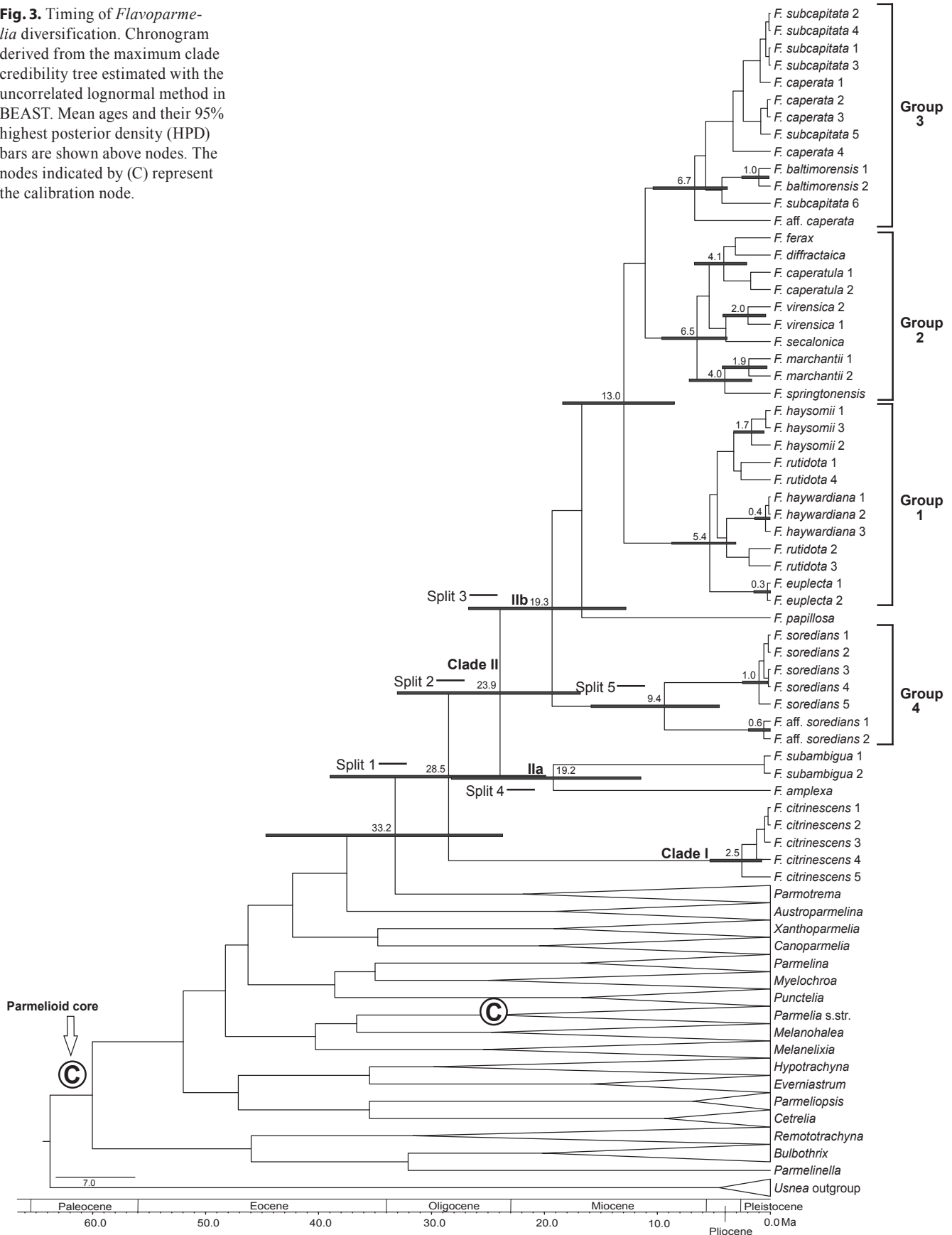
^a See Fig. 1 for details of the taxa included in each node

Table 2. Mean and range of divergence time estimations for the main clades of *Flavoparmelia*, obtained using partitioned dataset (consisting of two loci: ITS and nuLSU) BEAST analyses with two calibration points.

Clade	Mean age [Ma]	Height 95% HPD [Ma]
<i>Flavoparmelia</i> – <i>Parmotrema</i> split	33.22	23.70–44.67
<i>Flavoparmelia</i> crown		
Split 1: clade I–clade II	28.49	19.88–38.98
Split 2: clade IIa–clade IIb	23.93	16.82–33.03
Split 3: group 4–groups 1/2/3	19.33	12.78–26.71
Split 4: <i>F. amplexa</i> – <i>F. subbambigua</i>	19.22	11.46–28.23
Split 5: <i>F. soredians</i> – <i>F. aff. soredians</i>	9.37	4.52–15.87
<i>F. caperata</i> complex (group 4) crown	6.69	3.80–10.38
Australasian group 2 crown	6.49	3.83–9.61
Australasian group 1 crown	5.40	3.06–8.73
<i>F. citrinescens</i> (clade I) crown	2.52	0.77–5.35

HPD, highest posterior density

Fig. 3. Timing of *Flavoparmelia* diversification. Chronogram derived from the maximum clade credibility tree estimated with the uncorrelated lognormal method in BEAST. Mean ages and their 95% highest posterior density (HPD) bars are shown above nodes. The nodes indicated by (C) represent the calibration node.



■ DISCUSSION

Phylogenetic analysis. — The tree topology (Fig. 1) reveals more geographical structure within *Flavoparmelia* than previously recognized. Species clustering in groups 1 and 2 are restricted to Australasia, while the early diverging lineages are restricted to South America (*F. citrinescens*) and the Southern Hemisphere (clade IIa). Species in groups 3 and 4, however, have wider distributions, such as the subcosmopolitan *F. soredians*. However, also in these groups some lineages, such as *F. aff. soredians* (South Africa) and *F. baltimorensis* (North America) have restricted distribution ranges. In a number of cases, species identified using phenotypical characters (presence of soralia, chemistry), do not form monophyletic groups, but are para- or polyphyletic. This includes *F. caperata*, *F. rutidota*, *F. soredians*, and *F. subcapitata*. This is consistent with results in other groups of lichen-forming fungi and supports the notion that phenotypical species delimitation often underestimates true diversity (Crespo & Perez-Ortega, 2009; Crespo & Lumbsch, 2010; Lumbsch & Leavitt, 2011). Our sampling was not designed to address species delimitation in *Flavoparmelia* and additional data are needed to draw further conclusions regarding species boundaries in this group.

The species delimitation in group 3 seems especially troublesome with two of the three species being non-monophyletic. Further, morphological characters between and within currently accepted species are variable and sometimes ambiguous, partly due to the frequent lack of apothecia, leading to a major difficulty for delimiting species. *Flavoparmelia subcapitata* (Knudsen & al., 2005) is distinguished by having diffused soralia that are not capitate as found in *F. caperata*, while the secondary chemistry of those two species is identical. The monophyletic *F. baltimorensis* is distinguished in having pustulate isidia and containing at least traces of gyrophoric acid, and mainly grows on rocks. Our preliminary results indicate that a study with a broader sampling focusing on species delimitation in group 3 is necessary to resolve these issues.

Among specimens phenotypically identified as *F. soredians*, two well-supported sister clades were found. While both lineages occur in South Africa, only one clade (*F. soredians* s.str.) has been found in other continents as well. We interpret *F. aff. soredians* as a cryptic species distinct from *F. soredians*, which was originally described from southern France (Nylander, 1873). Cryptic species are frequently found in Parmeliaceae, with ca. 80 estimated cryptic lineages (Molina & al., 2011a, b; Nunez-Zapata & al., 2011).

Historical biogeography. — On the basis of our divergence time analyses, we hypothesize that *Flavoparmelia* originated at 33.2 Ma in southern South America. The estimated date from our analysis is approximately four million years older than that found in a previous study (Amo de Paz & al., 2011), which is most probably due to the inclusion of the early diverging South American lineage (*F. citrinescens*) that was absent in that study. The split of *Flavoparmelia* from the sister lineage *Parmotrema* with a subtropical distribution center (Lumbsch & al., 2008) coincides with the Eocene–Oligocene transition. This transition is referred to as Oi-1 glaciation (400,000 year long glacial

cycle), when the ice-sheet rapidly expanded on the Antarctic continent (Zachos & al., 2008). Around that time also the Antarctic Circumpolar Current (ACC) formed (Barker & al., 2007). The ACC led to global climate change by steepening the latitudinal temperature gradient, leading to the onset of glaciation in Antarctica (Lawyer & Gahagan, 2003). Such climate fluctuations are claimed to have caused increased rates of turnover and speciation, and global shifts in distribution of terrestrial and marine biota (Dynesius & Jansson, 2000; Zachos & al., 2008). Our time estimate for the split of *Flavoparmelia* from *Parmotrema* would be consistent with plate tectonics playing a role with *Flavoparmelia* originating in South America. There are compelling examples of lineage divergences during this Eocene–Oligocene transition, such as the split of *Allosyncarpia* S.T. Blake from *Eucalyptopsis* C.T. White between ca. 35 and 37 Ma (Crisp & al., 2004) in angiosperms.

Within *Flavoparmelia*, the major radiation began in the late Oligocene ca. 28.5 Ma, and five major lineage splits occurred at different times from 28.5 to 9.3 Ma, shaping the diversity of the main lineages within the genus (Fig. 3: Table 2). The basal split occurred ca. 28.5 Ma when *F. citrinescens* (clade I), diverged from the rest of *Flavoparmelia* (clade II). Subsequently, around 23.9 Ma at the Oligocene–Miocene boundary, a period known as Mi-1 glaciation, *F. amplexa* and *F. subambigua* diverged from the remaining species (clade IIb). Group 4, including samples of *F. soredians*, diverged from the rest of *Flavoparmelia* during mid-Miocene, ca. 19.3 Ma. Our AAR results are inconclusive for the ancestral areas of groups 3, 4, 5, and 6 (Fig. 2). The splits *F. amplexa*–*F. subambigua* and *F. aff. soredians*–*F. soredians* occurred during the mid-Miocene, ca. 19.2 Ma and late Miocene ca. 9.3 Ma, respectively (see Fig. 3 and Table 2).

The South American ancestor of *Flavoparmelia* originated (33.2 Ma, 95% HPD = 23.7–44.6 Ma) before the separation of southern South America and Australia from Antarctica, suggesting that vicariance could have played a role in shaping this distribution. We hypothesize that vicariance explains the ranges of the endemic Australasian taxa included in groups 1 and 2 (Fig. 3). Both groups clustered in a monophyletic lineage with unsupported sister relationships. The origin of the MRCA of these two groups is estimated ca. 13 Ma, indicating that the clade consisting of the Australasian endemic species existed in the continent but that the recent diversity in Australia resulted from recent diversification at the Miocene–Pliocene boundary (Table 2). Group 2 began to diversify at 6.5 Ma and group 1 at 5.5 Ma. Recent diversification in Australia is well known from other groups with rapid radiations of sclerophyllous taxa, including genera such as *Banksia* L. f., *Allocasuarina* L.A.S. Johnson and pea-flowered legumes (Crisp & al., 2004). More recently, the onset of severe aridity (mid-Pliocene, ca. 3 Ma) has triggered rapid radiation of taxa in Chenopodiaceae, Brassicaceae and *Gossypium* L. (Kadereit & al., 2003; Crisp & al., 2004; Mummenhoff & al., 2004). Hence, we propose that the aridification of the Australian climate has played a role for recent rapid radiation of Australasian lineages of *Flavoparmelia*. Group 3 is estimated to have originated in the Cape region of South Africa during the late Miocene (ca. 7 Ma). Our results

indicate that long-distance dispersal can account for the current distribution of *F. soredians*.

Lichen-forming fungi are widely distributed in all continents including Antarctica, and long-distance dispersal has traditionally been seen as the likely explanation for their diversification, simply because lichens have easily dispersible propagules. Wide disjunctions in distributional ranges have been attributed to long-distance dispersal (e.g., *Austroparmelina*, Crespo & al., 2010a; *Remototrachyna* Divakar & A. Crespo, Divakar & al., 2010; *Leptogium* (Ach.) S.F. Gray, Otálora & al., 2010), and it has been supposed that frequent dispersal would have weakened any signal of vicariance. In Parmeliaceae, however, evidence for vicariance has also been shown (Amo de Paz & al., 2011). Our results suggest that both long-distance dispersal and vicariance have played important roles in shaping the distributional ranges of *Flavoparmelia* species.

■ ACKNOWLEDGEMENTS

Financial support by the Ministerio de Ciencia e Innovación (CGL2010-21646) is gratefully acknowledged. PKD was funded by a Ramón y Cajal grant (RYC02007-01576). Sequencing was carried out at the Unidad de Genómica (Parque Científico de Madrid, UCM) and M. Isabel Garcia Saez is especially thanked for assistance.

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Appendix 1. Specimens used in the study, with location, reference collection detail, and GenBank accession numbers. Newly obtained sequences for this study are in bold face.

Austroparmelina conlabrosa (Hale) A. Crespo & al.; Australia, *Elix 38801* (CANB), GU183183, GU183176. *A. elixii* (Argüello & A. Crespo) A. Crespo & al.; Australia, *Elix s.n.* (MAF-Lich 13962), DQ273859, DQ268557. *A. endoleuca* (Taylor) A. Crespo & al.; Australia, *Elix 38805* (CANB), GU183184, GU183177. *A. labrosa* (Zahlbr.) A. Crespo & al.; South Africa, *Crespo & al. s.n.* (MAF-Lich 15877), GU183186, GU183179. *A. norpruinata* (Elix & J. Johnst.) A. Crespo & al.; Australia, New South Wales, *Elix 31571* (MAF-Lich 15376), **KF688963**, **KF688964**. *A. pruinata* (Müll. Arg.) A. Crespo & al.; Australia, *E. McCrum s.n.* (MAF-Lich 14270), EF042905, EF042914. *A. pseudorelicina* (Jatta) A. Crespo & al. 1; Australia, *Amo de Paz & Elix s.n.* (MAF-Lich 16115), GU183188, GU183181. *A. pseudorelicina* 2; Australia, *Amo de Paz & Elix s.n.* (MAF-Lich 16114), GU183189, GU183182. *Flavoparmelia* aff. *caperata*; South Africa, Cape Region, *Crespo & al. s.n.* (MAF-Lich 17572), **KF017377**, **KF017409**. *F. aff. soredians* 1; South Africa, Cape Region, *Crespo & al. s.n.* (MAF-Lich 17574), **KF017378**, **KF017416**. *F. aff. soredians* 2; South Africa, Eastern Cape, *Divakar s.n.* (MAF-Lich 17573), **KF017379**, **KF017417**. *F. amplexa* (Stirt.) Hale; South Africa, Cape Region, *Crespo & al. s.n.* (MAF-Lich 17571), **KF017381**, **KF017419**. *F. baltimorensis* (Gyeln. & Föriss) Hale 1; U.S.A., Maryland, *Molina s.n.*

Appendix 1. Continued.

(MAF-Lich 7660), AY586559, AY584832. *F. baltimorensis* 2; U.S.A., Maryland, *Molina s.n.* (MAF-Lich 10174), AY586560, AY584833. *F. caperata* (L.) Hale 1; China, Yunnan, *Crespo & al. s.n.* (MAF-Lich 10370), HM014182, **KF017407**. *F. caperata* 2; Kenya, Western Province, *Divakar & al. s.n.* (MAF-Lich 16412), HM014209, **KF017408**. *F. caperata* 3; China, Yunnan, *Crespo & Blanco s.n.* (MAF-Lich 10175), AY586561, AY584834. *F. caperata* 4; Spain, Teruel, *Crespo & al. s.n.* (MAF-Lich 6045), AY581059, AY578922. *F. caperatulula* (Nyl.) Elix & al. 1; Australia, Western Australia, *Elix 31814* (MAF-Lich 15367), HM010925, **KF017398**. *F. caperatulula* 2; Australia, Western Australia, *Elix 31749* (MAF-Lich 15371), HM010926, **KF017399**. *F. citrinescens* (Gyeln.) O. Blanco & al. 1; Argentina, Bariloche, *Messuti s.n.* (MAF-Lich 15521), GU994550, GU994596. *F. citrinescens* 2; Argentina, Bariloche, *Messuti s.n.* (MAF-Lich 19155), **KF017383**, **KF017420**. *F. citrinescens* 3; Argentina, Bariloche, *Messuti s.n.* (MAF-Lich 19156), **KF017382**, **KF017421**. *F. citrinescens* 4; Chile, National Park of Conguillio, *Amo & al. s.n.* (MAF-Lich 17570), **KF017384**, **KF017422**. *F. citrinescens* 5; Chile, National Park of Conguillio, *Ortega & Mendoza 326b* (MAF-Lich 17757), **KF017385**, **KF017423**. *F. diffracta* Elix & J. Johnst.; Australia, Western Australia, *Elix 31816* (MAF-Lich 15379), **KF017371**, **KF017400**. *F. euplecta* (Stirt.) Hale 1; Australia, New South Wales, *Elix 31547* (MAF-Lich 15375), HM010927, **KF017393**. *F. euplecta* 2; Australia, New South Wales, *Elix 36390* (MAF-Lich 16437), HM010928, **KF017394**. *F. ferax* (Müll. Arg.) Hale; Australia, South Australia, *Elix 31202* (MAF-Lich 15366), HM010929, **KF017395**. *F. haysomii* (C.W. Dodge) Hale 1; Australia, New South Wales, *Elix 42637* (MAF-Lich 7518), DQ299904, –. *F. haysomii* 2; Australia, New South Wales, *Elix 30717* (MAF-Lich 10496), HM010930, GU994597. *F. haysomii* 3; New Zealand, Gondallu, *Sancho s.n.* (MAF-Lich 16423), HM010931, **KF017392**. *F. haywardiana* Elix & J. Johnst. 1; New Zealand, Kuirau Park, *Elix 28568* (MAF-Lich 15370), HM010932, **KF017386**. *F. haywardiana* 2; New Zealand, Kerikeri, *Sancho s.n.* (MAF-Lich 16435), HM010934, **KF017387**. *F. haywardiana* 3; New Zealand, Kerikeri, *Sancho s.n.* (MAF-Lich 16425), HM010933, **KF017388**. *F. marchantii* Elix & al. 1; Western Australia, Nyamup, *Elix s.n.* (MAF-Lich 104929), DQ299905, GU994598. *F. marchantii* 2; Australia, Western Australia, *Elix 28988* (MAF-Lich 16438), HM010935, –. *F. papillosa* (Lyngby ex Gyeln.) Hale; Uruguay, Montevideo, *Osorio 9721* (MVM), HM010936, **KF017410**. *F. rutidota* (Hook. f. & Taylor) Hale 1; Australia, Western Australia, *McCrum s.n.* (MAF-Lich 15377), HM010937, **KF017389**. *F. rutidota* 2; Australia, Capital Territory, *Elix 28260* (MAF-Lich 15380), HM010938, **KF017390**. *F. rutidota* 3; Australia, New South Wales, *Elix 42638* (CANB), DQ299906, –. *F. rutidota* 4; Australia, New South Wales, *Elix 36095* (MAF-Lich 16436), HM010939, **KF017391**. *F. secalonica* Elix & J. Johnst.; Australia, Western Australia, *Elix 31649* (MAF-Lich 15368), HM010940, **KF017401**. *F. soledians* (Nyl.) Hale 1; Australia, New South Wales, *Elix 31555* (MAF-Lich 10497), HM010941, **KF017411**. *F. soledians* 2; South Africa, Cape region, *Crespo & al. s.n.* (MAF-Lich 16419), HM010944, **KF017412**. *F. soledians* 3; Spain, Canary Island, Tenerife, *Armas s.n.* (MAF-Lich 16433), HM010943, **KF017413**. *F. soledians* 4; Portugal, Abrantes, *Crespo s.n.* (MAF-Lich 16422), HM010942, **KF017414**. *F. soledians* 5; Kenya, Kijiado district, *Crespo & Divakar s.n.* (MAF-Lich 16411), HM010945, **KF017415**. *F. springtonensis* (Elix) Hale; Australia, Flinders Ranges, *Elix 31200* (MAF 14271), EF042907, EF042916. *F. subambigua* (Hale) O. Blanco & al. 1; Argentina, National Park of Calilegua, *Amo de Paz s.n.* (MAF-Lich 17575), **KF017380**, **KF017418**. *F. subambigua* 2; Argentina, National Park of Calilegua, *Amo de Paz s.n.* (MAF-Lich 15520), GU994551, GU994599. *F. subcapitata* (Nyl. ex Hasse) Hale ex DePriest & B.W. Hale 1; U.S.A., San Diego, California, *Knudsen 6462* (MAF-Lich 17576), **KF017372**, **KF017402**. *F. subcapitata* 2; U.S.A., San Diego, California, *Knudsen 10003* (MAF-Lich 17577), **KF017373**, **KF017403**. *F. subcapitata* 3; U.S.A., Tiburon Uplands Natural Preserve, California, *Sánchez-Mata s.n.* (MAF-Lich 19157), **KF017374**, –. *F. subcapitata* 4; U.S.A., San Diego, California, *Knudsen s.n.* (MAF-Lich 17578), **KF017375**, **KF017404**. *F. subcapitata* 5; U.S.A., California, *Knudsen s.n.* (MAF-Lich 16440), HM014208, **KF017405**. *F. subcapitata* 6; U.S.A., San Diego, California, *Knudsen s.n.* (MAF-Lich 17579), **KF017376**, **KF017406**. *F. virensica* Elix & al. 1; Australia, Western Australia, *Elix 32411* (MAF-Lich 15378), HM010946, **KF017396**. *F. virensica* 2; Australia, Western Australia, *Elix 33743* (MAF-Lich 16439), HM010947, **KF017397**.