

The evolutionary history of *Eugenia* sect. *Phyllocalyx* (Myrtaceae) corroborates historically stable areas in the southern Atlantic forests

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Received: 17 June 2016 Returned for revision: 28 July 2016 Accepted: 26 August 2016

- **Background and Aims** *Eugenia* sect. *Phyllocalyx* Nied. includes 14 species endemic to the Neotropics, mostly distributed in the Atlantic coastal forests of Brazil. Here the first comprehensive phylogenetic study of this group is presented, and this phylogeny is used as the basis to evaluate the recent infrageneric classification in *Eugenia sensu lato* (*s.l.*) to test the history of the evolution of traits in the group and test hypotheses associated with the history of this clade.
- **Methods** A total of 42 taxa were sampled, of which 14 were *Eugenia* sect. *Phyllocalyx* for one nuclear (ribosomal internal transcribed spacer) and four plastid markers (*psbA-trnH*, *rpl16*, *trnL-rpl32* and *trnQ-rps16*). The relationships were reconstructed based on Bayesian analysis and maximum likelihood. Additionally, ancestral area analysis and modelling methods were used to estimate species dispersal, comparing historically climatic stable (refuges) and unstable areas.
- **Key Results** Maximum likelihood and Bayesian inferences indicate that *Eugenia* sect. *Phyllocalyx* is paraphyletic and the two clades recovered are characterized by combinations of morphological characters. Phylogenetic relationships support a link between Cerrado and south-eastern species and a difference in the composition of species from north-eastern and south-eastern Atlantic forest. Refugia and stable areas identified within unstable areas suggest that these areas were important to maintain diversity in the Atlantic forest biodiversity hotspot.
- **Conclusion** This study provides a robust phylogenetic framework to address important historical questions for *Eugenia s.l.* within an evolutionary context, supporting the need for better taxonomic study of one of the largest genera in the Neotropics. Furthermore, valuable insight is offered into diversification and biome shifts of plant species in the highly environmentally impacted Atlantic forest of South America. Evidence is presented that climate stability in the south-eastern Atlantic forest during the Quaternary contributed to the highest levels of plant diversity in this region that acted as a refugium.

Key words: Biome shifts, diversity patterns, Myrtaceae, Pleistocene.

INTRODUCTION

Biogeography of the Neotropical flora, Myrtaceae, the Atlantic forest and refugia

The isolation of South America from Central America and Africa during the Mesozoic (250–65 Mya) left a strong imprint on the flora of the Neotropics, resulting in high levels of endemism in its humid forests (Burnham and Graham, 1999). Myrtaceae is one of the most important plant families in the Atlantic forest biome, considered one of five UNESCO priority biodiversity hotspots (Oliveira-Filho and Fontes, 2000). The evolutionary history of Myrteae, the neotropical Myrtaceae tribe, coincides with the evolution of the Atlantic forest and may be useful to understand other evolutionary aspects of the biome (Lucas and Bünge, 2015). A brief history of the evolution of Myrtaceae in the Neotropics follows. Palynostratigraphic evidence indicates that after its palaeotropical origin, the clades of contemporary Myrtaceae evolved and dispersed as far north

as North America, reaching as far south as Antarctica by the Cretaceous–Paleogene boundary (65.5 Mya; Systma *et al.*, 2004). Fossil pollen of the genus *Myrtaceidites* are found from the Late Eocene onwards in Australia, South America, New Zealand, Antarctica, Africa, North America, Europe and China (Thornhill and Macphail, 2012). The parent clade distribution then appears to contract into the Southern Hemisphere but is accompanied by an increase in morphological diversity and relative pollen abundance (Thornhill *et al.*, 2012). Regarding the historical distribution of Myrteae, Lucas *et al.* (2007) used dispersal–vicariance analysis (DIVA 1.1; Ronquist, 1997) to hypothesize a Gondwanan origin for Myrteae followed by migration to South America. From an infrageneric point of view, these authors found southern and western South America to be ancestral distribution areas of one of the richest clades in Myrteae, the ‘*Eugenia* group’, before northwards movement and dispersal to South Asia and Africa. In south-eastern South

America, the formation of the Atlantic forest was most influenced by the uplift of the Serra do Mar during the Oligocene, after the expansion of the *Eugenia* group. This geological event caused regional micro-regions and micro-climates to develop (Almeida and Carneiro, 1998) and has probably influenced the diversification of lineages.

During the Paleocene–Early Eocene, climatic conditions were probably warm enough to allow the expansion of megathermal angiosperms towards middle and high latitudes (Palazzesi and Barreda, 2007). After this interval, a progressive decline in temperature forced the migration of megathermal elements towards lower latitudes (Troncoso and Romero, 1998), e.g. south-eastern and north-eastern Brazil.

Based on the presence of presumed primitive species in the Atlantic forests, Gentry (1982) suggested that it could be a source area of Gondwanan taxa. The Atlantic forest flora includes early diverging lineages such as the Poaceae subfamily Anomochlooideae (Judziewicz and Clark, 2007), *Goniorrhachis* and *Barnebydendron*, the first diverging branches of the Detarieae clade of Leguminosae (Bruneau et al., 2008). Other Atlantic forest lineages appear to result from more recent colonization from other South American source areas such as birds (Cracraft and Prum, 1988), small mammals (Costa, 2003) and the genus *Pagamea* – Rubiaceae (Vicentini 2007). Based on these distributions, Pennington et al. (2006) conclude that the Atlantic forest flora comprises elements from both old and recently diverged lineages.

The southern portion of the Atlantic forest, an extensive centre of endemism that coincides with the Serra do Mar and Serra da Mantiqueira mountain ranges (Prance, 1982; da Silva et al., 2004), appears to be influenced more strongly by elements from extra-Amazonian biomes. As an example, some Andean-centred taxa can be found in the South Atlantic forest but are absent in the North Atlantic forest (from Rio Grande do Norte to northern Espírito Santo states), e.g. *Myrceugenia* (Landrum, 1981).

Within the Atlantic forest biodiversity hotspot, areas of higher angiosperm diversity and endemism are thought to correspond to areas of glacial refugia (Carnaval and Moritz, 2008; Staggemeier et al., 2015), where stable biota have contributed disproportionately to the evolutionary process, behaving as ‘museums’ and subsequently providing taxa to adjacent biomes (Keppel et al., 2012). An alternative explanation for high numbers of species found in so-called refugia is that soil conditions rather than climate change strongly influenced coastal ecosystems such as mangroves, sand banks and rain forests of south-east Brazil during the geological past (Sheel-Ybert, 2000). More recently, the refuge hypothesis is challenged by studies that predict historically unstable areas showing climatic stability during the Quaternary (Carnaval et al., 2014; Leite et al., 2016).

Eugenia as a case study for the refuge hypothesis

Angiosperms, the largest phylum of the Plant Kingdom, have approx. 250 000 species distributed in approx. 13 700 genera (Judd et al., 2008); only 57 of these genera contain >500 species (Frodin, 2004). The phenomenon of large genera and the fact that most large Angiosperm genera remain poorly known, particularly in terms of patterns of morphological and

geographic diversification, represents a formidable challenge for botanists (Frodin, 2004). Reasons to overcome these challenges include the potential for large and geographically widespread genera to reveal more comprehensive geographical structure within phylogenies than has been possible to date, allowing examination of relationships to ecological processes (Schrire et al., 2005). Large genera also require better basic understanding to resolve long-standing taxonomic issues, allowing generic diversity to be the basis of future study in related fields such as ecology, evolution, physiology and conservation.

Eugenia sensu lato (*s.l.*) (*sensu* Mazine et al., 2014) comprises approx. 1100 species (Govaerts et al., 2015); it is both the largest Neotropical Myrtaceae genus and the most species-rich genus in Brazil (Sobral et al., 2014). So circumscribed, and as suggested by previous authors (Niedenzu, 1893; Kiaerskou, 1893; Proença, 1991; Sobral, 2003; Holst et al., 2003), *Eugenia s.l.* includes four previously accepted genera: *Calycorectes* O. Berg, *Hexachlamys* O. Berg, *Phyllocalyx* O. Berg and *Stenocalyx* O. Berg.

Taxonomic classification in *Eugenia* has traditionally emphasized inflorescence morphology (Berg, 1856). Recent molecular study (Mazine et al., 2014) shows that *Eugenia* comprises nine clades, not always supported by inflorescence architecture. The clades identified by Mazine et al. (2014) are to be treated as sections (F. F. Mazine et al., unpubl. res.). One of these corresponds to *Eugenia* sect. *Phyllocalyx* Nied. and comprises 14 species. This section is widely distributed through the Atlantic forest, from eastern Brazil to Paraguay with some species in the Cerrado and Amazon biomes. The section is characterized by peduncles with leaf-like bracts and showy sepals that are proportionally larger than the flowers (Berg, 1856).

The centre of *Eugenia* diversity is in Brazil from where approx. 388 taxa are recorded, of which 313 are endemic (Sobral et al., 2014), *Eugenia* occurs in all phytogeographic domains. The greatest species richness is found in the Atlantic forest and Amazonia, with 250 and 91 taxa, respectively, while 74 species are reported from the Cerrado (savanna-like vegetation). The importance of Myrtaceae and *Eugenia s.l.* as structural and ecological components of Atlantic rain forest of Brazil makes this group a good proxy for the diversity of other important angiosperm groups in the Atlantic rain forest (Oliveira-Filho and Fontes, 2000; Murray-Smith et al., 2009; Lucas and Bünger, 2015).

This study presents a molecular phylogeny of *E.* sect. *Phyllocalyx* including representatives of all other infrageneric groups (‘clades’ *sensu* Mazine et al., 2014). The main goals of this study were to (1) test the monophyly of *E.* sect. *Phyllocalyx* (*sensu* Berg, 1856; Niedenzu, 1893); (2) provide a hypothetical chronology for major clade diversification within *Eugenia*; (3) test the Atlantic forest as the ancestral area for *E.* sect. *Phyllocalyx*; and (4) examine the evolutionary history of *E.* sect. *Phyllocalyx* in the context of stable Atlantic forest areas in the Quaternary.

MATERIALS AND METHODS

Taxonomic and molecular sampling

Forty-two Myrteae taxa were sampled including 28 *Eugenia s.l.*, of which 12 (from 14 species) were *Eugenia* sect.

TABLE 1. PCR conditions

Molecular marker	Primers	Conditions
ITS	AB101 AB102	5 min at 94 °C followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, 1 min at 72 °C and a last stage of 7 min at 72 °C
<i>psbA-trnH</i>	<i>psbA</i> (F) <i>trnH</i> (R)	5 min at 94 °C followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, 1 min at 72 °C and a last stage of 7 min at 72 °C
<i>rpl16</i>	rpL16F71 rpL16R1516	5 min at 94 °C followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, 1 min at 72 °C and a last stage of 7 min at 72 °C or 5 min at 80 °C; 35 × (95 °C, 1 min; 50 °C, 1 min with a ramp of 0.3 °C s ⁻¹ ; 65 °C, 5 min) and a last stage of 4 min at 65 °C
<i>trnL-rpl32</i>	<i>trnL</i> (UAG) <i>rpl32</i>	5 min at 94 °C followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, 1 min at 72 °C and a last stage of 7 min at 72 °C
<i>trnQ 5'-rps16</i>	<i>trnQ 5' MytrnQ Myrps16 rps16</i>	5 min at 94 °C followed by 28 cycles of 1 min at 94 °C, 1 min at 48 °C, 1 min at 72 °C and a last stage of 7 min at 72 °C

Phyllocalyx, encompassing as much morphological and geographical variation as possible within the group (Supplementary Data Fig. S2). Approx. 85 % of *E. sect. Phyllocalyx* was represented. Thirteen additional genera from tribe Myrteae were additionally included. *Myrtus communis* was used as outgroup in all analyses following Lucas *et al.* (2011). Species names and nomenclature follow Sobral *et al.* (2014). The internal transcriber spacer (ITS) of the ribosomal nuclear region and four plastidial regions: *psbA-trnH*, *rpl16*, *trnL-rpl32* and *trnQ-rps16* were used (Table 1). The final analysis included 588 sequences, 57 % generated for this study, with the remaining 43 % obtained from published and unpublished works (Lucas *et al.* 2007; J. Q. Faria-Junior, UnB, Brasilia, Brazil, unpubl. res.; F. F. Mazine, Ufscar, Sorocaba, Brazil, unpubl. res.; M. F. Santos, Usp, Sao Paulo, Brazil, unpubl. res.).

DNA sequencing

Total DNA was extracted from 0.3 g of silica gel-dried leaf material using a modified version of the 2 × cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). Total DNA was further purified for long-term storage in RBG Kew's DNA & Tissue Collections by equilibrium centrifugation in CsCl-ethidium bromide gradients (1.55 g mL⁻¹) followed by butanol extraction of the ethidium bromide and dialysis to remove the caesium chloride. Amplification and purification of target DNA regions were executed according to the protocols outlined in Lucas *et al.* (2007, 2011) and Shaw *et al.* (2007) using internal primers for *trnL-rpl32* and *trnQ-rps16*. The PCR conditions are listed in Table 1. Sequencing was performed according to Lucas *et al.* (2007). DNA sequences were assembled using MUSCLE (Edgar, 2004) and edited in Geneious 7.9 with subsequent manual adjustment when necessary. All sequences are deposited in GenBank; DNA samples are stored in RBG Kew's DNA & Tissue Collections (see Appendix 1). Due to the inheritance of the plastid genome as a single linked unit, the four resulting plastid DNA matrices were combined into a single data set of 4190 bp. The nuclear and combined plastid regions were then analysed independently and simultaneously.

Phylogenetic analyses

Phylogenetic reconstruction using maximum likelihood (ML) and Bayesian inference was performed on all data sets. Models of nucleotide substitution were selected for each sequence partition with MrModeltest2, version 2.2 (Nylander, 2004). The model recommended under the Akkaike Information Criterion for the ITS, *trnL-rpl32* and *trnQ-rps16* region was GTR + G + I, and that for *psbA-trnH* and *rpl16* was GTR + G. Data sets were analysed in combination with these model parameters fitted independently to each partition.

Maximum likelihood analyses were performed with RAXML v7.6.3 (Stamakis, 2006) using the rapid bootstrap algorithm with 1000 replicates, combined with a search of the best-scoring ML tree under default parameters. Bayesian analyses were performed using MrBayes version 3.2.1 (Ronquist *et al.*, 2012). Two independent analyses each of four chains were conducted with 5 million generations of Monte Carlo Markov Chains (MCMCs) and a sampling frequency of 1000. Results were examined with Tracer v. 1.6 (Drummond and Rambaut, 2007), to ensure that the analyses reached convergence and that the effective sample size of each parameter was >200. A consensus tree with posterior probabilities (PPs) was generated using the 'sumt' option in MrBayes with the default burn-in of 10 % (500 trees in our case). The consensus tree and PPs were visualized with FigTree 1.4 (Rambaut, 2012).

Analyses conducted for each partition returned topologies inspected by eye and found to be congruent for major clades. The combined data set provided greater resolution and higher levels of support; our results and discussion therefore focus on the combined results. Phylogenetic reconstructions obtained with ML and Bayesian results are highly congruent.

To estimate temporal evolution of *E. sect. Phyllocalyx*, we used a Bayesian inference approach implemented in the package BEAST v.1.8.0 (Drummond *et al.*, 2012) using the combined data set and applying the same partition and models as previously described for the Mr Bayes analyses (see above). An uncorrelated relaxed molecular clock assuming a lognormal distribution of rates and a Yule speciation model were applied. Four runs of 30 million generations were performed, sampling one tree every 1000 generations. Parameter convergence was confirmed by examining their posterior distribution in Tracer 1.6 (Drummond and Rambaut, 2007). MCMC sampling was

TABLE 2. Molecular data and parsimony-based tree characteristics

	ITS	<i>psbA-trnH</i>	<i>rpl16</i>	<i>trnL-rpl32</i>	<i>trnQ-rps16</i>	Plastid	Combined molecular data sets
Aligned characters (bp)	738	466	915	807	1274	3462	4190
No. of variable characters (%)	256 (34.7)	159 (34.1)	117 (12.8)	178 (22.0)	271 (21.3)	725 (21.0)	560 (13.4)
No. of potentially parsimony-informative characters (%)	123 (16.7)	67 (14.4)	31 (3.4)	79 (9.8)	87 (6.9)	296 (8.6)	421 (10.0)
Trees	1315	2757	7626	3407	7626	156	24
Length	553	253	140	257	370	1066	1677
Consistency index	0.61	0.76	0.95	0.83	0.84	0.89	0.77
Retention index	0.57	0.83	0.95	0.80	0.78	0.86	0.71

ITS, internal transcribed spacer region.

TABLE 3. Records used per species in the ecological model modelling (ENM)

Species	Records
<i>Eugenia elongata</i> Nied.	2
<i>Eugenia</i> sp. nov. Bünger & Sobral	2
<i>Eugenia expansa</i> Spring. ex Mart.	48
<i>Eugenia glandulosa</i> Cambess.	3
<i>Eugenia involucrata</i> DC.	451
<i>Eugenia itacarensis</i> Mattos	9
<i>Eugenia luschnathiana</i> (O.Berg) Klotzsch ex B.D.Jacks.	46
<i>Eugenia macrobracteolata</i> Mattos	6
<i>Eugenia magnibracteolata</i> Mattos & D. Legrand	7
<i>Eugenia membranifolia</i> Nied.	15
<i>Eugenia puberula</i> Nied.	5
<i>Eugenia regia</i> Bünger & Sobral	3
<i>Eugenia ruschiana</i> Bünger & Mazine	1
<i>Eugenia selloi</i> B.D. Jacks.	11
Total records	609

considered sufficient when the effective sample size of each parameter was >200. All analyses were performed on the CIPRES portal (Miller et al., 2010). A maximum clade credibility tree with median branch lengths and 95 % highest posterior density interval on nodes was built using TreeAnnotator 1.8 (Drummond et al., 2012) based on the remaining set of trees after burn-in (for each run, a burn-in period of 3 million generations was applied). Calibration was performed using the fossil *Paleomyrtinae princetonensis* from the Paleocene (Crane et al., 1990; Pigg et al., 1993) to early Eocene (Manchester, 1999) of North America, comprising well-preserved fruits and seed probably related to *Psidium* and/or *Mosiera* (Pigg et al. 1993). The most recent common ancestor of Myrteae and the outgroup taxa (crown group of Myrteae) was constrained, following the recommendations of Forest (2009), using a lognormal distribution with a median of 55.8 Mya (corresponding to the lower bound of the Eocene), lower quartile (2.5 %) of 54.94 Mya and superior quartile of (97.5%) 61.9 Mya, achieved using an offset value of 54.8 Mya.

Ancestral area analysis

The ‘core *Phyllocalyx*’ clade identified by the phylogenetic analysis contained 12 species. A matrix was compiled indicating the presence or absence of each *Eugenia* species sampled for the phylogeny, in one of four geographic areas. The areas used were designed to indicate movement of species of the ‘core *Phyllocalyx*’ clade between the biomes in which they are

found (Atlantic forest and Cerrado) but also included areas in which the other *Eugenia* species sampled occurred (Amazon and ‘not tropical America’). Dispersal Extinction Cladogenesis (DEC; Ree and Smith 2008) was implemented using the package RASP (Yu et al., 2015) run constraining range to two biogeographic units and dispersal between adjacent units (Atlantic/Amazon forest and Cerrado) set to 1.0, non-adjacent, proximal units (Atlantic and Amazon forest) set to 0.5 and non-adjacent distant units (Atlantic/Amazon forest/Cerrado and ‘not tropical America’) set to 0.1. The RASP output reports ancestral area probabilities as pie charts.

Ecological niche models (ENMs)

Data set. To access how the whole ‘core *Phyllocalyx*’ clade identified by the phylogenetic analysis could be a model group to identify a new refugium area during the Quaternary, the 14 species were modelled (the 12 from the phylogeny framework plus the two missing, i.e. *Eugenia elongata* Nied. and *E. itacarensis* Mattos). The floristic data set was extracted from NeoTropTree (Oliveira-Filho, 2015), a database of tree species (defined as free-standing woody plants >3 m in height) checklists for geo-referenced Neotropical sites compiled from the literature and herbarium specimen records. Data gathered for this study from multiple herbaria were also used (BHCB, BR, CEPEC, ESA, G, HB, HPL, HRCB, HUFU, K, LE, M, MBM, MBML, OUPR, OX, P, R, RB, SP, SPF, UEC and UPCB). In total, 609 records were included, encompassing all known occurrences (Supplementary Data Fig. S3) of *E.* sect. *Phyllocalyx*; the number of records per species is shown in Table 3.

Bioclimatic variables. For all sites, we obtained the value at 1 km spatial resolution, of the 19 standard BIOCLIM variables, from the WorldClim database (Hijmans et al., 2005). We cropped the bioclimatic layers to span from 10°N to 40°S and from 70°W to 20°W (Fig. S3). This covers all known occurrences as well as the potentially accessible area for the species (Fig. S3); ‘core *Phyllocalyx*’ is actually restricted to South America, mostly to the coastal Atlantic forest in Brazil, with some species growing in rain forests from Argentina, Bolivia, Paraguay and Uruguay, and Cerrado from Brazil.

After assessing correlations between the bioclimatic variables, we retained ten of 19 variables, eliminating those with less biological relevance from groups of strongly inter-related variables ($r > 0.9$). This procedure was done to avoid over-parametrization of our modelling with redundant variables. The

final selected variables were: annual mean temperature, mean diurnal range, isothermality, maximum temperature of warmest month, temperature annual range, mean temperature of driest quarter, annual precipitation, precipitation of wettest month, precipitation of wettest quarter and precipitation of warmest quarter.

Model construction. Ecological niche models (ENMs) usually involve the determination of associations between environmental conditions and information on the occurrence of species to identify areas critical to the maintenance of species populations (Peterson *et al.*, 1999; Warren *et al.*, 2010; Peterson and Soberón, 2012), and this technique can be used to analyse the limiting factors or regulators that determine the spatial distribution of plants and animals (Guisan and Zimmermann, 2000).

We modelled the ecological niche of the 14 species (Table 3) using Maxent ver. 3.3 (Phillips *et al.*, 2006). It has been demonstrated that Maxent is often critical to the maintenance of species populations (Elith *et al.*, 2006, 2011; Pearson *et al.*, 2006; Phillips and Dudík, 2008; Pena *et al.*, 2014). In addition, an important reason for choosing Maxent was that it allowed us to use presence-only species data, which is of great utility because the vast majority of the biotic data available, including those used here, come in this form (Elith *et al.*, 2006, Phillips and Dudík, 2008).

To calibrate and evaluate the quality of the models, we divided the data for each species into a training set (75 % of occurrences) and a test or validation set (25 % of occurrences). We constructed models five times and averaged the output to produce the final results used in downstream analyses. Next, for each species, we defined a threshold value above which grid cells were considered to have environmental characteristics suitable for the maintenance of viable populations of the species (Pearson *et al.*, 2006). We used the ‘minimum training presence’ as the threshold selection method because it assumes that the species presence is restricted to sites at least as suitable as those at which the species has been observed so far (Pearson *et al.*, 2006).

In order to produce models to infer the palaeodistribution of the ‘core *Phyllocalyx*’ species, we produced projections of the suitability of occurrence during the current (0 ka pre-industrial), mid-Holocene (6000 years ago – 6 kyr BP), Last Glacial Maximum (LGM; 21 000 years ago – 1 kyr BP) and Last Interglacial (LIG; 120 000 years ago – 120 kyr BP) time periods based on climatic simulations (Hijmans *et al.*, 2005).

Palaeoclimatic data represent downscaled climate data from simulations with Global Climate Models (GCMs) based on the Coupled Model Intercomparison Project Phase 5 (CMIP5; Taylor *et al.*, 2012). For the LIG model, we used the Otto-Bliesner *et al.* (2006) approach, while for LGM and Holocene we employed the Community Climate System Model – CCSM4 (Gent *et al.*, 2011). All geographic information system (GIS) analyses were performed in ArcGIS v.10 (ESRI, 2011).

To indicate potential areas of stability for the species of *E. sect. Phyllocalyx* throughout the Quaternary, similar protocols to those used in recent studies for other Neotropical domains were adopted (Carnaval and Moritz, 2008; Werneck *et al.*, 2011, 2012; Bueno *et al.*, 2016) where converted models were produced from continuous outputs into presence/absence maps by applying the lowest presence threshold for each model. This

approach maximizes agreement between observed and modelled distributions, balancing the cost arising from an incorrect prediction against the benefit gained from a correct prediction (Pearson *et al.*, 2006). By summing the presence/absence maps obtained for current conditions, mid-Holocene (6000 years ago – 6 kyr BP), LGM (21 000 years ago – 21 kyr BP) and LIG (120 000 years ago – 120 kyr BP) projections, a map of areas showing historical stability was generated. This combined map depicted areas potentially occupied by *E. sect. Phyllocalyx* species during the climatic oscillations of the Quaternary. These historically stable areas, or refugia, were defined as those grid cells for which the presence of all species was inferred in all models and time projections.

We also used a threshold-independent method of model validation, the receiver operating characteristic (ROC) curve analysis. The ROC curve is obtained by plotting sensitivity values (the true-positive fraction) on the y-axis against their equivalent specificity values (1 – specificity, the false-positive fraction) on the x-axis for all possible thresholds (Fielding and Bell, 1997). The ROC analysis characterizes the predictive performance of a model at all possible thresholds by a single number, the area under the curve (AUC) (Fawcett, 2003; Phillips *et al.*, 2006). A single AUC value was calculated for each species, representing the average across the five iterations of model construction. The value of the AUC can fall between 0.5 and 1.0. If the value is 0.5, the model is no better than random, while models with values >0.75 are generally considered potentially useful, and models with a value near one are considered to be strongly supported (Fielding and Bell, 1997; Elith, 2002; Rushton *et al.*, 2004; Phillips *et al.*, 2006).

RESULTS

Phylogenetic relationships

Molecular marker statistics are summarized in Table 1; of the 3462 characters included in the plastid analysis, 466, 915, 807 and 1274 bp are from *psbA-trnH*, *rpl16*, *trnL-rpl32* and *trnQ-rps16*, respectively. The ITS partition is more variable (16.7 % informative characters) than the four plastid regions (8.6 % informative characters for combined plastids; Table 2). Inspection of the topologies by eye and using the incongruence length difference (ILD) test ($P > 0.001$; partition-homogeneity test with heuristic search), performed using PAUP, indicates that the two partitions give congruent results. Differences between the resulting topologies were negligible; therefore, the combined ML tree is provided as Supplementary Data Fig. S1 and the Bayesian results (Fig. 1) form the basis of the discussion presented here.

Eugenia sect. *Phyllocalyx sensu* Berg emerges as a paraphyletic group (Fig. 1). The clade containing most species previously placed inside *E. sect. Phyllocalyx*, and the type-species of the section (*Eugenia involucrata* DC.) emerge as a well-supported monophyletic group (PPBAYES, 0.99; PPBEAST, 0.97; ML, 0.75). This clade corresponds to *Eugenia* sect. *Phyllocalyx*, and its taxonomic sense can be conserved; from hereon the name ‘core *Eugenia* sect. *Phyllocalyx*’ is used. A second well-supported clade (PPBAYES, 1; PPBEAST, 1; ML, 100) also included four species previously included in *Eugenia* sect. *Phyllocalyx* and was recently published as *Eugenia* sect.

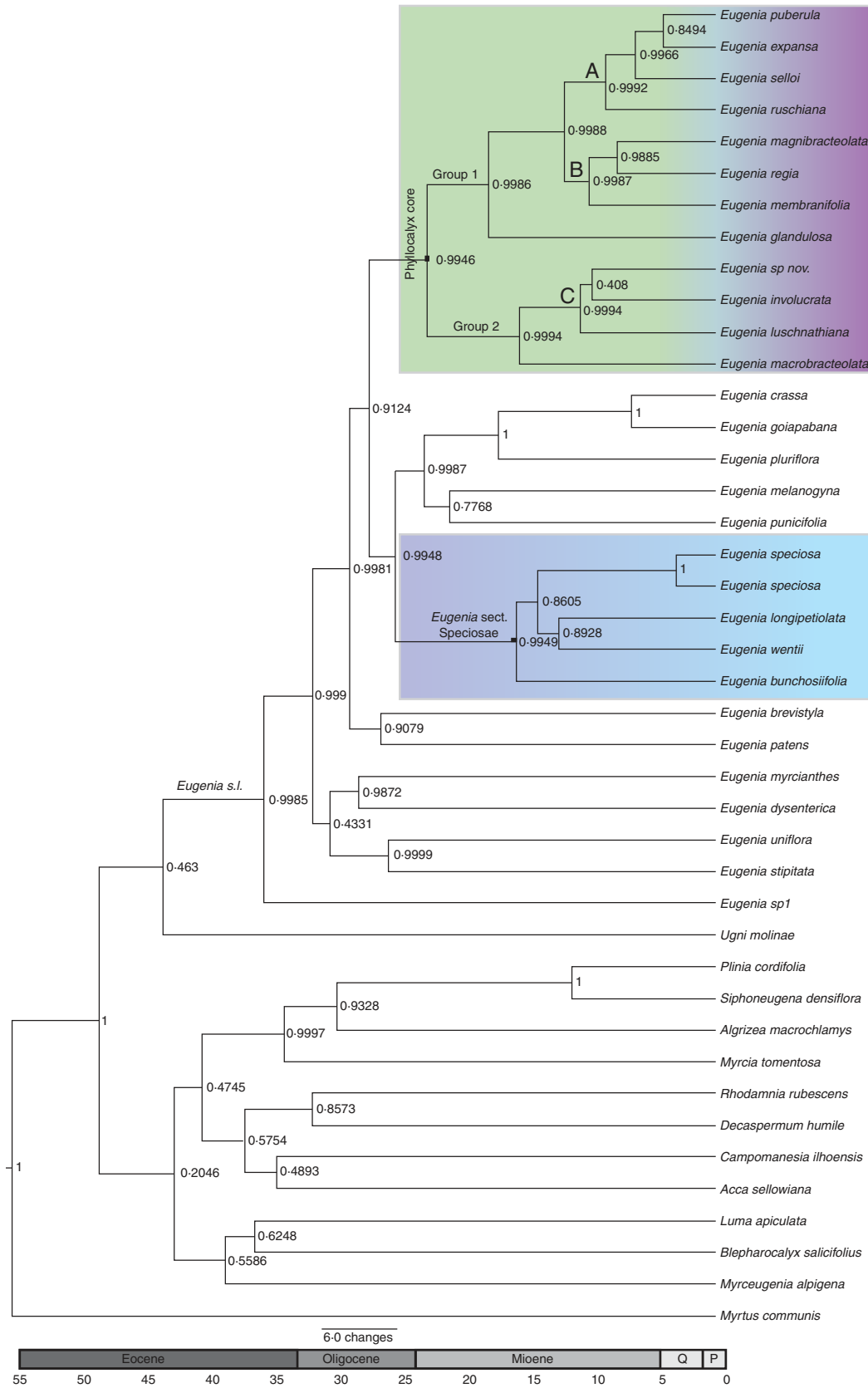


FIG. 1. Maximum credibility clad tree with divergence time estimates and posterior probabilities obtained from BEAST analyses of *Eugenia* section *Phyllocalyx* based on combined analyses of nuclear (ITS) and plastid (*psbA-trnH*, *rpl16*, *trnL-rpl32*, *trnQ-rps16*) regions and using *Paleomyrtinaea princetonensis* as a calibration point at the root of the tree (i.e. crown node of tribe Myrteae; 55.8 Mya). Posterior probability values are indicated on branches. P, Pliocene; Q, Quaternary.

Speciosae Bünger & Mazine (Bünger *et al.*, 2016). The new section emerges with high support (PPBAYES, 0.99; PPBEAST, 0.99; ML, 72) as sister to *Eugenia* sect. *Umbellatae* (F. F. Mazine *et al.*, unpubl. res.). These clades correspond entirely, or with minor exception, to combinations of macro-morphological characters. Often such characters taken individually are not sufficiently informative to place a species in a particular group, but, when taken together, the sub-clades become morphologically diagnosable. Other clades identified but poorly supported in Mazine *et al.* (2014) are represented here with relationships between them generally receiving higher statistical support.

Within core *Eugenia* sect. *Phyllocalyx*, ‘Group 1’ receives good support (PPBAYES, 0.99; PPBEAST, 0.99; ML, 97) and includes sub-clades A, B and *E. glandulosa* Cambess. ‘Group 2’ also receives high support (PPBAYES, 1; PPBEAST, 0.99; ML, 100) and includes sub-clade C and *E. macrobracteolata* Mattos. *Eugenia* sect. *Speciosae* includes *Eugenia wentii* Amsh. (the only Amazonian species from what was *E. sect. Phyllocalyx sensu Berg*), *E. bunchosifolia* Nied., *E. longipetiolata* Mattos and *E. speciosa* Cambess.; relationships within *Eugenia* sect. *Speciosae* are poorly supported.

In core *Eugenia* sect. *Phyllocalyx*, sub-clades A and B are composed of species exclusively from coastal Atlantic forest. *Eugenia glandulosa* appears sister to these clades and is exclusively found in Cerrado. Sub-clade C is composed of species from both Atlantic forest and dry biomes. *Eugenia luschnathiana* grows in the *restingas* of Northeast Brazil. *Eugenia* sp. nov. is from *campos rupestres* from Bahia and *E. involucrata* is widespread through Atlantic forest and other dry biomes (Cerrado, *restinga* and *campos rupestres*). *Eugenia macrobracteolata* from the Atlantic Forest is sister to sub-clade C.

Dating

Trees obtained from the BEAST analyses agree well with those from the Bayesian and ML phylogenetic hypotheses. The mean age for *Eugenia s.l.* is 35 million years (Mya) with 95 % confidence intervals (CIs) of 45–24 Mya (mid-Eocene to Early Miocene; Fig. 1). Core *Eugenia* sect. *Phyllocalyx* dates from the mid-Miocene (20.6 Mya; 95 % CI 27.5–14 Mya; mid-Oligocene to mid-Eocene; Fig. 1). Species endemic to the Atlantic forest (sub-clades A and B) are reported as younger (12.0 Mya; 95 % CI 16.0–8.0 Mya; mid-Miocene to Late Miocene) than species from Cerrado (*E. glandulosa*; 16.6 Mya; 95 % CI 23.0–10.0 Mya) and as older than others from dry biomes (sub-clade C; 10.6 Mya; 95 % CI 15.0–5.0 Mya). *Eugenia* sect. *Speciosae* dates from Miocene (16.9 Mya; 95 % CI 23–10 Mya).

Ancestral area analysis

Most likely ancestral areas are indicated in Fig. 2.; ancestral area probabilities for key nodes are summarized in Supplementary Data Table S1. The most likely ancestral area of *Eugenia s.l.* (*sensu Mazine et al.*, 2014) is seen to be the Atlantic forests (node 80: Atlantic forest, 58.4; Atlantic/Amazon forest, 32.37; Atlantic forest/Cerrado, 9.23). The majority of internal *Eugenia* nodes are reported to have originated

exclusively (probability 100) in the Atlantic forest including *Eugenia* sect. *Speciosae* (node 61), the *Phyllocalyx* core (node 73), Groups 1 (node 68) and 2 (node 72). Within the *Phyllocalyx* core, only node 69, the ancestor of Group 1 plus *Eugenia glandulosa*, has the probability to have arisen in the Cerrado (Atlantic forest, 55.3; Atlantic forest/Cerrado, 44.7).

Modelling *Eugenia* sect. *Phyllocalyx*

The quality of the models, according to the AUC values, were significant for all, confirmed by the correct assignment of testing data under the models, indicating that the models performed well in distinguishing species occurrence in relation to bioclimatic variables. The palaeomodelling suggest changes in distribution ranges of core *Phyllocalyx* across the Quaternary (Fig. 3) but also show a constant area with high adequacy in south-eastern Brazil in contrast to the refugia hypothesis of Carnaval and Moritz (Fig. 4). However, the core *Phyllocalyx* clade appears to have experienced a remarkable change in its whole distribution during the LGM (Fig. 3B). Except during the LGM, the distribution ranges of core *Phyllocalyx* across the Quaternary appear very similar to current-day distributions. Areas that show a high probability of occurrence of core *Phyllocalyx* are in the north-eastern and south-eastern Atlantic coast (Fig. 3A, C, D) with species appearing in the sub-montane rainforests and *restingas*.

DISCUSSION

Although the molecular framework was based on five markers and does not represent a complete evolutionary history, results presented here provide a first comprehensive understanding of phylogenetic relationships within *Eugenia* sect. *Phyllocalyx sensu Berg*. The study uses the 14 species of *Eugenia* sect. *Phyllocalyx* set within a small but representative sample of the 1100 total global *Eugenia* species, to investigate evolutionary and ecological patterns in the genus. Myrtaceae sub-taxa have been used successfully as proxies for other groups (Murray-Smith *et al.*, 2009; Lucas and Bünger, 2015), allowing the results presented here to be reasonably interpreted in this way. It is clear that the results presented here cannot be completely representative of the genus as a whole and that different *Eugenia* groups would have responded to different pressures or differently to the same pressures; however, this study represents the first biogeographical hypothesis of the genus and provides evolutionary and ecological models for consideration and testing by subsequent works, and data for inclusion in subsequent studies. Myrtaceae taxa have been used successfully as proxies for other groups (Murray-Smith *et al.*, 2009; Lucas and Bünger, 2015), allowing the results presented here to be reasonably interpreted in this way.

The results are significant from a variety of perspectives, first suggesting that the classical taxonomy requires some rearrangement. According to Mazine *et al.* (2014) and F. F. Mazine *et al.* (unpubl. res.), *Eugenia wentii* (not treated as a group by Mazine *et al.*, 2014) was more closely related to *Eugenia* clade 9 *sensu Mazine et al.* (2014) (= *Eugenia* sect. *Umbellatae*). Our results confirm this relationship and furthermore find the species to comprise part of a separate section inside *Eugenia s.l.*

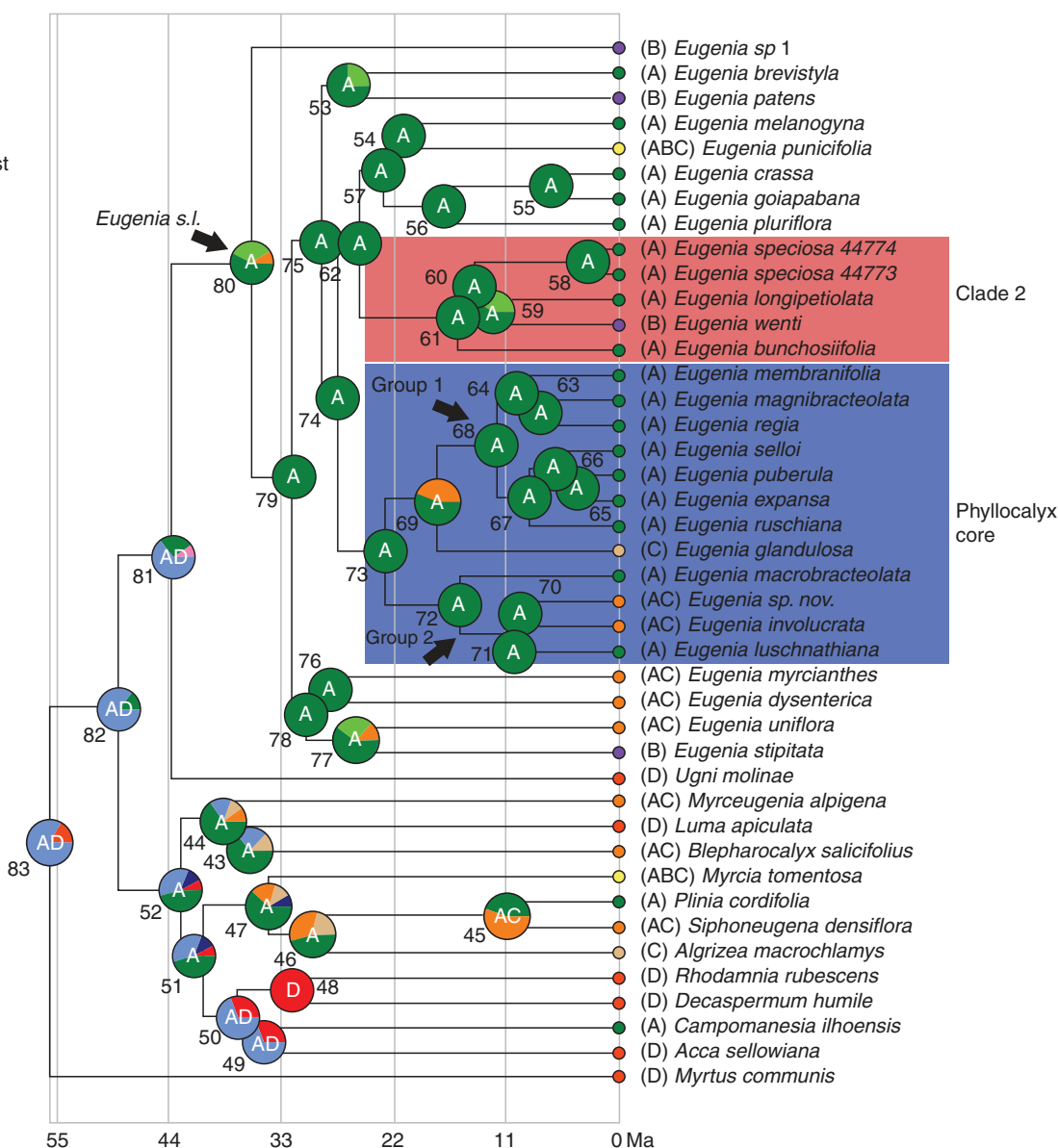


Fig. 2. Ancestral area analysis (DEC) represented on the maximum credibility tree. Letters within the pie charts are the single or pair of most probable ancestral areas (A, Atlantic forest; B, Amazon forest; C, Cerrado; D, 'non-tropical America'). Numbers adjacent to the pie charts identify each node. The range of each species is listed in parentheses next to the species name.

This section contains species from both the Atlantic and Amazon forests and supports suggested links between Amazonian and Atlantic forests (McVaugh, 1956). Although these two clades do not emerge in a monophyletic group, they share the floral characters of showy sepals and bracteoles that could be homoplastic characters in *Eugenia* s.l. *Eugenia* sect. *Speciosae* could be distinguished from the core *Phyllocalyx* by its linear sepals and bracteoles not persistent at anthesis. Further details and discussion are provided by Bünger et al. (2016).

The focus of the remainder of this study is the core *Phyllocalyx* group; the following discussion concerns the 14 species that comprise this group (Table 3), of which 12 are represented in the phylogenetic framework. The affinities of the

missing species, based on morphological characters, are predicted as follows: *Eugenia elongata* and *E. itacarensis* both resemble *E. regia* (sub-clade B); shared characters include significantly larger flowers and sepals and larger bracteoles persistent at anthesis.

Dating and biogeography

Myrtaceae dates from the Cretaceous (86 Mya; Sytsma et al., 2004; Thornhill et al., 2012), apparently originating in paleotropical Africa (Thornhill and Macphail, 2012) or with a more general Gondwanan origin (Lucas et al., 2007). Results presented here suggest that *Eugenia* s.l. originated in the transition

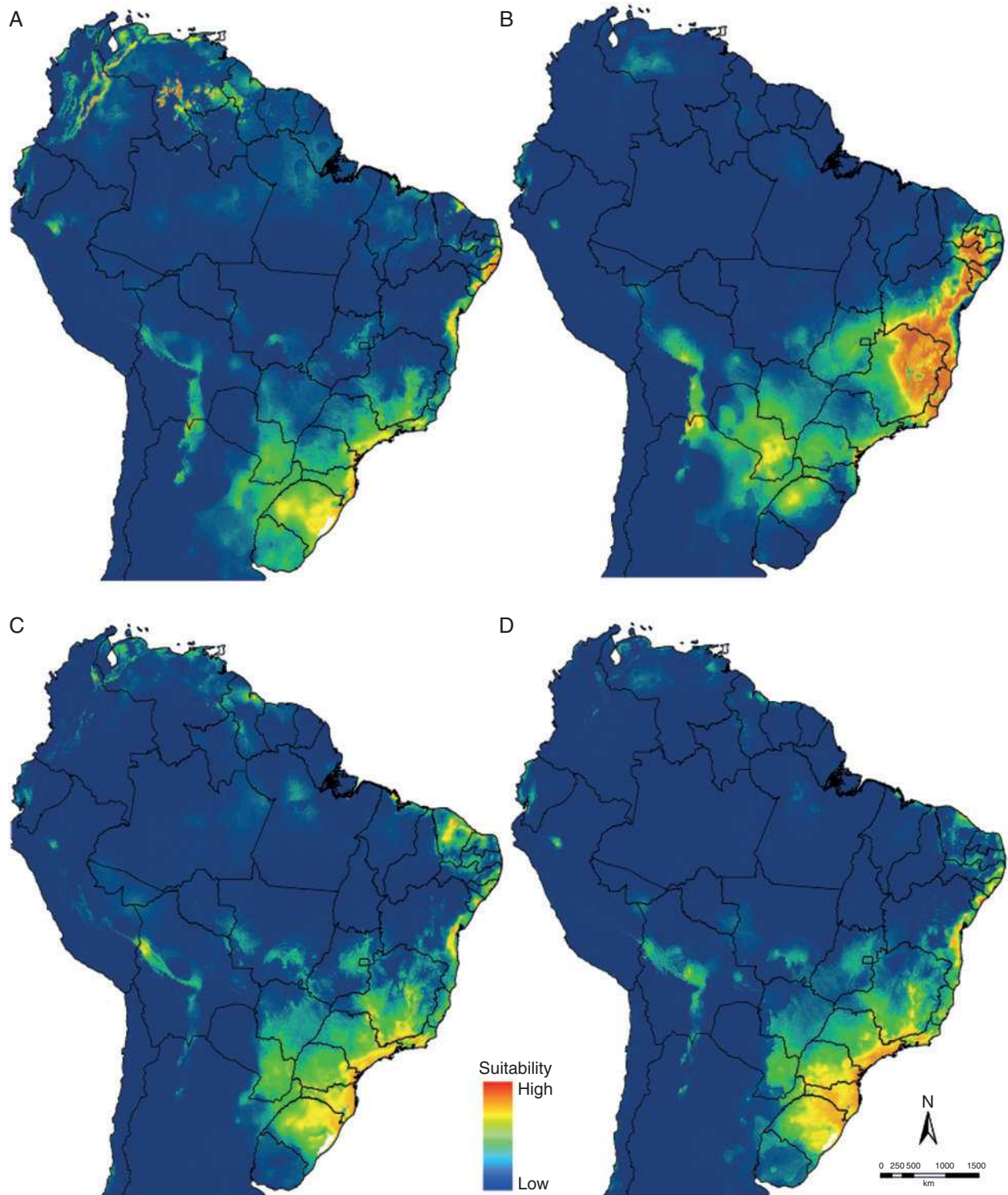


FIG. 3. Predicted occupancy of the *Phyllocalyx* clade across northern South America during: (A) the Last Interglacial (LIG; 130 ka BP); (B) the Last Glacial Maximum (LGM; 21 ka BP); (C) the mid-Holocene (6 ka BP); and (D) current climate (0 ka pre-industrial) scenarios. Warmer colours (red/yellow) of the logistic output correspond to regions with higher probability of occurrence.

between the Eocene and Oligocene (35 Mya; Fig. 2). The fossil record of Myrteae is poor as fleshy fruits are rarely well preserved compared with dry fruits from other members of the family (e.g. tribe Eucalypteae). Only a single reliable fossil was

available for the calibration procedure used here and, as a result, the biogeographical scenario outlined should be considered with some caution. Another putative Myrteae fossil, wood of *Myrceugenelloxylon antarcticus* from 72–66 Ma (Poole *et al.*,

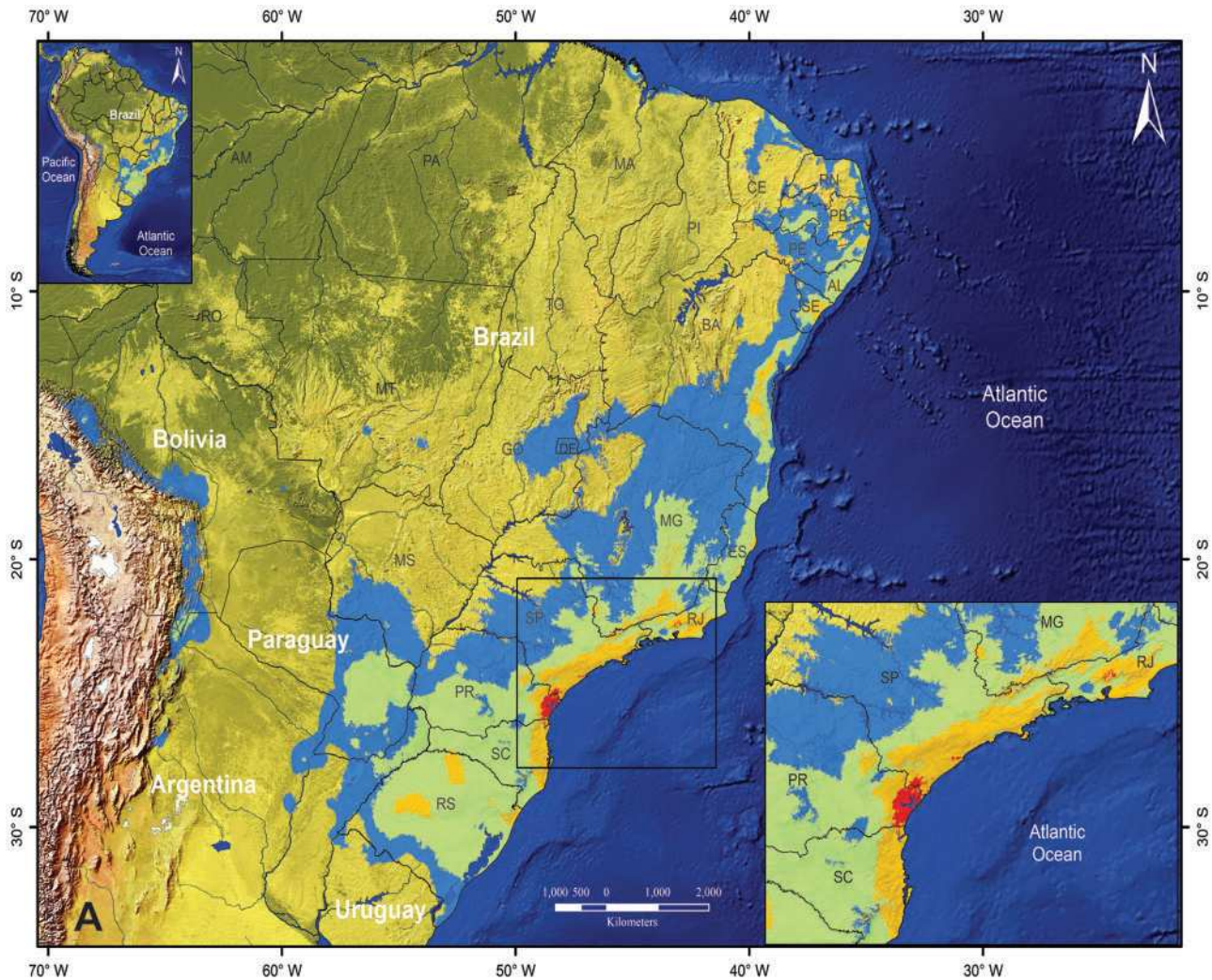


Fig. 4. Predicted regions of historical stability in the *Phyllocalyx* clade across the Quaternary, based on summed predicted occupancy of the clade across current (0 ka pre-industrial), mid-Holocene (6 ka BP), Last Glacial Maximum (LGM; 21 ka BP) and Last Interglacial (LIG; 130 ka BP) climatic scenarios. Areas in red are those where all species of the *Phyllocalyx* clade are predicted to occur during all four time periods and represent postulated refugial areas for the *Phyllocalyx* clade. Maps are for (a) South America, (b) central South America. Brazilian states are labelled as follows: Amazonas (AM), Bahia (BA), Ceará (CE), Distrito Federal (DF), Espírito Santo (ES), Goiás (GO), Maranhão (MA), Minas Gerais (MG), Mato Grosso (MT), Mato Grosso do Sul (MS), Pará (PA), Paraná (PR), Piauí (PI), Rio de Janeiro (RJ), Rio Grande do Norte (RN), Rio Grande do Sul (RS), Rondônia (RO), Santa Catarina (SC), São Paulo (SP), Tocantins (TO).

2003), was rejected due to doubts regarding its definite identification as Myrtaceae. The lack of an extensive, reliable fossil record might explain in part the discrepancies between results obtained here and those of Biffin *et al.* (2010) who found a mid-Miocene origin for *Eugenia* in a broader perspective.

Panti (2014) described Myrtaceae fossil leaves including those putatively, of *Eugenia* *s.l.*, from the Río Turbio formation in Argentina; this formation is dated from the mid-Eocene. Despite the lower reliability associated with assigning leaf fossils to extant taxa, these finds correspond to results presented here that suggest a late Eocene origin for *Eugenia* *s.l.* An earlier origin for *Eugenia* *s.l.* and the distribution of the leaf fossils fit the dispersion hypothesis of Lucas *et al.* (2007) who suggest an origin of *Eugenia* after the Drake Passage opened isolating South America from Antarctica 42–17 Mya (Upchurch, 2008).

At the subgeneric level in *Eugenia*, and based on the sample presented here, older Atlantic forest lineages are represented by more geographically widespread species (*Eugenia uniflora*, approx. 26 Mya; *E. brevistyla*, approx. 24 Mya; and *E. puniceifolia*, approx. 19.9 Mya). Lineages that appear more recently comprise species more likely to occur in centres of endemism such as the Serra do Mar in Paraná, São Paulo and Rio de Janeiro states (e.g. *Eugenia expansa* and *E. puberula*, approx. 4.3 Mya; *E. selloi*, approx. 6.5 Mya; and *E. magnibracteolata*, approx. 8 Mya). A large majority of core *Eugenia* sect. *Phyllocalyx* species are found only in the Atlantic forest, suggesting that the section originated in that biome (approx. 20 Mya). Within core *Eugenia* sect. *Phyllocalyx* (Fig. 1), two main groups emerge. Group 1 is comprised of almost all species from the Atlantic forest biome. Group 2 is comprised of species from drier biomes, *restinga* and Atlantic forest. Results from

the dating analysis show Group 2 to be slightly younger (approx. 15.6 Mya) than Group 1 (approx. 16.7 Mya), suggesting that Group 2 emerged after and from ancestors in the Atlantic forest (*Eugenia macrobracteolata* is mostly found in the Serra do Mar, but also rarely occurs in Espírito Santo, with one specimen from Rio Grande do Norte; A. R. Araújo, pers. comm.). This scenario is supported by ancestral area analysis (Fig. 2) that shows most clades of *Eugenia*, including core *Eugenia* sect. *Phyllocalyx* and *E.* sect. *Speciosae*, to arise in the Atlantic forest biome. Because the specific sample is limited outside of core *Eugenia* sect. *Phyllocalyx*, caution is required when interpreting results; indeed, more detailed studies of *Eugenia* biogeography (e.g. F. F. Mazine *et al.*, unpubl. res.) suggest a more pronounced role for the Amazon forest in early *Eugenia* diversification. However, for core *Eugenia* sect. *Phyllocalyx* and *E.* sect. *Speciosae*, sampling is almost complete (12 of 14 species and four of six species, respectively), and it is possible to be confident that these groups originated in the Atlantic forest biome with *E.* sp. nov. and *E. involucrata* subsequently expanding to drier environments. The pattern is supported by other studies that show Atlantic forest species colonizing the Cerrado at or around this time (Simon *et al.*, 2009) and suggests a biome shift (Crisp *et al.*, 2009; Donoghue and Edwards, 2014) between Atlantic forest and Cerrado. *Eugenia involucrata* occurs in multiple biomes; it is the most widespread species within core *Phyllocalyx* and emerges within Group 2. The most recent lineage in this group is composed of *Eugenia* sp. nov. and *E. luschnathiana*, species from the North Atlantic forest. The North Atlantic forest block (Thomas and Barbosa, 2008) is a relatively narrow strip of forest bounded to the west by the Caatinga domain. This result suggests that the two blocks (Thomas and Barbosa, 2008) of Atlantic forest form a ‘monophyletic’ group, unusual when previous biogeographic studies indicate the Atlantic forest to be a composite biogeographic area with significantly different species composition in the southern and northern blocks, suggesting relative evolutionarily independence of these areas (e.g. Cracraft and Prum, 1988; Costa, 2003; Perret *et al.*, 2006; Nihei and Carvalho, 2007; Santos *et al.*, 2007).

Eugenia glandulosa is the oldest lineage in Group 1 and is endemic to the Cerrado, suggesting the existence of the Cerrado biome approx. 16.7 Mya, or some precursor. This supports the findings of Ratter *et al.* (1997) who provide older dates for the origin of the Cerrado than the most recent hypotheses (e.g. Simon *et al.*, 2009). The oldest estimates of the Cerrado suggest the biome to be a precursor to the adjacent Amazonian and Atlantic rain forests, during the Holocene (Ledru, 2002). Simon *et al.* (2009) found fire-adapted Cerrado lineages of *Mimosa* to have evolved independently at least 11 times and more recently than core *Phyllocalyx* (from 9.8 Mya to 0.4 Mya; late Miocene to Pliocene vs. 16.7 Mya to 9.2 Mya; mid-Miocene to late Miocene). Simon *et al.* (2009) also suggest that these woody legume lineages coincide with a hypothesized expansion of C₄ grass-dominated savannah biomes that become ecologically dominant 4–8 Mya (Jaccobs *et al.*, 1999). This is in contrast to Christin *et al.* (2008) who demonstrate that C₄ grasses originated and began to diversify 32–25 Mya, coinciding more with the diversification time of core *Phyllocalyx*. If these hypotheses (an Atlantic forest origin for core *Phyllocalyx* and a biome shift between Atlantic forest and Cerrado) were

correct, it appears that core *Phyllocalyx* arrived in the Cerrado via two routes. This has been reported in other lineages [e.g. *Ruellia* in Tripp (2007) and *Stirax* in Fritsch (2001)], but core *Phyllocalyx* is much older than these groups. The confidence intervals of the dating analysis are large, and, as a result, certainty in dates is reduced. Within the confidence intervals, difference in age of Groups 1 and 2 may be insignificant (Fig. 1).

The Carnaval–Moritz (2008) hypothesis of biological refugia delimits the north-eastern Brazilian area limited by the rivers Rio Doce and São Francisco during the LGM (Pleistocene). Areas of elevated species diversity of *Myrcia* sect. *Aulomyrcia* (O.Berg) Griseb. (Myrtaceae) in the Atlantic forests were shown (Staggemeier *et al.*, 2015) to correspond to this hypothesis. However, the model here suggested indicates that south-east coastal regions of Brazil could comprise environmentally stable regions (Fig. 4) that acted as refuges for *Eugenia* sect. *Phyllocalyx* during the Quaternary period. The south-east coastal region from the southern state of São Paulo (SP) and northern state of Paraná (PR) comprise estuarine and lagoon complexes, with various micro-regions and micro-climates most probably influenced by the uplift of Serra do Mar during the Oligocene (approx. 70 Mya; Almeida and Carneiro, 1998). Palynological studies (Marchant *et al.*, 2002; Behling and Negrelli, 2006) also indicate the stability of south-east coastal vegetation, with Myrtaceae pollen widely representative of the stratigraphy. Recent studies using vertebrates as model groups show historically unstable areas (southern state of SP and northern state of PR) with climatic stability during the Quaternary (Carnaval *et al.*, 2014; Leite *et al.*, 2016). These stable forest refuges would have been responsible for maintaining the pattern of diversity and endemism observed today. In fact, eight out of 14 species of core *Phyllocalyx* occur in the southern SP/northern PR region; this emphasizes that these species should provide appropriate proxy records of forest changes through time.

Concluding remarks

Results presented here advance understanding of species relationships in *Eugenia* s.l., but much progress must be made before the group is completely understood. Amazonian species remain poorly studied, as do relationships between Amazonian and Atlantic forest species.

Conclusions discussed here must be considered in light of high confidence intervals returned; however, the presented results confirm the paraphyletic nature of *Eugenia* sect. *Phyllocalyx* *sensu* Berg and allowed a new infrageneric section to be proposed. Mazine *et al.* (2014) considered the emergence of *Eugenia wentii* close to *E.* sect. *Umbellatae* a likely error, but results presented here confirm this placement and demonstrate the importance of species sampling and molecular marker density in the phylogeny of large clades. Species niche modeling identified refuges within areas classically considered unstable during both the Quaternary and Pleistocene–Holocene–Present periods. As well as presenting further evidence for species diversity related to the north-eastern Brazilian refuge (Carnaval and Moritz, 2008; Staggemeier *et al.*, 2015), this study also suggests that high species diversity in southern SP and northern PR may be linked to an area of environmental

stability. This is supported by the analysis of Murray Smith *et al.* (2009) who reported centres of Myrtaceae species diversity in both the northern and southern blocks of the Atlantic. Evidence for refuges contributes to explanations of extreme plant species richness and endemism in the south and north-eastern coasts of Brazil and shows that plant diversification is not a simple random model with a constant rate of speciation and extinction (Donoghue and Sanderson, 2015). It is clear that multiple traits contribute to the success of both *Eugenia* and angiosperms in the Atlantic forest; these include morphological innovations, environmental changes and geographic movement, all traceable along the branches of a phylogenetic tree (Donoghue and Edwards, 2014).

Biodiversity data typically contribute to conservation planning by allowing compilation of species lists and distributions that ultimately define an area as a diversity ‘hotspot’ (Myers *et al.*, 2000), or otherwise labelled special (e.g. World Wildlife Fund ecoregions, 2015). Recently, evolutionary processes have also been implicated in conservation strategy, particularly in light of global climate change (Mace *et al.*, 2003). Increasing understanding of Myrtaceae evolution in Neotropical forests, as well as biodiversity data produced (Lucas and Bünger, 2015), provides both streams of diversity data. The fact that *Eugenia* is the most species-rich genus of Brazilian angiosperms (Forzza, 2015) and one of the most ecologically important tree species taxa in the Atlantic forests (Oliveira-Filho and Fontes, 2000; Murray-Smith *et al.*, 2009) compounds the significance of this information. For these reasons and in summary, this study provides a rich source of information for *in situ* conservation strategies under current and future scenarios of environmental change and anthropic pressure.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: maximum likelihood and Bayesian tree. Figure S2: plate of *Eugenia* sect. *Phyllocalyx* features and habitats. Figure S3: geographic distribution of *Eugenia* sect. *Phyllocalyx*. Table S1: ancestral area probabilities per numbered node of Fig. 2.

ACKNOWLEDGEMENTS

We are grateful to Laszlo Csiba, Dion Devey, Jim Clarkson, Laura Martinez, Rhina Duque, Jimmy, Ernesto and Becky for help in the lab, and Petra Broddle for help in the herbarium at Kew. Luis Palazzesi, Matheus Santos, Tânia Moura and Vanessa Staggemeier are thanked for discussion of and help with dating phylogenies; Marcos Sobral is gratefully acknowledged for all discussions throughout the period of the PhD of the first author. Herbarium curators that permitted examination of *Eugenia* specimens are thanked, as is Danilo Neves for discussion of modelling analyses, as well as all colleagues of the Laboratório de Sistemática from UFMG. This work was supported by CAPES and CNPq [014/2012] and a sandwich fellowship from MOB to develop part of this research at RBG Kew, London, UK [14045/2013-03]. M.L.B. thanks CNPq for a Post-doctoral scholarship (151002/2014-2).

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APPENDIX 1. VOUCHERS OF DNA SAMPLES USED FOR PHYLOGENETIC ANALYSIS, WITH GENBANK NUMBERS

Species	Collector	Voucher	ITS	<i>psbA-trnH</i>	<i>rpl16</i>	<i>trnL-rpl32</i>	<i>trnQ-rps16</i> *
<i>Acca sellowiana</i> (O. Berg) Burret	Chase 10349	K	10349	10349	10349	10349	10349
<i>Algrizea macrochlamys</i> (DC.) Proença & Nic Lugh.	Giulietti 1648	K	16833	16833	16833	16833	16833
<i>Blepharocalyx salicifolius</i> (Kunth) O. Berg	Lucas 78	K	KX789287	KX789292	KX789317	KX789346	KX910667
<i>Campomanesia ilhoensis</i> Mattos	Ibrahim 122	K	34650	34650	34650	34650	34650
<i>Decaspermum humile</i> (Sweet ex G. Don) A.J. Scott	Belsham M82	OTA	AM234128	19052	AM489824	19052	19052
<i>Eugenia brevistyla</i> D. Legrand	Mazine 993	ESA, K	KJ187614	20683	KJ469663	20683	20683
<i>Eugenia bunchosii</i> Nied.	Faria 2513	BHCB, UB	KX789268	KX789298	KX789323	KX789352	KX910673
<i>Eugenia involuocrata</i> DC.	Bünger 551	BHCB	KX789281	KX789294	KX789319	KX789348	KX910669
<i>Eugenia</i> sp1	Holst 9516	K	36243	36243	36243	36243	36243
<i>Eugenia crassa</i> Sobral	Giacomin 1860	BHCB	KX789269	KX789296	KX789321	KX789350	KX910671
<i>Eugenia expansa</i> Spring ex Mart.	Bünger 634	BHCB, K	KX789279	KX789297	KX789322	KX789351	KX910672
<i>Eugenia dysenterica</i> DC.	Mazine 466	ESA, K	KJ187620	20844	KJ469669	20844	20844
<i>Eugenia</i> sp. nov.	Moraes 600	BHCB	KX789284	KX789295	KX789320	KX789349	KX910670
<i>Eugenia glandulosa</i> Cambess.	Faria 37	BHCB, UB	KX789277	KX789299	KX789324	KX789353	KX910674
<i>Eugenia goiapabana</i> Sobral & Mazine	Bünger s/n	BHCB	KX789270	KX789300	KX789325	KX789354	KX910675
<i>Eugenia longipetiolata</i> (O. Berg) Mattos	Bünger 626	BHCB, K	KX789285	KX789301	KX789326	KX789355	KX910676
<i>Eugenia luschnathiana</i> (O. Berg) Klotzsch ex B.D. Jacks.	Faria 3140	BHCB, UB	KX789272	KX789302	KX789327	KX789356	KX910677
<i>Eugenia macrobracteolata</i> Mattos	Faria 3050	UB	KX789283	KX789303	KX789328	KX789357	KX910678
<i>Eugenia magnibracteolata</i> Mattos & D. Legrand	Bünger 627	BHCB, UB	KX789271	KX789304	KX789329	KX789358	KX910679
<i>Eugenia melanogyna</i> (D. Legrand) Sobral	Mazine 969	ESA, K	KJ187624	KX789305	KJ469673	20694	20694v
<i>Eugenia membranifolia</i> Nied.	Duarte 85677	BHCB, ESA	20942	KX789306	KX789330	KX789360	KX910680
<i>Eugenia myrcianthes</i> Nied.	Faria 2850	UB	44019	44019	44019	44019	44019
<i>Eugenia patens</i> Poir.	Lucas 104	ESA, K	KJ187633	20947	KJ469681	KX789361	KX910681
<i>Eugenia pluriflora</i> DC.	Mazine 961	ESA, K	KJ187636	Mazine unpubl.	KJ469684	Fiorella	Fiorella
<i>Eugenia puberula</i> Nied.	Bünger 629	BHCB, K	KX789282	KX789293	KX789318	KX789347	KX910668
<i>Eugenia puniceifolia</i> (Kunth) DC.	Mazine 1065	ESA, K	KJ187638	–	KJ469686	KX789361	KX910682
<i>Eugenia regia</i> Bünger & Sobral	Bünger 578	BHCB	KX789276	KX789307	KX789333	KX789362	KX910683
<i>Eugenia selloi</i> (O. Berg) B.D. Jacks.	Bünger 566	BHCB, RB	KX789278	KX789308	KX789334	KX789363	KX910684
<i>Eugenia ruschiana</i> Bünger & Mazine	Bünger 618	BHCB, K	KX789280	KX789309	KX789335	KX789364	KX910685
<i>Eugenia speciosa</i> Cambess.	Bünger 585	BHCB	KX789274	KX789310	KX789336	KX789365	KX910686
<i>Eugenia speciosa</i> Cambess.	Mota 2477	BHCB	KX789275	KX789311	KX789337	KX789366	KX910687
<i>Eugenia stipitata</i> McVaugh	Holst 8872	SEL	KJ187645	35648	KJ469694	35648	35648
<i>Eugenia uniflora</i> L.	Lucas 207	Cultivated K	AM234088	–	AM489828	KX789367	KX910688
<i>Eugenia wentii</i> Amshoff	Holst 9421	K	KJ187651	35646	KJ469701	KX789368	KX910689
<i>Luma apiculata</i> (DC.) Burret	Chase 17313	K	KX789288	KX789312	KX789340	KX789369	17313
<i>Myrceugenia alpigena</i> (DC.) L.R. Landrum	Lucas 167	K	KX789289	KX789313	19066	KX789370	19066
<i>Myrcia tomentosa</i> (Aubl.) DC.	Savassi s/n (ESA 85681)	K	20697	20697	20697	20697	20697
<i>Myrtus communis</i> L.	Chase 10347	K	KX789286	KX789314	KX789342	KX789371	KX910690
<i>Plinia cordifolia</i> (D. Legrand) Sobral	Mazine 957	ESA, K	KX789291	KX789315	20679	KX789372	20679
<i>Rhodamnia rubescens</i> (Benth.) Miq.	Belsham M83	OTA	AM234127	†	AM489879	†	†
<i>Siphoneugena densiflora</i> O. Berg	Mazine 1050	ESA, K	KX789290	KX789316	20681	KX789373	20681
<i>Ugni molinae</i> Turcz.	J. Murillo 4213, Belsham M69	CONC, OTA	JN660933	JN660982	AM489885	JN661081	JN661131

*Two internal primers for region *trnQ-rps16* were used; see Table 1.

†Missing data.

Abbreviations: Institution/State: BHCB, Universidade Federal de Minas Gerais, MG, Brazil; CONC, Universidad de Concepción/Concepción, Chile; ESA, Instituto de Botânica/SP, Brazil; OTA, University of Otago/Dunedin, New Zealand; SEL, Marie Selby Botanical Gardens/Florida, USA; UB, Universidade de Brasília/DF, Brazil.

