

Phylogenetic analysis in *Myrcia* section *Aulomyrcia* and inferences on plant diversity in the Atlantic rainforest

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• **Background and Aims** *Myrcia* section *Aulomyrcia* includes ~120 species that are endemic to the Neotropics and disjunctly distributed in the moist Amazon and Atlantic coastal forests of Brazil. This paper presents the first comprehensive phylogenetic study of this group and this phylogeny is used as a basis to evaluate recent classification systems and to test alternative hypotheses associated with the history of this clade.

• **Methods** Fifty-three taxa were sampled out of the 120 species currently recognized, plus 40 outgroup taxa, for one nuclear marker (ribosomal internal transcribed spacer) and four plastid markers (*psbA-trnH*, *trnL-trnF*, *trnQ-rps16* and *ndhF*). The relationships were reconstructed based on Bayesian and maximum likelihood analyses. Additionally, a likelihood approach, 'geographic state speciation and extinction', was used to estimate region-dependent rates of speciation, extinction and dispersal, comparing historically climatic stable areas (refugia) and unstable areas.

• **Key Results** Maximum likelihood and Bayesian inferences indicate that *Myrcia* and *Marlierea* are polyphyletic, and the internal groupings recovered are characterized by combinations of morphological characters. Phylogenetic relationships support a link between Amazonian and north-eastern species and between north-eastern and south-eastern species. Lower extinction rates within glacial refugia suggest that these areas were important in maintaining diversity in the Atlantic forest biodiversity hotspot.

• **Conclusions** This study provides a robust phylogenetic framework to address important ecological questions for *Myrcia s.l.* within an evolutionary context, and supports the need to unite taxonomically the two traditional genera *Myrcia* and *Marlierea* in an expanded *Myrcia s.l.* Furthermore, this study offers valuable insights into the diversification of plant species in the highly impacted Atlantic forest of South America; evidence is presented that the lowest extinction rates are found inside refugia and that range expansion from unstable areas contributes to the highest levels of plant diversity in the Bahian refugium.

Key words: *Aulomyrcia*, Atlantic forest, diversification rates, diversity patterns, extinction, GeoSSE, *Marlierea*, *Myrcia*, Myrtaceae, speciation.

INTRODUCTION

'The distinction between *Myrcia* and *Marlierea* is a somewhat nebulous one, and it is probable that *Marlierea* comprises a phylogenetically diverse assemblage of species which have been arbitrarily assigned to the genus because of the character of the irregularly splitting calyx.' (McVaugh, 1956, page 166).

Myrcia s.l. (*sensu* Lucas *et al.*, 2007) comprises 753 species (Govaerts *et al.*, 2014); representing the second-largest Neotropical genus of Myrtaceae after *Eugenia* (1038 species; Govaerts *et al.*, 2014). Taxonomic classification in *Myrcia* has traditionally emphasized characters of the calyx and mode of opening of the flower (McVaugh, 1956; Berg, 1855–1856); as described, molecular studies show that these traits do not group species naturally (Lucas *et al.*, 2007, 2011).

The most recent phylogenetic analysis of *Myrcia s.l.* (Lucas *et al.*, 2011) recovered nine major clades that have been treated as nine informal groups. *Myrcia* section *Aulomyrcia* (Lucas *et al.*, unpubl. res.) represents one of these groupings. This

section occurs primarily in rainforests from two of the most threatened biomes in the world, with ~80 species in Amazonia and related forests of Central and northern South America and ~40 species in the Atlantic coastal rainforests of Brazil (Lucas *et al.*, unpubl. res.). According to Lucas *et al.* (2011), representatives of this clade are characterized by a closed calyx and a combination of flower, inflorescence and venation characters (Supplementary Data Fig. S1), such as: (1) inflorescence axes emerging from a single terminal whorl and primary axes irregularly or asymmetrically branched; (2) calyx lobes free to partially fused, irregularly tearing vertically through the hypanthium; (3) hypanthium extended into a tube flared beyond the ovary, but inconspicuous after deep tearing; and (4) a (usually) bilocular ovary, with two ovules per locule.

As currently circumscribed, *Myrcia s.l.* encompasses four genera that were recognized during most of the 20th century: *Calypttranthes*, *Gomidesia*, *Myrcia* and *Marlierea*. However, earlier studies (Lucas *et al.*, 2011) have shown that the first

two genera are monophyletic and nested in the paraphyletic *Myrcia* and *Marlierea*, indicating that the current circumscription of these genera needs to be re-evaluated. McVaugh (1956) suspected the artificiality of *Marlierea* but maintained the genus in his studies on Tropical American Myrtaceae based on the presence of persistent bracts and the abortion of the primary axis of the inflorescence.

The importance of *Myrcia* s.l. as a structural and ecological component of the Atlantic rainforest of Brazil makes this group a good proxy for the diversity of other important angiosperm groups in the Atlantic rainforest (Murray-Smith *et al.*, 2009), one of the biodiversity hotspots (Myers *et al.*, 2000). Within this hotspot there are areas of higher angiosperm diversity and areas of elevated *Myrcia* diversity (Murray-Smith *et al.*, 2009) that may correspond to refugia (Carnaval and Moritz, 2008). Refugia (Haffer, 1969) are thought to act as ‘museums’ for the maintenance of biodiversity due to their expected lower extinction rates and/or higher speciation rates, when compared with unstable areas, resulting in a positive net diversification balance. Refugia can act as ‘safe havens’ (Keppel *et al.*, 2012) where biota would be protected for a long time, contributing important pieces of information for a better understanding of the evolutionary processes that may have driven modern species diversity.

This study aimed to test the monophyly of *Myrcia* section *Aulomyrcia* and further test its phylogenetic placement in the genus and to examine phylogenetic relationships within this section. This phylogenetic framework was then used as a basis to make inferences on the evolutionary history of *Myrcia* section *Aulomyrcia*, especially concerning speciation and extinction dynamics along the Atlantic forest.

MATERIAL AND METHODS

Taxonomic and molecular sampling

We sampled 93 taxa of tribe Myrteae, including 83 *Myrcia* s.l., of which 55 were from *Myrcia* section *Aulomyrcia*, encompassing as much as possible of the morphological and geographical variation observed in the group (Appendix; Supplementary Data Fig. S1). Approximately 40 % of species in section *Aulomyrcia* (Lucas *et al.*, unpubl. res.) were represented. Representatives of all four traditionally recognized genera of *Myrcia* s.l. (*Calyptanthes*, *Gomidesia*, *Myrcia* and *Marlierea*), representative of all nine clades and subclades of section *Aulomyrcia* of Lucas *et al.* (2011), were included. Ten additional genera from Myrteae were also studied. *Myrtus communis* was used as the outgroup taxon in all analyses, following Lucas *et al.* (2011). To encompass morphological and geographical variation of widely distributed species, multiple individuals were included for those taxa. For example, we included phenotypes from the morphological range of *Myrcia racemosa*; specimens from Bahia have significantly thicker leaves and inflorescences than those from São Paulo. Species names and nomenclature used in this work follow Sobral *et al.* (2014). The internal transcribed spacer (ITS) of the ribosomal nuclear region and four plastid markers (*psbA-trnH*, *trnL-trnF*, *trnQ-rps16* and *ndhF*) were used (Supplementary Data Table S1), leading to a total of 461 sequence accessions (Appendix), 64 % of which were generated for this study and 36 % were

obtained from other studies (Lucas *et al.*, 2007, 2011; MF Santos, USP, São Paulo, Brazil, unpubl. res.; C. Wilson, RBG Kew, UK, unpubl. res.).

DNA sequencing

Total DNA was extracted mainly from 0.3 g of silica-gel-dried leaf material (in some cases from 0.2 g when using herbarium specimens) using a modified version 2× cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). Total DNA was further purified for long-term storage in the RBG Kew DNA and Tissue Collections by equilibrium centrifugation in caesium chloride–ethidium bromide gradients (1.55 g ml⁻¹) followed by butanol extraction of ethidium bromide and dialysis to remove caesium chloride. Amplification and purification of target DNA regions were executed according to the protocols outlined in Lucas *et al.* (2007, 2011) using internal primers for some regions when necessary (Appendix). PCR conditions are listed in Supplementary Data Table S2. Sequencing was performed according to Lucas *et al.* (2007). DNA sequences were assembled using MUSCLE (Edgar, 2004) and edited in GENEIOUS 7.0.2 with subsequent manual adjustments when necessary. Any doubtful base calls and all phylogenetically informative base changes were compared with the general consensus and individually checked. A paralogous copy of ITS was identified in *Myrcia riodocensis* and this sequence was excluded. All sequences have been deposited in GenBank and the DNA samples are deposited in the RBG Kew DNA and Tissue Collections (Appendix). Due to the inheritance of the plastid genome as a single linked unit, the resulting four plastid DNA matrices were combined into a single dataset of 3477 bp. The molecular datasets, 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 datasets. For the combined dataset, one partition was applied for the nuclear region and another for all four plastid regions combined. The best nucleotide substitution model estimated for each partition according to the Akaike information criterion (AIC) using JModeltest 2 (Darriba *et al.*, 2012) was GTR + gamma + invariable sites.

Maximum likelihood analyses were performed with RAxML v7.6.3 (Stamatakis, 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 5 000 000 generations of Monte Carlo Markov chains (MCMCs) and a sampling frequency of 1000. Results were examined with Tracer version 1.5 (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).

To estimate the temporal evolution of *Myrcia s.l.* we used the Bayesian inference approach implemented in the package BEAST version 1.8.0 (Drummond et al., 2012) using the combined data set and applying the same partitions and models as previously described for the MrBayes analyses (see above). An uncorrelated relaxed molecular clock assuming a lognormal distribution of rates and a Yule speciation model were applied. Four runs of 30 000 000 generations were performed, sampling one tree every 1000th generation. Parameter convergence was confirmed by examining their posterior distributions in TRACER version 1.5 (Drummond and Rambaut, 2007). The MCMC sampling was considered sufficient when the effective sampling size of each parameter was >200, as assessed using TRACER version 1.5. All analyses were performed on the CIPRES portal (Miller et al., 2010). A maximum clade credibility tree with median branch lengths and a 95 % highest posterior density interval on nodes was built using TreeAnnotator version 1.8 (Drummond et al., 2012) based on the remaining set of trees after burn-in (for each run a burn-in period of 3 000 000 generations was applied). Calibration was performed using the fossil *Paleomyrinaea princetonensis* from the Palaeocene (Crane et al., 1990; Pigg et al., 1993) to the early Eocene (Manchester, 1999) of North America, comprising well-preserved fruits and seeds, which is probably related to *Psidium* and/or *Mosiera* (Pigg et al., 1993). The most recent common ancestor of Myrteae and the outgroup taxa (crown node of Myrteae) was constrained 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.

Diversification analyses

We used a likelihood approach, geographic state speciation and extinction (GeoSSE) (Goldberg et al., 2011), as implemented in the R package ‘diversitree’ (FitzJohn, 2012), to estimate region-dependent rates of speciation, extinction and dispersion from the calibrated phylogenetic trees generated with BEAST. We removed outgroup taxa and pruned the tree to only include species occurring in the Atlantic forest, each represented by one accession to avoid potential bias from overrepresentation of intraspecific variation. The resulting tree contained 31 terminals (~60 % of *Myrcia* section *Aulomyrcia* species known for this biome, plus nine undetermined species that will be taxonomically treated in other studies). The majority of the 40 % of species of *Aulomyrcia* not sampled here are species known only from the type, some of which may eventually be synonymized with currently described species (Lucas et al., unpubl. res.). However, we evaluated the possible bias of incomplete molecular sampling in GeoSSE, and taking this into account using the ‘sampling.f’ function of ‘diversitree’ package did not qualitatively change the results, although there was an increase in support of the estimated rates, thus reinforcing the interpretations proposed here based on the assumption of complete sampling. The results from the incomplete molecular sample are available from the first author upon request.

Occurrence records were obtained for each species from herbarium collection records from the CRIA database

(*speciesLink*, 2014) and filtered to remove clearly erroneous localities and identifications as recommended by Giovanni et al. (2012). Each species was scored (see below) in accordance with forest refugium hypothesis proposed for the Atlantic forest by Carnaval and Moritz (2008). These authors identified refugia as historically stable areas where forest presence was inferred by hindcasting the distribution of the biome using ecological niche modelling including two Atlantic forest definitions, one narrower and other broader. Stable areas were defined if a given place belonged to the biome in past projections using two methods (BIOCLIM and MAXENT) and two time frames (6 and 21 ka). Using this approach, Carnaval and Moritz (2008) inferred two refugia areas in the Atlantic forest: a large area in the central corridor (Bahia) along the Brazilian coast, extending from the Rio Doce northward to the Rio São Francisco (Fig. 1), and a geographically restricted and smaller area in the state of Pernambuco. The Bahian refugium hypothesis was more robust, being inferred from all analyses, whereas the Pernambuco region was returned in incongruent positions related to the definition (broader or narrower) of Atlantic forest that was applied. In addition, the Bahian refugium encompasses a larger area, more suitable for macroecological analyses; for these reasons we used the Bahian refugium only in our GeoSSE analyses.

In the GeoSSE model each species is coded for three states (Fig. 1): endemic to a refugium (‘A’), endemic to unstable areas (‘B’) or widespread, occurring in both regions (‘AB’). In this two-region model, range expansions are considered transitions from A or B to AB, occurring with per-lineage dispersal rates dA and dB , respectively. The reverse process, range contraction from AB to A or B, occurs with per-lineage extinction rates xB and xA , respectively. Extinction from a region is independent of presence in the other regions, so global extinction of species from states A and B also occurs at per-lineage rates xA and xB , respectively. Lineage extinction in the GeoSSE model thus depends on range size and location, because more events are required for extinction of an AB species. The method does not allow instantaneous transitions between A and B or immediate global extinction of AB because each of these requires two events. All model rates are constant over time and across lineages. Speciation occurs within each region (sA and sB) and when a widespread species produces two new species, one in each different region (sAB). We consider sAB not to be negligible in our scenario because study species with small ranges occur in the transition of refugium and unstable areas; these species might therefore give rise two new species, one in A and other in B as a result of a single cladogenetic event.

We constructed two likelihood functions representing different evolutionary models for the relationship between occurrence and diversification rate using the ‘subplex’ algorithm to search the likelihood space (R package ‘diversitree’; FitzJohn, 2012). We estimated the full model (all parameters were allowed to vary) and a model with free extinction ($sA \sim sB \sim sAB$; $dA \sim dB$). The parameters were estimated by ML and we used the AIC ML to compare adjustment between the models. The best model is the one with the lowest AIC score but models scoring no more than 2 units higher still have ‘substantial’ support (Burnham and Anderson, 2002). All tests were repeated for each of the 1000 phylogenetic trees sampled randomly from the Bayesian posterior distribution produced by BEAST to represent variation among dated trees (uncertainty of phylogenetic

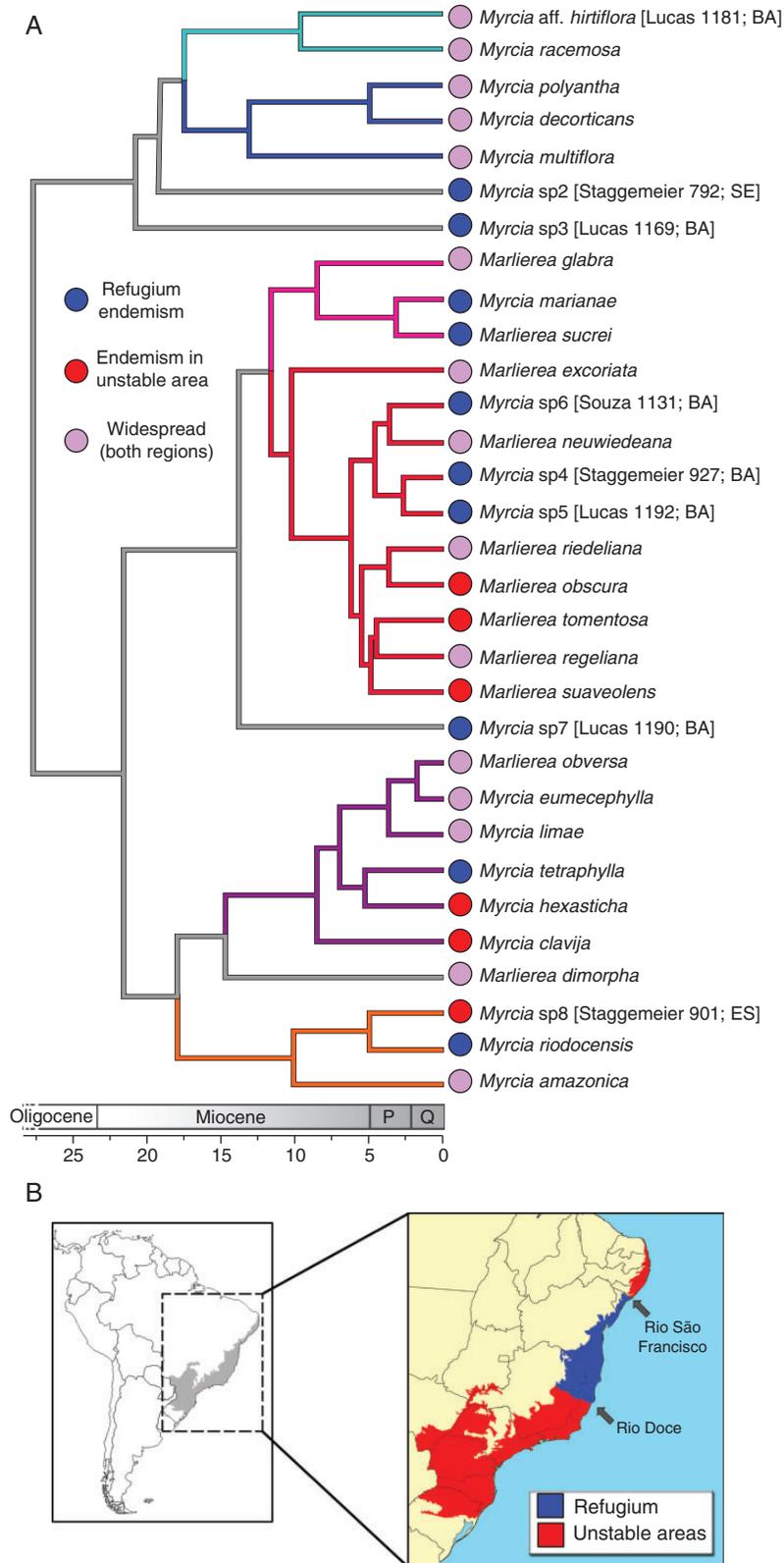


FIG. 1. Chronogram showing phylogenetic relationships, divergence times and geographical distribution of the 31 species of section *Aulomyrcia* (A) found in the Atlantic Forest (B; grey area). The Bahia refugium (blue; see text) and unstable areas (red), as proposed by [Camaval and Moritz \(2008\)](#). Branches for subclades are coloured to identify the informal clades described here (Subclades are detailed in Fig. 2 and 3)

Table 1. Sequence statistics summary for ITS and the combined plastid region (CP) used to infer phylogenetic relationships in *Myrcia* section *Aulomyrcia*

	CP (%)	ITS (%)
Number of taxa	93	90
Aligned length (bp)	3477	748
Variable characters	567	229
Potentially parsimony-informative characters (%)	226 (6.5)	137 (18.3)
Mean GC content (%)	25.0	50.1

hypothesis and calibration) that could yield wide confidence intervals for clade ages.

In accordance with the refugium hypothesis (Haffer, 1969) we would expect to find the highest diversification balance within refugia due to lower extinction rates in these areas ($x_A < x_B$). In contrast we would expect lower net diversification and higher extinction rates in historically unstable regions.

RESULTS

Phylogenetic relationships

The ITS partition presented greater variability (18.3 % potentially informative characters) than the four combined plastid regions (6.5 ± 1.6 %; Table 1). Analyses conducted for each partition (nuclear and plastid) returned topologies inspected by eye and found to be congruent for major clades. The combined dataset provided greater resolution and higher levels of support; our results and discussion therefore focus on the combined results. The phylogenetic reconstructions obtained with ML and Bayesian results were highly congruent. The ML tree is available as Supplementary Data Fig. S2 and the Bayesian results are used here for further discussion.

Myrcia section *Aulomyrcia* emerges as a monophyletic group, but with low support (PP_{BEAST} , 0.88; PP_{BAYES} , 0.50). Inside *Myrcia* section *Aulomyrcia*, seven subclades were identified (Figs 2 and 3) that correspond entirely or with minor exceptions to consistent combinations of morphological characters (Fig. 4). Usually these characters, taken individually, are not informative to place a species in a particular subclade, but when they are taken altogether, the seven subclades become diagnosable morphologically (Fig. 4). The other sections identified in Lucas *et al.* (2011) were well supported here (PP_{BEAST} , 1.0; PP_{BAYES} , 0.91) except for clade 8, as only one species was included and its position was unstable between analyses. The relationships among clades were not always well supported.

The first group in section *Aulomyrcia* includes subclades A, B and C plus seven species in the BEAST analysis (or five species in Bayesian analysis) (PP_{BEAST} , 0.97; PP_{BAYES} , 0.43; Group 1, Fig. 3). The two additional species, *Marlierea* aff. *montana* (Hoffman 945) and *Myrcia minutiflora*, emerge with a second group composed of subclades D, E, F and G (PP_{BEAST} , 0.99; PP_{BAYES} , 0.5; Group 2, Fig. 3) after Bayesian analysis.

Species from subclade A are from the Amazon region and are divided into two further species groups: *Marlierea umbraticola*, *Myrcia* sp1 (Prévost 4749) and *Myrcia saxatilis* emerge as sister to *Marlierea caudata* plus *Marlierea* aff. *montana* (Holst 9384). Subclade B is composed of *Myrcia racemosa* plus

Myrcia aff. *hirtiflora*. Subclade C includes *Myrcia multiflora*, the type species of *Myrcia* section *Aulomyrcia* [*Myrcia multiflora*] plus *Myrcia decorticans* and *Myrcia polyantha*.

The second group is composed mainly of species from the Atlantic rainforest. Subclades D and E comprise species currently described as *Marlierea* due to their wholly or partially fused calices, morphological traits of this traditional genus (*sensu* Cambessèdes, 1829). *Marlierea sucrei* emerges in subclade D with *Marlierea glabra* and *Myrcia marianae*, a recently described (Staggemeier and Lucas, 2014) species from Una, Bahia. Subclade G includes multiple accessions of *Myrcia amazonica* from northern and southern Brazil, a further Amazonian species, *Myrcia* aff. *subobliqua* and two species from Espírito Santo (*Myrcia riocensis* and Staggemeier 901). In summary, subclades B, C and G are composed exclusively of *Myrcia sensu* DC., whereas D and E are exclusively comprised of *Marlierea sensu* Cambessèdes; clades A and F contain species currently described as both taxa.

Dating

Trees obtained from the BEAST analyses agree well with those from the Bayesian and ML phylogenetic hypotheses. The mean age for *Myrcia s.l.* is 31.4 million years ago (Mya) with 95 % confidence limits (95 % CI) of 22.3–41.0 Mya (Late Miocene to Early Eocene; Fig. 3B). The average age of *Myrcia* section *Aulomyrcia* (Lucas *et al.*, unpubl. res.) is 27.9 Mya (95 % CI 19.3–36.3 Mya; Late Miocene to Mid Oligocene; Fig. 3B). Section *Aulomyrcia* species endemic to the Amazonian and Guiana Shields (subclade A) are older (21.4 Mya, 95 % CI 14.0–29.0 Mya) than Atlantic species (Fig. 3B). Subclade E (excluding *Marlierea excoriata*) exhibits short branch lengths and younger age estimates; there is no congruence inside this subclade between the Bayesian and BEAST analyses, probably due to low phylogenetic signal within the subclade due to recent speciation 6.2 Mya (95 % CI 3.7–9.3 Mya; Mid Pleistocene to Early Miocene).

Diversification

Inside refugium, lineage evolution rates are lower than in unstable areas, a pattern supported in >70 % of trees examined (Fig. 5A–C). Speciation in refugium is three times lower than in unstable areas (median s_A , 0.03; Fig. 5A), whereas extinction is 2.2 times lower (x_A , 0.0049×10^{-3} ; Fig. 5B). Results suggest that dispersal from refugium to unstable areas is rare ($>1 \times 10^{-4}$; Fig. 5C), supported by 68.3 % of trees sampled.

Moreover, within refugium net diversification is lower than elsewhere (median A , 0.04; median B , 0.08; Fig. 5D), supported in 74.2 % of trees (Fig. 5E). However, when all rates (diversification plus dispersal) are taken into account, the diversity balance is positive and higher inside refugium; ~80 % of trees support the highest diversity in refugium (Fig. 5F). Dispersal events from unstable areas to refugium are common (median d_B , 0.18; Fig. 5C).

Considering our initial hypothesis under the simplest model, holding dispersal and speciation constant and allowing only extinction to vary, 92.9 % of the trees had lower AIC than for the full model. In addition, the extinction-free model was

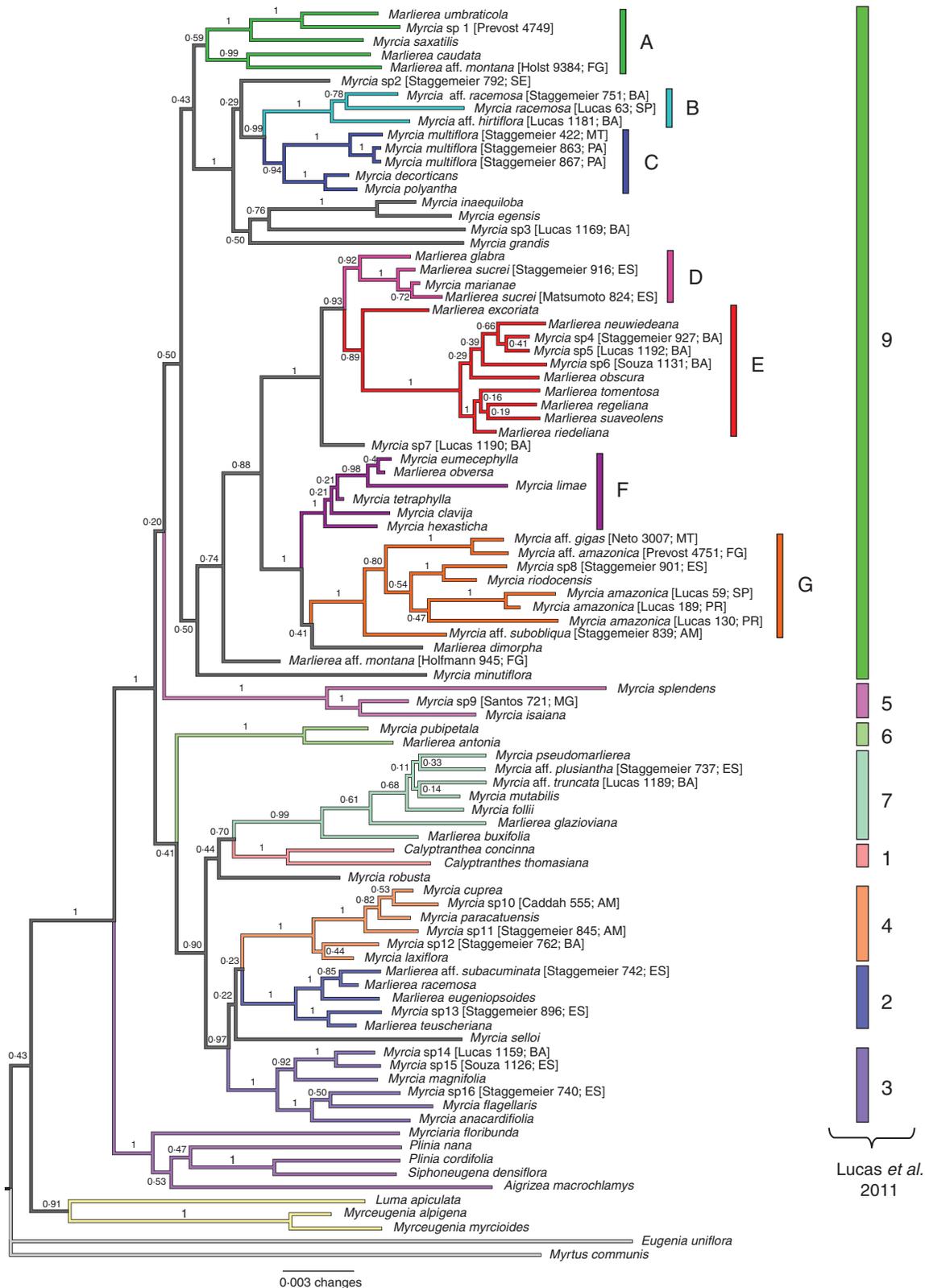


Fig. 2. Phylogenetic hypothesis for *Myrcia* section *Aulomyrcia*, maximum credibility tree and branch lengths generated from the Bayesian inference based on a combined dataset of nuclear and plastid DNA markers (ITS, *psbA-trnH*, *trnL-trnF*, *trnQ-rps16*, *ndhF*). Clades from Lucas et al. (2011) are indicated by the numbers 1–9 on the right of the tree. Subclades in section *Aulomyrcia* are coloured to identify informal clades as defined here. Values on branches are posterior probabilities.

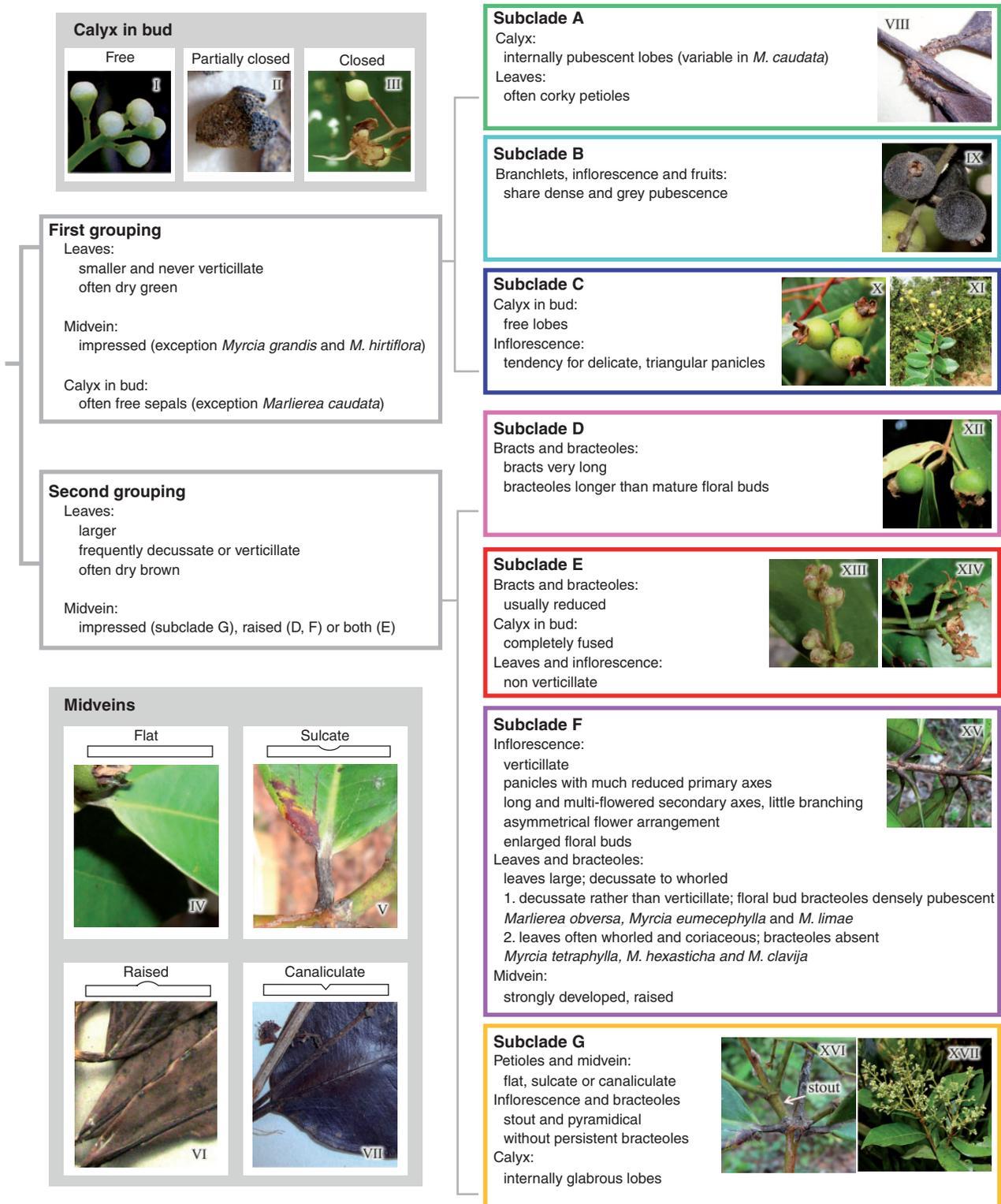


Fig. 4. Combinations of morphological traits supporting the results of the phylogenetic analyses based on molecular data in *Myrcia* section *Aulomyrcia*. Branch colours identify informal clades within section *Aulomyrcia* as defined here. Shape of calyx in the bud: *Myrcia multiflora* (I), *Marlierea obversa* (II), *Marlierea glabra* (III); midveins: *Myrcia marianae* (IV), *Myrcia* aff. *subobliqua* (V), *Marlierea obversa* (VI), *Myrcia inaequiloba* (VII); corky petioles in *Marlierea umbraticola* (VIII); grey dense pubescence in the mature fruits of *Myrcia racemosa* (IX); free calyx lobes in immature fruits of *Myrcia polyantha* (X); triangular panicles in *Myrcia decorticans* (XI); long bracts in *Myrcia marianae* (XII); buds of *Marlierea newwiedana* (XIII); old flower illustrating irregularly splitting calyx lobes in *Marlierea excoriata* (XIV); verticillate arrangement in the leaves in *Myrcia tetraphylla* (XV); stout inflorescence in *Myrcia* aff. *subobliqua* (XVI); pyramidal inflorescence in *Myrcia amazonica* (XVII).

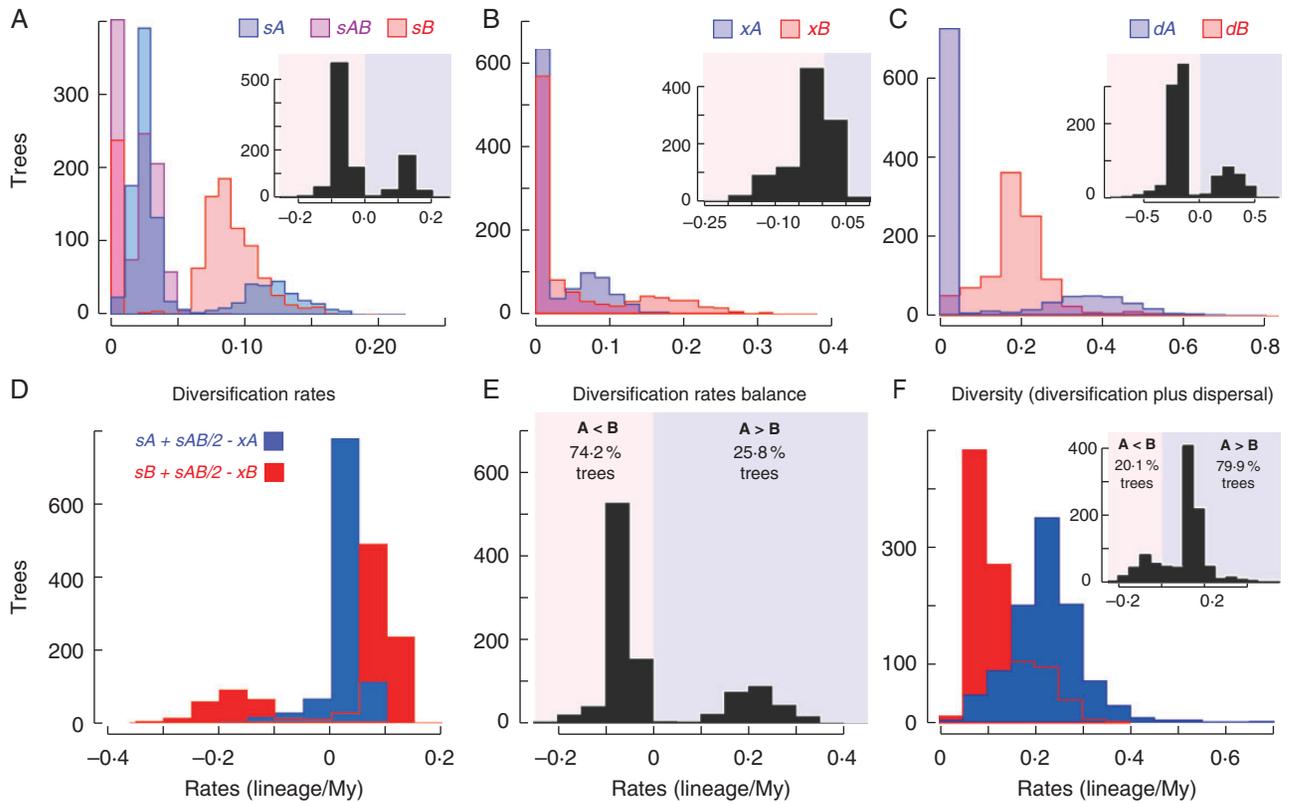


Fig. 5. Frequency histograms of estimated speciation (A), extinction (B) and dispersal (C) rates (events per lineage per million years) in the full model for *Myrcia* section *Aulomyrcia* in the Bahian refugium (blue bars; A) versus the unstable areas (red bars; B). Rates of speciation, extinction and dispersal were combined to calculate diversification rates (speciation minus extinction rates; D) and their balance (E); and net increased diversity (diversification plus dispersal rates; F). Inset histograms show the differences in rates between areas (rates A minus B).

substantially better ($|\Delta\text{AIC}| > 2$) in 55 % of trees (Fig. 6A). Extinction rates inside refugium tend to zero, whereas in other regions they are higher (x_B , 0.04 ± 0.01 ; Fig. 6B), corroborating our initial hypothesis that the highest extinction rates are found in climatically unstable areas; this pattern was strongly supported (100 % of trees; Fig. 6B).

DISCUSSION

In this study we reconstruct phylogenetic relationships within *Myrcia* section *Aulomyrcia* (Lucas *et al.*, unpubl. res.) based on 53 taxa from this section plus 40 outgroups and using one nuclear and four plastid DNA regions. Our results provide the first comprehensive understanding of phylogenetic relationships in *Myrcia* section *Aulomyrcia*, further supporting the need to unite taxonomically the two traditional genera *Myrcia* and *Marlierea* in an expanded *Myrcia s.l.* The morphological distinction of *Myrcia* and *Marlierea* described by McVaugh (1956) is that the former has a calyx with five (rarely four) distinct lobes, whereas *Marlierea* Cambessèdes has a closed or barely open bud in which the calyx splits irregularly at anthesis. This distinction was suspected to be false as some species in each genus have partially fused calyx lobes. Results presented here demonstrate molecular affinity between species until now believed to be in separate genera, confirming the suspicions (e.g. McVaugh

1956, 1968) that these traits cannot be relied on to support such a division.

Our results also supported ideas put forward by McVaugh (1956) regarding the distribution of species of Myrtaceae in South America and their relationship in tropical forests. McVaugh (1956) emphasized the importance of studying species occurring in the Amazon and Atlantic forests to understand the distribution of the family better because species composition from the north-eastern states of Brazil are often distinct from those of southern Brazil and usually distinct from those of the Amazon, but may show affinities to both. In our phylogenetic study, two main arrangements were identified: Amazonian plus north-eastern Atlantic species in subclades A–C (including the widespread species *Myrcia multiflora* and the widely distributed Atlantic forest species *Myrcia racemosa*); and north-eastern plus south-eastern species in subclades D–G except for two Amazonian species emerging in subclade G, one of which is the widely distributed *Myrcia amazonica*.

Myrtaceae date back to the Cretaceous (86 Mya; Sytsma *et al.*, 2004) and our results suggest that *Myrcia s.l.* probably first evolved at the transition between the Eocene and Oligocene (Fig. 3). Unfortunately, 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

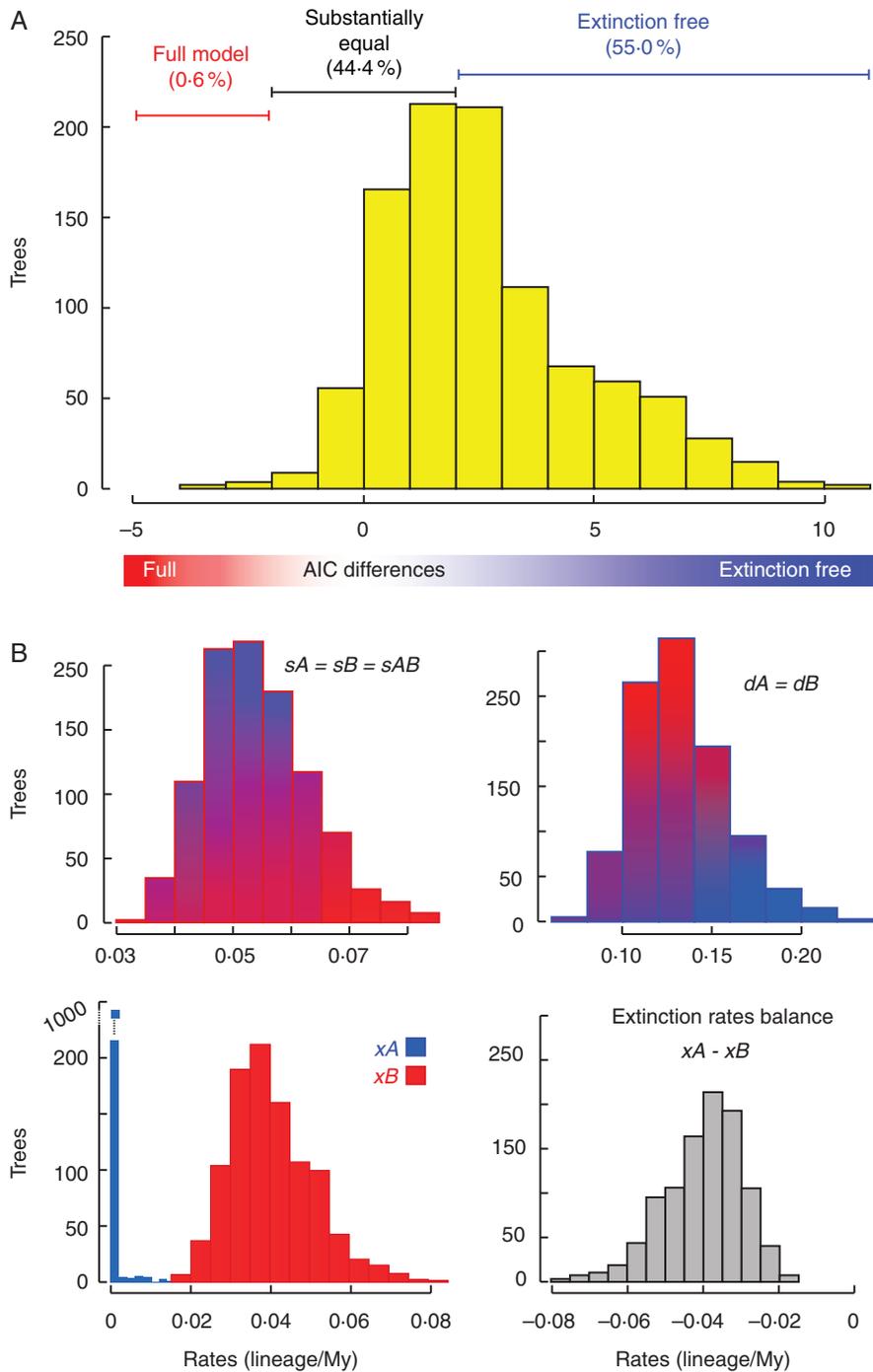


FIG. 6. (A) GeoSSE results for AIC differences between full and free extinction models. (B) Speciation, dispersal and extinction rates in free extinction model.

calibration procedure and as a result the scenario outlined here to explain the origin and biogeographical history of *Myrcia* section *Aulomyrcia* should be treated with caution. The lack of an extensive fossil record and the different calibration node might explain in part the discrepancies between these results and those obtained by Biffin *et al.* (2010) (a mid-Miocene origin for *Myrcia*), rather than differences in sampling density for Myrteae. However, dates for subclade A were older (Fig. 3B)

than those for other clades (around 8 million of years of divergence), suggesting an Amazonian origin for *Myrcia* section *Aulomyrcia*. The highest diversity in this region (twice that in the Atlantic forest) would confirm this origin if speciation rates are assumed to be equivalent throughout the phylogenetic tree.

The emergence of Amazonian and southern species in subclade G supports suggestions of a link between the Amazonian and Atlantic forests. The first-diverging species in subclade G

is *Myrcia* aff. *subobliqua*, an Amazonian species sister to seven species which can be assigned to two groups. *Myrcia gigas* and *Myrcia amazonica* aff. *amazonica* (Prévost 4751 from French Guyana), also from the Amazon, compose the first group, which emerges as a sister to a group including species from Espírito Santo plus three accessions of *Myrcia amazonica* from southern Brazil (São Paulo and Paraná). These relationships indicate that the link between these two tropical forest biomes probably took place via the cerrado biome, supported by the presence of the widely distributed species *Myrcia amazonica* in this clade, which occurs in cerrado but not in caatinga.

It is remarkable that *Myrcia inaequiloba*, *Myrcia egensis*, *Myrcia grandis* (all Amazonian species) and another probably new species from Bahia (Lucas 1169) emerge in a weakly supported subclade that is sister to a further arrangement formed of subclades B and C because these species were presumed, based on shared morphology, to be more closely related to species found in subclade A, comprising only Amazonian species. In subclades B and C, the widespread *Myrcia multiflora* and the widely distributed Atlantic forest species *Myrcia racemosa* are sister to four north-eastern species (*Myrcia* aff. *hirtiflora*, Staggemeier 792, *M. decorticans* and *M. polyantha*) supporting McVaugh's suggestion that Amazonian species are more closely related to north-eastern rather than south-eastern species of the Atlantic forest. More recently, Batalha-Filho et al. (2013) discussed connections between the Amazonian and Atlantic forests in birds and suggested two distinct connections between these two forests, through the southern portions of cerrado (Mato Grosso and Mato Grosso do Sul) and in the north linking to the north-eastern forests through the caatinga.

There are few *Marlierea* species in the first group of clades and they have only slightly fused calyx lobes. The majority of species with closed calices occur in the second group of clades, apparently a younger group. The occurrence of completely closed calices throughout clades D and E, with incompletely fused lobes a feature of clade F (and therefore species described as a mix of *Myrcia* or *Marlierea*) and free calyx lobes in clade G, suggests that the closure of the calyx was a more recent, secondary event and that the ancestor of *Myrcia* section *Aulomyrcia* had an open calyx. The path of calyx closure in the second group of clades is unclear; a hypothesis for future testing is that the ancestor of the second group had free calyx lobes, then full or partial calyx closure occurred independently in clades D + E and in clade F, respectively. Alternatively, the common ancestor of the second group may have had a completely or incompletely fused calyx and the free calyx lobes of clade G results from a subsequent loss of the closed bud.

In recent years, increased investigation of *Myrcia* s.l. molecular data and morphology has meant that consistently diagnosable species groups are becoming the norm rather than the exception (Lucas et al., 2007, 2011). However, the morphological and molecular data are not always congruent, as shown in some cases, such as in subclade D, where *Myrcia marianae*, a new species represented by Staggemeier 764 (Staggemeier and Lucas, 2014) emerges between two accessions of *Marlierea sucrei* (Figs 2 and 3). This new species does not share many morphological traits with *Marlierea sucrei*, but on a molecular basis they are indistinguishable (Staggemeier and Lucas, 2014). These results demonstrate that the five molecular markers

employed here are not sufficiently divergent to separate all morphologically distinct species within this group.

Lucas et al. (2011) found that *Marlierea sucrei* emerges with six species originally described in *Marlierea* subsection *Clausae* sensu Legrand (1962). However, we found *Marlierea sucrei* to be related to the new species *Myrcia marianae* (Staggemeier 764) and *Marlierea glabra*, whereas the other six species from Lucas et al. (2011) are grouped with four more from subclade E. Clades D and E are defined by completely closed buds (Supplementary Data Fig. S1 and Fig. 4) and, except for *Marlierea neuwiediana* and *Marlierea riedeliana*, all species were previously assigned to *Marlierea* subsection *Clausae*. Clade E exhibits short branch lengths, very low internal support (Figs 2 and 3) and appears to be the youngest clade in section *Aulomyrcia* (Fig. 3B).

Marlierea dimorpha emerges with low support as sister to subclade G in the MrBayes analysis (Fig. 2), but as sister to subclade F in the BEAST analysis (Fig. 3). This species resembles those of subclade F (presence of bracts, terminal inflorescence, big flower buds and leaves with a raised midvein).

Myrcia micropetala, included in Lucas et al. (2011), was not included in our analysis as we were unable to amplify every DNA marker; however, in an exploratory analysis with ITS, *trnL-trnF* and *psbA-trnH* (not presented here) we found a well-supported arrangement between *Myrcia micropetala* and *Myrcia* aff. *hirtiflora* emerging as sister to *Myrcia racemosa*. Morphological comparison of material from all taxa included in subclade B demonstrates a striking morphological gradient from thinner forms of leaves and inflorescences in the south of the Atlantic forest to thicker in the north. The type specimen of *Myrcia racemosa* is from Rio de Janeiro; from there to southern Brazil this species has thin, sometimes membranaceous leaves that become thicker in the more northern part of its distribution (i.e. Bahia). In the geographical region where the distributions of *Myrcia racemosa* and *Myrcia hirtiflora* overlap (Bahia) the distinction based on morphology can sometimes be laborious. A similar situation is observed with *Myrcia hirtiflora* and *Myrcia micropetala*, where the latter species is encountered with small leaves and inflorescences. Molecular similarity among species in the well supported subclade B is reflected by strong morphological similarities that have produced a wealth of misidentified collections for these three species in many Brazilian and international herbaria. However, although a continuum of certain characters exists between these three species, they are clearly distinguishable entities based on a combination of other traits such as venation and hairs.

Misidentified collections are also common for *Myrcia decorticans*. Some taxonomists identified many specimens that are morphologically matched with Prévost 4749 as *Myrcia decorticans*, in line with McVaugh (1969). However, the type for this species is described from Bahia (*Martius* s.n.) and matches Staggemeier 799 from north-eastern Brazil. The specimen Prévost 4749 could instead be assigned to *Marlierea gleasonii*; a thorough review of this complex is required.

Lucas et al. (2011) note that clade 8 (*Myrcia tomentosa*, *Myrcia selloi*, *Myrcia laruoteana* in their sample) shares morphological features such as asymmetrical panicles, a glabrous staminal disc and the hypanthium somewhat extended beyond the ovary, with species from clade 9. These species were at some time treated by authors as *Aulomyrcia*. As noted above,

the analysis presented here includes only a single sample of *Myrcia selloi* but its position is unstable and it repeatedly emerges elsewhere than clade 9; apparently these shared characteristics have evolved more than once in *Myrcia s.l.* The species of clade 8 consistently exhibit morphological characters quite different from species in clade 9 (e.g. completely free calyx lobes acutely reflexed in the fruit, buds constricted beneath the ovary, and flowering and vegetative branchlets emerging from a single point). It is therefore clear that these groups of species have independent origins.

Elsewhere in *Myrcia s.l.* the feature of the closed calyx occurs in clades other than *Myrcia* section *Aulomyrcia*. A partially closed calyx is also found in the clade containing species of or matching the diagnosis of *Eugeniopsis* (clade 2; Lucas *et al.* 2011), a genus now in the synonymy of *Marlierea*, and in a further clade of species previously described in both *Myrcia* and *Marlierea* (clade 7; Lucas *et al.*, 2011). *Calyptanthus* spp. also have a completely fused calyx (clade 1; Lucas *et al.*, 2011). These clades also demonstrate consistent morphologies otherwise quite separate from the species of *Myrcia* section *Aulomyrcia*. In terms of the clades of Lucas *et al.* (2011), clade 1 and clade 7 have markedly sympodial branching, strongly cymose inflorescences, long, internally glabrous turbinate buds with a dehiscent calyptra or short calyx lobes partially tearing horizontally from the rim of the hypanthium, respectively. Clade 2 has partially fused buds that tear longitudinally at anthesis, symmetrical panicles and characteristic lenticles covering the bark and often the underside of the leaves. The clear multiple origins of the closed bud throughout *Myrcia s.l.* must now be explained by further study of the anatomy of various closed calyx groups alongside the environmental implications of bud closure. Such results, with those presented here, will allow confirmation or otherwise of hypotheses of the timing and significance of calyx closure.

Diversification

The Carnaval–Moritz hypothesis (Carnaval and Moritz, 2008) appears realistic for a representation of biome dynamics when compared with other refugial scenarios proposed for the Atlantic forest (e.g. Thomé *et al.*, 2010; Tonini *et al.*, 2013). The Carnaval–Moritz hypothesis was the only one that modelled the occurrence of the entire forest; others were taxon-specific to fauna (especially anurans) and may be linked with biological requirements of these species rather than the dynamics of the biome as a whole.

Evidence suggests that diversification rates differ in species of *Myrcia* section *Aulomyrcia*, with the lowest rates occurring within refugium. However, the tendency of range expansion in unstable areas resulted in multiple species colonizing adjacent refugia. Consequently, range expansion contributes to higher plant diversity in the Bahian refugium. Results indicate that this area acts as a biodiversity museum and centre of species accumulation, maintaining the high diversity of *Myrcia* section *Aulomyrcia* in the central corridor of the Atlantic forest. This analysis indicates a positive balance of diversity based on diversification rates and dispersal, in climatically more predictable areas, i.e. refugia. Reasons for higher speciation rates in unstable areas remain to be explored in future studies.

Concluding remarks

This study provides a basic phylogenetic background with which to address ecological and evolutionary questions based on the hyper-diverse clade of *Myrcia s.l.*, an ecologically important Neotropical genus (~750 species). The inclusion of more species and regions of genome will be important in understanding relationships between the species of *Myrcia* section *Aulomyrcia* better. For example, the inclusion in future studies of further species predicted to emerge in clade F (e.g. *Marlierea verticillaris* and *Myrcia insularis*) will help clarify the placement of *Marlierea dimorpha*.

Despite advances presented here to improve understanding of relationships inside *Myrcia s.l.*, much work remains to be done. Amazonian species remain poorly investigated and the relationships between Amazonian and Atlantic forest species still need attention. Insights into these would improve understanding of the early evolution of *Myrcia* and species disjunctions between some of the most threatened biomes on Earth.

Our GeoSSE analysis was based on a single section of *Myrcia s.l.* with good representation from the Atlantic forest, but this dataset does not include the complete diversity in the genus. Despite uncertainty in some of the species relationships, the patterns recovered allow conclusions to be drawn about the diversification pattern of species in an area of angiosperm endemism located in a relatively poorly known and highly threatened area of the Atlantic rainforest of north-eastern Brazil. More specifically, significantly lower rates of extinction and accumulation of species inside refugia corroborate the importance of these processes in maintaining diversity in a region that is well known for its great biological richness. Studies evaluating similar questions in an evolutionary context in other taxonomic groups (e.g. birds, mammals, reptiles and other angiosperm families) are greatly needed so that the mechanisms associated with the origin, maintenance and evolution of biodiversity can be deciphered. Finally, based on the ecological importance of this group in Neotropical forests, we expect that the results presented here will provide a basis for further taxonomic work (e.g. Lucas *et al.*, unpubl. res.) and ecological studies investigating drivers of speciation of large genera in tropical forest communities.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Fig. S1: images of species of *Myrcia* section *Aulomyrcia*. Fig. S2: maximum likelihood tree. Table S1: primers used for PCR. Table S2: PCR conditions.

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APPENDIX

Vouchers for DNA samples used in the phylogenetic analysis, with GenBank numbers (two letters plus six numbers) or the RBG Kew's DNA and Tissue Collections numbers (five numbers)

Species	Collector	Voucher	ITS	<i>psbA-trnH</i>	<i>trnL-trnF</i>	<i>trnQ-rpS16^a</i>	<i>ndhF</i>
<i>Algrizea macrochlamys</i> (DC.) Proença & NicLugh.	Giulietti, A.M. 1648	K	AM234126	AM489809	JN091320	KP722283	16833
<i>Calyptanthes concinna</i> DC.	Lucas, E. 74	K, ESA	KP722378	AM489817	KP722334	KP722231	KP722454
<i>Calyptanthes thomasi</i> O.Berg	Pollard, B.J. 1195	K	AM234106	AM489820	JN091325	KP722211	KP722434
<i>Eugenia uniflora</i> L.	Lucas, E. 207	K	AM234088	AM489828	KP722326 ^a	KP722202	KP722418
<i>Luma apiculata</i> (DC.) Burret	Lucas, E. 208	K	AM234101	AM489843	KP722331	KP722209	KP722433
<i>Marlierea</i> aff. <i>montana</i> (Aubl.) Amshoff	Hoffmann, B. 945	US	43531	43531	43531	–	43531
<i>Marlierea</i> aff. <i>montana</i> (Aubl.) Amshoff	Holst, B. 9384	–	KP722377	KP722285 ^a	KP722333 ^a	KP722229	KP722453
<i>Marlierea</i> aff. <i>subacuminata</i> Kiaersk.	Staggemeier, V.G. 742	K, UB	KP722397	KP722305	KP722355	KP722252	KP722475
<i>Marlierea antonia</i> (O.Berg) D.Legrand	Santos, M.F. 840	SPF, K	43245	43245	43245	KP722277	43245
<i>Marlierea buxifolia</i> Amshoff	Clarke, H.D. 5707	US	–	43536	–	–	43536
<i>Marlierea caudata</i> McVaugh	Zappi, D. 1506	K	42097	42097	42097	KP722232	KP722455
<i>Marlierea dimorpha</i> O.Berg	Folli, D.; 6649	K	KP722416 ^a	KP722324	KP722374	KP722271	KP722494
<i>Marlierea eugenioisoides</i> (D.Legrand & Kausel) D.Legrand	Lucas, E. 61	K	AM234107	AM489845	JN091327	KP722205	KP722429
<i>Marlierea excoriata</i> Mart.	Matsumoto, K. 825	UEC	JN091203	JN091394	JN091328	KP722226	KP722449
<i>Marlierea glabra</i> Cambess.	Staggemeier, V.G. 935	UB, K, RB, IAN, UFG, HUFJSJ	KP722391	KP722299	KP722349	KP722245	KP722469
<i>Marlierea glazioviana</i> Kiaersk.	Matsumoto, K. 799	UEC	JN091204	JN091395	JN091329	KP722275	KP722451
<i>Marlierea neuwiedea</i> (O.Berg) Nied.	Staggemeier, V.G. 793	UB, K, UFG, RB	KP722402	KP722310	KP722360	KP698774	KP722480
<i>Marlierea obscura</i> O.Berg	Matsumoto, K. 836	UEC	JN091205	JN091396	JN091330	KP722228	KP722452
<i>Marlierea obversa</i> D.Legrand	Matsumoto, K. 820	UEC	JN091206	JN091397	JN091331	KP722227	KP722450
<i>Marlierea regaliana</i> O.Berg	Matsumoto, K. 814	UEC	JN091208	JN091399	JN091333	KP722225	KP722448
<i>Marlierea riedeliana</i> (O.Berg) D.Legrand	Lucas, E. 88	K	AM234109	AM489847	KP722330	KP722208	KP722432
<i>Marlierea suaveolens</i> Cambess.	Lucas, E. 85	K	AM234108	AM489846	KP722329	KP722207	KP722431
<i>Marlierea subacuminata</i> Kiaersk.	Lucas, E. 225	K	JN091207	JN091398	JN091332	KP722218	KP722443
<i>Marlierea sucrei</i> G.M.Barroso & Peixoto	Matsumoto, K. 824	UEC	JN091209	JN091400	JN091335	KP722222	KP722445
<i>Marlierea sucrei</i> G.M.Barroso & Peixoto	Staggemeier, V.G. 916	UB, K	KP722388	KP722295	KP722345	KP722242	KP722465
<i>Marlierea teuscheriana</i> (O. Berg.) D. Legrand	Lucas, E. 633	K	43712	43712	43712	KP722280	43712
<i>Marlierea tomentosa</i> Cambess.	Matsumoto, K. 798	UEC	JN091210	JN091401	JN091336	KP722224	KP722447
<i>Marlierea umbraticola</i> (Kunth) O.Berg	Souza, M.A.D. sn	INPA	KP722392	KP722300	KP722350	KP722246	KP722470
<i>Myrcogenia alpigena</i> (DC.) Landrum	Lucas, E. 167	K	AM234098	AM489854	KP722376	JN661090	KP722441
<i>Myrcogenia myrcioides</i> (Cambess.) O.Berg	Lucas, E. 82	K	AM234097	AM489853	16821	KP722281	16821
<i>Myrcia</i> aff. <i>amazonica</i> DC.	Prévost, M.F. 4751	K	JN091214 ^a	JN091405	JN091339	KP722215 ^b	KP722439
<i>Myrcia</i> aff. <i>amazonica</i> DC.	Neto, L.A.; 3007	INPA	KP722417	KP722325	KP722375	KP722272	KP722495
<i>Myrcia</i> aff. <i>hirtiflora</i> DC.	Lucas, E. 1181	K	KP722409	KP722317	KP722367	KP722264	KP722487
<i>Myrcia</i> aff. <i>plusiantha</i> Kiaersk.	Staggemeier, V.G. 737	K, UB, UFG, RB, IAN	KP722395	KP722303	KP722353 ^a	KP722250	KP722473
<i>Myrcia</i> aff. <i>subobliqua</i> (Benth.) Nied.	Staggemeier, V.G. 839	K, UB, UFG, RB, IAN, INPA	KP722396	KP722304	KP722354	KP722251	KP722474
<i>Myrcia</i> aff. <i>truncata</i> Sobral	Lucas, E. 1189	K	KP722412	KP722320	KP722370	KP722267	KP722490
<i>Myrcia amazonica</i> DC.	Lucas, E. 130	K	JN091215	JN091406	JN091340	–	–
<i>Myrcia amazonica</i> DC.	Lucas, E. 59	K	JN091213	JN091404	JN091338	KP722240	KP722422
<i>Myrcia amazonica</i> DC. [as <i>detergens</i>]	Lucas, E. 189	K	JN091212	JN091403	JN091337	KP722213	KP722437
<i>Myrcia anacardifolia</i> Gardner	Nadruz, M. 999	K	JN091212	JN091407	JN091341	KP722210	KP722419
<i>Myrcia clavija</i> Sobral	Lucas, E. 244	K	JN091220	JN091411	KP722332	KP722217	KP722442
<i>Myrcia cuprea</i> (O.Berg) Kiaersk.	Staggemeier, V.G. 862	K, UB	KP722394 ^a	KP722302	KP722352 ^a	KP722248	KP722472
<i>Myrcia decorticans</i> DC	Staggemeier, V.G. 799	UB, K	KP722383	KP722290	KP722339 ^a	KP722237	KP722460
<i>Myrcia egenis</i> (O.Berg) McVaugh	Araújo, M.H.T. 311	SPF, INPA	43272	43272	43272	–	43272
<i>Myrcia eumecephylla</i> (O.Berg) Nied.	Matsumoto, K. 803	UEC	JN091223	JN091414	JN091349	KP722223	KP722446
<i>Myrcia flagellaris</i> (D.Legrand) Mattos	Lucas, E. 83	K	AM234113	AM489836	JN091350	KP722206	KP722430
<i>Myrcia follii</i> G.M.Barroso & Peixoto	Staggemeier, V.G. 907	UB, K, CVRD, UFG, RB	KP722384	KP722291	KP722340 ^a	KP722238	KP722461
<i>Myrcia grandis</i> McVaugh	Staggemeier, V.G. 850	UB, K, UFG, RB, INPA	KP722385	KP722292 ^a	KP722341 ^a	KP698772	KP722462
<i>Myrcia hexasticha</i> Kiaersk.	Lucas, E. 194	K	JN091227	JN091418	JN091354	KP722214	KP722438

(continued)

Continued

Species	Collector	Voucher	ITS	<i>psbA-trnH</i>	<i>trnL-trnF</i>	<i>trnQ-rpS16</i> ^a	<i>ndhF</i>
<i>Myrcia inaequiloba</i> (DC.) Lemée	Lucas, E. 105	K	JN091228	JN091419	JN091355	KP722204	KP722428
<i>Myrcia isaiana</i> G.M.Barroso & Peixoto	Lucas, E. 60	K	JN091229	JN091420	JN091356	KP722249	KP722423
<i>Myrcia laxiflora</i> Cambess.	Meirelles, J. 307	RB	KP722403 ^a	KP722311	KP722361 ^{a,b}	KP722257	KP722481
<i>Myrcia limae</i> G.M.Barroso & Peixoto	Cordeiro, M.J. 310	RB	43210 ^a	KP722284	–	–	–
<i>Myrcia magnifolia</i> (O.Berg) Kiaersk.	Lucas, E. 1182	K	KP722411	KP722319	KP722369	KP722266	KP722489
<i>Myrcia marianae</i> Staggemeier & Lucas	Staggemeier, V.G. 764	UB, K, UFG, RB, SPF, IAN, HRCB	KP722381	KP722288	KP722337	KP722235	KP722458
<i>Myrcia minutiflora</i> Sagot	Sasaki, D. 2394	K	KP722399	KP722307	KP722357 ^a	KP722254	KP722477
<i>Myrcia multiflora</i> (Lam.) DC.	Staggemeier, V.G. 422	UB, IAN, HUFSJ	KP722379 ^a	KP722286	KP722335 ^a	KP722233	KP722456
<i>Myrcia multiflora</i> (Lam.) DC.	Staggemeier, V.G. 863	UB, K	KP722386 ^a	KP722293	KP722342 ^a	KP722239	KP722463
<i>Myrcia multiflora</i> (Lam.) DC.	Staggemeier, V.G. 867	UB	KP722387	KP722294	KP722343	KP698771	KP722464
<i>Myrcia mutabilis</i> (O.Berg) N.Silveira	Mazine, F. 1052	ESA	JN091233	JN091424	KP722344	KP722241	KP722435
<i>Myrcia paracatuensis</i> Kiaersk.	Mello-Silva, R. 1713	K	AM234118	AM489859	KP722328 ^a	KP722230	KP722421
<i>Myrcia polyantha</i> DC	Staggemeier, V.G. 797	UB, K	KP722400	KP722308 ^a	KP722358	KP722255	KP722478
<i>Myrcia pseudomarlierae</i> Sobral	Souza, M.C. 1139	RB	KP722404	KP722312	KP722362 ^a	KP722258	KP722482
<i>Myrcia pupipetala</i> Miq.	Lucas, E. 86	K	AM234114	AM489855	JN091364	KP722273	KP722426
<i>Myrcia racemosa</i> (O.Berg) Kiaersk.	Lucas, E. 63	K	AM234120	AM489861	JN091366	KP722259	KP722424
<i>Myrcia racemosa</i> (O.Berg) Kiaersk.	Staggemeier, V.G. 751	UB	KP722380	KP722287	KP722336 ^a	KP722234	KP722457
<i>Myrcia riodocensis</i> G.M.Barroso & Peixoto	Staggemeier, V.G. 917	UB, K, UFG, RB, CVRD	–	KP722296	KP722346	KP722243	KP722466
<i>Myrcia robusta</i> Sobral	Lucas, E. 727	K	36229	36229	36229	–	36229
<i>Myrcia saxatilis</i> (Amshoff) McVaugh	Lucas, E. 98	K	AM234119	AM489860	JN091370	KP722203	KP722427
<i>Myrcia selloi</i> (Spreng.) N.Silveira	Lucas, E. 110	K	JN091240	JN091431	JN091371	KP722212	KP722436
<i>Myrcia</i> sp1	Prévost, M.F. 4749	K	JN091221	JN091412	JN091347	KP722216 ^b	KP722440
<i>Myrcia</i> sp2	Staggemeier, V.G. 792	UB, K, UFG	KP722382	KP722289 ^a	KP722338 ^a	KP722236	KP722459
<i>Myrcia</i> sp3	Lucas, E. 1169	K	KP722410	KP722318	KP722368	KP722265	KP722488
<i>Myrcia</i> sp4	Staggemeier, V.G. 927	UB, K, UFG, IAN	KP722390 ^a	KP722298	KP722348	KP722244	KP722468
<i>Myrcia</i> sp5	Lucas, E. 1192	K	KP722414	KP722322	KP722372	KP722269	KP722492
<i>Myrcia</i> sp6	Souza, M.C. 1131	RB	KP722405	KP722313	KP722363 ^a	KP722260	KP722483
<i>Myrcia</i> sp7	Lucas, E. 1190	K	KP722413	KP722321	KP722371	KP722268	KP722491
<i>Myrcia</i> sp8	Staggemeier, V.G. 901	UB, K, UFG	KP722401	KP722309	KP722359 ^a	KP722256	KP722479
<i>Myrcia</i> sp9	Santos, M.F. 721	SPF, K	43267	43267	43267	KP722279	43267
<i>Myrcia</i> sp10	Caddah, M.K. 555	SPF, INPA	–	43260	43260	KP722278	–
<i>Myrcia</i> sp11	Staggemeier, V.G. 845	UB, K, UFG, IAN	KP722398	KP722306	KP722356	KP722253	KP722476
<i>Myrcia</i> sp12	Staggemeier, V.G. 762	K, UB, UFG	KP722393	KP722301	KP722351	KP722247	KP722471
<i>Myrcia</i> sp13	Staggemeier, V.G. 896	UB, K, UFG, IAN	KP722407	KP722315	KP722365 ^a	KP722262	KP722485
<i>Myrcia</i> sp14	Lucas, E. 1159	K	KP722415	KP722323	KP722373	KP722270	KP722493
<i>Myrcia</i> sp15	Souza, M.C. 1126	RB	KP722406 ^a	KP722314	KP722364 ^a	KP722261	KP722484
<i>Myrcia</i> sp16	Staggemeier, V.G. 740	UB	KP722408	KP722316	KP722366	KP722263	KP722486
<i>Myrcia splendens</i> (Sw.) DC.	Lucas, E. 73	K	AM234122	AM489863	JN091374	KP722274	KP722425
<i>Myrcia tetraphylla</i> Sobral	Staggemeier, V.G. 926	UB, K, UFG, HUFSJ, RB, R, CVRD	KP722389	KP722297	KP722347	KP698773	KP722467
<i>Myrciaria floribunda</i> (H.West ex Willd.) O.Berg	Mazine, F. 796	K	AM234094	AM489870	16827	KP722282	16827
<i>Myrtus communis</i> L.	Lucas, E. 211	K	AM234149	AM489872	KP722327	KP722221	KP722420
<i>Plinia cordifolia</i> (D.Legrand) Sobral	Mazine, F. 957	K	AM489411	AM489570	20679	KP722219	20679
<i>Plinia nana</i> Sobral	Mazine, F. 662	K	35640	35640	35640 ^a	KP722276	35640
<i>Siphoneugena densiflora</i> O.Berg	Mazine, F. 1050	K, ESA	AM489412	AM489571	JN091389	KP722220 ^b	KP722444

^aFor these accessions we used internal primers (see details Table S1). For *psbA-trnH* we were occasionally unable to amplify the *trnH* region; we therefore designed an internal primer to complement the forward *psbA* primer (Table S1) and overlapped these two strands. The *trnL* intron and *trnL-F* spacers were generally amplified in one reaction using primers c and f, but in some cases, the intron (primers c and d) and the intergenic spacer (primers e and f) were amplified separately. Two internal primers for region *trnQ-rpS16* were used.

^bFor these regions we obtained just partial sequences.

Abbreviations – Institution/State: CVRD - Companhia Vale do Rio Doce/ES; EAFM - Instituto Federal de Educação, Ciência e Tecnologia do Amazonas/AM; ESA - Universidade de São Paulo - ESALQ/SP; HRCB - Herbário Rio Clarence/SP; HUFSJ - Universidade Federal de São João del Rei/MG; IAC - Instituto Agronômico de Campinas/SP; IAN - Instituto Agronômico do Norte - Embrapa Oriental/AM; INPA - Instituto Nacional de Pesquisas Amazônicas/AM; K - Royal Botanic Gardens - Kew/UK; R - Museu Nacional/RJ; RB - Jardim Botânico do Rio de Janeiro/RJ; SP - Instituto de Botânica/SP; SPF - Universidade de São Paulo/SP; UB - Universidade de Brasília/UB; UEC - Universidade Estadual de Campinas/SP; UFG - Universidade Federal de Goiás/GO.