Molecular Phylogenetics and Evolution 109 (2017) 113–137

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Myrteae phylogeny, calibration, biogeography and diversification patterns: Increased understanding in the most species rich tribe of Myrtaceae

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ARTICLE INFO

Article history:
Received 8 August 2016
Revised 29 November 2016
Accepted 4 January 2017
Available online 6 January 2017

Keywords:
Eugenia
Evolution
Myrcia
Myrtus
Psidium
Systematics

ABSTRACT

Myrteae (c. 2500 species; 51 genera) is the largest tribe of Myrtaceae and an ecologically important groups of angiosperms in the Neotropics. Systematic relationships in Myrteae are complex, hindering conservation initiatives and jeopardizing evolutionary modelling. A well-supported and robust phylogenetic hypothesis was here targeted towards a comprehensive understanding of the relationships within the tribe. The resultant topology was used as a base for key evolutionary analyses such as age estimation, historical biogeography and diversification rate patterns. One nuclear (*ITS*) and seven chloroplast (*psbA-trnH*, *matK*, *ndhF*, *trnl-trnF*, *trnQ-rps16*, *rpl16* and *rpl32-trnL*) DNA regions for 115 taxa representing 46 out of the 51 genera in the tribe were accessed and analysed using maximum likelihood and Bayesian inference tools for phylogenetic reconstruction. Dates of diversification events were estimated and contrasted using two distinct fossil sets (macro and pollen) in BEAST. The subsequent dated phylogenies were compared and analysed for biogeographical patterns using BioGeoBEARS and diversification rates using BAMM. Myrteae phylogeny presents strong statistical support for three major clades within the tribe: Australasian group, *Myrtus* group and Main Neotropical Lineage. Dating results from calibration using macrofossil are an average of 20 million years older and show an early Paleocene origin of Myrteae, against a mid-Eocene one from the pollen fossil calibration. Biogeographic analysis shows the origin of Myrteae in Zealandia in both calibration approaches, followed by a widespread distribution throughout the still-linked Gondwana continents and diversification of Neotropical endemic lineages by later vicariance. Best configuration shift indicates three points of acceleration in diversification rates, all of them occurring in the Main Neotropical Lineage. Based on the reconstructed topology, several new taxonomic placements were recovered, including: the relative position of *Myrtus communis*, the placement of the

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http://dx.doi.org/10.1016/j.ympev.2017.01.002

Blepharocalyx group, the absence of generic endemism in the Caribbean, and the paraphyletism of the former *Pimenta* group. Distinct calibration approaches affect biogeography interpretation, increasing the number of necessary long distance dispersal events in the topology with older nodes. It is hypothesised that biological intrinsic factors such as modifications of embryo type and polyploidy might have played a role in accelerating shifts of diversification rates in Neotropical lineages. Future perspectives include formal subtribal classification, standardization of fossil calibration approaches and better links between diversification shifts and trait evolution.

1. Introduction

Myrtaceae is a large family of woody flowering plants represented by around 5500 accepted species, classified in 144 genera and 17 tribes (Wilson et al., 2005; Wilson, 2011; WCSP, 2016). Myrtaceae represents an old, mid-Cretaceous lineage within the order Myrtales (c. 85 mya, Berger et al., 2016) and is characterized by a strong southern-hemisphere, Gondwanan distribution (Thornhill et al., 2015). Myrtaceae is an important floristic component in the areas where it is most species diverse, especially in the forests of Southeast Asia, Australia and South America (e.g. Johnson and Briggs, 1981; Kochummen et al., 1990; Oliveira-Filho and Fontes, 2000; Flora of Brazil, 2016). In Neotropical environments, all Myrtaceae diversity (excluding a single species from tribe Metrosidereae, *Metrosideros stipularis*, restricted to Chile, Pillon et al., 2015) is represented by a sole lineage: tribe Myrteae (Wilson et al., 2005; Lucas et al., 2007). Myrteae is the most diverse tribe within Myrtaceae both in number of species (c. 2500) and genera (51), representing half of the family's biodiversity (Wilson, 2011; WCSP, 2016). Myrteae species are ecologically important in many Neotropical environments due to the fleshy berries eaten by birds and mammals and the white generalist flowers that supply pollen and resources to a variety of bee species (Mori et al., 1983; NicLughadha and Proença, 1996; Gressler et al., 2006, see Fig. 1). Due to its ecological importance, a growing interest has been addressed by researchers using Myrteae as a model group for evolutionary, ecological and conservation studies in Neotropical biomes (e.g. Murray-Smith et al., 2009; Lucas and Bünger, 2015; Staggemeier et al., 2015; Giaretta et al., 2015).

1.1. Myrteae systematics and diversity

A common barrier encountered by those wishing to study Myrteae is the problematic systematics of the group. The homogeneous morphology of flowers, fruits and vegetative characters between even distantly related Myrteae species makes taxonomy in the tribe a tiresome process even for specialists and until recently resulted in its neglect (McVaugh, 1968; Landrum and Kawasaki, 1997; Lucas et al., 2005). Recent phylogenetic systematic studies and taxonomic revision of individual clades within the tribe has improved the understanding of relationships and characterization of smaller groups (e.g. Landrum, 1981; Landrum, 1986; Proença, 1990; Grifo, 1992; Lucas et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015). However, narrower distributed genera not sampled at the molecular level until now remain phylogenetically unplaced. To place such taxa in a broader phylogenetic system is central to improve the understanding of relationships and evolution within this ecologically important tribe.

Although morphologically similar, Myrteae lineages have an uneven, heterogeneous distribution of biodiversity in terms of species per genus. Two thirds of the diversity of described species occurs in only two genera, *Eugenia* s.l. (*sensu* Mazine et al., 2014) and *Myrcia* s.l (sensu Lucas et al., 2011), which are also two of the largest angiosperm genera (Frodin, 2004) with c. 1000 and 700 species, respectively (WCSP, 2016). Furthermore, these two genera have been consistently proved to be sister to species poor lineages in the tribe (Lucas et al., 2007, this study), increasing the extant diversity disparity between closely related clades.

1.2. Myrteae global geographic distribution

Although most extant biodiversity of Myrteae is restricted to the Neotropics, at least 15 genera (Wilson, 2011) and ca. 450 species are found in other continents. These are predominantly from Southeast Asia, Northeast Australia and the Pacific islands, including New Caledonia and New Zealand (Scott, 1978; Snow, 2000; Wilson, 2009; Snow et al., 2011; WCSP, 2016). A few species of *Eugenia* are also found in Africa, Madagascar and Mauritius (Van Wyk et al., 1982; van der Merwe et al., 2005; Snow, 2008) and an additional genus, *Myrtus*, represents the only European/Northern African lineage (Lucas et al., 2007; Migliore et al., 2012). On the American continent, most species diversity is found in the rainforests and savannah of central and eastern Brazil, the Guiana shield and Caribbean (McVaugh, 1968; Mori et al., 1983; Oliveira-Filho and Fontes, 2000; Holst et al., 2003; Murray-Smith et al., 2009); less but still significant biodiversity is found in continental Central America and the low-land Amazon basin (Landrum, 1992; WCSP, 2016). Species diversity is relatively low in the subtropical and temperate areas of southern South-America (Patagonia) and the high altitude Andes, but these areas boast a significant array of endemic genera (e.g. *Ugni*, *Amomyrtus*, *Legrandia, Luma;* Landrum, 1981, 1986, Landrum and Grifo, 1988).

Previous phylogenetic analyses consistently showed *Myrtus* representing a sister clade to all of the extant Myrteae (Lucas et al., 2005, 2007; Biffin et al., 2010; Thornhill et al., 2015). In these studies, most Australasian genera also group in a distinct clade, sister to the that containing all Neotropical clades (Lucas et al., 2005, 2007). The relative position of these clades in the tribe, in addition to biogeographical analysis in a broader Myrtaceae context (Thornhill et al., 2015) shows that Australia represents the most likely ancestral range in the family and that Neotropical genera are likely a result from a more recent event of vicariance between Australia and South America, while the distribution of *Myrtus* is attributed either to a previous wider distribution of the tribe or to an old long distance dispersion and establishment (henceforward coined LDDE) event.

1.3. Study aims

Despite recent progress in understanding relationships within Myrteae using molecular tools (e.g. Lucas et al., 2011; Snow et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015; Santos et al., 2016), available studies have focused mainly on smaller clades and still lack complete generic sampling, ultimately preventing proper examination of relationships within the tribe. Improving taxonomic and DNA sampling when building phylogenetic trees is known to solve controversial relationships in plants (e.g. APG IV, 2016). Results from such

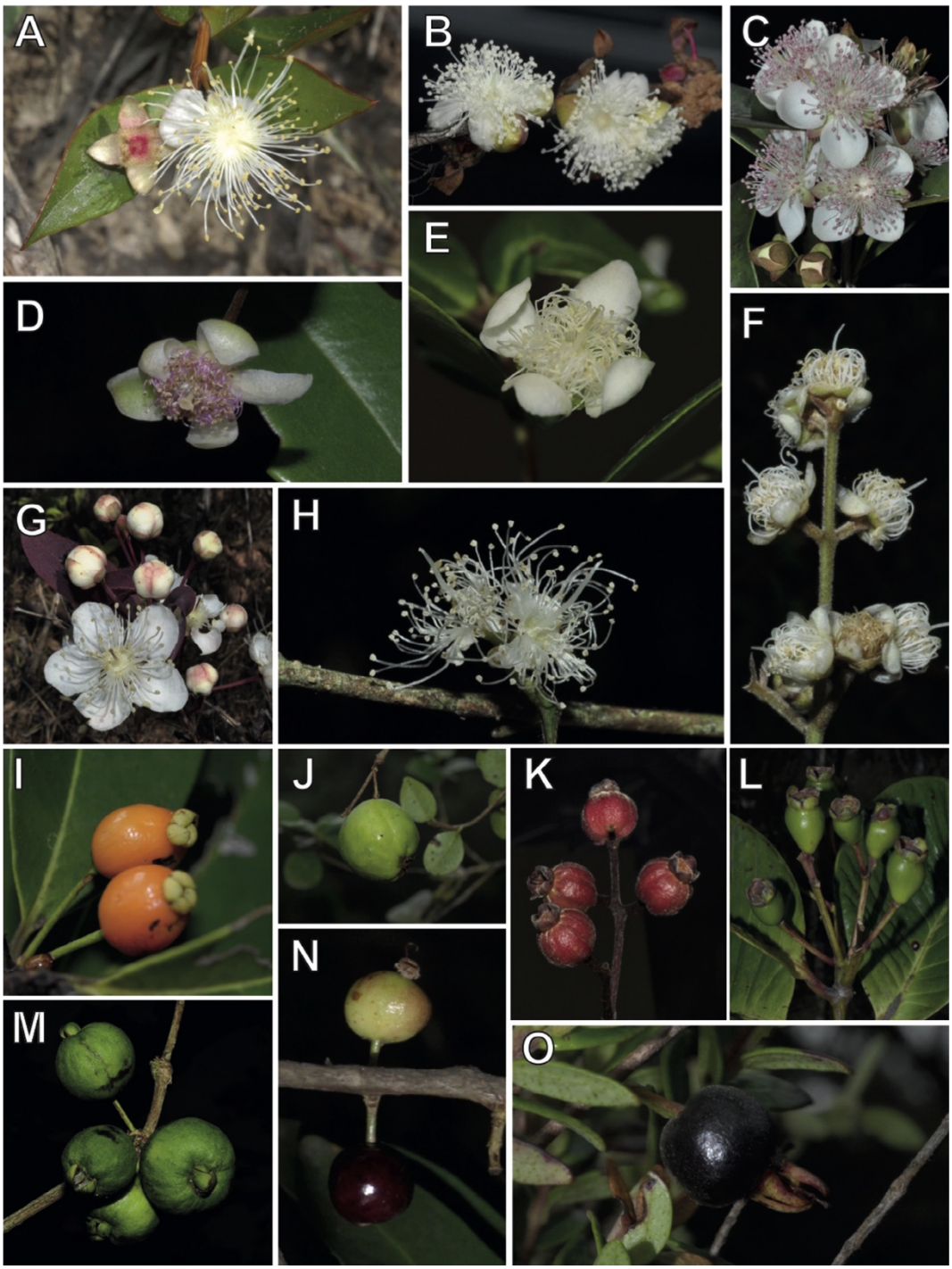

Fig. 1. Biodiversity of Myrteae represented by the characteristic polystemonous white flowers (A–H) and fleshy, berry-like fruits (I–O). (A) *Accara elegans*; (B) *Calyptrogenia cuspidata*; (C) *Eugenia involucrata*; (D) *Archirhodomyrtus turbinata*; (E) *Luma apiculata*; (F) *Myrcia splendens*; (G) *Campomanesia adamantium*; (H) *Myrciaria floribunda*; (I) *Eugenia punicifolia*; (J) *Hottea neibensis*; (K) *Myrcia sp1* (voucher T. Vasconcelos 307); (L) *Gossia clusioides*; (M) *Chamguava schippii*; (N) *Siphoneugena densiflora* (O) *Myrtastrum rufopunctatum*. Size of reproductive structures varies between c. 0.5 and 3 cm. Pictures by R. Aguilar (M) and T. Vasconcelos (all besides M).

improved phylogenies are key to elucidating systematic problems and also to detect consistent evolutionary patterns as low statistically supported and unbalanced phylogenetic trees may present unreliable branching patterns, branch lengths and substitution models, all of which are ultimately misleading when estimating dates or any other subsequent analysis. Improved phylogenetic resolution in Myrteae will allow more reliable systematic, biogeographic and evolutionary hypotheses of diversity in the tribe. Therefore, the aims of this study are to:

(1) Develop a well-supported and robust phylogenetic chronogram for Myrteae including all main lineages (46 out of 51 genera and all main clades within large genera).
(2) Propose a biogeographical hypothesis of evolution of the tribe allowing detection of variation (shifts) in ancestral geographical ranges within a global perspective.
(3) Estimate diversification rate variation to understand the evolution of heterogeneous diversity among closely related lineages.

2. Methods

2.1. Taxonomic sampling

The selected sample includes a large range of lineages and geographical distributions within Myrteae. In the case of the megadiverse genera *Myrcia s.l.* and *Eugenia s.l.*, at least one species was sampled from each informal group (soon to be recognized as formal sections, Mazine et al. in prep, Lucas et al. in prep.) in each genus, following the clade classifications of Lucas et al. (2011) for the nine *Myrcia* s.l. clades and Mazine et al. (2014) and Bünger (2015) for the ten *Eugenia* s.l. clades (clades 1 to 9 and section *Speciosae*). Fieldwork was conducted in Brazil, Jamaica, Costa Rica, Dominican Republic, New Caledonia, Singapore and Malaysia to collect missing taxa for DNA extraction. Samples was supplemented from the living collection of the Royal Botanic Gardens Kew (K). Duplicate vouchers were deposited in local herbaria and in the Kew herbarium.

The final sample comprises 115 terminals representing 114 species. These include 99 species representing 46 of the 51 genera of Myrteae, 16 genera more than the previous published sample (Lucas et al., 2007). *Blepharocalyx salicifolius* was sampled twice, due to inconsistent placement in past studies (Lucas et al., 2005; Lucas et al., 2007; Murillo-A et al., 2012; de-Carvalho, 2013). Fifteen species were chosen as outgroups based on previous phylogenetic works (Lucas et al., 2007; Biffin et al., 2010; Thornhill et al., 2015). These represent five tribes of Myrtaceae: Leptospermeae (*Leptospermum scoparium*, defined as the furthermost outgroup in all analysis), Eucalypteae (*Eucalyptus perriniana*), Metrosidereae (*Metrosideros perforata, M. stipularis* and *M. nervulosa*), Tristanieae (*Xanthostemon compacta* and *X. montivaga*) and Syzygieae (*Syzygium jambos, S. maire, S. gustavioides, S. buxifolium, S. paniculatum, S. amplifolium, S. muellerii* and *S. guineense*). Previous studies provide evidence that Metrosidereae, Syzygieae and Tristanieae are closely related to Myrteae (part of the BKMMST clade *sensu* Biffin et al., 2010). See Appendix for a full list of sampled species and vouchers.

2.2. Extraction and sequencing

DNA extraction followed the CTAB extraction protocol for long term DNA storage (Doyle and Doyle, 1987, with modifications following Lucas et al., 2007, and Staggemeier et al., 2015). Approximately 200 mg of leaf tissue were used for each extraction. Eight DNA regions were selected for sequencing based on their informative quality evidenced in previous Myrtaceae studies (Lucas et al., 2005; Lucas et al., 2007; Snow et al., 2011; Murillo-A et al., 2012; Mazine et al., 2014; Staggemeier et al., 2015). These are the nuclear region ITS and seven chloroplast regions: *psbA-trnH*, *matK*, *ndhF*, *trnl-trnF*, *trnQ-rps16*, *rpl16* and *rpl32-trnL*. Sequencing was performed using traditional Sanger sequencing protocol, following Lucas et al. (2007). Information on primers and PCRs conditions are available in Supplementary Material 1 and 2. Raw sequences were imported and assembled using Geneious (v. 9, Kearse et al., 2012). Resulting contigs were aligned separately for each region using Muscle (Edgar, 2004) implemented in Geneious and adjusted manually. A total of 535 new sequences were generated in this study. Sequences sourced from Genbank are listed in Appendix.

2.3. Phylogenetic analysis

The seven chloroplast regions were concatenated resulting in a matrix of 6453 base pairs, hereafter referred to as the 'cpDNA dataset'. This and the 'nuclear dataset', including only the ITS region (916 base pairs), were used to run two independent Bayesian Inference (BI) phylogenetic analysis**.** The best evolutionary model was estimated prior to phylogenetic reconstruction using jModelTest 2 (Darriba et al., 2012). Estimation resulted in a best model of GTR gamma + inv for both nuclear and cpDNA datasets. Models were then implemented in MrBayes on XSEDE V. 3.2.6 (Ronquist and Huelsenbeck, 2003) executed in Cipres and run for 15,000,000 generations using default parameters. After visual comparison between phylogenies based on nuclear and cpDNA datasets separately (see Section 3.1: *Phylogenetic tree analysis - Grouping and Main lineages*), both nuclear and cpDNA matrices were concatenated resulting in a final matrix of 7369 base pairs, hereafter referred to as the 'combined dataset'. For this matrix, Maximum Likelihood (ML) and BI were run independently to compare topologies and node support (bootstrap vs. posterior probabilities, respectively). For the ML analysis, the final concatenated alignment (available in Supplementary Material 3) was converted into a simplified Nexus file in Mesquite v3.04 (Maddison and Maddison, 2015) and sourced as input to RAxML-HPC2 (Stamatakis, 2014) analysis implemented in Cipres (Miller et al., 2010). Outputs of all phylogenetic analysis were read using Figtree v1.4.2 (Rambaut, 2014).

2.4. Fossil calibration and dating

Dates of Myrteae diversification events are controversial. Myrtaceae and Myrteae phylogenies have been dated using fossil calibration and molecular clock approaches in at least seven previous studies (Sytsma et al., 2004; Biffin et al., 2010; Thornhill et al., 2012a, 2015; Murillo-A et al., 2016; Staggemeier et al., 2015; Berger et al., 2016 – see Supplementary Material 4). Except on the occasions where studies were conducted by the same research group, most obtain different dates for similar nodes, sometimes extremely (e.g. Berger et al. (2016) date the crown node of Myrteae at 18 million years old, while Murillo-A et al., 2016 date the same node at 92 million years old). The differences in dates appear partially related to phylogeny sample size and balance, but distinctly dependent on the fossils selected and their position in calibration analysis. Because phylogenetic node age is key to interpretation of historical biogeography, reliable fossil selection, calibration and dating analysis is critical; it is discouraging to realise that these decisions are so subjective and open to interpretation. In dating estimation using fossil calibration the standard protocol is to place the estimate minimum date of a fossil on the stem node of a related extant monophyletic taxa in the phylogeny (Forest, 2009). A survey of the oldest fossil records with affinity to Myrteae was conducted and a relatively good fossil record was found assigned to the tribe in the literature. Many fossil descriptions tentatively link them to modern genera (see Supplementary Material 5) however, in reality it is very difficult to identify individual Myrteae genera based on only a few morphological characters. For this reason, the safest approach is to choose the oldest fossil remains confidently described as any genus in Myrteae and place them in the deepest nodes of the tribe.

The oldest fossil records of Myrteae are represented by macrofossil from the upper Cretaceous of Antarctica and represent remains of wood (*Myrceugenelloxylon antarcticus*) and leaves (*Myrciophyllum santacruzensis*) that are similar to extant *Luma* and *Myrcia* respectively (Poole et al., 2003). Other wood and leaf fossils from the Paleocene at extreme southern latitudes show affinity in form and distribution to modern genera (e.g. Ragonese, 1980; Troncoso et al., 2002). The most popular fossil from this period used for calibration of Myrteae studies, however, is *Paleomyrtinae*, a fossil fruit with affinity to *Psidium* or *Mosiera* recorded far from any other Myrteae records, in Northern North America (Pigg et al., 1993). Recently, another Paleocene/Eocene macrofossil from the northern hemisphere was described and placed in Myrteae:

Myrtineoxylon maomingensis, from China (Oskolski et al., 2013). This is stated to be similar to extant Australasian group genera (*sensu* Lucas et al., 2007). Macrofossils assigned to Myrteae found in Eocene deposits are also common and show similar distribution to modern Myrteae (see Supplementary Material 5).

Pollen fossil in Myrteae is, contrariwise, only found in more recent, mid-late Eocene deposits. Myrtaceae pollen fossil (represented by the genus *Myrtacedeites*) was recently reviewed by Thornhill and Macphail (2012) and even though these are found in deposits as old as the Cretaceous, only one species, *M. verrucosus*, shows morphology that undoubtedly places it as Myrteae. Myrteae pollen morphology is conservative (Thornhill et al., 2012b) and in this sense, *Myrtacedeitees verrucosus* represents the most reliable fossil record for Myrteae. At least two varieties of *Myrtaceideites verrucosus* are found in late Eocene deposits of Australia, New Zealand, Patagonia and Panama, suggesting Myrteae was an already widespread and diverse group during that period. *Myrtacedeites verrucosus* is not however, found in deposits of earlier periods (Thornhill and Macphail, 2012).

An important and antagonistic reasoning arises here; pollen fossil of Myrtaceae was recently reviewed and is found to be up to 90 million years old (Thornhill and Macphail, 2012), however, the morphotype that closely matches Myrteae only appears and apparently diversifies in mid Eocene deposits. Added to the hypothesis that pollen is usually the first structure to fossilize when an angiosperm group diversifies (Sauquet et al., 2012), it appears that Myrteae had not diversified before the mid Eocene. Alternatively, if identification of the late cretaceous and Paleocene macrofossils assigned to Myrteae are correct, then Myrteae has to be older than the dates showed by fossil pollen. Furthermore, it is not possible to combine pollen and macrofossil datasets in this case, because they would be placed on similar nodes or represent paradoxal calibration (e.g. if the fossil *Myrceugenia chubutenses* is used to calibrate the stem node of *Myrceugenia* at 66 mya, the oldest *Myrtacedeites verrucosus* remains cannot be used to calibrate the whole of the Neotropical Myrteae at 37 mya, because the first represents a shallower node in the phylogeny than the second). The solution adopted by this study is to compare two calibration approaches using two distinct fossil sets: a macrofossil set, based on the oldest fossil remains assigned to Myrteae in the literature; and a pollen fossil set, based on different records of *Myrtacedeites verrucosus* remains. The macrofossil approach referred to as Approach A, considered three fossil records: *Myrceugeneloxylon antarticus*, the oldest fossil in Myrteae, was placed on the crown node of Myrteae calibrating it at 66 million years ago (mya). The following fossils were placed based on their geographical distribution: the crown of the Australasian group was calibrated at 41 mya, based on the minimum age estimate of *Myrtineoxylon maomingensis*, a fossil remain from China with affinity to *Octamyrtus*. *Paleomyrtineae princetonensis* from the Paleocene was used to calibrate the crown node of the Myrtus group + Main Neotropical Lineage clade at 56 mya, given its reported affinities to modern *Psidium* and *Mosiera* and its distribution closer to extant Neotropical Myrteae.

The second approach is referred to as Approach B and considers three distinct records of *Myrtacedeites verrucosus* (revised by Thornhill and Macphail, 2012) and additional secondary calibration points. The placement of the three remains of *M. verrucosus* was geographically based, following a similar protocol to that of Thornhill et al. (2012a). The oldest record of the pollen in the Neotropics (*Myrtacedeites verrucosus* from the mid-Eocene of Panama and Argentina) was placed on the crown node of the *Myrtus* group + Main Neotropical Lineage clade, calibrating it at 37 mya. The oldest *Myrtacedeites verrucosus* recorded for Australia was placed on the crown node of the Australasian group, calibrating it at 35 mya. Finally, *Myrtacedeites verrucosus* remains found in New Zealand from 23 mya was used to calibrate the crown node of the *Myrteola* group, the only clade currently found in New Zealand (Lucas et al., 2007, this study). Secondary calibration points from the broader Myrtaceae analysis of Thornhill et al. (2012a, 2015) were used to calibrate the crown of Myrteae at 41 mya and the crown of the BKMMST clade (Myrteae + sister tribes, sensu Biffin et al., 2010) at 66 mya. In both approaches A and B, the root of the family was constrained to be no older than 85 mya (following Berger et al. 2016). A summary of the calibration points used and the rate parameters applied in Beast are summarized in Table 1. Both approaches A and B were used to produce dated phylogenies using a lognormal relaxed clock set for Birth-Death speciation and 50,000,000 generations in BEAST v.1.8.3. (Drummond et al., 2012). Two analyses were run for each approach, results were checked for convergence in Tracer v1.6.0 (Rambaut et al., 2013), burnin was selected as 0.1% of total trees and final chronograms (dated phylogenies) were visualised in Figtree v1.4.2 (Rambaut, 2014).

2.5. Historical biogeography inference

BioGeoBEARS (Matzke, 2013) implemented in R (R Core Team, 2016) was used to analyze ancestral geographical range variation over resulting chronograms (Approaches A and B). BioGeoBEARS allows implementation of a third free parameter "j" (founder event/jump speciation) that permits a daughter lineage to have a different area from the direct ancestor a feature that improves the log likelihood of resulting inferences of ancestral areas in comparison to a model with only two free parameters (e.g. dispersion/extinction only in Lagrange, Ree and Smith, 2008). BioGeoBEARS does not work well when many possible ancestral areas are implemented unless the maximum number of areas any species may occupy is reduced. Range area per terminal in the phylogeny was therefore coded in relation to species distributions, not genera. In this way, most terminals are restricted to single area. Area coding aimed to consider the current distribution of the group and historical geology and tectonics. The seven areas chosen were: (A) South

Table 1
Summary of two fossil sets and secondary calibration points selected to estimate diversification rates in Myrteae. Rate (normal or lognormal) is based on Beast parameters. For fossil reference see Supplementary Material 5.

	Node	Age (in million years ago)	Rate
Approach A: Macrofossil			
Myrceugenelloxylon antarcticus	Myrteae crown	66 (late-Cretaceous)	Lognormal
Myrtineoxylon maomingensis	Australasian group crown	40 (Mid-Eocene)	Lognormal
Paleomyrtinae princetonensis	Neotropical lineage crown	56 (late-Palaeocene)	Lognormal
Approach B: Pollen fossil			
Secondary calibration point – Thornhill et al. (2012a, 2012b)	Crown BKMST	63.1 (early-Paleocene)	Normal
Secondary calibration point – Thornhill et al. (2012a, 2012b)	Crown Myrteae	41 (early-Eocene)	Normal
Myrtaceideites verrucosus (Panama, Argentina)	Neotropical lineage crown	37.2 (late-Eocene)	Lognormal
Myrtaceideites verrucosus (Australia)	Australasian group crown	35 (late-Eocene)	Lognormal
Myrtaceideites verrucosus (New Zealand)	Myrteola group crown	23 (late-Oligocene)	Lognormal
Both approaches:			
Secondary calibration point – Berger et al. (2016)	Myrtaceae crown	85 (Cretaceous)	Normal

America, (B) Central + North America (including the greater Antilles in the Caribbean), (C) Australia and New Guinea (referred to as Australia + NG), (D) New Caledonia and New Zealand (referred to as NCNZ, representing the Zealandia plate, Trewick et al. (2007)), (E) Africa (here including Madagascar), (F) Mediterranean Europe and (G) Southeast Asia (referred to as SEAsia). Distribution ranges, time slice matrices and values of area adjacency through time are available as Supplementary Material 6.

2.6. Diversification rates analysis

Configuration shifts in diversification rates were calculated using speciation/extinction model type analysis in BAMM (Rabosky et al., 2014). BAMM works with incomplete phylogenetic datasets and allows a certain degree of phylogenetic uncertainty (see BAMM documentation). Missing taxa per tip or clade in the phylogenetic tree was estimated using previously published works (Wilson et al., 2005; Wilson, 2011; Lucas et al., 2007; Lucas et al., 2011; Mazine et al., 2014; Staggemeier et al., 2015; Santos et al., 2016; WCSP, 2016). In the largest genera, *Myrcia* s.l. and *Eugenia s.l.*, the numbers of species per clade was estimated by specific studies (Mazine et al., 2014) and unpublished data (Lucas et al., in prep, Faria Júnior, 2014; Bünger, 2015). Priors for the BAMM control file were generated using the dated phylogenetic tree input into the function *setBAMMpriors* in the package *BAMMtools* v2.5.2 implemented in R (R Core Team, 2016), estimating 2500 species in Myrteae. The control file was set for 100,000,000 generations and the analysis was run twice as recommended (see BAMM documentation), giving similar results. Resultant MCMC Log likelihoods were tested against generation number for convergence using the *coda* package implemented in R (R Core Team, 2016). All other outputs contained in the "*event_data*" file were analysed using *BAMMtools* in R. A recent paper casted doubt in the reliability of results produced by BAMM (Moore et al., 2016), but the criticism concerning the priors used by the software were adjusted in the latest version (see BAMM documentation). Other problems cited by that study can be applied to most macroevolutionary methods (e.g. estimation of extinct clades) and in this sense BAMM was not considered better or worse than similar software. Priors and proportion of samples per clade are given in Supplementary Material 7.

3. Results

3.1. Phylogenetic tree analysis – Grouping and main lineages

Phylogenetic analysis shows Myrteae to be a coherent, well defined group with >0.95 posterior probability and 100% bootstrap support in cpDNA, nuclear and combined datasets analyses (node A, Fig. 2, Supplementary Materials 8 and 9). The next deepest node in the tribe's phylogeny (node B, Fig. 2) is poorly supported by all datasets while the two following nodes (nodes C and D, Fig. 2) are recovered with strong posterior probability (>0.95) and high bootstrap support (>70) in the combined and cpDNA datasets. Four lineages result from divergences at these four nodes (A, B, C and D). One of them represents a single, ungrouped monotypic genus (*Myrtastrum*) and the other three are here informally coined: the Australasian group, the *Myrtus* group and the Main Neotropical Lineage (color coded in Fig. 2 as orange, blue and green respectively).

The backbone of the Main Neotropical Lineage is poorly supported in all dataset analyses, but eight major clades with high bootstrap (>70) and/or posterior probability (>0.95) supports are recovered in the combined dataset and here informally named: the *Eugenia*, *Pimenta*, *Myrteola*, *Myrceugenia*, *Myrcia*, *Plinia*, *Blepharocalyx* and *Psidium* groups. These eight clades are also recognized with similar representing taxa and support in the cpDNA dataset analysis (Supplementary Material 8). The nuclear dataset analysis presents poor support for most of the deepest nodes in the phylogeny and is mostly non-informative to analyse relationship between and within these clades. The relationship between *Plinia* sp1 as sister to *Myrrhinium atropurpureum* is the only strongly supported arrangement in the nuclear dataset analysis that differs from the cpDNA and combined datasets (Supplementary Material 9). In the next sections, relationships within each of the ten clades (the eight clades within the Main Neotropical Lineage plus *Myrtus* and Australasian groups) and two ungrouped genera (*Myrtastrum* and *Amomyrtus*) are discussed based on the combined dataset (Fig. 2). Diversity estimates per clade are taken from WCSP (2016) and Wilson (2011).

3.1.1. The Australasian group

The Australasian group (in orange, Fig. 2) has similar configuration to the informal Australasian group *sensu* Lucas et al. (2007). It is positioned as sister to the *Myrtus* group + Main Neotropical lineage clade and includes species within the genera *Gossia*, *Uromyrtus*, *Rhodamnia*, *Austromyrtus*, *Decaspermum*, *Octamyrtus*, *Rhodomyrtus*, *Kanakomyrtus*, *Pilidiostigma* and *Archirhodomyrtus*. This lineage comprises genera restrictedly distributed in Southeast Asia, Australia and Pacific islands (Fig. 3A) and an estimated c. 250 accepted species. Supports both from ML and BI analysis are high (>70 bootstrap and/or 0.95 posterior probability) for most internal nodes in the clade, except for the positions of *Austromyrtus*.

3.1.2. The Myrtus group

The *Myrtus* group (in blue, Fig. 2) contains the only European genus *Myrtus* and three Neotropical genera: *Accara*, *Chamguava* and *Calycolpus*. This group is recovered in all molecular dataset analyses, although relationships within the group vary slightly depending on the dataset under examination and the type of phylogenetic analysis (ML or BI). The main distinction is the placement of *Accara* and *Myrtus* that swap positions between sister to the rest of the group or to *Chamguava*. The two species of *Calycolpus* always appear as a strong supported group. Based on these results, *Myrtus* group present a peculiar discontinuous distribution throughout Mediterranean and Neotropical areas (Fig. 3B) and an estimated diversity of c. 20 species.

3.1.3. Main Neotropical lineage

The Main Neotropical Lineage (in green, Fig. 2) presents eight well supported (PP > 0.95, BS > 70) clades: the *Blepharocalyx*, *Psidium*, *Pimenta*, *Myrteola*, *Myrceugenia*, *Plinia*, *Myrcia*, *Eugenia* groups. The latter five are very similar to the circumscription of Lucas et al. (2007). With the exception of the consistently well supported relationship between the *Plinia* and *Myrcia* groups, the relationship between these groups is poorly resolved within the Neotropical lineage. The *Blepharocalyx* group is endemic to the Neotropics (Fig. 3C) and includes *Blepharocalyx salicifolius* and *B. eggersii*. *Blepharocalyx* is a genus of only four accepted species and future additions to the phylogeny may also place *Blepharocalyx myriophyllus* (the only unsampled *Blepharocalyx* species in this study) in this group increasing diversity to three accepted species. Currently accepted *Blepharocalyx cruckshanksii* is nested in the *Myrceugenia* group. The *Psidium* group includes the genera *Mosiera*, *Myrrhynium*, *Psidium* and at least one species of the polyphyletic *Calyptrogenia* (*C. biflora*).

The *Pimenta* group includes the genera *Curitiba*, *Acca* (*A. sellowiana*), *Campomanesia*, *Legrandia*, *Pimenta* and at least one species of *Eugenia* (*Eugenia yumana*), nested within *Pimenta*. Taken in this sense, the group is endemic to the Neotropics (Fig. 3C) and includes an estimated c. 50 species. The *Myrteola* group

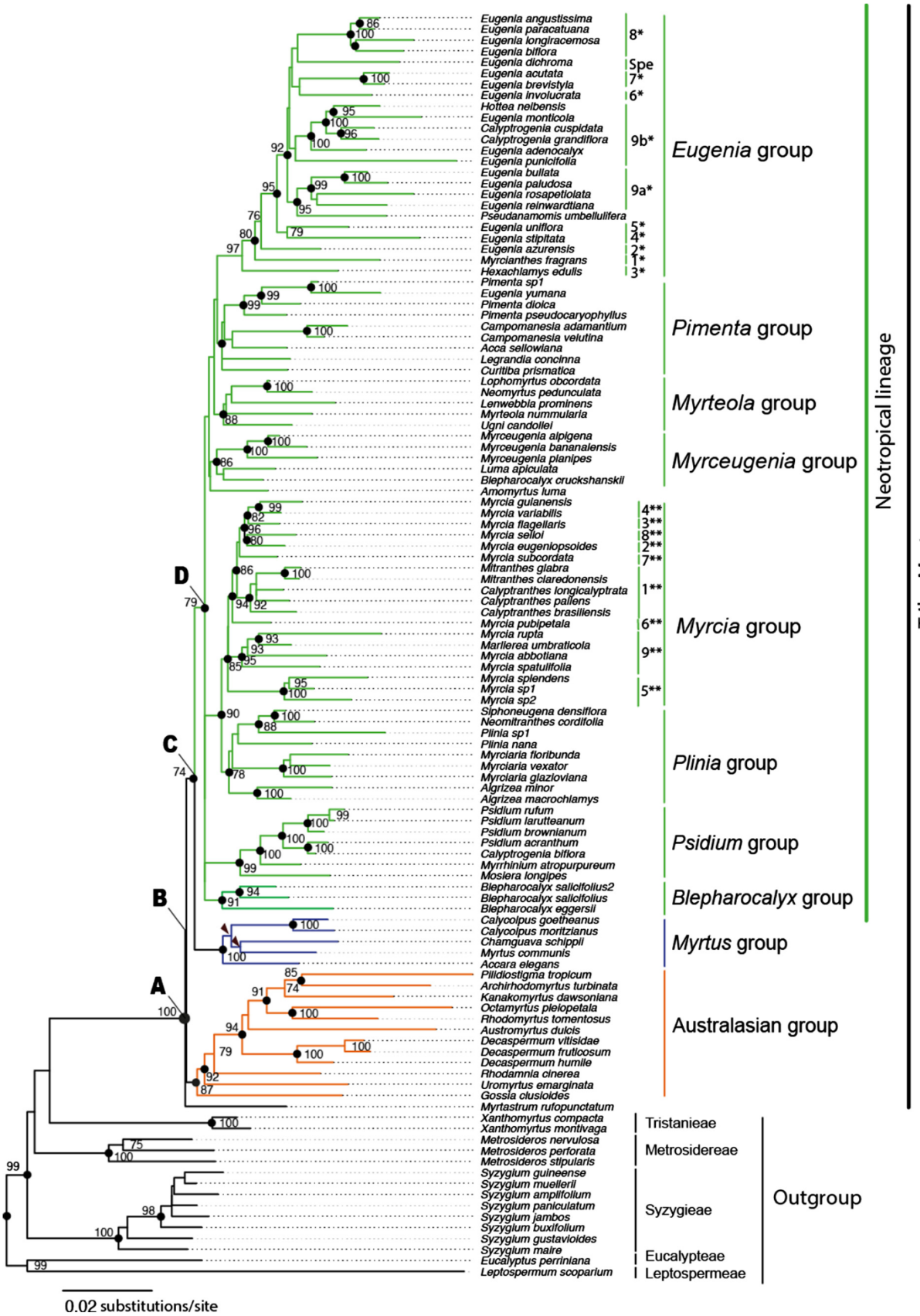

Fig. 2. Myrteae ML phylogenetic tree resulting from the combined dataset analysis. Bootstrap percentages greater than 50 are shown above branches; clades receiving posterior probabilities greater than 0.95 in equivalent BI analysis are indicated by black dots. Arrows indicate clades that were not recovered in BI analysis. *Clade numbers *sensu* Mazine et al. (2014). **Clade numbers *sensu* Lucas et al. (2011). 'Spe': section Speciosae sensu Bünger et al. (2016).

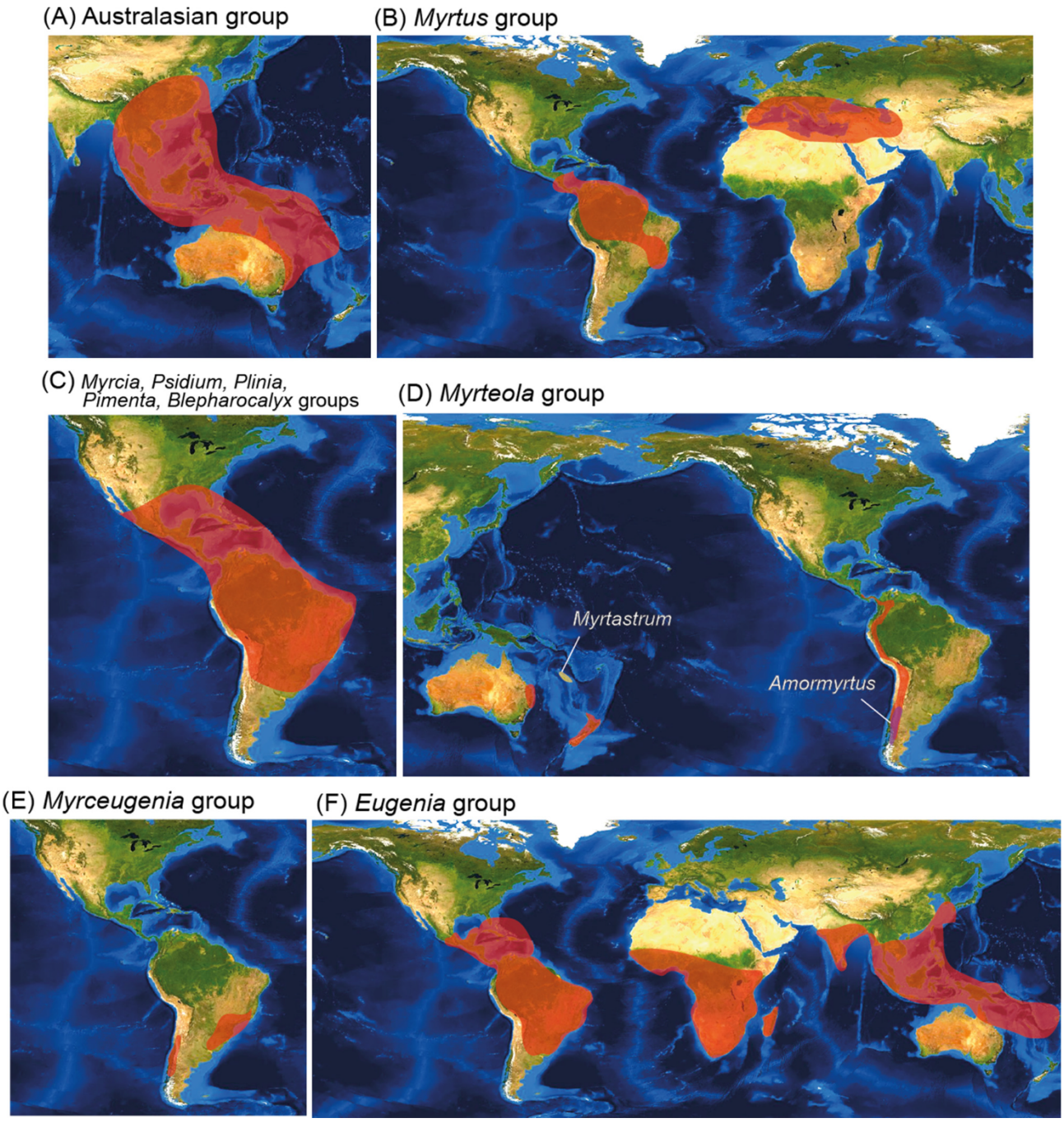

Fig. 3. Global species distribution of Myrteae, as sourced from WCSP (2016).

includes the genera *Lophomyrtus*, *Neomyrtus*, *Myrteola*, *Ugni* and *Lenwebbia*, and contains c. 15 species. This group presents an atypical geographical distribution within the tribe, with two genera (*Ugni* and *Myrteola*) endemic to Patagonia and the alpine biomes of South and Central America, one genus endemic to Australia (*Lenwebbia*) and two genera endemic to New Zealand (*Neomyrtus* and *Lophomyrtus*) (Fig. 3D). The *Myrceugenia* group includes the genera *Luma*, *Myrceugenia* and one species of the polyphyletic *Blepharocalyx* (*B. cruckshanksii*); an estimated c. 50 species are assigned here. This group presents a somewhat restricted distribution to subtemperate and subtropical biomes of South America, mainly Chile and Southern Brazil (Fig. 3E). The *Plinia* group includes the genera *Plinia* (emerging paraphyletic), *Algrizea*, *Myrciaria*, *Siphoneugena* and *Neomitranthes* and an estimated diversity of c. 120 species. The *Myrcia* group includes four genera: *Mitranthes, Myrcia*, *Marlierea* and *Calyptranthes.* This group is estimated to include around 700 species. Both *Plinia* and *Myrcia* groups are endemic to the Neotropics (Fig. 3C). The *Eugenia* group includes the genera *Myrcianthes, Hottea, Pseudanamomis*, and *Calyptrogenia*. Clade 9 (sensu Mazine et al., 2014) appears polyphyletic in our analysis with all old world species (including *Eugenia roseopetiolata*, *E. reinwardtiana*, *E. bullata* and *E. paludosa*, here defined as clade 9a) appearing monophyletic in an unrelated, well supported clade. The *Eugenia* group is the most diverse and widespread group in Myrteae, with around 1000 species and a pantropical distribution (Fig. 3F).

3.1.4. Ungrouped genera: Myrtastrum and Amomyrtus

Two genera, *Myrtastrum* and *Amomyrtus*, appear ungrouped in the combined dataset. *Myrtastrum*, a monotypic genus endemic to New Caledonia (shown in orange, Fig. 3D), appears either isolated as sister to all extant Myrteae in the combined and nuclear datasets, or as sister to *Myrtus* group + Main Neotropical lineage, in the cpDNA dataset analysis. *Amomyrtus*, a genus of two species endemic to Patagonia (shown in purple, Fig. 3D), appears as sister to *Myrceugenia* group in both the cpDNA and combined dataset, though this relationship presents a poor support in the latter. This relationship is not supported by the nuclear dataset, where it appears as sister to *Legrandia,* again with a low support.

3.2. Dating inference

Fig. 4 contrasts results from calibration using the two fossil datasets (approaches A and B). Relationships between the *Eugenia*, *Pimenta* and *Myrteola* groups receive high statistical support (PP > 0.95) in the chronograms compared to the lower support returned from the ML and BI analysis. Other aspects of the topology, including outgroup relationships, show discreet differences between chronograms where node support is low.

Because the macrofossil ages are older, approach A returns older dates for all nodes within Myrteae. In this analysis, the stem node of Myrteae (Fig. 4A "a") is estimated as being from the late-Cretaceous (80.72 mya) and the crown node (Fig. 4A "b") from the Cretaceous-Paleocene boundary (KT boundary, 65.55 mya). Approach A also suggests that the three major clades within Myrteae (the Australasian group, *Myrtus* group and the Main Neotropical Lineage) split soon after initial Myrteae diversification, in the Paleocene and early-Eocene, between 63 mya and 53 mya (highlighted in Fig. 4A). The diversification of all major clades within the Main Neotropical Lineage are estimated in this analysis to have taken place in the Eocene, between 52 and 39 mya. The oldest crown nodes in this analysis are: the Australasian group (59.05 mya), the *Eugenia* group (44.42 mya) and the *Pimenta* group (44.41 mya). The youngest crown nodes in this analysis are: the *Plinia* group (39.61 mya), the *Myrcia* group (39.19 mya) and the *Psidium* group (39.12 mya).

Myrteae pollen fossil is younger than the macrofossils and consequently ages estimated from this fossil set (approach B, Fig. 4B) are younger than those from approach A. In this approach, the stem node of Myrteae (Fig. 4B "a") is estimated from the late-Paleocene (58.96 mya) and the crown node (Fig. 4B "b") dates to the mid-late Eocene (40.76 mya), around 25 mya younger than the same nodes in approach A. In approach B the three major clades within Myrteae (Australasian and *Myrtus* groups and the Main Neotropical Lineage) again split immediately after initial Myrteae diversification (highlighted in Fig. 4B) but these events are estimated to have occurred between 40 mya and 35 mya, in the late Eocene. In this approach the diversification of all major clades within the Main Neotropical Lineage are estimated to have taken place between the late-Eocene and Oligocene. The oldest and youngest crown nodes in this analysis are similar to approach A but between 15 mya and 20 mya younger. The oldest groups in this analysis are: the Australasian group (36.88 mya), the *Pimenta* group (29.40 mya) and the *Eugenia* group (29.29 mya). The youngest crown nodes in this analysis are: the *Psidium* group (25.62 mya), the *Myrcia* group (25.58 mya) and the *Myrteola* group (23.39 mya). Median age estimates and 95% confidence intervals (CI) for diversification dates of the main nodes of both analysis are plotted and contrasted in Table 2.

3.3. Biogeographical patterns

BioGeoBEARS was applied to chronograms resulting from both calibration approaches (Fig. 5). In each case results indicate a higher value of log likelihood for three parameters (DEC + j, LnL = −156.72 and LnL = −161.48 for approaches A and B respectively) in comparison to two parameters (DEC, LnL = −202.75 and LnL = −207.92 for approaches A and B respectively) showing jump speciation (i.e. dispersal between non-adjacent areas) as an important pattern in range variation of Myrteae. The most probable ancestral areas for the stem and crown nodes of Myrteae (Fig. 5 "a", "b" respectively) is NCNZ in both analyses.

In the Australasian group the ancestral range of the crown node also has high probability of being NCNZ in both dating approaches but subsequent nodes show multiple shifts from NCNZ to Australia + NG and SEAsia and back to NCNZ. These shifts are estimated to date from the Eocene-Oligocene (shifts 2–7, Fig. 5A) in approach A and from the Oligocene to late Miocene (shifts 2–7, Fig. 5B) in approach B. The clade composed of the *Myrtus* group + Main Neotropical Lineage share a most likely ancestral area of South America for both approaches shifting from a previous NCNZ range (shift 1, Fig. 5) during the Paleocene (approach A) or the late-Eocene (approach B). The estimate of ancestral range for the stem and crown node of the *Myrtus* group presents an important difference between approaches A and B. In approach A an early South American range shifts to Central + North America range during the late Paleocene (shift 8, Fig. 5A) influenced by the distribution of *Chamguava* on the latter tectonic plate. This then shifts to the Mediterranean during the mid-Eocene for *Myrtus* (shift 9, Fig. 5A) and to South America for *Calycolpus* and *Accara* in the late-Eocene to early-Oligocene (shifts 10 and 11, Fig. 5A). In dating approach B, the crown node of the *Myrtus* group presents high probability of ancestral range in South America, shifting from there to the Mediterranean area during the late Oligocene for *Myrtus* (shift 8, Fig. 5B) and to Central + North America in the early Miocene for *Chamguava* (shift 9, Fig. 5B). In the Main Neotropical Lineage the most likely areas of ancestral range for both Approaches A and B is South America. In approach A, nine shifts from South to Central + North America (shifts 12, 14, 16, 18, 19, 23, 25, 27, 29, Fig. 5A) and seven shifts back to South America (shifts 13, 15, 16, 20, 24, 26, 28, Fig. 5A) are detected in this lineage. These occurred during the Eocene-Oligocene time slice and are observed in all clades with the exceptions of the *Myrceugenia* and *Myrteola* groups. In approach B, the same nine shifts from South to Central + North America are detected in the same groups (shifts 10, 11, 12, 13, 14, 17, 18, 19, 23, Fig. 5B). In approach B however, these shifts are no older than the early Miocene and no shifts back to South America are observed. Events of dispersion from the Neotropics (areas A and B) to the region of Australia + NG and NCNZ (areas C and D) are observed in the *Myrteola* and in *Eugenia* groups. In the *Myrteola* group this event is estimated in approach A to have occurred from South America to Australia + NG in the late Eocene (in *Lenwebbia*, shift 21, Fig. 5A) and afterwards to NCNZ (in *Neomyrtus* + *Lophomyrtus,* shift 22, Fig. 5A). In approach B, the same event is estimated to have occurred in the late Oligocene and with a higher probability for the route NCNZ to Australia + NG than the other way around (shifts 15 and 16, Fig. 5B). The *Eugenia* group presents a more complex series of dispersion events. In both approaches A and B, a shift from the Central + North America region to NCNZ is observed in the common ancestor of the clade containing the Australasian and African species (shift 29 in Fig. 5A and 20 in Fig. 5B). This lineage subsequently disperses to Africa + Madagascar (represented by *Eugenia rosapetiolata,* shift 30 in Fig. 5A and 21 in Fig. 5B) and to Southeast Asia (represented by *Eugenia reinwartdiana,* shift 31 in Fig. 5A and 22 in Fig. 5B). Even though the geographic sequence of events in this *Eugenia* clade is the same, the estimated date for these dispersion events in approach A is the late Oligocene, while in approach B it is at least 10 million years later, in the Miocene.

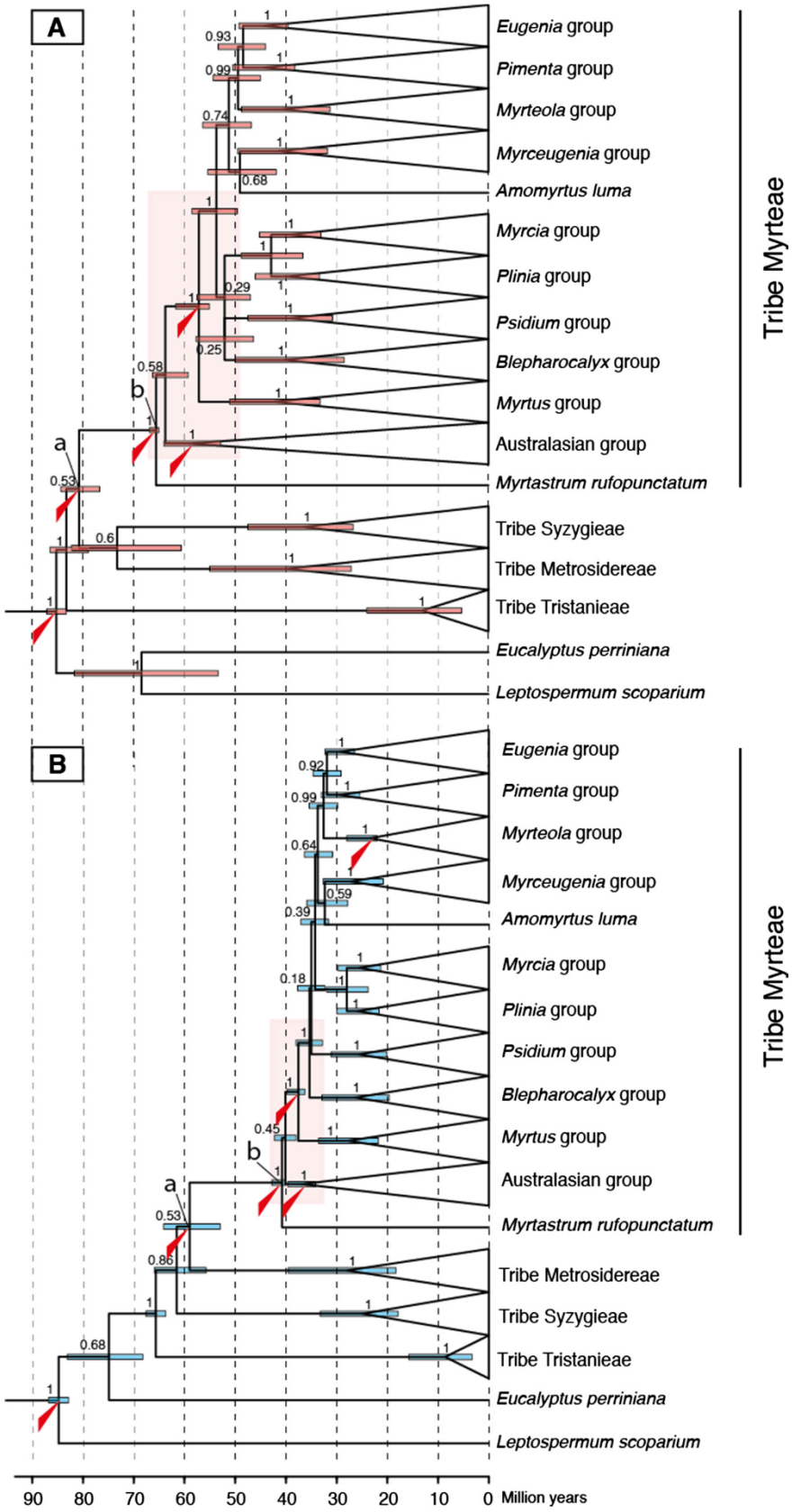

Fig. 4. Comparative dating analysis in Myrteae generated by Beast and based on two distinct fossil sets. (A) Calibration using macrofossil dataset (approach A). (B) Calibration using microfossil dataset (approach B). "a" and "b" indicate Myrteae stem and crown nodes respectively. Highlighted areas show divergence between the three major clades (Australasian and *Myrtus* groups and the Main Neotropical lineage) in each calibration. Fossil placements used to calibrate each chronogram are marked with red arrows and refer to estimations presented in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Median age estimations and 95% confidence intervals (CI) for dates of the main Myrteae nodes based on BEAST analysis.

	Approach A (Macrofossil) Age (95% HPD) in million of years		Approach B (Microfossil) Age (95% HPD) in million of years	
Clade	Stem	Crown	Stem	Crow
Myrteae	80.72 (76.64–84.27)	65.55 (65.03–66.80)	58.96 (53.00–64.07)	40.76 (40.03–42.76)
Australasian Lineage (Australasian group)	63.73 (59.25–66.24)	59.05 (52.80–63.96)	40.09 (38.01–42.22)	36.88 (34.16–39.62)
Myrtus group	57.09 (55.06–61.68)	42.34 (33.20–51.04)	37.56 (36.27–39.73)	27.78 (21.80–33.60)
Psidium group	52.03 (46.33–57.60)	39.12 (30.75–47.47)	35.01 (32.34–37.70)	25.62 (20.14–31.07)
Blepharocalyx group	52.03 (46.33–57.60)	40.15 (28.49–49.95)	35.36 (32.80–38.03)	26.38 (19.64–32.90)
Myrcia supergroup	42.85 (36.57–48.76)	39.19 (33.04–45.17)	27.99 (23.83–31.98)	25.58 (21.32–29.73)
Myrceugenia group	49.00 (41.84–55.34)	41.40 (31.72–49.42)	32.32 (27.85–35.86)	27.33 (20.83–32.62)
Plinia group	42.85 (36.57–48.76)	39.61 (33.35–46.00)	27.99 (23.83–31.98)	25.86 (21.66–29.93)
Eugenia supergroup	48.36 (44.01–53.22)	44.42 (39.58–49.17)	31.93 (29.16–34.63)	29.29 (26.55–32.29)

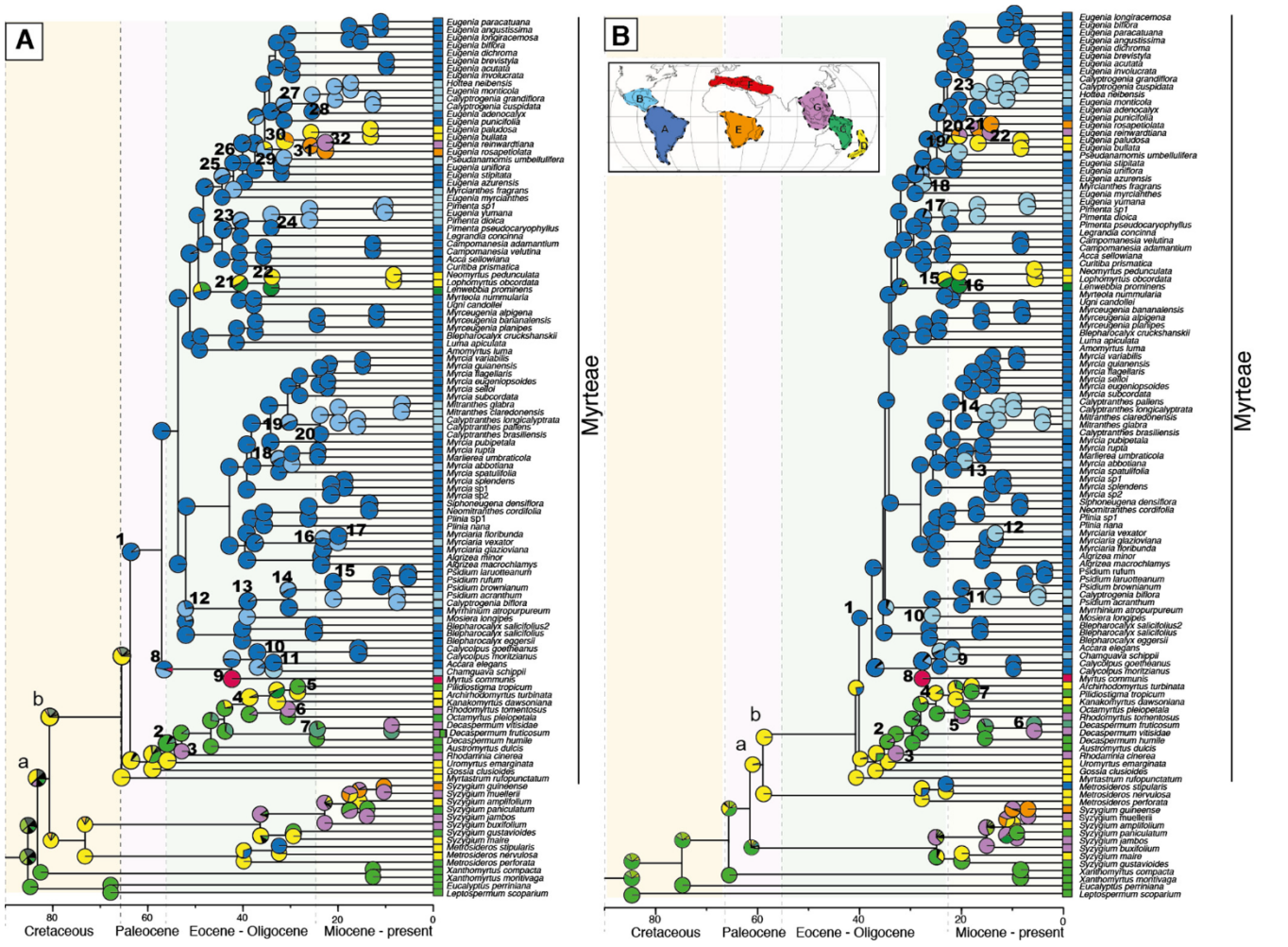

Fig. 5. Biogeographic inference recovered from BioGeoBEARS analysis in phylogenies dated with (A) Macrofossil dataset ($j = 0.0574$; LnL = −156.72), and (B) pollen fossil data set ($j = 0.055$; LnL = −161.48). "a" and "b" represent Myrteae stem and crown node respectively. Range shifts are numerated above pie charts.

3.4. Diversification rate shifts

Number of configuration shifts and log likelihood were higher than 1000 (significantly more than the recommended minimum of 200) after burnin for all BAMM analyses. Convergence between log likelihood and number of generations was observed in analysis with both callibrations (Approach A and B). The 95% credible set of rate shift configurations sampled with BAMM included 91 distinct shift configurations for approach A and 73 for approach B, of which the configurations with the highest probability included two or three shifts for both approaches. Posterior probability for a null model (i.e. no diversification rate shifts) was lower than could be estimated in both cases, therefore a Bayes factor was not calculated (see BAMM documentation). Thus, diversification rate heterogeneity is clear in the dataset. Mean phylorate through time is plotted for both chronograms in Fig. 6. In both approaches, the best configuration shift indicates three points of increasing diversification rates, all of which occur in the Main Neotropical Lineage. The highest shift configuration probability shows three shifts towards acceleration of diversification rates positioned in similar branches

in the two analyses: one in the common ancestor of most extant species of *Eugenia*, (Fig. 6Aa, Ba), one in the crown node of *Psidium* (Fig. 6Ab, Bb) and one in the common ancestor between *Plinia* and *Myrcia* groups (Fig. 6Ac, Bc). In approach A, shifts in the *Eugenia* and *Plinia* + *Myrcia* groups occurred at the mid or late-Eocene, while that in *Psidium* occurred at the Oligocene/Miocene boundary. In approach B, both shifts in the *Eugenia* and *Plinia* + *Myrcia* groups occurred at the Oligocene, while the one in *Psidium* dates to the mid-Miocene. Due to its younger dating estimation, approach B presents higher diversification rates through the tribe than approach A.

4. Discussion

4.1. Systematic implications

The phylogeny of Myrteae resulting from the combined dataset was reconstructed by a more informative molecular matrix and has considerably broader lineage sampling and higher statistical support in the deep nodes than those in previous works (e.g. Wilson et al., 2005; Lucas et al., 2005; Lucas et al., 2007; Murillo-A et al., 2012; Thornhill et al., 2015) and can be used to understand the systematics, evolution and ecology of the tribe more accurately. Low support in most branches from the nuclear database makes it difficult to evaluate potential incongruence between nuclear and cpDNA trees. There is not enough evidence to detect, for example, the role of ancient hybridization events in Myrteae history, usually noted by incongruence between these genomes (e.g. Soltis and Kuzoff, 1995). The only clear incongruence, the position of *Plinia* sp1 as sister to *Myrrhinium atropurpureum*, has to be investigated but may be an artefact of the sequencing process (e.g. contamination).

One of the main differences between this and previous phylogenetic hypotheses is the relative position of the three main lineages: the Australasian and *Myrtus* groups and the Main Neotropical Lineage. In the first phylogenetic works focused on the tribe (Lucas et al., 2005; Lucas et al., 2007), *Myrtus communis* appeared as the sister lineage to all extant Myrteae and the Australasian clade appeared sister to the equivalent Main Neotropical Lineage clade. With this broader sample however, it is evident that *Myrtus* forms part of a predominantly Neotropical lineage. Within the Main Neotropical lineage, novel subtribal relationships are the inclusion of the *Blepharocalyx* group, formally ungrouped (Lucas et al., 2005, 2007; Murillo-A et al., 2012) or placed next to *Pimenta* (de-Carvalho, 2013) and the position of *Algrizea*, previously unplaced (Lucas et al., 2007), within *Plinia* group (also shown but not discussed in Staggemeier et al., 2015). Another novelty is the division of the former *Pimenta* group genera (sensu Lucas et al., 2007) into two groups, the *Pimenta* group and the new *Psidium* group, and one ungrouped species *Amomyrtus luma*. The placement of *Amomyrtus luma* fluctuates, but the high support of the relationship between *Amomyrtus* and the *Myrceugenia* group in the cpDNA sataset, in addition to similar geographical distribution, might mean that this genus will be treated as *Myrceugenia* group in the future. Further analysis to better place this genus within Myrteae is desirable.

Genera that will require nomenclatural adjustment include: *Hottea*, *Pseudanamomis* (both nested inside *Eugenia*), *Calyptrogenia* (polyphyletic, with species nested in *Eugenia* and *Psidium*), *Mitranthes* (nested within *Myrcia* s.l.), *Eugenia* (polyphyletic, with at least

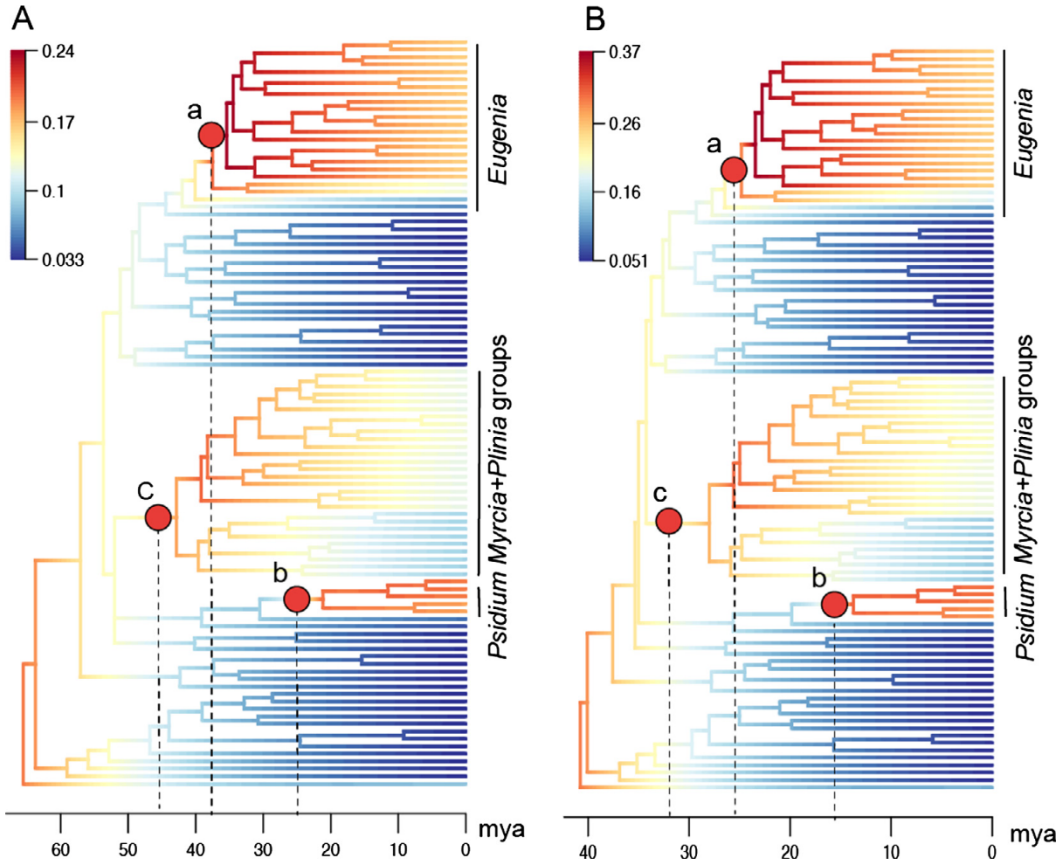

Fig. 6. Phylorate showing the single best shift configuration recovered from BAMM in chronograms resulting from (A) macrofossil calibration and (B) pollen fossil calibration. Three accelerating shifts on diversification rates (marked by "a", "b" and "c") are detected in each case. Color coding (blue to red) is in scale of species per million years. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

one species nested in *Pimenta*) and *Plinia* (paraphyletic). *Blepharocalyx* is known to be polyphyletic since the first molecular works in the tribe, likely requiring the resurrection of the genus *Temu* for *Blepharocalyx cruckshanksii* (see Lucas et al., 2007). *Calyptrogenia biflora* is noted to strongly resemble the continental America species *Psidium amplexicaule* Pers., but formal synonimization is required. A further important result from this phylogenetic topology is that it seems that the Caribbean, previously considered home to four endemic genera, apparently has no generic endemism in Myrteae, *as Hottea, Calyptrogenia, Mitranthes, and Pseudanamomis* are all nested inside larger widespread genera.

Of the five here unsampled, accepted genera in Myrteae (based on Wilson 2011), *Meteroromyrtus* has recently been shown to be nested in *Eugenia* (Wilson and Heslewood, 2016). The remaining four (*Myrtella* from New Guinea, Andean *Amomyrtella*, *Lithomyrtus* from Australia and *Stereocaryum* from New Caledonia) are still to be placed. These four genera present straight stamens in the bud, so based on this consistent morphological character it is likely that their positions will be other than within the *Myrcia*, *Plinia* or *Blepharocalyx* groups, in which stamens are consistently incurved (Vasconcelos et al., 2015). These results, in addition to the already proven polyphyletism of the classical subtribal classification based on embryo morphology (Lucas et al., 2007) brings consistency to the current understanding of Myrteae and its classification.

4.2. Comparative dating analysis

Results from comparative fossil calibration show important distinctions between estimated crown node ages using different approaches. Thornhill et al. (2012a) also contrast macro and microfossil calibration in Myrtaceae, combining the two fossil sets in a third calibration analysis. The fossils selected in the study presented here however, had to be placed on the same nodes so a combined dataset was not possible. Since calibration was performed with fossils of different ages on similar nodes in each approach, the resulting date distinction is expected but it is useful to demonstrate subjectivity when choosing fossil placement and how this influences interpretation of dates. Even though dates stabilize towards shallower nodes, especially when considering confidence intervals, overlap between dates from approaches A and B is still low (see Fig. 7).

Approach A, using only macrofossil data finds estimated dates similar to Sytsma et al. (2004) and Staggemeier et al. (2015), suggesting a first event of Myrteae diversification in the Paleocene. An estimated age near the KT boundary might link increased Myrteae species diversity to increased mammal and bird diversity following dinosaur extinction (Cracraft, 2001; Penny and Phillips, 2004). A preference of mammals and birds for fleshy berries may have provided a selective advantage over the capsular fruits of closely related tribes of Myrtaceae (Friis et al., 1987; Biffin et al., 2010). On the other hand, approach B finds a similar dates to Biffin et al. (2010) and Thornhill et al. (2012a), suggesting a first event of Myrteae diversification in the Eocene. In this approach, the explanation for the KT boundary above could be applied to the BKMSST clade (Myrteae and sister tribes, *sensu* Biffin et al., 2010) as this clade has other fleshy fruited Myrtaceae tribes and appears in approach B to date from the KT boundary (Thornhill et al., 2012a). In further support of approach B, the younger dates returned better explain the current distribution of Myrteae with less necessary LDDE events (see section below).

4.3. Biogeographical inference

The biogeographical analyses presented here provides a hypothesis of how Myrteae acquired its present Pantropical geographical distribution. Thornhill et al. (2015) and Berger et al. (2016) using a smaller Myrteae sample, recovered Australia as the most likely ancestral area of early diversification for Myrtaceae. The present study infers NCNZ as the ancestral range of Myrteae, with high probability in both approaches A and B (Fig. 5 "a" and "b"). There is evidence, however, that large portions of Zealandia, including New Caledonia and New Zealand, were underwater between the Eocene and Oligocene (Gibbs, 2004), casting doubt on a potential NCNZ Eocene origin suggested by the more recent dates of approach B. Some hypothesis, however, indicate that other adjacent land portions of the Zealandia continent were above sea level when NCNZ was submerged; these neighbouring islands could have acted as refugia, preserving representative biodiversity in Zealandia from lineages that have since undergone extinction in other continents (e.g. Australia) even when NCNZ was submerged (e.g. Condamine et al., 2016). This pattern would explain the survival and present distribution of *Myrtastrum*, a monotypic genus endemic to New Caledonian and sister to the rest of Myrteae. Even though a possible NCNZ origin can be explained, the safest conclusion may be that Myrteae shows an eastern Gondwana ancestral area that today is represented by NCNZ and also Australia + NG. Reasons for this include the proximity of the Zealandia and Australian plate during that period (Trewick et al., 2007), the possibility that NCNZ species diversity observed today is a relict of more widespread lineages (as reasoned above) and the possibility that incomplete sampling of some deeper-node genera is biasing the analysis (*Gossia* and *Uromyrtus*, for instance are also diverse in Australia + NG (WCSP, 2016) but area coding according to species distribution influenced the reconstruction towards NCNZ).

Approaches A and B show similar area shifts (numbered in Fig. 5), but occurring during distinct time periods. The older age estimation of approach A causes it to present more area shifts (32 in comparison with 23 from approach B), perhaps due to area adjacencies of different time slices (see Supplementary Material 6). The dating divergences between approaches also affect the number

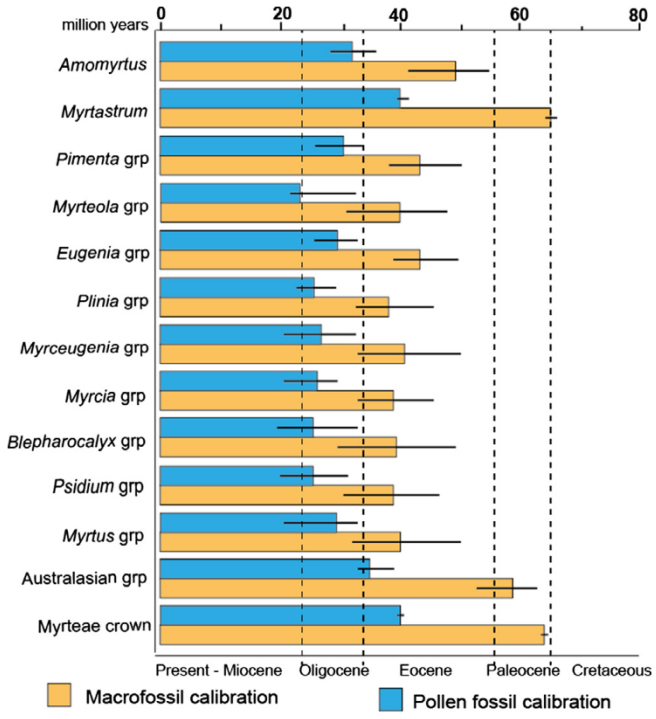

Fig. 7. Graph comparing crown node ages of macrofossil calibration (orange) and pollen fossil calibration (blue). Bars show confidence intervals per node. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of LDDE events necessary to explain the current distribution in Myrteae (see summary in Table 3). Although events of LDDE are an important process in angiosperm biogeography (Crisp et al., 2011), long transmarine diversification events are considered less likely than short distance dispersion and diversification by vicariance or continental population isolation (Howe and Smallwood, 1982). The first area shift recorded in both approaches A and B is the transition from NCNZ to South America from the stem to the crown node of the clade containing *Myrtus* group and the Main Neotropical Lineage (shift 1, Fig. 5A and B). LDDE is unlikely here as until around 40 mya, South America was still linked to portions of eastern Gondwana, forming a single continent connected by Antarctica (McLoughlin, 2001). It is possible that, after initial diversification in eastern Gondwana, Myrteae became widespread throughout Antarctica and South America; there is evidence that global temperature was much warmer in the early Cenozoic (Huber et al., 1995) and that rainforest vegetation covered Antarctica until around 30 mya (Francis and Poole, 2002; Francis et al., 2008). Abundant Myrtaceae fossil records found at high latitudes in South America, southern Patagonia and nearby Antarctica (Supplementary Material 5, Eklund, 2003; Hayes et al., 2006; Francis et al., 2008) also provide evidence for this hypothesis. The scenario of a widespread Myrteae throughout these continents, followed by their late-Eocene disconnection (McLoughlin, 2001) and Miocene Antarctica glaciation (Kennett et al., 1975) with consequent vicariance between the Australasian group and *Myrtus* group + Main Neotropical Lineage on distinct sides of the globe is likely in both dating scenarios.

In the Australasian group, most area shifts between SE Asia, Australia + NG and NCNZ, in both approaches, occurred in a period range where proximity between these continents did not require LDDE events. The only exception is *Rhodamnia cinerea* that shifts from Australia + NG to SE Asia (shift 3, Fig. 5A and B) in the Eocene to early Oligocene; this may only be explained by LDDE, given the distance between these areas in that period (McLoughlin, 2001). In both approaches A and B, there is evidence for a quick northerly vertical expansion into the whole of South America soon after initial diversification in that continent. In approach A, a series of shifts back and forth South America and Central + North America are observed occurring mostly from the early Eocene to the late Oligocene. Such area shifts, however, would require multiple LDDE events, because these two continents were too far apart during that period (McLoughlin, 2001). Similar area shifts in approach B are estimated to have occurred much more recently, mostly during the Miocene, when South and North America were closer together or connected by the Panama Isthmus (Montes et al., 2015) suggesting short distance dispersion events. The only exception is the diversification of *Myrcianthes fragrans* to the greater Antilles that would require an LDDE event in both approaches.

Based on past phylogenic position and northern hemisphere distribution, past studies proposed that the current geographical range of *Myrtus* might be a relic from a much wider distribution of Myrteae (Berry, 1915; Thornhill et al., 2015). However, the highly supported sister relationship of *Myrtus* to exclusively Neotropical genera, including Central American *Chamguava*, provides evidence of vertical movement through the American continents towards the Mediterranean, perhaps by relatively short distance dispersal via what is today Greeenland and northern Europe, under a warmer paleo-climatic regime (Zachos et al. 2001). Possible evidence for this event is the presence of the *Paleomyrtineae* fossil from this period in North Dakota (Pigg et al., 1993). The diversification of the *Myrtus* group from South to Central + North America in the Paleocene as estimated by approach A (shift 8, Fig. 5A) is possible without LDDE events due to the Nicoya island complex, which linked present day Ecuador and Central America during that period (Dengo, 1975; Gentry, 1982). In approach B, the shift between South America to Central + North America in the stem node of the *Myrtus* group is not recovered. In this approach, the estimated shift occurs from South America straight to Mediterranean Europe (shift 8, Fig. 5B). Nevertheless, much later dates for this shift in this approach means that a similar route from South to Central + North America and Europe would be possible without LDDE events, because of the proximity of these continents in the Miocene. *Myrtus* genetic diversification varies however, from the east to west of its range (Migliore et al., 2012), not congruent with vertical movement through the American continent. This complex pattern requires future research.

Two clades (*Myrteola* and *Eugenia* groups) within the Main Neotropical Lineage also have representatives in Australia + NG, SE Asia and Africa, but these colonisation events likely occurred in different periods and by different processes. Antarctica remained habitable and in proximity to NCNZ and South America until the late Oligocene (Francis et al., 2008). In both approaches A and B (when considering upper confidence interval limits), the shift in ancestral area in the *Myrteola* group from South America to NCNZ and Australia + NG occurred before this bridge was severed by ice-sheet formation, suggesting the possibility of terrestrial migration or Antarctic colonization followed by vicariance, giving the *Myrteola* group a *Nothofagus*-like distribution (van Stenis, 1971, Swenson et al., 2001). Adaptations that may have allowed this group to achieve this range and survival in Antarctica until later than sister lineages even in colder climates, include their shrubby habit, winter seed dormancy (Smith-Ramirez et al., 1998) and likely frost resistant wood anatomy (Schmid and Baas, 1984), uncommon in other Myrteae (Lucas et al., 2007).

Due to stabilization of dates at the shallower nodes and considering the confidence intervals, Australasian and African *Eugenia* events of dispersion are estimated to have occurred at similar dates, around the late Oligocene-early Miocene, in both dating approaches. Considering an ancestral area of Central + North America for the clade and that Antarctica was already covered by ice-sheets and no longer habitable (Zachos et al., 1991; Ivany et al., 2006) at the Miocene, the only scenario possible to explain Eugenia's current pantropical distribution is a series of LDDE events (similar to other plant groups such as *Psychotria*, Matzke, 2013, and Simaroubaceae, Clayton et al., 2009). The picture proposed by the results of biogeographic analysis is that this event was towards the east, from the Caribbean (in *Pseudanamomis*) colonizing first NCNZ, then Africa and lastly SE Asia, but a larger *Eugenia* sample from these regions may prove otherwise. Particular abilities of the *Eugenia* lineage that underwent long-distance dispersal, to cross marine boundaries, might explain why species of this group are also found in many islands of the Indian and Pacific oceans. Many (possibly all) South African species of *Eugenia* are cryptically dioecious, a character unrecorded for the genus out of Africa (van der Merwe et al., 2005, Vasconcelos pers. obs.). Dioecy is linked to small green or white flowers, generalistic pollination systems and to island floras where in extreme cases, such as Hawaii, over a quarter of the species can be dioecious (Bawa, 1980). It is possible that dioecy of extant South African *Eugenia* species is a legacy of island-hopping ancestors. Further research focused on innovative reproductive characteristics necessary for such dispersal, such as co-evolution with migratory birds, seed resistance and self-compatibility (Baker, 1955) will be necessary to better understand the unique distribution patterns of this group.

4.4. Changes in diversification rates, key innovations and mega-diverse genera

This study demonstrates heterogeneity of diversification rates in Myrteae. Both dating approaches return similar results in this case: the three main accelerating shifts of diversification rates

Table 3
Summary of most likely events responsible for area shifts in Myrteae based on age period and confidence intervals. LDDE events were considered when distance between areas are recorded as 0.1 or 0.5 for the time slice (see Supplementary Material 6).

Shift Number (Fig. 5)	Approach A shifts	Area shift	Age (CI 95%)	Geological time	Likely nature of event inferred by period age
1	Neotropical stem - crown	NCNZ - South America	63.73 (59.25–66.24)	early-Paleocene	Land migration and vicariance
2	Australasian group - first shift to Australia	NCNZ - Australia + NG	55.93 (49.52–61.56)	early-Eocene	Short distance dispersal and/or vicariance
3	Australasian group - *Rhodamnia*	Australia + NG - SE Asia	52.89 (46.14–58.78)	Early-Eocene	LDDE only
4	Australasian group - shift to Zealandia	Australia + NG - NCNZ	43.96 (37.16–50.39)	Mid-Eocene	Short distance dispersal and/or vicariance
5	Australasian group - second shift to Australia	NCNZ - Australia + NG	28.64 (20.27–36.84)	Early-Oligocene	Short distance dispersal and/or vicariance
6	Australasian group - *Rhodomyrtus*	NCNZ - SE Asia	30.76 (22.17–38.85)	Early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and/or vicariance
7	Australasian group - *Decaspermum*	Australia + NG - SE Asia	24.52 (15.79–33.66)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and/or vicariance
8	*Myrtus* group - North American shift	South America to Central + North Am	57.08 (55.06–61.68)	Late-Paleocene	Short distance dispersal and/or vicariance
9	*Myrtus* group - *Myrtus*	South America to Mediterranean EU	42.34 (33.19–51.04)	Mid-Eocene	Short distance dispersal and/or vicariance
10	*Myrtus* group - South America shift (*Calycolpus*)	Central + North Am to South America	37.37 (28.58–46.19)	Late-Eocene	LDDE only
11	*Myrtus* group - South America shift (*Accara*)	Central + North Am to South America	33.56 (24–42.78)	Early-Oligocene	LDDE only
12	*Psidium* group - stem	South America to Central + North Am	52.03 (46.33–57.6)	Early-Eocene	LDDE, but upper CI limit also allows short distance dispersal and/or vicariance
13	*Psidium* group - first shift to South America	Central + North Am to South America	39.12 (30.75–47.47)	Mid-Eocene	LDDE only
14	*Psidium* group - Caribbean *Psidium*	South America to Central + North Am	30.5 (22.7–38.74)	Early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal or vicariance
15	*Psidium* group - second shift to South America	Central + North Am to South America	21.15 (14.66–28.9)	Early-Miocene	Short distance dispersal or vicariance
16	*Plinia* group - *Myrciaria*	South America to Central + North Am	23.15 (15.89–31.29)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal or vicariance
17	*Plinia* group - *Myrciaria*	Central + North Am to South America	20.23 (12.97–28.33)	Early-Miocene	Short distance dispersal and/or vicariance
18	*Myrcia* group - first North American shift	South America to Central + North Am	32.98 (26.47–40.14)	Early-Oligocene	LDDE only
19	*Myrcia* group - shift to South America	Central + North America to South America	30.59 (22.72–37.25)	Early-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
20	*Myrcia* group - second North American shift	South America to Central + North Am	23.79 (16.89–30.79)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
21	*Myrteola* group - New Zealand	South America to NCNZ	40.64 (31.28–48.68)	Mid-Eocene	Short distance dispersal and/or vicariance
22	Myrteola group - Australia	NCNZ - Australia + NG	34.14 (23.40–43.89)	Late-Eocene	Short distance dispersal and/or vicariance
23	Pimenta group - North American shift	South America to Central + North Am	41.58 (34.48–48.24)	Mid-Eocene	LDDE only
24	Pimenta group - Pimenta pseudocaryophyllus	Central + North Am to South America	34.08 (26.07–41.98)	Late-Eocene	LDDE only
25	Eugenia crown - Myrcianthes	South America to Central + North Am	44.42 (39.58–49.17)	Mid-Eocene	LDDE only
26	Eugenia - shift back SA	Central + North Am to South America	42.01 (37.38–46.86)	Mid-Eocene	LDDE only
27	Eugenia - shift Umbellatae caribbean	South America to Central + North Am	31.38 (26.55–36.41)	Early-Oligocene	LDDE only
28	Eugenia - shift Umbellatae back to SA	Central + North Am to South America	25.7 (20.33–30.93)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance

(*continued on next page*)

29	Eugenia - Pseudanamomis	South America to Central + North Am	35.42 (31.02–39.08)	Late-Eocene	LDDE only
30	Eugenia - NCNZ	Central + North Am to NCNZ	31.24 (25.69–36.73)	Early-Oligocene	LDDE only
31	Eugenia - Africa	NCNZ to Africa	25.72 (20.04–31.55)	Late-Oligocene	LDDE only
32	Eugenia - SA Asia	Africa to SE Asia	22.75 (16.15–28.88)	Early-Miocene	Land migration

Shift Number	Approach B shifts	Nature and timing of tested geological event	Age (HPD 95% interval)	Geological time	Likely nature of event inferred by age
1	Neotropical stem - crown	NCNZ to South America	40.09 (38.01–42.21)	Late-Eocene	Land migration and vicariance
2	Australasian grp - first Australia shift	NCNZ to Australia + NG	35.15 (31.99–38.61)	Late-Eocene	Short distance dispersal and/or vicariance
3	Australasian grp - Rhodamnia	Australia + NG to SE Asia	33.37 (29.81–36.96)	Early-Oligocene	LDDE only
4	Australasian grp - shift to Zealandia	Australia + NG to NCNZ	25 (21.07–29)	Late-Oligocene	Short distance dispersal and/or vicariance
5	Australasian grp - Rhodomyrtus	Australia + NG to SE Asia	19.85 (14.64–24.64)	Early-Miocene	Short distance dispersal and/or vicariance
6	Australasian grp - Decaspermum	Australia + NG to SE Asia	5.87 (2.75–9.9)	Late-Miocene	Short distance dispersal and/or vicariance
7	Australasian grp - Pilidiostigma	NCNZ to Australia + NG	18.23 (13.35–23.15)	Early-Miocene	LDDE, but upper CI limit also allows short distance dispersal and vicariance
8	Myrtus group - Myrtus	South America to Mediterranean EU	27.78 (21.79–33.60)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
9	Myrtus group - Chamguava	South America to Central + North Am	22.03 (15.88–28.22)	Early-Miocene	Short distance dispersal and/or vicariance
10	Psidium group - Moseira	South America to Central + North Am	25.62 (20.14–31.07)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
11	Psiidum group - Caribbean Psidium	South America to Central + North Am	13.73 (9.38–18.58)	Mid-Miocene	Short distance dispersal and/or vicariance
12	Plinia group - Myrciaria	South America to Central + North Am	13.55 (8.38–18.86)	Mid-Miocene	Short distance dispersal and/or vicariance
13	Myrcia group - M. abbotiana	South America to Central + North Am	19.59 (14.70–24.39)	Early-Miocene	Short distance dispersal and/or vicariance
14	Myrcia group - Calyptranthes	South America to Central + North Am	12.73 (8.27–17.35)	Mid-Miocene	Short distance dispersal and/or vicariance
15	Myrteola group - Australia	South America to Australia + NG	23.39 (22.04–28.02)	Late-Oligocene	Land migration and vicariance
16	Myrteola group - New Zealand	Australia + NG to NCNZ	20.45 (14.55–26.16)	Early-Miocene	LDDE, but upper CI limit also allows short distance dispersal and vicariance
17	Pimenta group - North American shift	South America to Central + North Am	22.52 (17.52–27.46)	Early-Miocene	Short distance dispersal and/or vicariance
18	Eugenia - Myrcianthes	South America to Central + North Am	27.72 (24.83–30.71)	Late-Oligocene	LDDE only
19	Eugenia - shift three - Pseudanamomis	South America to Central + North Am	23.44 (21.88–27.99)	Late-Oligocene	LDDE, but lower CI limit also allows short distance dispersal and vicariance
20	Eugenia - NCNZ	Central + North Am to NCNZ	20.69 (17.24–24.1)	Early-Miocene	LDDE only
21	Eugenia - Africa	NCNZ to Africa	16.87 (12.07–20.43)	Early-Miocene	LDDE only
22	Eugenia - SE Asia	Africa to SE Asia	14.96 (10.82–19.06)	Mid-Miocene	Land migration
23	Eugenia - shift two - Umbellatae	South America to Central + North Am	16.93 (13.58–20.36)	Early-Miocene	Short distance dispersal and/or vicariance

occurred in the Main Neotropical lineage. This explains why species diversity of the tribe in this continent is ten times higher than in the Old World (Lucas et al., 2007; WCSP 2016). In evolutionary biology, some of the most plausible explanations for changes in diversification rates are related to acquisition of new biological traits in the lineage (e.g. key-innovations, Donoghue, 2005). This is a reasonable hypothesis for Myrteae: differences in characters related to embryo morphology in *Myrcia, Plinia* and *Eugenia* have been proposed as adaptive advantages for these groups (Landrum, 1986; Landrum and Stevenson, 1986). The *Plinia* and *Eugenia* groups, with independent origins, present homogeneous cotyledons that have been related to seedling starch storage (Landrum, 1986) while *Myrcia* have leaf-like, well developed embryos that allow faster germination. These embryo forms are different from extant Myrteae that do not exhibit these specialisations.

The accelerating diversification rate shift in *Psidium* however, is less likely to be linked to the embryo as in this group it is similar to those found in the Australasian and *Pimenta* groups (Landrum and Stevenson, 1986). A possible explanation for the success of *Psidium* may be linked to cytogenetic events: *Psidium* is the Myrteae lineage with the highest documented cases of polyploidy (Costa et al., 2008), frequently associated with increased fitness (Wood et al., 2009; Madlung, 2013). The bony *Psidium* testa opening via an operculum (a synapomorphy of the genus) through which germination occurs (Landrum and Stevenson, 1986) may also be a factor, promoting mechanical seed dormancy conducive to success in seasonal environments. It is also notable that all invasive species of Myrteae are *Psidium* (Richardson and Rejmanek, 2011), showing adaptive features of this lineage that might be linked to its higher diversification rate.

5. Conclusions remarks and future directions

This work provides an up to date phylogeny to be used as a base for further systematic and modelling studies in Myrteae. The dating, biogeography and diversification patterns analyses clarify the evolutionary picture of the most diverse tribe in Myrtaceae, but also raise a number of avenues for future studies. These include, for instance: a better resolution for the relationships in the backbone of the main Neotropical lineage; nomenclatural changes in poly and paraphyletic genera; formalization of subtribal nomenclature; detailed biogeographical analysis of individual clades; the importance of high southern latitudes in early Myrteae diversification events; and better links between acceleration shifts in diversification rates and trait evolution. Results from the comparative dating approaches using macro and microfossil separately show how the choice of fossil set and placement interpretation affects all interpretation of subsequent evolutionary analysis. Calibration using pollen fossil evidence (approach B) requires less LDDE events to explain current Myrteae distribution. This, in addition to the reasoning provided in the Section 2.4 (*Fossil calibration and Dating*), suggests that this dating approach is more reliable and should be preferred by future studies in Myrteae.

Acknowledgements

We thank CAPES, CNPq, Reflora and the Emily Holmes Memorial Scholarship for funding laboratorial work and fieldtrips. For assistance in the field and collection permits we thank A. Veloz (Dominican Republic), D. Bogarín (Costa Rica), L. Barrabé (New Caledonia), J. Meikle, T. Commock (Jamaica), N. Taylor, E. Velautham (Singapore), and J. Nais (Sabah - Malaysia). We also thank the institutions that issued collection permits: IBAMA, SISBIO (Brazil), NEPA (Jamaica), Sinac, Conagebio (Costa Rica), JBSD herbarium (Dominican Republic), Assemblee de la Priovince Nord, Assemblee de la Priovince Sud (New Caledonia), NParks (Singapore) and Sabah Biodiversity Centre (Sabah – Malaysia). Laboratorial assistance is kindly thanked to L. Csiba, P. Malakasi, D. Devey, R. Duque-Thues and L.L. Santos. Analysis help is thanked to F. Forest. For valuable ideas and insights, we thank M. Sobral and A. Franc. We also would like to thank A. Thornhill and two anonymous reviewers for useful comments in the manuscript.

Appendix A

Sample list, collection localities and Genbank accession numbers for the species used in the phylogenetic analysis. *Accession numbers represent different vouchers from those indicated in the voucher column (see Genbank for more information). Blank spaces represent missing data in the molecular matrix.

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Acca sellowiana (O.Berg) Burret	E. Lucas 205	RBG Kew (cultivated)	AM234067	AM489973	This study	AM489807			This study	
Accara elegans (DC.) Landrum	T. Vasconcelos 485	Brazil (Minas Gerais)	This study	This study	This study	This study	This study	This study	This study	
Algrizea macrochlamys (DC.) Proença & NicLugh.	A. Giulietti 1648	Brazil (Bahia)	AM234126	AM489975	This study	AM489809	This study	This study	JN091320	KP722283
Algrizea minor Sobral, Faria & Proença	J.E.Q. Faria 4157	Brazil (Bahia)	This study		This study	This study	This study	This study		This study
Amomyrtus luma (Molina) D. Legrand & Kausel	RBGE 1996-1065	RBG Edinburgh (cultivated)	AM234073	KM065305*	This study	AM489811		This study	This study	
Archirhodomyrtus turbinata (Schltr.) Burret	J. Soewarto HB 11	New Caledonia	This study		This study	This study	This study	This study	This study	This study
Austromyrtus dulcis (C.T.White) L.S. Sm.	S. Belsham M77	Australia (Queensland)	This study	AM489977	This study	AM489813				This study
Blepharocalyx cruckshanksii (Hook. & Arn.) Nied. in H.G.A.Engler & K.A. E.Prantl	RBGE 1998-073D; [a]Murillo 4219	RBG Edinburgh (cultivated)	AM234070	AM489978	This study	AM489814	JN660956[a]	JN661055[a]		JN661105[a]
Blepharocalyx eggersii (Kiaersk.) Landrum	T. Vasconcelos 458	Brazil (Bahia)	This study	This study	This study	This study	This study	This study	This study	This study
Blepharocalyx salicifolius (Kunth) O. Berg	E. Lucas 78	Brazil (São Paulo)	AM234084	AM489979	This study	AM489815	JN660984*	JN661083*	This study	JN661133*
Blepharocalyx salicifolius (Kunth) O. Berg	T. Vasconcelos 482	Brazil (Minas Gerais)	This study	This study	This study	This study	This study		This study	This study
Calycolpus goetheanus (Mart. ex DC.) O.Berg	T. Vasconcelos 332	Brazil (Amazonas)	This study	This study	This study	This study	This study	This study	This study	This study
Calycolpus moritzianus (O.Berg) Burret	(all from GenBank)	Colombia	KU945986	KU945991		KU945999				
Calyptranthes brasiliensis Spreng.	E. Lucas 930	Brazil (Espirito Santo)	This study		This study	This study	This study	This study		
Calyptranthes longicalyptrata B. Holst & M.L. Kawas.	T. Vasconcelos 523	Costa Rica			This study		This study		This study	This study
Calyptranthes pallens Griseb.	T. Vasconcelos 534	Costa Rica	This study	This study	This study	This study	This study		This study	This study
Calyptrogenia biflora Alain	T. Vasconcelos 565	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Calyptrogenia cuspidata Alain	T. Vasconcelos 593	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Calyptrogenia grandiflora Burret	T. Vasconcelos 588	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Campomanesia adamantium (Cambess.) O.Berg	T. Vasconcelos 474	Brazil (Minas Gerais)	This study		This study	This study	This study	This study	This study	This study
Campomanesia velutina (Cambess.) O.Berg	T. Vasconcelos 507	Brazil (Distrito Federal)	This study		This study	This study	This study	This study	This study	This study

Appendix A (*continued*)

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Chamguava schippii (Standl.) Landrum	D. Aguilar 9833	Costa Rica	This study	This study	This study	This study	This study	This study	This study	This study
Curitiba prismatica (D.Legrand) Salywon & Landrum	D.F. Lima 551	Brazil (Paraná)	This study	This study	This study	This study	This study	This study	This study	This study
Decaspermum fruticosum J.R.Forst. & G.Forst	T. Vasconcelos 730	Malaysia (Sabah)	This study	This study	This study	This study	This study			This study
Decaspermum humile (Sweet ex G. Don) A.J.Scott	S. Belsham M82	RGB Melbourne (cultivated)	AM234128	This study	AY498780*	AM489824	This study		This study	
Decaspermum vitis-idaea Stapf	T. Vasconcelos 729	Malaysia (Sabah)	This study		This study	This study	This study	This study	This study	
Eucalyptus perriniana F.Muell. ex Rodway	E. Lucas 283	RBG Kew (cultivated)	AM234139	AM489985	This study	AM489825	This study	This study	This study	This study
Eugenia acutata Miq.	T. Vasconcelos 506	Brazil (Distrito Federal)	This study		This study	This study	This study	This study		This study
Eugenia adenocalyx DC.	A. Giaretta 1441	Brazil (Roraima)	This study		This study	This study	This study	This study		This study
Eugenia angustissima O.Berg	T. Vasconcelos 405	Brazil (Goias)	This study		This study	This study	This study	This study	This study	This study
Eugenia azurensis O.Berg	J.E.Q. Faria 4186	Brazil (Bahia)	This study		This study	This study	This study	This study	This study	
Eugenia biflora (L.) DC.	F.F. Mazine 1075	Brazil	KJ187610	This study	This study	KJ469659			This study	
Eugenia brevistyla D.Legrand	F.F. Mazine 993	Brazil	KJ187614		This study	KJ469663			This study	
Eugenia bullata Pancher ex Guillaumin	T. Vasconcelos 608	New Caledonia	This study		This study	This study	This study	This study	This study	This study
Eugenia bunchonsiifolia Nied.	T. Vasconcelos 466	Brazil (Espirito Santo)	This study		This study	This study	This study	This study		This study
Eugenia involucrata DC.	T. Vasconcelos 256	Brazil (Distrito Federal)	This study		This study	This study	This study	This study	This study	This study
Eugenia longiracemosa Kiaersk.	T. Vasconcelos 310	Brazil (Amazonas)	This study		This study	This study	This study	This study		This study
Eugenia monticola (Sw.) DC.	T. Vasconcelos 566	Dominican Republic	This study	JQ588481*	This study	This study	This study	This study	This study	This study
Eugenia myrcianthes Nied.	Savassi ESA 85681	Brazil	KJ187652	This study	AY498784	KJ469702	This study	This study		This study
Eugenia paludosa Pancher ex Brongn. & Gris	T. Vasconcelos 646	New Caledonia	This study		This study	This study	This study	This study		This study
Eugenia paracatuana O.Berg	P.O. Rosa 1399	Brazil (Goias)	This study			This study	This study	This study		This study

(*continued on next page*)

Appendix A (*continued*)

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Eugenia punicifolia (Kunth) DC.	F.F. Mazine 1065	Brazil (Mato Grosso)	This study		This study	AM489827*			This study	
Eugenia reinwardtiana (Blume) DC.	B. Holst 8870	MSBG (cultivated)	This study	KM894685*	This study		AY463131*		This study	
Eugenia roseopetiolata N.Snow & Cable	T. Vasconcelos s.n.	RBG Kew (cultivated)	This study		This study	This study	This study	This study	This study	This study
Eugenia stipitata McVaugh	T. Vasconcelos 677	Singapore BG (cultivated)	This study		This study	This study	This study	This study	This study	
Eugenia uniflora L.	E. Lucas 207	RBG Kew (cultivated)	AM234088	AM489986	This study	AM489828	AF215627*		KP722326	KP722202
Eugenia yumana Alain	T. Vasconcelos	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Gossia clusioides (Brongn. & Gris) N. Snow	J. Soewarto HB 14	New Caledonia	This study		This study	This study	This study	This study	This study	This study
Hottea neibensis Alain	T. Vasconcelos 590	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Kanakomyrtus dawsoniana N.Snow	T. Vasconcelos 639	New Caledonia	This study		This study	This study	This study	This study		
Legrandia concinna (Phil.) Kausel	RBGE 1999–0656	RBG Edinburgh (cultivated)	AM234072	AM489990	This study	AM489839				
Lenwebbia prominens N.Snow & Guymer	N. Snow 7463	Australia (Queensland)	This study	AY521538*		This study		This study		
Leptospermum scoparium J.R.Forst. & G.Forst.	E. Lucas 284		AM234142	AM489991	AM235423	AM489840	AM235459		KF591267	
Lophomyrtus obcordata (Raoul) Burret	S. Belsham M41	New Zealand	AM234146	AM489993	This study	AM489842	This study	This study		
Luma apiculata (DC.) Burret	E. Lucas 208	RBG Kew (cultivated)	AM234101	AM489995	AY498795	AM489843	JN660959*	This study	KP722331	KP722209
Marlierea umbraticola (Kunth) O. Berg	M.A.D. Souza s. n.	Brazil (Amazonas)	KP722392		KP722470	KP722300	This study	This study	KP722350	KP722246
Metrosideros nervulosa C.Moore & F. Muell.	(all from GenBank)		JF950784	DQ088535	AY498802		DQ088395		JF950929	
Metrosideros perforata (J.R.Forst. & G.Forst.) Druce	E. Lucas 209	RBG Kew (cultivated)	AM234141	AM489998	This study	AM489848	This study	This study	This study	
Metrosideros stipularis (Hook. & Arn.) Hook.f.	(all from GenBank)		AM234071	AF368222		AM489884				
Mitranthes clarendonensis (Proctor) Proctor	T. Vasconcelos 511	Jamaica	This study		This study	This study	This study		This study	This study
Mitranthes glabra Proctor	E. Lucas 1224	Jamaica	This study		This study	This study	This study	This study	This study	This study
Mosiera longipes (O.Berg) Small	Salywon 1183	U.S.A. (Florida)	This study		This study	This study	This study	This study	This study	
Myrceugenia alpigena (DC.) Landrum	E. Lucas 167	Brazil (Minas Gerais)	AM234098	JN660991	KP722441	AM489854	JN660941.	This study	KP722376	JN661090

Appendix A (*continued*)

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Myrceugenia bananalensis Bezerra & Landrum	J.E.Q. Faria 4049	Brazil (Distrito Federal)	This study		This study	This study	This study	This study	This study	This study
Myrceugenia planipes (Hook. & Arn.) O.Berg	L. Landrum s.n.	Chile	This study	JN661027*	This study	This study	This study	This study		
Myrcia abbotiana (Urb.) Alain	T. Vasconcelos 571	Dominican Republic	This study				This study	This study		
Myrcia rupta M.L.Kawas. & B. Holst	T. Vasconcelos 311	Brazil (Amazonas)	This study		This study	This study	This study	This study		This study
Myrcia eugeniopsoides (D.Legrand & Kausel) Mazine	E. Lucas 61	Brazil (Sao Paulo)	AM234107	AM489996	KP722429	AM489845	This study	This study	JN091327	KP722205
Myrcia flagellaris (D.Legrand) Sobral	E. Lucas 83	Brazil (Sao Paulo)	AM234113	AM489989	KP722430	AM489836	This study	This study	JN091350	KP722206
Myrcia guianensis (Aubl.) DC.	Harley 50307	Brazil	JN091225	This study	This study	This study	This study		JN091351	
Myrcia pubipetala Miq.	E. Lucas 86	Brazil (Sao Paulo)	AM234114	AM490001	KP722426	AM489855	This study	This study	JN091364	KP722273.
Myrcia selloi (Spreng.) N.Silveira	E. Lucas 110	Brazil	JN091240	JN091315	KP722436	JN091431	This study	This study	JN091371	KP722212
Myrcia sp2	J.E.Q. Faria 4193	Brazil (Bahia)	This study		This study	This study	This study	This study		
Myrcia sp1	T. Vasconcelos 307	Brazil (Amazonas)	This study		This study	This study	This study	This study	This study	This study
Myrcia spathulifolia Proença	J.E.Q. Faria 4214	Brazil (Bahia)	This study		This study	This study	This study	This study		This study
Myrcia splendens (Sw.) DC.	T. Vasconcelos 587	Dominican Republic	This study		This study	This study	This study	This study	This study	
Myrcia subcordata DC.	M. Santos 586	Brazil (Minas Gerais)	This study		This study	This study	This study	This study	This study	This study
Myrcianthes fragrans (Sw.) McVaugh	B. Holst 8862	Guyane	KJ187655	KJ772955	AY498803*	KJ469705				
Myrciaria floribunda (H.West ex Willd.) O.Berg	T. Vasconcelos 388	Brazil (Amazonas)	This study		This study	This study	This study	This study	This study	This study
Myrciaria glazioviana (Kiaersk.) G.M. Barroso ex Sobral	T. Vasconcelos 413	Brazil (Bahia)	This study		This study	This study	This study	This study	This study	This study
Myrciaria vexator McVaugh	T. Vasconcelos 709	Singapore BG (cultivated)	This study	AY521544*	This study	This study	This study	This study	This study	This study
Myrrhinium atropurpureum Schott in K.P.J.Sprengel	Costa, I.R. 594	Brazil (Rio de Janeiro)	This study		This study	This study	This study	This study	This study	This study
Myrtastrum rufopunctatum (Pancher ex Brongn. & Gris) Burret	J. Soewarto HB 10	New Caledonia	This study	This study	This study	This study	This study	This study	This study	This study
Myrteola nummularia (Lam.) O.Berg	RBGE 1996–1096	RBG Edinburgh (cultivated)	AM234068	AM490008	This study	AM489871	This study	This study	This study	This study
Myrtus communis L.	E. Lucas 211	RBG Kew (cultivated)	AM234149	AM490009	This study	AM489872	JN660939*	This study	KP722327	KP722221
Neomitranthes cordifolia (D.Legrand) D.Legrand	Forster 1011	Brazil	AM489410			AM489569	This study	This study	JN091386	This study

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Appendix A (*continued*)

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Neomyrtus pedunculata (Hook.f.) Allan	S. Belsham M42	New Zealand	AM234144	AM490010		AM490637	This study			
Octamyrtus pleiopetala Diels	R. Johns s.n.	New Guinea	AM234130		This study	AM489873	This study	This study	This study	
Pilidiostigma tropicum L.S.Sm.	Forster 27636	Australia (Queensland)	This study		This study	This study		This study		This study
Pimenta dioica (L.) Merr.	E. Lucas 212	RBG Kew (cultivated)	AM234081	AM490011	This study	AM489874	This study	This study	This study	
Pimenta pseudocaryophyllus (Gomes) Landrum	E. Lucas 161	Brazil	AM234083	AM490013	This study	AM489876	This study	This study	This study	This study
Pimenta sp1	T. Vasconcelos 576	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Plinia nana Sobral	F.F. Mazine 662	Brazil (Minas Gerais)	This study			This study	This study	This study	This study	This study
Plinia sp1	B. Holst 9482	French Guiana	This study		This study	This study	This study	This study	This study	
Pseudanamomis umbellulifera (Kunth) Kausel	T. Vasconcelos 572	Dominican Republic	This study		This study	This study	This study	This study	This study	This study
Psidium acranthum Urb.	T. Vasconcelos 578	Dominican Republic	This study		This study	This study	This study	This study		This study
Psidium brownianum Mart. ex DC.	T. Vasconcelos 465	Brazil (Bahia)	This study		This study	This study	This study	This study	This study	This study
Psidium laruotteanum Cambess.	J.E.Q. Faria 2362	Brazil (Bahia)		This study	This study	This study	This study	This study	This study	This study
Psidium rufum Mart. ex DC.	J.E.Q. Faria 4270	Brazil (Minas Gerais)	This study		This study	This study	This study	This study	This study	
Rhodamnia cinerea Jack	T. Vasconcelos 672	Singapore	This study	KJ709064*	This study	This study	This study	This study	This study	This study
Rhodomyrtus tomentosa (Aiton) Hassk	T. Vasconcelos 678	Singapore BG (cultivated)	This study	AF105093*	This study	This study	This study	This study	This study	This study
Siphoneugena densiflora O.Berg	F.F. Mazine 1050	Brazil	AM489412		KP722444	AM489571	This study	This study	JN091389	KP722220
Syzygium amplifolium L.M.Perry	(all from GenBank)		EF026620	DQ088556	DQ088381		DQ088416			
Syzygium buxifolium Hook. & Arn.	(all from GenBank)		KP093045	KP093852	DQ088491	KJ687225	DQ088424		AB817604	
Syzygium guineense (Willd.) DC.	(all from GenBank)		EF026628	DQ088581	DQ088500		DQ088432			
Syzygium gustavioides (F.M.Bailey) B.Hyland	(all from GenBank)		AY187194	DQ088582	DQ088501		DQ088433			
Syzygium jambos (L.) Alston in H. Trimen	E. Lucas 214	RBG Kew (cultivated)	AM234135	AM490017	This study	AM489882	DQ088434*	This study	This study	
Syzygium muellerii (Miq.) Miq.	(all from GenBank)		EF026634	DQ088593	DQ088511		DQ088439			
Syzygium maire (A.Cunn.) Sykes & Garn.-Jones	NZFRI29089	New Zealand	KM064865	KM065310	DQ088508	AM489883	DQ088438			

Appendix A (*continued*)

Species	Voucher	Collection locality	ITS	*matK*	*ndhF*	*psbA-trnH*	*rpl16*	*rpl32-trnL*	*trnL-trnF*	*trnQ-rps16*
Syzygium oblatum (Roxb.) Wall. ex A.M.Cowan & Cowan	(all from GenBank)		KR532632	AB924759		KR532989				
Syzygium paniculatum Gaertn.	(all from GenBank)		KM065112	KM065271	DQ088515		DQ088441			
Ugni candollei (Barnéoud) O.Berg	T. Vasconcelos s.n.	RBG Kew (cultivated)	This study	This study	This study	This study	This study	This study	This study	This study
Uromyrtus emarginata (Pancher ex Baker f.) Burret	T. Vasconcelos 628	New Caledonia	This study	This study	This study	This study	This study	This study		
Xanthomyrtus compacta (Ridl.) Diels	P. Edwards 4214A	New Guinea	AM234148		This study	AM489887	This study	This study	This study	This study
Xanthomyrtus montivaga A.J.Scott	E. Lucas 16	New Guinea	AM234147		This study	AM489886	This study	This study		

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.01.002.

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