Divergence times, historical biogeography, and shifts in speciation rates of Myrtales

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ABSTRACT

We examine the eudicot order Myrtales, a clade with strong Gondwanan representation for most of its families. Although previous phylogenetic studies greatly improved our understanding of intergeneric and interspecific relationships within the order, our understanding of inter-familial relationships still remains unresolved; hence, we also lack a robust time-calibrated chronogram to address hypotheses (e.g., biogeography and diversification rates) that have implicit time assumptions. Six loci (rbcL, ndhF, matK, matR, 18S, and 26S) were amplified and sequenced for 102 taxa across Myrtales for phylogenetic reconstruction and ten fossil priors were utilized to produce a chronogram in BEAST. Combretaceae is identified as the sister clade to all remaining families with moderate support, and within the latter clade, two strongly supported groups are seen: (1) Onagraceae + Lythraceae, and (2) Melastomataceae + the Crypteroniaceae, Alzateaceae, Penaeaceae clade along with Myrtaceae + Vochysiaceae. Divergence time estimates suggest Myrtales diverged from Geraniales during the Aptian of the Early Cretaceous. The crown date for Myrtales is estimated at 116 Ma (Albian–Aptian). BioGeoBEARS showed significant improvement in the likelihood score when the “jump dispersal” parameter was added. South America and/or Africa are implicated as important ancestral areas in all deeper nodes. BAMM analyses indicate that the best configuration included three significant shifts in diversification rates within Myrtales: near the crown of Melastomataceae (67–64 Ma), along the stem of subfamily Myrtoideae (Myrtaceae; 75 Ma), and along the stem of tribe Combretaeae (Combretaceae; ~50–45 Ma). Issues with conducting diversification analyses more generally are examined in the context of scale, taxon sampling, and larger sets of phylogenetic trees.

1. Introduction

Resolving deep-level phylogenetic relationships has often been difficult due to confounding issues of ancient, rapid lineage diversification, a lack of clear morphological synapomorphies, a propensity for morphological and molecular homoplasy, and organizational extinction (e.g., Davis et al., 2005; Deng et al., 2015; Givnish et al., 2009; Schönberger et al., 2005). A lack of a well-resolved phylogenetic framework has made it difficult to estimate biogeographic ancestral ranges and assess shifts in species diversification rates within such groups. However, increased amounts of phylogenetic/phylogenomic data and improved analytical tools to evaluate these data are providing more robust phylogenetic frameworks for hypothesis testing (Davis et al., 2014; Ruhfel et al., 2014; Soltis et al., 2011; Zeng et al., 2014; Magallón et al., 2015). In turn, model-based approaches have been developed to more rigorously estimate biogeographic history (Lagrange: Ree et al., 2005; BEAST: Drummond et al., 2006; BioGeoBEARS: Matzke, 2013) and to assess shifts in diversification rates (MEDUSA: Alfaro et al., 2009; BAMM: Rabosky, 2014; Rabosky et al., 2014). The use of these advances has allowed more detailed insight into clades lacking resolution of early diverging lineages (e.g., Asteraceae: Panero et al., 2014), with histories shaped by ancient intercontinental disjunctions (e.g., campanulids; Beaulieu et al., 2013), and with lineage specific shifts in speciation and/or extinction rates (e.g., Bromeliaceae: Givnish et al., 2014; hummingbirds: McGuire et al., 2014).
Hemisphere affinity and whose current distributions, shifts into different habitat or biome types, and thus rates of species diversification may be influenced by the continental breakup of Gondwana during the Cretaceous (Crisp et al., 2009; Donoghue and Edwards, 2014). The distribution patterns and implied biogeographical events of Southern Hemisphere organisms have been vigorously debated since the late 1960s, but a strong vicariance voice (e.g., Axelrod, 1970; Cracraft, 1988; Raven and Axelrod, 1974; Rosen, 1978) had emerged for explaining present-day disjunct patterns in a wide diversity of plant and animal groups (e.g., Edwards and Boles, 2002; Haddrath and Baker, 2001; Murphy et al., 2001; Sequeira and Farrell, 2001; Swenson et al., 2001; Vinther and Bremer, 2001). Recent molecular phylogenetic studies that have included dating methods have provided support for the vicariance model for some Southern Hemisphere groups (e.g., Bukoita et al., 2014; Korall and Pryer, 2013; Mao et al., 2012; Murienne et al., 2013; Wilf and Escapa, 2014). However, others have questioned the sole reliance on Gondwanan vicariance as explanatory for many plant and animal clades showing Southern Hemisphere disjunct patterns (Crisp and Cook, 2013; Sanmartin and Ronquist, 2004). These studies have included support for the Boreotropics Hypothesis (Lavin and Luckow, 1993) or Laurasian rather than Gondwanan origin of some of these plant clades (Baker and Couvreur, 2013; Davis et al., 2002; Renner et al., 2001; Zerega et al., 2005). Most studies, however, have revealed clade dates that are indicative of long-distance dispersals (sometimes associated with vicariance) rather than solely vicariance for Southern Hemisphere disjunctions of both plants and animals [Armstrong et al., 2014; Barker et al., 2007; Beaulieu et al., 2013; Chacón and Renner, 2014; Christenhusz and Chase, 2013; Cook and Crisp, 2005; Friedman et al., 2013; Gallaher et al., 2014; Gamble et al., 2011; Givnish and Renner, 2004; Givnish et al., 2000, 2004; Knapp et al., 2005; Müller et al., 2015; de Queiroz, 2005; Rowe et al., 2010; Sytsma et al., 2004; Thomas et al., 2014).  

1.2. Myrtales as a model group for assessing Southern Hemisphere biogeography and rates of species diversification

Here we examine the eudicot order Myrtales, a clade that has strong Gondwanan representation for most of its families, although one family (Onagraceae) is most diverse in Laurasia. APGIII (2009) recognizes nine families in Myrtales, including: Melastomataceae (188 genera/4618 species), Myrtaceae (131/5638), Onagraceae (22/667), Lythraceae (31/522), Combretaceae (14/570), Vochysiaceae (7/217), Penaeaceae (9/29), Crypteroniaceae (3/10), and Alzateaceae (1/2). Four large families (Melastomataceae, Myrtaceae, Lythraceae, and Combretaceae) are pantropical in distribution, while the Vochysiaceae possess an amphi-Atlantic disjunct distribution pattern. The order is the third most species-rich lineage of the Superrosidae clade of angiosperms (12,264 spp.), varies tremendously in habit (including herbaceous herbs, lianas, trees, and mangroves), floral form, and fruit type (berry, capsule, drupe and samara), and exhibits high species diversification in several fleshy-fruited and dry-capsular clades (e.g., within Melastomataceae and Myrtaceae) (Dahlgren and Thorne, 1984). Despite this ecological and morphological variation, as well as the presence of species rich subclades within Myrtales, no detailed analysis of diversification rates, shifts in these rates, or correlation with evolutionary traits has been performed across the order or within family clades.

A well-resolved and temporally calibrated phylogenetic framework of Myrtales is essential to address questions and hypotheses relating to ancestral range estimation, importance of vicariance and/or dispersal models, and character evolution relative to diversification. All previous molecular phylogenetic studies, either focusing on the order or including placeholder taxa, support a monophyletic Myrtales regardless of the generic region used (see Solis et al., 2011; Sytsma et al., 2004). Although previous phylogenetic studies greatly improved our understanding of intergeneric and interspecific relationships within the order, our understanding of inter-familial relationships still remains unresolved, and thus, we also lack a strong time-calibrated chronogram to address hypotheses (e.g., biogeography and diversification rates) that have implicit time assumptions. An earlier r8s (Sanderson, 2002) analysis for the order used multiple fossil calibration points and a two-genome data set, but did not account for time estimate variation at nodes and was based on a single fixed topology (Sytsma et al., 2004). Advances in the last decade for estimating chronograms, availability of considerably more molecular data, and documentation of many more Myrtales fossils mandate a more thorough analysis of the Myrtales as done here.

Especially problematic in Myrtales has been the placement of the pantropical Combretaceae (Conti et al., 1996, 1997; Sytsma et al., 2004). Two alternative hypotheses exist: (1) Combretaceae is sister to the clade comprising Onagraceae + Lythraceae, which is then sister to the rest of Myrtales (Conti et al., 1996, 1997); or (2) Combretaceae is sister to all other members of Myrtales (Sytsma et al., 2004). The placement of Combretaceae is of considerable importance because its position directly influences clade ages; hypotheses addressing dispersal/vicariance scenarios, species diversification rates, and character reconstructions. Since Conti et al. (1996, 1997) proposed the first interfamilial relationships of Myrtales based on rbcL, most ordinal level phylogenetic studies have continued to utilize a limited number of taxa and gene regions (often the same GenBank accessions) as placeholders for Combretaceae (e.g., Magallon, 2010; Rutschmann et al., 2007; Sytsma et al., 2004; Wang et al., 2009). The continued use of the same 2–5 taxa and 1–2 plastid regions has limited our ability to place Combretaceae and confidently address other hypotheses. To circumvent this issue (as summarized by Sanderson et al., 2010), our analysis increases sampling, both in terms of number of taxa and breadth across the family.

Thus, we develop here a more rigorous time-calibrated phylogenetic framework for Myrtales based on a three-genome approach with nearly 98% coverage of gene regions for each sampled taxon. Taxa sampling is designed to cover all major clades within each family. We then use this new historical framework of Myrtales to: (1) evaluate phylogenetic relationships within and among the nine families; (2) estimate when and where major lineages of Myrtales originated with BEAST and BioGeoBEARS analyses; (3) examine the biogeographic processes that may have contributed to extant distributions, especially several different disjunct patterns in the Southern Hemisphere; and (4) test for shifts in speciation and extinction rates across Myrtales and within each of the five major family clades using BAMM analyses.

2. Material and methods

2.1. Taxon and gene sampling, and phylogenetic analyses

Sampling was performed in an effort to maximize diversity across the nine currently recognized families (APGIII, 2009); thus, we based our sampling of 102 taxa on available sequences and previous phylogenetic studies (see Supplementary Information Table S1). Outgroups (15 species) were selected from Vitales (Vitis), Crossosomatales (Crossoxoma), Malvales (Thymelaeeae), Brassicales (Arabidopsis, Curica), and Geraniales (California, Erodium, Francoa, Geranium, Hypseocharis, Melianthus, Monsonia, Pelargonium, Viviana). Geraniales is the sister order to Myrtales, with the Crossoxomatales, Malvales, and Brassicales representing three orders of
the Malvid clade within the Rosidae sister to these two orders (APGIII, 2009; Soltis et al., 2011). Vitis was used as the ultimate outgroup in all phylogenetic reconstructions. When possible, the same species, and often the same DNA samples from previous studies, were used to expand gene sampling. When unavailable, sequences of closely related species were generated or obtained from GenBank to serve as generic placeholders. Species, voucher-/collection information, and GenBank accession numbers are provided in SI Table S1.

Six loci (rbcl, ndhF, matK, matR, 18S, and 26S) were amplified and sequenced for taxa across Myrtales and Geraniales using primers shown in SI Table S2. Each locus was selected based on utility in large-scale studies across angiosperms (see Soltis et al., 2011), the Rosidae (Wang et al., 2009; Zhu et al., 2007), and family-wide studies in Myrtales (Biffin et al., 2010; Bult and Zimmer, 1993; Clauing and Renner, 2001; Conti et al., 1993, 1996, 1997, 2002; Gadek et al., 1996; Huang and Shi, 2002; Graham et al., 2005; Levin et al., 2003, 2004; Lucas et al., 2005; Maurin et al., 2010; Rutschmann et al., 2004, 2007; Schönenberger and Conti, 2003; Sytsma et al., 2004; Tan et al., 2002; Thornhill et al., 2012; Wilson et al., 2005). 307 sequences from GenBank were added to the dataset to improve sampling and to serve as placeholders for key lineages essential for biogeographic reconstruction and assessing rates of species diversification.

Total genomic DNA was extracted from silica-dried leaf material and herbarium specimens using a DNeasy™ Plant Mini Kit (Qiagen, Valencia, California). All samples received an extended heat treatment (30 min at 65°C instead of 10 min) to reduce secondary compound co-precipitation. PCR methods followed those described previously (Wilson et al., 2001; Huang and Shi, 2002; Sytsma et al., 2004). PCR products were sequenced using BigDye Terminator kits (Applied Biosystems, Foster City, California) on an Applied Biosystems 3730xl automated DNA sequencer at the University of Wisconsin-Madison Biotechnology Center. Contiguous alignments were edited and assembled using Geneious v.6.1.8 (http://www.geneious.com, Kearse et al., 2012). Sequences were aligned initially using MAFFT v.6.864 (Katoh and Toh, 2008) with subsequent adjustments made by eye in Geneious. Gaps were treated as missing data.

Best fitting models of sequence evolution for each locus were determined using the Bayesian Information Criterion (BIC) in jModeltest v.2.1.4 (Darriba et al., 2012; Guindon and Gascuel, 2003). The BIC was used for model selection based on its ability to outperform other model-selection criteria (Luo et al., 2010). Models selected included TVM+I+G for rbcl, TVM+G for matK, matR, and ndhF, GTR+I+G for 26S, and SYM+I+G for 18S. Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using the CIPRES Science Gateway v.3.3 (www.phylo.org; Miller et al., 2010). ML analyses were conducted using default parameters in GARLI v.2.01 (Zwickl, 2006). One thousand bootstrap (BS) replicates were conducted using the same parameters applied for ML searches. BI was performed using MrBays v.3.2.3 (Alteker et al., 2004; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Preliminary BI analyses revealed topological variation in the placement of relationships of outgroup taxa (data not shown), especially with regard to Geraniales; therefore, the sister relationship of Myrtales to Geraniales was constrained (APGIII, 2009; Ruhfel et al., 2014; Soltis et al., 2011). All BI analyses were run for 15,000,000 generations with four chains in two parallel runs sampling every 1500 generations. Proper mixing was determined using Tracer v.1.6 (Rambaut et al., 2014) and 20% of trees were discarded as burn-in prior to constructing a majority rule consensus tree using TreeAnnotator v.1.8.0 (Drummond et al., 2012).

Congruence among plastid (rbcl, ndhF, matK), mitochondrial (matR), 18S rDNA, and 26S rDNA was assessed by estimating concordance factors (CF; Baum, 2007) using BUCKy v.1.4.3 (Target et al., 2010). BUCKy uses the Bayesian concordance approach (Ane et al., 2007), which integrates over gene tree uncertainty without speculating what causes discordance. For the BUCKy analysis, we used the posterior distribution of the chloroplast, mitochondrial, and nuclear ribosomal gene trees produced with MrBayes. The 18S and 26S data sets were analyzed separately as a conservative approach given the differing best models of sequence evolution. The primary concordance tree and concordance factors were estimated with the discordance parameter (τ) of 1 and 100. Both analyses were run with two Markov chain Monte Carlo searches (MCMC) of 100,000 generations with 10,000 generations burn-in. Both runs converged on the same primary concordance tree with identical concordance factors. The final analysis was conducted with τ = 1 and two MCMC chains of 1,000,000 generations following a burn-in of 100,000 generations.

2.2. Fossil priors and BEAST analyses

Divergence time estimates were performed in BEAST v.1.8.0 (Drummond et al., 2012), incorporating an uncorrelated lognormal clock and Yule speciation process. In order to accommodate rate heterogeneity, chloroplast markers and matR were partitioned into three codon positions. We ran two independent analyses of 50,000,000 generations each. To verify effective sampling of all parameters and to assess convergence of independent chains, we examined output log files in Tracer v.1.6 (Rambaut et al., 2014). After removing 20% of samples as burn-in, independent runs were combined and a maximum clade-credibility (MCC) tree was constructed using TreeAnnotator v.1.8.0 (Drummond et al., 2012). We offset the minimum ages of 14 nodes across the phylogeny using a combination of fossil (see below) and secondary priors (Table 1). All fossil priors were placed under a lognormal distribution with a mean of 1.5 and a standard deviation of 1, allowing for the possibility that these nodes are considerably older than the fossils themselves. Secondary priors were placed under a normal distribution and the root under a uniform distribution. Because of the aforementioned topological variation with regard to Myrtales and Geraniales, we constrained the topology using the most likely tree obtained from GARLI. No age offset was placed on this prior.

Eleven fossil priors were utilized in this study. In Melastomaceae, we used a leaf fossil (Hickey, 1977) to offset the most recent common ancestor (MRCA) of Melastomataceae s.s. (minus tribe Kibessiaceae) and a seed fossil as a Melastomaceae crown prior (Clausing and Renner, 2001; Renner et al., 2001; Renner, personal communication; Rutschmann et al., 2007; Sytsma et al., 2004; Wehr and Hopkins, 1994). The placement of the second fossil was supported by morphological characters, as extant Melastomaceae exhibit cochleate seeds with a round operculum and tuberculate testa, whereas all other Melastomaceae have straight or cuneate seeds that lack operculi and differ in ornamentation (Renner et al., 2001). In Myrtaceae, we used two Myrtaceous (=Syncaloritites) fossilized pollen grains to place priors on or near the crown of the family. Myrtaceites lisimaevae is the oldest fossil in Myrtaceae (from Gabon, Africa; Boltenhagen, 1976; Muller, 1981). While the assignment of M. lisimaevae to the Myrtaceae is clear, its affinity to either subfamily is ambiguous and likely represents a stem lineage placement (Rutschmann et al., 2007; Sytsma et al., 2004); therefore, we placed it conservatively on the family of the crown. We placed Myrtaceites mesonesus on the first node in from the crown of subfamily Myrtoideae (i.e., excluding Lophostemon; M. mesonesus) following the suggestion of Thornhill et al. (2012). The third prior across Myrtaceae, placed on the stem node of Eucalyptus, represents the earliest record of meso-fossils attributed to the eucalypt clade from South America (Gandolfo et al., 2011; Thornhill et al., 2012). In Lythraceae, we used fossilized pollen...
grains attributed to *Lythrum* and *Peplis* as an offset for the crown of the two lineages (Grímsson et al., 2011). We also used the oldest described *Lagerstroemia* leaf impression fossil, the silicified wood of *Punicoxylon*, and the oldest confirmed *Sonneratiaoxylon* wood as conservative offsets on the stem nodes of *Lagerstroemia*, *Punica*, and *Sonneratia*, respectively (Graham, 2013). In Onagraceae, fossil pollen of *Koninidites aspis* (the type palynomorph of *Diporites aspis* was recently found to be a fungal sporophyte, thus, new typological nomenclature was assigned; Berry et al., 1990; Lee et al., 2013) was used to offset the split between Old World and New World *Fuchsia* (Berry et al., 2004; Rutschmann et al., 2007; Sytsma et al., 2004).

Although previous large-scale studies (Bell et al., 2010; Smith et al., 2011; Wikström et al., 2001) have included putative Combreteaceae fossils assigned to *Esqueiria* (Fries et al., 1992; Takahashi et al., 1999) to offset the Combreteaceae node, the fossil differs significantly from extant taxa, especially with regard to stylar branches, and its placement cannot be assigned confidently (E.M. Fries, personal communication; Fries et al., 1992; Magallón et al., 1999). In lieu of *Esqueiria*, we use the fin-winged fruits of *Dilcherocarp combretoides* (Manchester and O’Leary, 2010) as an offset on the stem node of tribe Combreteae. Morphologically, the epigynous fruits resemble those of extant *Combretum* and some *Terminalia* (Gere, 2014; Maurin et al., 2010), possessing four lateral wings arising in two perpendicular planes. The presence of abundant parallel veins and the absence of a fimbrial vein on the fruit is an extremely rare combination of characters outside of the family (Manchester and O’Leary, 2010). Although winged fruits are reported in all genera of Combreteae (Johnson and Briggs, 1984), the occurrence of winged fruits in the genus *Strephonomia* (subfamily *Strephonomatoideae*) has not been confirmed and the fruits of tribe Laguncularieae appear to be secondarily derived from bracteoles (Stace, 2007).

### Table 1

<table>
<thead>
<tr>
<th>Fossil (clade)</th>
<th>Minimum age (Ma)</th>
<th>Coalescent node</th>
<th>Reference(s)</th>
<th>Prior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root*</td>
<td>100</td>
<td>Vitis + Osbeckia</td>
<td>See Sytsma et al. (2014)</td>
<td>Uniform 125–100</td>
</tr>
<tr>
<td>Caricaceae/Brassicaceae crown*</td>
<td>98.65</td>
<td>Arabidopsis + Carica</td>
<td>Cardinal-McTeague et al. (unpublished)</td>
<td>Normal 98.65</td>
</tr>
<tr>
<td>Geraniales crown*</td>
<td>87</td>
<td>Viviania + Geranium</td>
<td>Sytsma et al. (2014)</td>
<td>Normal 87.0</td>
</tr>
<tr>
<td>Pelargonium crown*</td>
<td>28.4</td>
<td>Pelargonium + Pelargonium</td>
<td>Sytsma et al. (2014)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Eucalyptus freguelliana Gandolfo &amp; Zamaloa, sp. n.</td>
<td>51.69</td>
<td>Stockwellia + Eucalyptus</td>
<td>Gandolfo et al. (2011)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Myrtaceae clade</td>
<td>83.5</td>
<td>Heteropyxis + Myrtus</td>
<td>See Sytsma et al. (2004)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Melastomeae seeds</td>
<td>61.7</td>
<td>Osbeckia + Myrtus</td>
<td>Couper (1960)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Acrovena laevis Hickey</td>
<td>23.0</td>
<td>Rhezia + Osbeckia</td>
<td>See Renner et al. (2001)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Koninidites aspis Pocknall &amp; Mildenhall</td>
<td>21.4</td>
<td>Fuchsia pa + Fuchsia c</td>
<td>Berry et al. (1990); Lee et al. (2013)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Lythrum elkenis sp. n./Pepis englensis sp. n.</td>
<td>81.0</td>
<td>Lythrum + Peplis</td>
<td>Grímsdóttir et al. (2011)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Lagerstroemia patellii Kuhlm. in Melastomataceae</td>
<td>56.0</td>
<td>Lagerstroemia + Duabanga</td>
<td>See Graham (2013)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Punicoxylon ecocenicum Privé-Gill</td>
<td>40.4</td>
<td>Punica + Galpinia</td>
<td>See Graham (2013)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Sonneratiaaxylon preapetalum Awasthi</td>
<td>63.8</td>
<td>Sonneratia + Trapa</td>
<td>See Graham (2013)</td>
<td>Lognormal 1.5</td>
</tr>
<tr>
<td>Dilcherocarp combretoides gen. et. sp. n.</td>
<td>93.5</td>
<td>Laguncularia + Terminalia k</td>
<td>Manchester and O’Leary (2010)</td>
<td>Lognormal 1.5</td>
</tr>
</tbody>
</table>

### 2.3. Inserting additional terminals for biogeographical and diversification analyses

To improve lineage sampling for biogeographical and diversification analyses, we added five tips to the MCC tree from BEAST. These five taxa were too incomplete for our gene sampling, but their phylogenetic placements within families are well known. The five new tips added included: (1) *Xylonastra* Donn. Sm. & Rose in Onagraceae (Levin et al., 2003, 2004); (2) *Syncarpia* Ten. and (3) *Xanthostemon* F. Muell. in Myrtaceae to represent tribes Syncarpeae and Xanthostemoneae, respectively (Thornhill et al., 2012); and (4) *Astronia* Blume and (5) *Merianthera* Kuhl. in Melastomataceae to represent the Astronieae and the clade comprised of “Merianthera and its allies” + “Bertoloniaceae 2” + Cyphostylidaceae (Goldenberg et al., 2012). The taxa were added to the BEAST tree using the bind.tip function of the *phytools* package (Revell, 2012) for R (R Development Core Team, 2014). This function rescales an ultrametric tree after new tips are added so the tree remains ultrametric. Similarly, we added these five tips to a random subset of 100 trees obtained from the posterior distribution of post-burnin BEAST trees for further biogeographical analyses.

### 2.4. Ancestral range estimation in BioGeoBEARS

Ancestral range estimation (ARE) for Myrttales was done using the nested DEC and DECj models in BioGeoBEARS (Matzke, 2013, 2014) in R v3.1.1. Similar to the Dispersal-Extinction-Cladogenesis (DEC) program LaGrange (Ree and Smith, 2008; Ree et al., 2005), the DEC model in BioGeoBEARS evaluates ML parameters for ana
genetic events involving range expansion and extinction and for cladogenetic events involving sympathy and vicariance. Unlike LaGrange, however, BioGeoBEARS with the DECj model can also parameterize cladogenetic “founder-events” (Templeton, 1980).
by incorporating the J parameter for “jump-dispersals”. This J parameter allows for a daughter lineage to immediately occupy via long-distance dispersal a new area that is different from the parental lineage. DECj models have been shown to be significantly better than DEC models for island groups (Matzke, 2014) and for inter-continental distributions, but not for some more localized continental groups (e.g., western North American Salvia; Walker et al., 2015).

We identified six broad geographic areas, modified after Buerki et al. (2011), important in the context of the distributions of the families in Myrtales (see Fig. 3): (1) Eurasia (from western Europe, Mediterranean Africa, to temperate Asia); (2) Sub-Saharan Africa including Madagascar; (3) Southeast Asia, including India, Indochina, the Malaysian Peninsula, the Philippines, Sumatra, Borneo and the Inner Banda Arc; (4) Australia, including New Guinea, New Caledonia and New Zealand, as well as the Pacific Islands (e.g., Hawaii); (5) North America; and (6) South America, including most of Mexico, Central America and the West Indies. Because our study focused on family-level relationships, all tip taxa within Myrtales were coded as present or absent for each of the six areas based on extensive literature available for each family. In instances where a tip taxon represented a more diverse and widespread lineage, we coded these tips to cover the maximal distribution of the lineage. Due to the difficulty of scoring under-sampled outgroup orders for geographic areas, we restricted outgroup taxa only to the Geraniales as the sister clade to Myrtales. Geraniales has an ancestral area of Africa, South America, or combined Africa + South America (Fitz et al., 2008; Palazzesi et al., 2012; Sytsma et al., 2014). Our sampling of three families in the order included all possibilities: African Melianthaceae, South American Vivianiaceae, and likely combined African + South American Geraniaceae.

In BioGeoBEARS we allowed the inferred ancestors to occupy up to all six areas. Dispersal probabilities between pairs of areas and areas allowed were specified for four separate time slices (SI Table S3) based on known geological events that have been similarly analyzed elsewhere (e.g., Buerki et al., 2011; Drew and Sytsma, 2012; Sessa et al., 2012; Sulman et al., 2013). We estimated ancestral ranges on the MCC tree from BEAST, pruned of all outgroups except Geraniales, with the addition of five terminals. The resulting ML score for the more parameter-rich DECj model was tested for significance against the resulting ML score of the DEC model. We explored the degree to which differences in tree topology and branch lengths yield different ARE for nodes by developing a script to run BioGeoBEARS on the 100 randomly selected PP trees from the BEAST analysis (with five species added for each) under both DEC and DECj models. Additionally, we conducted biogeographical stochastic mapping analyses in BioGeoBEARS on the single best BEAST tree (with five species added) under both DEC and DECj models. This permitted measuring the probability of each class of cladogenetic event (vicariance, sympatry, subset-sympatry, and jump dispersals) given the DEC and DECj models, distribution data, and phylogeny.

2.5. Estimating speciation and extinction rates and identifying rate shifts in species diversification in BAMM

We used the program BAMM (Rabosky, 2014; Rabosky et al., 2014) to: (1) estimate rates of speciation, extinction, and net diversification for clades, (2) conduct rate-through-time analysis of these rates, and (3) identify and visualize shifts in species rates across the Myrtales phylogeny. BAMM is a Bayesian approach that uses reversible jump Markov chain Monte Carlo (RJMCMC) sampling to explore shifts in macro evolutionary regimes assuming they occur across the branches of a phylogeny under a compound Poisson process, and explicitly accommodates diversification rate variation through time and among lineages. BAMM is both time-sensitive and diversity-dependent, allowing rate shifts to occur anywhere on a branch based on the posterior tree density. BAMM accounts for non-random and incomplete taxon sampling in the phylogenetic trees by allowing all non-sampled species to be associated with a particular tip or more inclusive clade. Terminals and proportion of extant species sampled for each are provided in SI Table S4. For example, only one of eight species of Circaea (Onagraceae) was sampled, and thus BAMM recognized sampling at this tip as only 0.125. Tips were assigned to the smallest possible taxonomic unit, which often corresponded to genera or tribes within families. For example, all 873 species representing a number of genera of the tribe Eucalyptae (Myrtaceae) were identified as belonging to a clade defined by the most recent common ancestor of our three sampled genera (Eucalyptus, Angophora, and Stockwellia). Species numbers were obtained from published sources (Forster, 1994; Jordaen et al., 2011; Maurin et al., 2010; Renner, 1993; Stace, 2007; Stevens, 2001; The Plant List, 2014). Terminals and proportion of extant species sampled for each are provided in SI Table S4. A total of 12,264 species in Myrtales are recognized, updating previous numbers from Stevens (2001) and Solis et al. (2011).

Priors for BAMM were generated using the R package BAMMtools v.2.0.2 (Rabosky, 2014) by providing the MCC tree from BEAST and total species numbers across the order (12,264). Two independent MCMC chains of 100,000,000 generations were run in BAMM and convergence was assessed by computing the effective sample sizes of log likelihoods, as well as the number of shift events present in each sample using the R package coda v. 0.16-1 (Plummer et al., 2006). After removing 10% of trees as burn-in, we analyzed the BAMM output using BAMMtools and computed the 95% credible rate shift configurations using the Bayes factors criterion for including nodes as core shifts set to 5. This procedure computes Bayes factors evidence associated with a rate shift for every branch in the phylogeny and excludes all unimportant nodes using the Bayes factor criterion (see BAMM documentation). Large Bayes factors values are taken as strong evidence for a shift on a branch in the tree. We also estimated the rate shift configuration with the highest maximum a posteriori (MAP) probability (“the best shift configuration”) after having excluded all none core shifts.

Additionally, we ran independent BAMM analyses on each of the five largest families within Myrtales (Combretaceae, Lythraceae, Melastomataceae, Myrtaceae, and Onagraceae) to test if BAMM identified different shifts when each family was analyzed separately. The same parameters and analytical procedures were used for family-specific analyses except the number of generations was reduced to 50,000,000. Family level phylogenies were obtained by extracting the corresponding families from the Myrtales phylogeny.

3. Results

3.1. Phylogenetic relationships and timing of clade formation in Myrtales

A total of 362 sequences were generated for this study: 31 rbcl, 28 ndhf, 80 matK, 63 18S, 62 26S and 98 matR (see SI Table S1). The aligned, partitioned data set consisted of 7337 characters, with 1626 being parsimony-informative. ML and BI trees highlight several important phylogenetic results at the broad scale within Myrtales (Fig. 1). First, Combretaceae is identified as the sister clade to all remaining families with moderate support (ML-BS/BI-PP = 69/0.84). Second, within the latter clade, two strongly supported groups are observed: (1) Onagraceae + Lythraceae form a subclade with strong support (ML-BS/BI-PP = 100/1.0), and (2) Melastomataceae + the CAP clade and Myrtaceae + Vochysiaceae
Fig. 1. Maximum likelihood tree of Myrtales and Geraniales based on six loci shown as a cladogram (phylogram inset). Tree is rooted with four other Rosidae orders (Vitis is the ultimate outgroup). Support values are provided for each branch: (ML-BS/BI-PP). Bold black branches are ML-BS P 95% and BI-PP = 1.0. Aside from the node designating the divergence of Combretaceae from all other Myrtales, support values 6 70% ML-BS are designated by a (⁄). Support values 6 70% ML-BS and 6 0.9 BI-PP are collapsed.
are both highly supported (ML-BS/BI-PP = 100/1.0), as is the entire subclade (ML-BS/BI-PP = 100/1.0). Third, all families within Myrtales are monophyletic with high support (ML-BS/BI-PP = 100/1.0). And fourth, our sub-sampling scheme corroborates most large-scale studies addressing intergeneric relationships (see Section 4). Bayesian concordance analysis using BUCKY resulted in a primary concordance tree with the same overall topology as the BI and ML phylogenies (SI Fig. S1). Concordance factors were moderate to high for all families (>0.5) and these values decreased toward the backbone of the tree.

Divergence time estimates suggest Myrtales diverged from Geraniales ~123.6 Mya (Table 2; Fig. 2) during the Aptian of the Early Cretaceous. The crown date for Myrtales is estimated at ~116.4 Mya (Albian–Aptian). Stem lineages of the four major clades of Myrtales are all present by the end of the Albian (~109.3 Mya): (1) Combretaceae, (2) Lythraceae + Onagraceae, (3) Melastomataceae + the CAP clade, and (4) Myrtaceae + Vochysiaceae. Stem lineages of all extant families are present by ~90.7 Mya (mid-Turonian). Crown dates of modern families range from ~102.6 Mya for the Combretaceae (Albian) to ~38.8 Mya for the Vochysiaceae (late Eocene).

3.2. Biogeographical patterns of diversification in Myrtales

In BioGeoBEARS, significant improvement in the likelihood score of the model was seen when the “jump dispersal” parameter was added (DEC) vs. without (DEC), as indicated by a likelihood ratio test (DEC Lnθ = 341.97, DEC LnL = −287.70, df = 1, P = 2.05e−25). Because of the significant improvement in the likelihood of the model when the jump dispersal parameter is added, only ARE under the DECj model is presented. The resulting parameters of the DECj models included: anagenetic dispersal rate d = 0.0049; extinction rate e = 1.0e−08; cladogenetic dispersal rate j = 0.096. Biogeographical stochastic mapping, given the parameters of the DECj model, indicated that 60% of all cladogenetic events are sympatric, 32% are subset-sympatry, 7% are vicariance, and 1% involve founder-event dispersals.

ARE for Myrtales and Geraniales using BioGeoBEARS is shown in Fig. 3 (area or combined area with highest frequency shown for each node and corner). The chronogram illustrates ARE in the context of geological age and continental drift during the Cretaceous and Paleogene. The MCC tree indicates a combined South America and Paleogene radiation of subfamily Myroideae (Myrtaceae), Melastomataceae, Crypteroniaceae, and Combretaceae. Eurasia and Southeast Asia appear to play more minor and recent roles in most families, with the exception of an early diversification (Late Cretaceous) in Lythraceae in Eurasia. More recent migrations in the Paleogene over adjacent or still connected areas are seen between South America and Australia (e.g., Melastomataceae, Onagraceae, and Lythraceae) and between Australia, Southeast Asia, and Eurasia (Melastomataceae, Myrtaceae, and Combretaceae). Long distance dispersal and establishment (LDDE) events between South America and Africa and over oceanic water barriers during the Paleogene occur in the CAP clade, Vochysiaceae, and Combretaceae.

Most of the major family clades also have either South America (Melastomataceae, Vochysiaceae, Onagraceae, Lythraceae) or Africa (Myrtaceae, CAP clade) as ancestral. Australia, including islands east of the Wallace Line and Polynesia, is an important biogeographic area early in the Late Cretaceous or Paleocene radiation of subfamily Myroideae (Myrtaceae), Melastomataceae, Crypteroniaceae, and Combretaceae. Eurasia and Southeast Asia appear to play more minor and recent roles in most families, with the exception of an early diversification (Late Cretaceous) in Lythraceae in Eurasia. More recent migrations in the Paleogene over adjacent or still connected areas are seen between South America and Australia (e.g., Melastomataceae, Onagraceae, and Lythraceae) and between Australia, Southeast Asia, and Eurasia (Melastomataceae, Myrtaceae, and Combretaceae). Long distance dispersal and establishment (LDDE) events between South America and Africa and over oceanic water barriers during the Paleogene occur in the CAP clade, Vochysiaceae, and Combretaceae.

After discarding the burn-in, we confirmed convergence of the MCMC chains in the BAMM analyses, as well as effective samples sizes >900 for both the number of shifts and log likelihoods. BAMM analyses strongly supported a diversity-dependent speciation process across Myrtales with a net diversification rate (speciation minus extinction) of 0.065 species/Myr (Table 3). The highest speciation rates are seen at nodes within Combretaceae (0.69 species/Myr), Melastomataceae (0.45 species/Myr), and Myrtaceae (0.52 species/Myr) (Table 3). The highest extinction rates are seen at nodes within Combretaceae (0.76 species/Myr), and Lythraceae, Myrtaceae, and Combretaceae. Large distance dispersal and establishment (LDDE) events between South America and Africa and over oceanic water barriers during the Paleogene occur in the CAP clade, Vochysiaceae, and Combretaceae.

3.3. Speciation and extinction rates and identification of three rate shifts in species diversification in Myrtales

After discarding the burn-in, we confirmed convergence of the MCMC chains in the BAMM analyses, as well as effective samples sizes >900 for both the number of shifts and log likelihoods. BAMM analyses strongly supported a diversity-dependent speciation process across Myrtales with a net diversification rate (speciation minus extinction) of 0.065 species/Myr (Table 3). The highest speciation rates are seen at nodes within Combretaceae (0.69 species/Myr), Melastomataceae (0.45 species/Myr), and Myrtaceae (0.52 species/Myr) (Table 3). The highest extinction rates are seen at nodes within Combretaceae (0.76 species/Myr), and Lythraceae, Myrtaceae, and Combretaceae. Large distance dispersal and establishment (LDDE) events between South America and Africa and over oceanic water barriers during the Paleogene occur in the CAP clade, Vochysiaceae, and Combretaceae.

Table 2

<table>
<thead>
<tr>
<th>Node</th>
<th>Clade</th>
<th>BEAST age (95% HPD)</th>
<th>r8s age (Sytsma et al., 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stem</td>
<td>Crown</td>
<td>Stem</td>
</tr>
<tr>
<td>1</td>
<td>Myrtales</td>
<td>123.6 (124.8–122)</td>
<td>116.4 (118.8–113.7)</td>
</tr>
<tr>
<td>2</td>
<td>Combretaceae</td>
<td>116.4 (118.8–113.7)</td>
<td>102.6 (106.5–98.9)</td>
</tr>
<tr>
<td>3</td>
<td>Onagraceae</td>
<td>104.6 (109.1–100.2)</td>
<td>85.4 (96.2–73.3)</td>
</tr>
<tr>
<td>4</td>
<td>Lythraceae</td>
<td>104.6 (109.1–100.2)</td>
<td>95.5 (99.7–91.7)</td>
</tr>
<tr>
<td>5</td>
<td>Vochysiaceae</td>
<td>101.1 (107.0–95.1)</td>
<td>38.9 (51.8–28.0)</td>
</tr>
<tr>
<td>6</td>
<td>Myrtaceae</td>
<td>101.1 (107.0–95.1)</td>
<td>85.0 (87.6–83.6)</td>
</tr>
<tr>
<td>7</td>
<td>CAP clade</td>
<td>90.7 (98.8–82.4)</td>
<td>52.7 (66.4–40.4)</td>
</tr>
<tr>
<td>8</td>
<td>Melastomataceae</td>
<td>90.7 (98.8–82.4)</td>
<td>64.5 (74.81–56.1)</td>
</tr>
</tbody>
</table>
Fig. 2. Maximum clade credibility (MCC) tree of Myrtales obtained from BEAST analysis. Mean divergence time estimates are shown with 95% highest posterior density (HPD; blue bars). Clades I–VII represent crown radiations of extant families. Mean age plus 95% HPD are as follows: I – 64.5 Ma (74.8–56.1 Ma); II – 52.7 Ma (66.4–40.4 Ma); III – 85.0 Ma (87.6–83.6 Ma); IV – 38.8 Ma (51.8–28.0 Ma); V – 95.5 Ma (97.7–91.7 Ma); VI – 85.4 Ma (96.2–73.3 Ma); and VII – 102.6 Ma (106.5–98.9 Ma). Black circles indicate calibration points 1–7 (see Table 2 for additional information). The shaded box corresponds to the maximum/minimum age range for the divergence of all extant Myrtalean stem lineages (based on 95% confidence intervals).
Fig. 3. Ancestral range estimation (ARE) on the Myrtales chronogram with BioGeoBEARS (DEC+) model. Areas of tip species (or genera) are shown left of taxa names, color-coded for the six biogeographical areas shown on the map inset. Boxes at each node and corner are color coded for the area (or combined area, up to four allowed) with the highest ML probability. SI Fig. S2 provides all ARE per node and corner with pies designating probability of each area (or combined area). Numbers next to nodes indicate the proportion out of 100 PP trees (if less than 1.0) that the area (or combined area) had the highest ML probability. Inset globes depict continental positions at the beginning of the Late Cretaceous and the K–Pg boundary (modified after Scotese, 1998).
taceae (excluding Heteropyxis and Psiloxylon) received a Bayes factor of 300, which does not closely approach the 800 value of overwhelming support for a rate shift.

The family level analyses (Figs. 5 and 6) recovered shifts in speciation rates that are similar but not identical to the ordinal level analyses. Like in the large analyses, shifts were detected in the Combretaceae, Myrtaceae, and Melastomataceae. In Combretaceae, the 95% credible set of rate shift configurations sampled with BAMM included 2 distinct configurations of which the configurations with the highest probability included 1–2 core shifts. The best shift configuration included a single core shift on the same branch as the ordinal level analyses (Fig. 6). Bayes factor evidence for this shift was high (BF = 3158). In the Melastomataceae, the 95% credible set of rate shift configurations sampled included 3 distinct configurations of which the configurations with the highest probability included one or no core shifts. The best configuration detected a single shift within the tribe Miconieae, on the branch leading to the clade comprised of Clidemia, Miconia and Tococa (Fig. 6). Bayes factor evidence for this shift was low (BF = 116). Lastly in Myrtaceae, the 95% credible set of rate shift configurations sampled included 23 distinct configurations of which the configurations with the highest probability included one, two, or no core shifts. The best configuration included a single core shift on the crown of the Myrteae clade (Fig. 6). Bayes factor evidence for this shift was 84.

4. Discussion

4.1. Myrtales: a model Cenozoic clade for understanding the role of dispersal, vicariance, cladogenesis, and extinction in formation of Southern Hemisphere biodiversity

Our results, which are based on the largest assemblage of both taxa and loci for the order Myrtales, provide strong evidence for
Fig. 5. Speciation rates in Myrtales utilizing BAMM. Chronogram of Myrtales with branch lengths colored according to speciation rate. Red circles indicate significant increases in speciation rates present in the best shift configuration. Right panel shows speciation rates in seven family clades when each are evaluated separately. The blue curve is the speciation rate for the entire Myrtales, the respective colored curve is for the specific family.
phyletic relationships among and within the nine families, for the ancestral areas, timing, and biogeographical mode of this remarkable radiation, and for the specific subclades that have undergone significant shifts in speciation. We show for the first time, stronger support for the placement of Combretaceae as sister to all remaining Myrtales. This relationship and other early cladogenetic events within Myrtales signal the importance of both South America and Africa (western Gondwana) as ancestral areas in the early biogeographic diversification of the order during the early Cretaceous. This biogeographic diversification involves both vicariant and dispersal (including jump or founder) events among continents. Lastly, we find three significant shifts in speciation rates within three families (Myrtaceae, Melastomataceae, Combretaceae) and all on branches spanning the K–Pg boundary. With the wealth of previously published phylogenetic, fossil, biogeographical, ecological, and evolutionary studies, the Myrtales is now one of the better-known Southern Hemisphere dominant lineages of organisms.

4.2. Phylogenetic perspective of evolution within Myrtales

Our results provide new insight into phylogenetic relationships within Myrtales and support an ancient radiation of the order closer to the diversification of the eudicot angiosperms than has previously been suggested (Bell et al., 2010; Magallón and Castillo, 2009; Moore et al., 2010; Smith et al., 2010; Sytsma et al., 2004; Wang et al., 2009; Wikström et al., 2001). Seven major clades representing the major families (the CAP clade is considered a single lineage) are resolved with high support. Aside from the position of Combretaceae, all other interfamily relationships are congruent with previous studies.

Prior analyses of relationships within Myrtales have provided an improved understanding of both the phylogenetic relationships among the families and the biogeography of the order; however, the placement of Combretaceae has remained unresolved (Conti et al., 1996, 1997, 2002; Soltis et al., 2011; Sytsma et al., 2004). The lack of adequate taxon sampling of the Combretaceae (Figs. 1 and 2) contributed to the uncertainty in its phylogenetic placement in these previous studies. In our study, we explicitly improved sampling in Combretaceae by including representatives of Streplovea (subfam. Strephonematoideae; Stace, 2007), and multiple representatives from each of the largest subfamilies (subfam. Combretinae tribe Combretinae subtribe Combretinae and subtribe Terminaliinae; Stace, 2007). Based on the ML and BI analyses, Combretaceae is sister to the rest of the order with moderate support (Fig. 1). In addition to molecular phylogenetic results, the position of Combretaceae is supported by recent fossil evidence considered the earliest occurrence of Myrtales (Manchester and O’Leary, 2010).

Importantly, this new phylogenetic framework for Myrtales (or that based on sets of plausible PP trees) will be critically important in re-evaluating the prodigious wealth of morphological and anatomical data that has accumulated for the order, such as that found in the hallmark work of Johnson and Briggs (1984) who examined “cladistically” 77 characters across Myrtales. For example, they highlight the unusual and distinctive presence of fibrous seed exotegmen as an example of convergence between the Combretaceae and the Lythraceae + Onagraceae clades based on a morphology derived phylogram. However, under the new DNA-based framework with Combretaceae sister to the remaining families, this feature now can be interpreted as the plesiomorphic state retained in these three families but subsequently lost in the larger clade sister to Lythraceae + Onagraceae. We are now using updated Myrtales relationships to better understand morphospace evolution of quantitative features such as pollen size and shape (Kriebel et al., 2015).

4.3. Origin and biogeography of Myrtales

The biogeographical scenario presented here is the first for the entire order Myrtales. Several families or closely related clades have been examined in more detail, notably Myrtaceae, Vochysiaceae, Combretaceae, Melastomataceae, and the CAP clade (Berger, 2012; Conti et al., 2002; Gere, 2014; Maurin et al., 2010; Renner et al., 2001; Rutschmann et al., 2004; Sytsma et al., 2004). ARE at greater taxonomic depth presents both opportunities to gain biogeographical insight concerning early cladogenetic events but also issues due to the lack of sampling within families and thus loss of biogeographical signal. This trade-off is clearly observed with Myrtales where on one hand, ARE provides a consistent geographical area (South America and/or Africa) for the stem, crown, and other deeper nodes of the order (and sister order Geraniaceae), while on the other hand, a lack of dense taxonomic sampling...
pling within families, despite adequate coverage of major within-family subclades, generates ARE of some family crown nodes that are at odds with previous family-level studies (see below for further discussion). One possible solution to this biogeographic challenge, which we are now exploring, is to use a supermatrix-derived chronogram that is framed on the well-supported Myrtales topology presented here that uses a smaller data set but with nearly complete taxon-gene coverage. Additionally, it is increasingly recognized that extant taxa indicate persistence in time only, not necessarily in area, and that many clades have been subject to considerable extinction without an attendant fossil record (Crisp et al., 2011; Kooyman et al., 2014). Thus, the following discussion of biogeographic history in Myrtales concedes that limitations in full taxon sampling and in our knowledge of extinction events do not permit unambiguous interpretations.

4.3.1. Early historical biogeography of Myrtales

The biogeography of Myrtales is often attributed to a Gondwanan origin (Conti et al., 1997; Johnson and Briggs, 1984; Muller, 1981; Raven and Axelrod, 1974; Rutschmann et al., 2007; Sytsma et al., 2004). However, phylogenetic uncertainty (Conti et al., 1996, 1997; Sytsma et al., 2004), particularly with respect to the position of Combretaceae, has been a hindrance. Improved support for the placement of Combretaceae and other clades shown by this study provides a good phylogenetic framework for hypothesis testing and ancestral range estimation. Importantly, despite the use of a larger number of fossils, different genes, and different analytical approaches to estimate a chronogram, the dates we infer here are largely consistent with previous dates for Myrtales evolution (Table 2) based on fixed fossil constraints in rks (Sytsma et al., 2004). Most of the stem and crown dates obtained with rks, other than clades not comparably sampled at their bases, fall within the 95% credibility ages obtained with BEAST (Table 2). Thus, we argue that downstream analyses utilizing this Myrtales chronogram(s) (e.g., biogeography, species diversification, character evolution) have a stronger phylogenetic and temporal framework from which to interpret those results.

The BioGeoBEARS analyses support a west Gondwana (South America, Africa) origin of the Myrtales (stem age ~124 Mya, crown age ~116 Mya) and not east Gondwana (Antarctica, Australia, India, Madagascar). ARE does suggest other possible areas (or combined areas) at the oldest nodes within Myrtales, but the dominant ARE shown in Fig. 3 have fairly high likelihood scores. The west Gondwana origin for Myrtales has previously been suggested by Raven and Axelrod (1974), as well as Johnson and Briggs (1984), and is also seen in the Geraniaceae, which is the sister group to Myrtales (Sytsma et al., 2014). Diversification of all the major stem clades within Myrtales are also west Gondwana in origin and date to no younger than 85–90 Mya.

Although dates obtained in our analysis are all younger than age estimates for the initial breakup of Gondwana (180–150 Mya; Ali and Aitchison, 2008; Chatterjee et al., 2013; Jokat et al., 2003; McLoughlin, 2001; Scoteze et al., 1988; White et al., 2013) into west Gondwana and east Gondwana, subsequent continental connections, separations, and tectonic shifts allowed for persistent but intermittent contact and floral exchange into the Paleogene (Hallam, 1994). Raven and Axelrod (1974) argued that it appears likely that upwards of 80 large angiosperm families were widely distributed in tropical regions before the Gondwanan continents separated. According to Pitman et al. (1993), the beginning of the separation of Africa from South America occurred just before 135 Mya in the Early Cretaceous (Valanginian). The early phase of rifting lasted from 135 to about 106 Mya (middle Albanian) but, due to the rotation of Africa away from South America, tropical West Africa was still in contact with northeastern South America. Complete separation of the two continents was achieved sometime between 106 and 84 Mya. Thus, the early diversification of Myrtales, including all the major stem clades, are west Gondwana in origin, date to no younger than 85–90 Mya, and are largely supportive of vicariant explanations.

Recent fossil evidence assigned to Combretaceae (Manchester and O'Leary, 2010) represents the earliest occurrence of the order and establishes a minimum date for tribe Combretaceae that is older than the crown date used for the family in several previous studies (Bell et al., 2010; Magallon et al., 1999; Smith et al., 2010; Wikström et al., 2001). Our analyses estimate the stem age of Combretaceae (=crown of Myrtales) at ~116 Mya, which is closely aligned with the recent estimate for the crown of Myrtales obtained when examining flowering plant diversification (~117 Mya; Magallon et al., 2015). Of the remaining Myrtales lineages, two major clades were established shortly thereafter in the early Albanian–late Aptian (~115 Mya): (1) Onagraceae + Lythraceae and (2) Melastomataceae s.l. + CAP + Myrtaceae + Vochysiaceae. Separation of Melastomataceae s.l. + CAP from Myrtaceae + Vochysiaceae occurred mid-Albian (~109 Mya) with all major extant lineages represented by the Turonian (~91 Mya). As in previous studies (APGIII, 2009; Conti et al., 1996, 1997; Rutschmann et al., 2007; Soltis et al., 2011; Sytsma et al., 2004), Onagraceae + Lythraceae form a well-supported sister clade, which diverged ~105 Mya in the Albian. This date represents the oldest estimate for the clade by ~12 Myr (93 Mya in Sytsma et al., 2004) and may be attributed to new fossil findings, particularly of Lythrum and Paepli pollen from the Lower Campanian (~82–81 Mya; Grönvold et al., 2011). These estimates agree with ages obtained in other fossil-calibrated studies of major clades of eudicots, including the Canerunulids (Beaulieu et al., 2013), Saxifragales (Jian et al., 2008), Malpighiales (Davis et al., 2005), Geraniaceae (Sytsma et al., 2014), Sapindales (Buerki et al., 2010), and Dipsacales (Bell and Donoghue, 2005).

4.3.2. Historical biogeography of Combretaceae

Combretaceae began differentiating ~116 Mya with the split into two extant subfamilies occurring ~104 Mya. While the stem date of the Combretaceae is older than previously proposed ages for the crown of Myrtales (88.2 Mya, Magallon and Sanderson, 2001; 107 Mya, Wang et al., 2009; 107 Mya, Wikström et al., 2001; 108 Mya, Magallon and Castillo, 2009; 111 Mya, Sytsma et al., 2004), the estimates presented here better approximate the ages of the order and the family for three reasons: (1) the inclusion of the West African Stenophenema (subf. Stenophenomatoideae) allows more accurate estimation of the stem lineage of Combretaceae (Conti et al., 1996, 1997; Sytsma et al., 2004); (2) improved sampling provides support for Combretaceae as sister to the rest of the order; and (3) recent fossil descriptions attributed to Combretaceae from the Albanian–Cenomanian (112–93.5 Mya; Manchester and O'Leary, 2010) pushes back the crown date of the family to at least ~103 Mya.

Based on BioGeoBEARS analysis, the crown of Combretaceae was in an ancestral area comprising Africa + South America (Fig. 2), which is consistent with a Gondwanan vicariant origin. Subsequent diversification within the family involved repeated area shifts, often involving more recent dispersals based on continental separations (Fig. 3). We note that the long branch leading to the core Combretaceae (minus the African trees of Stenophenema and the mangrove clade) has an ARE of South America for both the stem and crown nodes. Recent studies with denser sampling within the core Combretaceae (Berger, 2012; Gere, 2014; Maurin et al., 2010) have ARE of Africa for the same nodes, suggesting that biogeographic signal for these nodes is impacted by taxon sampling. Family level ARE such as within Combretaceae, however, often cannot, or do not, take into account the third branch leading into the crown node – that coming from the sister clade. This might
be especially problematic in biogeographic studies centering on Combretaceae as our results provide support that the sister clade is the remainder of Myrtales.

4.3.3. Historical biogeography of Onagraceae + Lythraceae

While multiple phylogenetic studies have corroborated most tribal and generic relationships across Onagraceae (Bult and Zimmer, 1993; Conti et al., 1993, 2003, 2004), the biogeographical history of the family has remained speculative. A Gondwanan origin was suggested by Raven and Axelrod (1974) and Raven (1988) based on the pattern of extant plant distributions and plate tectonics. Conti et al. (1997) provided the first phylogenetic context invoking a West Gondwanan origin, while Sytsma et al. (2004) first estimated divergence times using Penalized Likelihood (PL) and suggested a Late Cretaceous origin for Onagraceae + Lythraceae during the Albian (99 Mya) with subsequent divergence of the two families by 93 Mya (Table 2). In contrast to Sytsma et al. (2004), our data support an earlier Early Cretaceous origin (~115 Mya) for the stem lineage of Onagraceae + Lythraceae with subsequent splitting of the families by the end of the Albian (~105 Mya). Both the timing of these events and the pattern of cladogenesis provide credence for a west Gondwanan origin of Onagraceae with LDDE to the humid Boreotropics of North America from South America during the Late Cretaceous to Paleocene likely playing a role in diversification of the clade (Wolfe, 1975). Within the family, two dispersal events back to South America from North America coincide with the cooling and degradation of the Boreotropical flora during the Early Eocene Climatic Optimum/Paleocene–Eocene Thermal Maximum (EECO/PETM), 55–50 Mya (Zachos et al., 2001). Additionally, as cooling of the planet and subsequent drying intensified with the onset of glaciation during the Oligocene (~33 Mya (McLoughlin, 2001), new lineages, including Cirsium and Fuchsia, evolved from a South American lineage (~29 Mya) and moved northward and southward, respectively. Unlike previous studies (Berry et al., 2004; Xie et al., 2009), we did not constrain the age of Cirsium and Fuchsia (41.5 Mya; based on Sytsma et al., 2004) and obtained an age estimate more than 10 Myr younger, but much more in context with bioclimatic events. Consistent with previous studies, Cirsium exhibits a North American–Eastern Asian disjunct pattern (Xie et al., 2009), while Fuchsia demonstrates a pattern of LDDE from South America to the South Pacific (Berry et al., 2004).

Previous phylogenetic studies of Lythraceae resolved most relationships among extant genera and supported an Old World origin of the family (Conti et al., 1997; Graham et al., 2005; Huang and Shi, 2002; Morris, 2007). However, deep-level relationships of the family have remained unresolved, likely reflecting an early, rapid radiation (Morris, 2007). While our sampling represents a subset of major lineages from the family, we do find evidence of rapid diversification near the base of Lythraceae. Lythraceae diverged from Onagraceae by the end of the Albian (~105 Mya) with the crown date for the family estimated at ~96 Mya with two major clades established within 5 Myr. The fossil record of Lythraceae is diverse (wood, leaves, flowers, pollen and seeds) and widespread in the northern hemisphere (Graham, 2013; Graham and Graham, 1971, 2014) indicative of a Boreotropical distribution. Biogeographical estimation provides strong support for a South American ancestor of the Onagraceae + Lythraceae clade, suggesting movement from South America into North America early in the history of the family (~90 Mya). Fossils attributed to the family supporting this distribution include (reviewed in Graham, 2013): Late Cretaceous (82–81 Mya) fossil pollen grains from Park County, Wyoming, USA, and western Siberia, Russia; leaf impressions and wood from the Deccan intertrappean beds of western India (Late Cretaceous or Early Paleocene 66–63.7 Mya); pollen grains from the Paleocene in France; fruits from the Eocene London Clay Flora; and seeds from the lower Eocene beds in England and Eocene Russia. While one of the major clades (Trapa, Sonneratia, Duabanga, Lagerstroemia, Ammania, Lawsonia, Lythrum, Peplis, and Decodon) provides clear signal of movement from North America into Africa, Eurasia, and eventually into the South Pacific for some lineages, the other (Galpinia, Punica, Cuphea, Adenanthera, and Hemia) indicates LDDE from South America into Sub-Saharan African with movement northward in Eurasia during the Eocene by a single lineage (Punica).

4.3.4. Historical biogeography of Melastomataceae + CAP clade

Renner et al. (2001) suggested a Paleogene (Paleocene/Eocene), non-Gondwanan (north of Tethys Sea) diversification for Melastomataceae s.s. based exclusively on ndhF. They estimated the crown diversification ~53 Mya with substantial diversification within the family beginning ~30 Ma. While an alternative biogeographic hypothesis based on plate tectonics was put forward suggesting a much older age of Melastomataceae (Morley and Dick, 2003), Renner (2004) provided additional evidence refuting most claims. Sytsma et al. (2004) further supported the findings of Renner et al. (2001) using rbcL + ndhF and suggested a crown date for Melastomataceae of 56 Mya. In both Renner et al. (2001) and Sytsma et al. (2004), Early Eocene fossil leaves (Hickey, 1977) were used to constrain the crown of Melastomataceae s.s. including tribe Kibessieae. However, based on the leaf description (Hickey, 1977) and personal correspondence (S. Renner, personal communication), we used the fossils to offset the crown of Melastomataceae s.s. without tribe Kibessieae; hence the older dates for the crown Melastomataceae s.l. should be viewed as minimum ages as opposed to maximum ages.

Although some phylogenetic uncertainty remains with regard to the earliest diverging lineages of the family (Pterandra or Mouriri + Memecylon), biogeographic reconstruction with just extant taxa included supports a South American origin for the family with its crown diversification at ~64 Mya. Across Melastomataceae, a minimum of five dispersal events occurred. The shift from South America to Australasia of the Pterandra clade occurred between 64 and 8 Mya. Whether this migration was facilitated by Southern Hemisphere connections of South America, Antarctica, and Australia or by more direct LDDE is unclear. The spread of Pterandra across tropical Australasia during the Paleogene was likely via dispersal of fleshy capsules by frugivorous bats (Bolmgren and Eriksson, 2010; Hodgkinson et al., 2003; Tan et al., 2000), a mammalian lineage undergoing diversification at this time (Gunnell and Simmons, 2005; O’Leary et al., 2013). During the Eocene (~38 Mya), widespread dispersal of the Medinilla lineage into sub-Saharan Africa, Madagascar, Eurasia, and Southeast Asia occurred, but limited sampling prevents any type of dispersal pattern from being recognized. Near the Oligocene–Miocene border (~27–24 Mya), movement of three lineages out of South America occurred, including Rhexia moving into North America (~27 Mya), a clade within tribe Melastomeae dispersing to the Old World (Sub-Saharan Africa, Eurasia vs. Southeast Asia are recovered equally as the most likely area), and LDDE of Memecylon from South America to the South Pacific (~21 Mya). Evidence previously presented by Conti et al. (2002, 2004) and Rutschmann et al. (2004, 2007) described the Gondwanan breakup as critical to the diversification of the CAP clade, with emphasis on the isolation and rafting of the Indian subcontinent and subsequent dispersal of Crypteroniaceae out of India. Our analyses strongly support (ML-BS/Bi-PP = 100/1.0) the relationship between the Southeast Asian Crypteroniaceae with the west Gondwanan clade, as well as the split between the South American Alzateaceae and South African Penaeaceae (including Rhynchocalycaceae and Olmiaceae; ML-BS/Bi-PP = 100/1.0). While the ideal for calibration is to assign a fossil as close to the node to be estimated as possible, no
current fossil evidence can confidently be assigned to the CAP clade (Mid-Miocene heterocolpate pollen assigned to Crypteroniaceae is difficult to distinguish from Melastomataceae s.l. and Panaeaceae; Muller, 1975). In lieu of this limitation, we view that the use of multiple fossils across Myrtales provides a robust context to better approximate the stem (~91 Mya) and crown (~53 Mya) dates of this lineage. Although our study used a subset of taxa from the CAP clade, the estimated stem age (~91 Ma) is compatible with previously published estimates that utilized more intensive taxon sampling (141–106 Mya, Conti et al., 2002; 109–62 Mya, Rutschmann et al., 2004; 87.25–72.15 Mya, Rutschmann et al., 2007) and thus supports the “out-of-India” hypothesis previously proposed.

4.3.5. Historical biogeography of Myrtaceae

Strong support (ML-BS/BP-PP = 100/1.0) for the inclusion of Heteroppyxis and Psiloxylon within Myrtaceae (now considered subfamily Psiloxylidoideae) agrees with previous results (Biffin et al., 2010; Conti et al., 1996, 1997; Gadek et al., 1996; Sytsma et al., 2004; Thornhill et al., 2012, 2015; Wilson et al., 2005). The split of subfamily Psiloxylidoideae from the rest of Myrtaceae (subfamily Myrtoideae) occurred ~85 Mya, which is in accordance with the estimate of ~83 Mya obtained by Sytsma et al. (2004). Similarity between the estimates for the crown date is likely due to the placement of the fossil Myrtaceae sensu (Myrtaceae sensu stricto) as a minimum date for the split between the two subfamilies; however, where Sytsma et al. (2004) used a fixed date with a tight confidence interval, we used a lognormal distribution to allow the possibility for the clade to be much older. We note that the use of M. lisimae at the crown of Myrtaceae or M. mesenesus at the crown of subf. Myrtoideae as suggested by Thornhill et al. (2012) (or both together) provides very similar dates for the Myrtaceae radiation.

Biogeographical estimations including subf. Psiloxylidoideae have been problematic for two reasons in Myrtaceae: (1) the restricted ranges of Heteroppyxis and Psiloxylon within Myrtaceae (now considered subfamily Psiloxylidoideae) agrees with previous results (Biffin et al., 2010; Conti et al., 1996, 1997; Gadek et al., 1996; Sytsma et al., 2004; Conti et al., 1996, 1997; Gadek et al., 1996; Sytsma et al., 2012) based on another set of fossils, although later than the ~75 Ma date from Biffin et al. (2010) and Thornhill et al. (2015). Dispersal events to Africa, the Mediterranean, and the Americas occurred much more recently during the Miocene. Although our phylogenetic analyses did not fully resolve relationships along the backbone of subf. Myrtoideae (Paleocene–Eocene), all nodal inferences clearly support an Australian/South Pacific origin. The rapid and extensive radiation of Myrtaceae within Australia (often unresolved in phylogenetic analyses) occurs from the Eocene into the Miocene as cooling and aridification intensified (Byrne et al., 2008; Crisp and Cook, 2013; McLoughlin, 2001). Our sampling of South American taxa of fleshy-fruited myrtoids is limited or includes genera of wider geographical distribution (e.g., multiple areas attributed to a single placeholder); hence, there is a lack of strong dispersal signal to the Neotropics relative to that seen in Sytsma et al. (2004), which sampled broadly across the family, and in Thornhill et al. (2015), which recognized up to nine LDDE events in lineages with succulent fruits. The two main fleshy-fruited lineages (Syzygiaceae and Myrtaceae) appear to achieve their pantropical distributions via dispersal during the Miocene. Phylogenetic relationships found strong support for tribal associations for Eucalyptaceae + (Leptospermeae + Chamelaucieae), Osbornieae + Melaleucaeeae, and Metrosideroeeae + Backhouseaeae, all of which are also supported by Thornhill et al. (2012).

4.3.6. Historical biogeography of Vochysiaceae

Vochysiaceae is one of the more intriguing groups within Myrtales because it is so morphologically distinct that it was not placed in the order until Quirk (1980) and van Vliet and Baas (1984) described the presence of bicolonolate vascular bundles and vented pits—two anatomical synapomorphies of Myrtales. This anatomical association was subsequently corroborated by earlier molecular phylogenetic studies (Conti et al., 1996, 1997; Sytsma et al., 2004). The amphi-atlantic distribution of the family has been of interest for decades and has prompted many discussions of vicariance (e.g., Axelrod, 1970) versus dispersal (e.g., Smith, 1973; Thorne, 1972, 1973) as explanatory for the disjunct pattern. Sytsma et al. (2004) provided the first phylogenetic context using a matrix + indel chronogram including six American taxa and one species of the African genus Erismadelphus. Those results indicated a separation from the Myrtaceae ~83 Mya with a crown group radiation for extant taxa ~36–33 Mya. Our data demonstrate a similar pattern of an ancient divergence from the Myrtaceae (~101 Mya) followed by a long period of time until the crown radiation ~39 Mya.
With regard to phylogenetic relationships, Sytsma et al. (2004) indicated a moderately supported sister relationship between American Erisma and African Erismadelphus (ML-BS = 85), which was then sister to a clade comprised of the remaining five Neotropical species (ML-BS < 50). The origin of African taxa out of a South American clade was consistent with previous morphological and molecular studies (Litt, 1999, 2003; Litt and Stevenson, 2003). In this study with more limited taxon sampling but expanded loci sampling, we found the African genus Erismadelphus sister to all Neotropical Vochysiaceae (BI-PP = 0.92). However, the African/American split at the base of the family (rather than more internal) is still in agreement with previous studies as the stem lineage of the family supports a South American origin. The timing of the South American – African split in the late Eocene indicates a LDDE event in Vochysiaceae across an Atlantic Ocean that was then almost 2/3rds formed, one of a number of similar South American–African disjunct patterns best explained by LDDE rather than vicariance (Givnish and Renner, 2004; Givnish et al., 2000, 2004).

4.4. Myrtales exhibits three significant shifts in species diversification rates

An important question now being addressed more critically in evolutionary biology is the nature of the processes that lead to significant shifts in speciation and/or extinction rates within clades (e.g., Alfaro et al., 2009; Antonelli et al., 2015; Biffin et al., 2010; Givnish et al., 2014; Linder et al., 2014; McGuire et al., 2014; Rabosky et al., 2014; Smith et al., 2011). Relevant issues in detecting significant rate shifts include incorporating extinction, phylogenetic uncertainty, phylogenetic scale, sampling density, correlation and/or causality of biotic or niche attributes driving the rate shifts. The program BAMM, as now implemented, can address a number of these issues. The largely Southern Hemisphere Myrtales is an ideal candidate for such studies considering its relatively old origins in the Cretaceous, a widespread biogeographical distribution, occupation of diverse habitats, considerable morphological diversity, the presence of both species-rich and depauperate clades, and a now well-supported and dated phylogeny. Our analyses were conducted at two scales: (1) across Myrtales with 102 species, but carefully chosen to represent all the major distinct clades within families, and (2) within individual families or family groups.

The baseline or average rate of net diversification for Myrtales is not striking (0.06–0.07 species/Myr; Table 3). This rate is very similar to those estimated by Magallon and Sanderson (2001) using different approaches (0.079–0.097 species/Myr), and comparable to average rates they report for Eudicots and Angiosperms. Antonelli et al. (2015) provided estimates for net diversification in Myrtales (and 16 other lineages) from a supermatrix Angiosperm tree, and while not strictly comparable to our study as they separated out rates based on biogeographical area, their results are in line with those presented here (in species/Myr): African tropics (0.111), Asian tropics (0.051), American tropics (0.009). In general, and demonstrated in rates-through-time analysis (Fig. 4), Myrtales exhibited long-term speciation rate decline for the first ~60 Myr and extinction rate increase throughout its entire ~115 Myr diversification. However, at ~65 Myr speciation (and net diversification) increases rapidly (Fig. 4). Around this time three significant rate shifts are seen within the Myrtales (Fig. 3) and, though they are placed at different ages (~76, ~64, ~43 Mya), all occur on branches that span the K–Pg boundary (66 Mya). Antonelli et al. (2015) presented intriguing evidence, based on their angiosperm-wide meta-analysis of net diversification rates in the context of biogeography, that a peak in angiosperm speciation and in biogeographical shifts between tropical regions is correlated to another climatic event – the Paleocene–Eocene Thermal Maximum at ~56.3 Mya. Whether either of these two major climatic events with profound vegetation changes (Jaramillo et al., 2010; McElwain and Punyasena, 2007) influenced the shifts in diversification within Myrtales remains unknown.

The first and oldest shift in speciation rates within Myrtales, which occurred ~76 Mya on the branch leading to the subf. Myrtoidae (after divergence of the African lineage comprising Heteropyxis and Psiloxylon), coincides with the likely LDDE of its ancestors to Australia from Africa (Fig. 3). A paralythene assemblage of largely Australian, capsular-fruit–, and arid-adapted tribes (Xanthostemoneae, Lophostemoneae, Osborneaee, Melaleu–

eae, Lindsayomyrtaceae, Synnapiae, Eucalypteae, Leptospermeae, and Chamelaucieae) are the first diverging clades within subf. Myrtoideae. Notably, a large, Southern Hemisphere clade containing all other tribes, including the fleshy-fruited mesic-adapted taxa, diverged in the Paleocene and rapidly diversified starting at the Eocene/Oligocene boundary. Thus, the shift in species diversification in Myrtaceae is correlated with a shift to a new biogeographic area and seemingly to one of the strongest biome/niche shifts in the Myrtales – tropical forest to arid communities. This niche shift, a pattern seen in other major radiations (Crisp et al., 2009; Donoghue and Edwards, 2014; Kissling et al., 2012; Linder et al., 2014), would be possible however only in the mid-Miocene (15 Mya) when initial aridification of Australia occurred after South America broke free of Antarctica and initiated the Antarctic circumpolar current (Byrne et al., 2008). Thus, our dates (late Cretaceous/early Paleocene) and even earlier dates of Ladiges et al. (2003), Thornhill et al. (2015) for the crown radiation of subf. Myrtoidae surprisingly suggest a rapid radiation of taxa all now largely adapted and confined to an arid biome that would not appear in Australia for at least another 40 Myr.

What biotic features of the Myrtaceae may be correlated with this shift in species diversification? Ancestral state reconstruction of fruit type onto the phylogeny of Myrtales (unpublished data) allowed us to test the hypothesis that birds or bats may have played a role in this dispersal event, which was then followed by the shift in speciation rate. We found no support for this hypothesis since the common ancestor of subf. Myrtoidae unambiguously had dry fruit, with fleshy fruits evolving much later some 30 million years ago. Other studies have also suggested the common ancestor and early divergent lineages of Myrtaceae to be dry fruited (Biffin et al., 2010). In that study focused on the subclade of the family with two, probably unrelated, fleshy fruit tribes, increased rates of speciation were correlated with the acquisition of fleshy, bird-dispersed fruits. A second set of factors that may be involved in the diversification of Myrtaceae include the evolution and radiation of the most diverse group of bees, the corbiculate (Martins et al., 2014), and subsequently ~50 Mya the alidioapines in Australia (Chenoweth and Schwarz, 2011). Bees are the most common pollinators of Australian Myrtaceae today (Beardsell et al., 1993) and further studies should explicitly test the impact of bee pollinators on subf. Myrtoidae. Interestingly, the shift in speciation rate detected in Myrtaceae when examining the entire order was not detected in the Myrtaceae-only analysis (Fig. 6). This is likely due to its occurrence toward the base of the Myrtaceae-only phylogeny. A supermatrix chronogram of Myrtaceae may be necessary to obtain sufficient taxon sampling to more rigorously test the number and placement(s) of significant rate shifts and examine the potential role of these and other biotic and abiotic factors in promoting diversification within the family.

The second shift in species rates within Myrtales occurred ~64 Mya (branch age of 64.5–62.2 Mya) at the base of Melastomataceae, after the split between the genus Pernandra (tribe Kibesieae) and the rest of the family (Fig. 4), and clearly occurred in a South American lineage based on our phylogenetic estimate and ARE (Fig. 3). This shift corresponds with the suggested synapomor-

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phy for the subfamily Melastomoideae of libriform fibers (Renner, 1993). This shift was not detected in the Melastomataceae only BAMM analysis that shows support for a shift within tribe Miconieae, after the divergence from Leandra. Both of these resulting shifts in speciation rates are somewhat unexpected because fleshy-fruiting clades such as the Miconieae are so diverse that we would have predicted an association between diversification rates and fleshy fruits within the Melastomataceae. This result could also be due to the effects of scaling, sampling and phylogenetic resolution discussed below, which can affect the placement of shifts such as the one we expected in the common ancestor of the tribe Miconieae. Future studies with higher taxon sampling in the Melastomataceae are needed to test this hypothesis.

A second possible explanation for the shift in speciation rate toward the base of the Melastomataceae is the evolution of poricidal anthers that provide a pollen reward via buzz-pollination and are visited by female bees (Buchmann, 1983; De Luca and Vallejo-Marin, 2013). Most of the 6000 species of Melastomataceae are thought to be buzz-pollinated and poricidal anthers were inferred to have evolved early in the evolution of the family (Clausing and Renner, 2001; Renner, 1989, 1993). Since only female bees can access the pollen reward offered by flowers in the family, buzz-pollination is considered a highly specialized mechanism of pollination and likely contributed to the radiation of the family. Such is the strength of the relationship between these melanotomes and their bee pollinators, that the Melastomataceae have been suggested to be trapped in an adaptive peak with their bee pollinators, comparable to what is suggested for the Malpighiaceae and Solanaceae (Biffin et al., 2010; Renner, 1989). Whether this close relationship between bees and the Melastomataceae has resulted in the observed species rate shift is unclear. Buzz pollination has rarely evolved within Myrtales aside from the Melastomataceae (but see Proença, 1992). For this reason, we presently lack statistical power to test for the relationship between poricidal anthers/buzz-pollination and an increase in speciation rate.

A recent study looking at shifts in diversification rates associated with the transition from the forest to the fynbos habitat in South Africa found a shift within the CAP clade that was not detected in our study (Onstein et al., 2014). The difference in shifts is likely due to the different strategies in taxon and gene sampling employed by both studies and highlights the sensitivity in detecting shifts in diversification rates associated with scale.

The final and third shift in speciation rate within Myrtales, and the best supported with Bayes factor evidence, occurred within Combretaceae at ~43 Mya. The shift occurred after the earlier divergence of the west African tree genus Strephehoma and the largely pantropical mangrove clade. Although placed at ~43 Mya, the branch exhibiting the rate shift spans from 94.5 to 33.2 Mya (Fig. 5). Our biogeographical reconstruction indicated that the shift occurred in South America, although Africa was important earlier in family differentiation. Greater taxonomic sampling of the pantropical Combretum and Terminalia clade (subtribes Combretinae and Terminalinae) derived from this shift in speciation rate suggests Africa as a more likely AKE for this branch (Berger, 2012; Gere, 2014; Maurin et al., 2010). If so, the three significant shifts within Myrtales at this scale involved three different Southern Hemisphere continents: Africa, South America, and Australia.

The Combretaceae occupy a diversity of ecological niches and exhibit striking habit diversity (liana, mangrove, shrub, tree). The shift in speciation rate occurred after the tree genus Strephehoma and a mainly mangrove clade had already diverged. The clade demonstrating the increased rate of speciation, the Combretum (272 spp.) and Terminalia (232 spp.) lineages, are rarely fleshy fruited (as in the Melastomataceae and Myrtaceae clades showing significant speciation rate shifts). However, one feature this clade shares is winged fruits, although lacking in some members and convergent with Macropeteranthes (Manchester and O'Leary, 2010) in the mangrove clade. Additionally, this clade shows pronounced levels of dispersal events between the three main tropical areas (Berger, 2012) and these events may have spurred repeated diversification in newly occupied areas.

The analyses presented here, both at the ordinal and family levels in Myrtales, reinforce the importance of scale, sampling, and phylogenetic resolution when examining and interpreting patterns of diversification. First, we demonstrate that scale influences the best shift configuration despite the same taxa being analyzed. For example, the rate shifts seen in Melastomataceae are different than that seen when the Melastomataceae are examined in the context of the entire Myrtales (Fig. 6). Second, increased taxon sampling (and thus larger numbers of terminals that can be scored for extant species richness) should provide more precise identification of rate shift placements. And third, most diversification analyses assume a single tree as representative of the group being studied. Specifically, Biffin et al. (2010) acknowledge this issue as they evaluated rate shifts in speciation among the fleshy-fruiting Myrtaceae, two main lineages whose relationships to each other are not yet known with certainty. Future studies should focus on two approaches. One is taking a supermatrix approach (e.g., Antonelli et al., 2015), although this approach entails assumptions (and thus trade-offs) using a single tree and estimating branch lengths with a high percentage of missing data. A second approach should examine PP distributions of trees (as we did in biogeographic analyses with BioGeoBEARS) and summarizing signal in rate shifts across many trees. We note that a BAMM analysis conducted on one of the most different 100 PP trees examined in this study (Combretaceae sister to Onagraceae + Lythraceae), the same three significant rate shifts in speciation were uncovered within Myrtales although at different points on their respective branches (data not shown). Thus, these results should be viewed as coarse-grained, and future studies using supermatrix approaches (e.g., Kriebel et al., 2015) and suites of alternative but likely trees may provide refinement on the placement of and processes leading to significant shifts in speciation rates within Myrtales.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.10.001.


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