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 ∼124 Mya during the Aptian of the Early Cretaceous. The crown date for Myrtales is estimated at ∼116 Mya (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 Mya), along the stem of subfamily Myrtoideae (Myrtaceae; ∼75 Mya), and along the stem of tribe Combreteae (Combretaceae; ∼50–45 Mya). Issues with conducting diversification analyses more generally are examined in the context of scale, taxon sampling, and larger sets of phylogenetic trees.

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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 organismal extinction (e.g., Davis et al., 2005; Deng et al., 2015; Givnish et al., 2009; Schönenberger 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).

1.1. Biogeographic hypotheses of Southern Hemisphere disjunct groups

Of particular interest for re-examination using these improved phylogenetic approaches are groups exhibiting a Southern

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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; Vinnersten 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., Bukontaite 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; Sanmartín 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 diversifications 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 genic region used (see Soltis 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., Magallón, 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 (Crossosoma), Malvales (Thymelaea), Brassicales (Arabidopsis, Carica), and Geraniales (California, Erodium, Francoa, Geranium, Hypseocharis, Melianthus, Monsonia, Pelargonium, Vivania). Geraniales is the sister order to Myrtales, with the Crossosomatales, Malvales, and Brassicales representing three orders of