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Calyx (con)fusion in a hyper-diverse genus: Parallel evolution of unusual flower patterns in *Eugenia* (Myrtaceae)

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ABSTRACT

Eugenia has a pantropical distribution and comprises ca. 1000 species found mostly in the Neotropics. Recent DNA based phylogenies show that unusual flower morphology of 'eugenioid' collections, e.g. fused calices that open by tearing, consistently emerged within *Eugenia*. These results emphasize a demand to revaluate flower morphology in a phylogenetic context within the genus. A reassessment of calyx fusion in *Eugenia* and traditionally related genera is here focused on clarification of the systematic relevance of this apparently recurrent characteristic. Twenty-four *Eugenia* species with some level of calyx fusion in the bud were newly used (one nuclear and four plastid markers) in conjunction with a representative sample of previously sequenced species to recover a time-calibrated *Eugenia* phylogeny of 86 accessions. Development of the fused calyx was analysed using scanning electron microscopy, differing patterns were re-coded and subsequently phylogenetic character reconstruction was performed. *Eugenia* was recovered as monophyletic including the traditionally segregated genera *Calycorectes* and *Catinga*. Ancestral character reconstruction uncovered free calyx lobes as the ancestral condition. Five development patterns leading to calyx fusion are reported in *Eugenia* including species with apparently six petals, which contrast with the standard tetramerous flowers. This condition is interpreted as the *petaloid* pattern, where two external fused calyx lobes cover the bud while two internal calyx lobes are free and petaloid. The fused calyx condition is homoplastic and evolved independently, several times in *Eugenia,* as did the different development patterns. Data presented here show that systematic incongruence resulting from multiple, independent origins of the fused calyx in *Eugenia* is further aggravated by an inability to distinguish parallelism and convergence within the recovered patterns.

1. Introduction

Classical authors recognized two or three tribes of Myrtaceae, circumscribed according to the presence of fleshy-berry or dry-capsular fruits (Candolle, 1828; Schauer, 1841; Niedenzu, 1898). Cladistic insight based exclusively on morphology (Johnson and Briggs, 1984), molecular data (Gadek et al., 1996; Lucas et al., 2005; Wilson et al., 2005) or both (Wilson et al., 2001), demonstrated that many characters presumed diagnostic in earlier classifications were, in fact, more homoplastic than previously appreciated. Myrtaceae is a large family with ca. 5500 species, and today classified into 17 tribes (Wilson et al., 2005; Wilson, 2011) based in many combined sets of morphological and molecular evidence (Wilson, 2011). Myrteae is the most diverse tribe comprising half of the species of the family, with 51 genera and ca. 2500 species (Wilson, 2011; WCSP, 2019) and includes the hyper-diverse genus *Eugenia* L.

Eugenia has a pantropical distribution and comprises ca. 1000 species, found mostly in the Neotropics (Wilson, 2011; Mazine et al., 2014; 2016). *Eugenia* has high ecological importance (Staggemeier et al., 2017) and is the richest tree genus in some regions of the Brazilian Atlantic coastal forest (Oliveira-Filho and Fontes, 2000). However, high levels of homogeneity of taxonomically important characters such as

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flowers and fruits make species identification notoriously difficult (Lucas and Bünger, 2015). At one hand, the classic *Eugenia* flower has four free calyx lobes, enabling easy genus recognition (e.g. Berg, 1857; Landrum and Kawasaki, 1997; Sobral, 2003). On the other hand, collections 'eugenioid' in every way apart from flowers with unusual morphology, e.g. fused calices that open by tearing, consistently emerged within *Eugenia* (Mazine et al., 2014, 2018; Giaretta et al., 2019) in DNA based phylogenies. These results emphasize a demand to reassess flower morphology in a phylogenetic context within the genus.

Previously, genera have been segregated from *Eugenia* on the basis of differences in degree of fusion and dehiscence of the calyx (Amshoff, 1951; McVaugh, 1969; Legrand and Klein, 1972). As a consequence, genera such as *Calycorectes* O.Berg and *Catinga* Aubl., including species that resemble *Eugenia* in all but possession of a fused calyx (also described as "calyx closed or almost closed in bud", McVaugh, 1968) are taxonomically controversial. Some authors segregate *Calycorectes* from *Eugenia* based on calyx morphology whereas a 'unified *Eugenia*' is supported by most contemporary authors (e.g. Holst, 2002; Flora do Brasil, 2020).

A seminal molecular phylogenetic framework focused on infrageneric classification in *Eugenia* confirmed that the genus can only be monophyletic if fused calyx species previously identified within *Calycorectes* are included (Mazine et al., 2014). The character of the fused calyx conveniently diagnosed *Eugenia* sect. *Schizocalomyrtus* (Kausel) Mattos (Mazine et al., 2014; Giaretta et al., 2018) but the variable degree and pattern of calyx fusion suggest that closer scrutiny of developmental aspects of this character might allow more accurate use. Vasconcelos et al. (2018) describe the most common pattern of calyx development in *Eugenia* species. However, there was no mention to or descriptions of any of the unusual forms of calyx fusion in that study. To fully understand the origins and significance of the closed calyx in *Eugenia*, a more representative morphological and molecular sample is required; only four closed calyx species were used in the most recent phylogenetic study (Mazine et al., 2018). It is here estimated that ca. 40 species of *Eugenia* undergo some degree of calyx fusion, most of which are included in what used to be recognized as *Calycorectes*.

Phylogenetic relationships based on molecular data have been investigated at various ranks in Myrteae, resulting in current, increasingly accurate, natural classifications (Lucas et al., 2007, 2011; Mazine et al., 2014; Staggemeier et al., 2015; Bünger et al., 2016; Vasconcelos et al., 2017b). However, recurring morphological homoplasy that historically misleads systematists and taxonomists still hampers the organization of diversity (e.g. Vasconcelos et al., 2017a). Insufficiently investigated morphological characters produce classifications that lack predictability and the necessary insight can be gleaned by morphological assessment. The approach used here integrates molecular phylogeny and a survey of calyx development patterns to (1) clarify systematic relationships among fused calyx species in the hyper-diverse genus *Eugenia*; (2) re-evaluate the fused calyx through careful investigation of flower morphology; (3) assess the evolutionary history of the fused calyx and; (4) diagnose fused calyx clades in *Eugenia*.

2. Material and methods

2.1. Molecular and morphological sampling

Material of 24 taxa of *Eugenia* and traditionally related genera were extracted and sequenced from 30 samples. The survey prioritized species that fit the current delimitation of *Eugenia* (see Mazine et al., 2016) with some level of calyx fusion in the bud, including as much morphological and geographical variation as possible. Taxonomy used here follows Flora do Brasil (2020) with complementary information from Mattos (2005). The survey corresponds to ca. 70% of *Eugenia* sect. *Calycorectes* (O.Berg) Mattos (sensu Mazine et al., 2016). A further 48 *Eugenia* s.l. and two *Myrcianthes* O.Berg (*Eugenia*'s closest related genus) were included to recover internal relationships among recognised clades. Six additional Myrteae genera were used as the outgroup. The internal transcribed spacer (ITS) of the ribosomal nucleus and the plastid regions *psb*A-*trn*H, *rpl*16, *trn*L-*rpl*32 and *trn*Q5′-*rps*16 were used. In total, 149 new sequences are provided with the remaining obtained from existing published (Lucas et al., 2007; Mazine et al., 2014, 2018; Bünger et al., 2016; Vasconcelos et al., 2017b) and unpublished work (J.E. Faria, UB, Brasília, Brazil, unpubl. res.). The molecular sample of 86 accessions is available in Appendix A.

Fused calyx development patterns were assessed from floral buds in as many development stages as possible, collected and conserved in 70% ethanol from field collections in Brazil and French Guiana. A complementary survey was based on buds from recent herbarium collection available at K; the material was rehydrated in boiling water for 10 min, left to cool overnight and then preserved in 70% ethanol. A total of 25 samples representing 18 species and all known variation between fused and free calices in *Eugenia* were surveyed in a comparative development analysis. At least three species were assessed for each development pattern. The only exception is the *longohypanthium* pattern found only in a single species. Descriptions of later stages of bud development were emphasized to facilitate recognition of development patterns on pre-anthetic flowers, as these are easier to manipulate in herbarium material. A list of analysed material using scanning electron microscopy (SEM) is provided in Appendix B.

2.2. DNA sequencing

Total DNA was extracted using QIAGEN® DNeasy® Plant Maxi Kits from until 0.2 g of silica-gel dried leaf material generating 1.5 ml of total DNA. Amplification and purification of DNA regions were performed according to protocols outlined in Lucas et al. (2007, 2011) and (Shaw et al., 2007) for *rpl*32-*trn*L and *trn*Q5′-*rps*16. PCR conditions were executed according to Bünger et al. (2016). Nucleotide sequencing follows the protocols outlined by Lucas et al. (2007). Sequences were assembled and aligned using MUSCLE (Edgar, 2004) and edited when necessary using Geneious v7.9 (Kearse et al., 2012). DNA samples are stored in the DNA Bank and Tissue Collections of Royal Botanic Gardens, Kew.

2.3. Phylogenetic analysis and estimation of divergence times

Four chloroplast-DNA regions (cpDNA) were combined resulting in a matrix of 3185 base pairs; the nuclear ITS partition comprised 671 base pairs. Independent Bayesian Inferences (BI) were performed on both the cpDNA and nuclear ITS regions (available in Appendix C, respectively). The best nucleotide substitution models were selected with jModeltest2 v2.2 (Darriba et al., 2012) through the Akaike Information Criterion (AIC); GTR + I + G was implemented for both ITS and cpDNA datasets. The model was implemented in MrBayes v3.2.1 (Ronquist et al., 2012) on XSEDE v.3.2.6. Two independent runs with four Markov Chains Monte Carlo (MCMC) each was performed with 10 million generations, sampling every 1000 trees. A 10% burn-in was used for tree annotation. Output was examined using Tracer v1.6 (Drummond and Rambaut, 2007) to confirm chains convergence through verification of the effective sample size (ESS) values of each parameter were > 200. Visual inspection of BI topologies detected no statistically supported incongruence (i.e. incongruences are found only in poorly supported clades). Concatenation of cpDNA and nuclear datasets resulted in a matrix of 3856 bp, that was then subjected to independent BI analysis implemented in CIPRES (Miller et al., 2010). Phylogenetic reconstruction using maximum likelihood (ML) was also performed but only for the combined dataset using RAxML v.7.6.3 and implemented in CIPRES, using the fast algorithm with 1000 bootstrap replicates and the remaining options set to default. *Myrtus communis* was used to root the phylogeny on BI and ML.

The phylogeny was time-calibrated using an uncorrelated relaxed molecular clock model, lognormal distribution of rates and Birth-Death

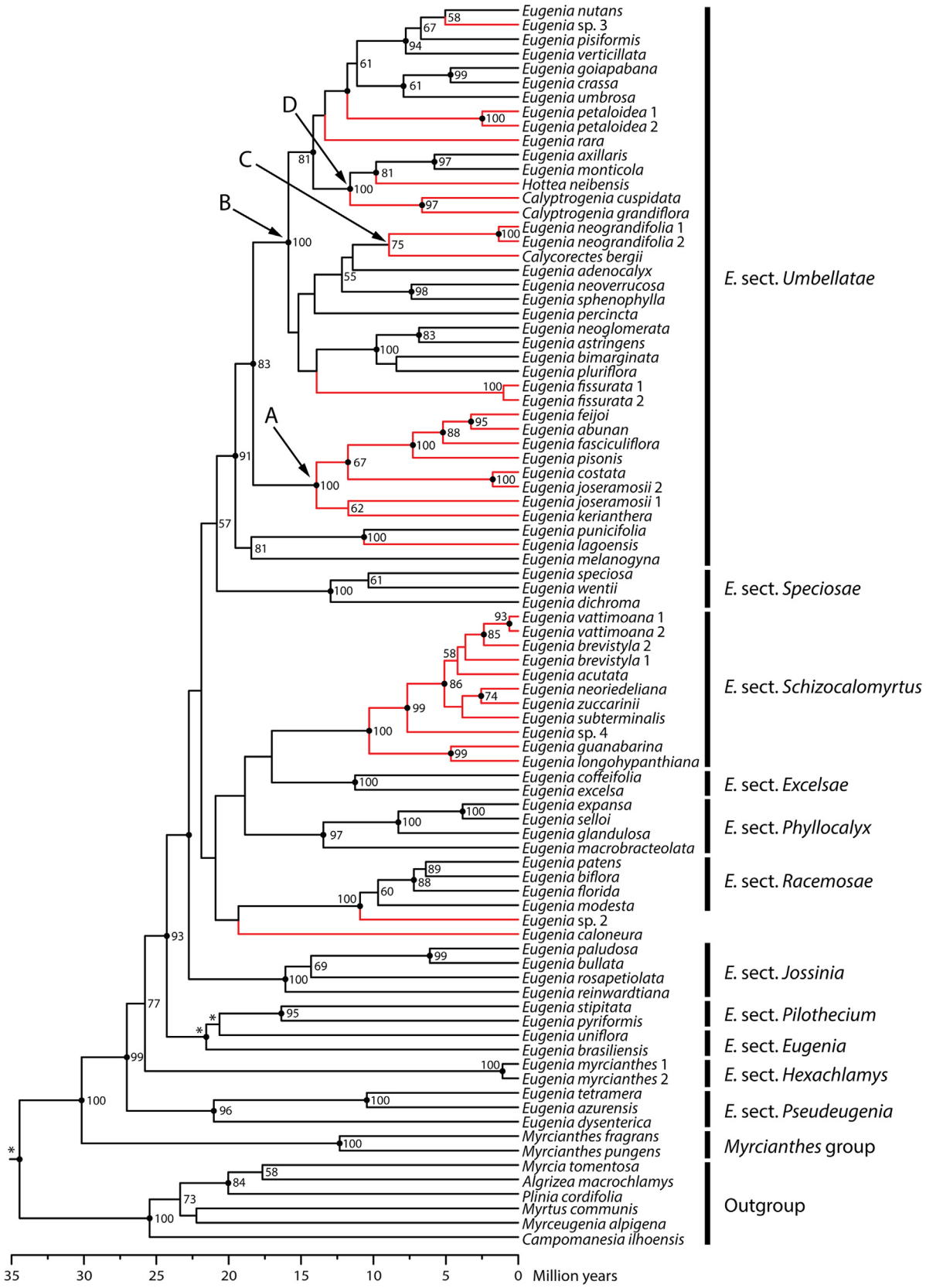

Fig. 1. Maximum clade credibility (MCC), time-calibrated phylogeny resulting from BEAST analysis of the combined dataset. Nodes receiving posterior probabilities greater than 0.95 are indicated by black dots; bootstrap percentages recovered by the Maximum Likelihood (ML), equal or greater than 50 are shown above branches. * indicates nodes not recovered in the ML. Red branches indicate lineages with fused calyx. (Node A) "*Eugenia feijoi* group". (Node B) *Eugenia* sect. *Umbellatae*. (Node C) "*Calycorectes* group". (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

model implemented using Bayesian inference in BEAST v1.8.4 (Drummond and Rambaut, 2007). The assumption was made that the *Eugenia* supergroup (*Eugenia* and sister *Myrcianthes*) was monophyletic, as recovered in the non-dated BI and ML. The partitions and nucleotide substitution models were the same as in the initial MrBayes analysis, using empirical base pair frequencies. Two secondary calibration points were taken from the phylogeny of Vasconcelos et al. (2017b). This study tested different sets of fossil calibrations to estimate ages for the main clades of Myrteae. In this sense, to reconstruct a more inclusive Myrteae phylogeny only with the purpose of accommodating fossil calibration was not considered necessary here. Crown nodes of the *Eugenia* supergroup and the Neotropical lineage were calibrated with normal prior means of 29.29 Ma and 35.36 Ma respectively, and a standard deviation of 1.4. The calibration procedure adopted here follows the recommendations of Forest (2009). Four independent MCMC runs of 50 million generations were performed sampling every 1000th. Output examination followed the procedure applied to non-dated BI. Results were combined in LogCombiner and maximum clade credibility (MCC) tree was built using TreeAnnotator (both distributed on BEAST v1.8), selecting median height nodes and a burn-in of 10%. Although discussion of divergence times is not the main purpose of this paper, inclusion of a temporal framework and estimations of branch length are relevant for robust reconstructions of character evolution (Forest, 2009; Litsios and Salamin, 2012). Thus, the following ancestral character estimations were based on the resulting MCC tree.

2.4. Floral development pattern analysis

Flower buds were dehydrated in an alcohol series and left overnight in 100% ethanol. The material was then brought to dehydration in a critical-point dryer using an Autosamdri-815B (Tousimis Research, Rockville, Maryland, USA). The dried material was mounted onto aluminum stubs, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinstead, UK) and examined in detail using Hitachi cold field emission Scanning Electron Microscopy S-4700-II (Hitachi High Technologies, Tokyo, Japan). Different stages of flower development of the same species were examined from different collections when necessary. Relative orientation among bracteoles and structural whorls was used as reference for developing structures. A total of 306 images were analysed.

2.5. Ancestral character reconstructions

Ancestral states of development patterns were reconstructed as discrete traits using two approaches to better scrutinise the view supported here. A maximum likelihood approach (MLA) was implemented using the package *ape* v4.1 (Paradis et al., 2004) and the 'ace' function. Bayesian stochastic character (BSC) mapping (Huelsenbeck et al., 2018) was performed using the 'make.simmap' function available in *phytools* v0.5–64 (Revell, 2012). Both analyses were implemented in R (R Core Team, 2018) using an "equal rates" model to recover the evolutionary history of the development patterns under investigation. Outgroup and terminals with more than one accession per taxon were removed from the analysis using the function 'drop.tip' from *ape* to prevent bias in character reconstruction. Terminals with fused calices were coded in six states (see Section 3), where "free lobes" is a modification of the flower development pathway proposed in *Eugenia punicifolia* by Vasconcelos et al. (2018). Stochastic character mapping was performed using 10,000 simulations. The resulting optimisation of characters incorporates branch lengths and timing of character states transitions, depicted using the function 'densityMap' in *phytools*. BSC provide transition rates which were used to build a transition matrix among patterns as input to the heatmap depicted using *pheatmap* v1.0.12. No relevant incongruence was detected between the MLA and BSC character reconstructions, therefore only results from the stochastic character mapping are presented (MLA resulting analysis is available in Supplementary Material 3).

3. Results

3.1. Phylogenetic reconstruction and divergence time

Bayesian inference (BI) and Maximum likelihood (ML) topologies recover *Eugenia* as a well-defined group in the combined (1 posterior probability/PP and 100% bootstrap/BS), nuclear (0.99 PP; 75 BS) and cpDNA datasets (0.96 PP; 97 BS). The combined Bayesian inference tree with the equivalent ML topology are available as Supplementary Data (1 and 2). Topologies of BI and ML recovered here are congruent with recent *Eugenia* phylogenies (Mazine et al., 2014, 2018; Bünger et al., 2016). The topology of the BEAST MCC tree (Fig. 1) is congruent with the BI and ML, therefore further discussion is based on the MCC tree. Divergence time estimations for the stem and crown node of *Eugenia* (27.7 to 32.6 Mya and 23.89 to 30.04 Mya, respectively) are similar to previous studies. Extant calyx fused lineages appeared ca. 14 Mya (10.76 to 17.34 Mya) with ten independent origins within *Eugenia* (marked in red); the following results and discussion focus on relationships between and within these species.

The phylogenetic relationships recovered in this study, most relevant to the evolution of the closed calyx in *Eugenia* can be summarised as follows: a strongly statistically supported clade (1 PP) emerges within *Eugenia* sect. *Umbellatae*, here informally called the "*Eugenia feijoi* group" (node A). A further informal group (0.91 PP) within *Eugenia* sect. *Umbellatae* called "*Calycorectes* group" (node C) includes *Eugenia neograndifolia* (previously *Calycorectes grandifolius*), the type species of *Calycorectes*, and *Calycorectes bergii*. Newly sampled *Eugenia vattimoana, E. acutata, E. cambucae, E. guanabarina, E. zuccarinii* and *E.* sp. 4 emerge in *Eugenia* sect. *Schizocalomyrtus* (1 PP). Other newly sampled, fused calyx species *Eugenia fissurata*, *E. lagoensis, E. rara, E. caloneura, E.* sp. 2, *E.* sp. 3 and *E. petaloidea* emerge throughout *Eugenia* sect. *Umbellatae*, all sister to species with free calyx lobes.

3.2. Development patterns of calyx fusion

Scanning electron microscopy revealed all *Eugenia* flowers examined to be tetramerous regardless their calyx fusion, with external sepals decussate relative to the bracteoles. The sepals are the first structures to initiate as independent organs (see Vasconcelos et al., 2018) and, in flowers with fused calyx, undergo late-congenital fusion inferred by remnants of free lobes on the bud apex. Petals are positioned between sepals. *Eugenia* with free sepals (Fig. 2A) were observed to have followed the flower development pattern of *Eugenia punicifolia* detailed in Vasconcelos et al. (2018). In species with calyx fusion, five distinct patterns are observed and are here considered in conjunction with other known Myrteae development pathways (e.g. *Myrcia* s.l., Vasconcelos et al., 2017a). These patterns are here described from later stages of bud development, however, future analyses of early stages of development are required to complete understanding of the patterns found. The five development patterns found in *Eugenia* fused calices are described as follows:

3.2.1. Heterosepalous pattern

Flowers that follow this pattern have four calyx lobes fused to a varying degree at the base. Lobes are most commonly fused along two-thirds of the length of the bud leaving an opening of ca. 1 mm diameter (Fig. 2B and D). Buds in which fusion is as little as one-third of bud length or as much as near-complete closure are also found. In this arrangement, the line of fusion is along the edge of each calyx lobe and results in heterogeneous thickness of the calyx that tapers into a fragile tissue between each sepal (Fig. 2C, E and F). The different degrees of fusion observed suggest that the timing of calyx fusion varies although it is most frequently observed early on in floral development. At anthesis the fragile calyx tissue splits into four regular lobes (Fig. 4A).

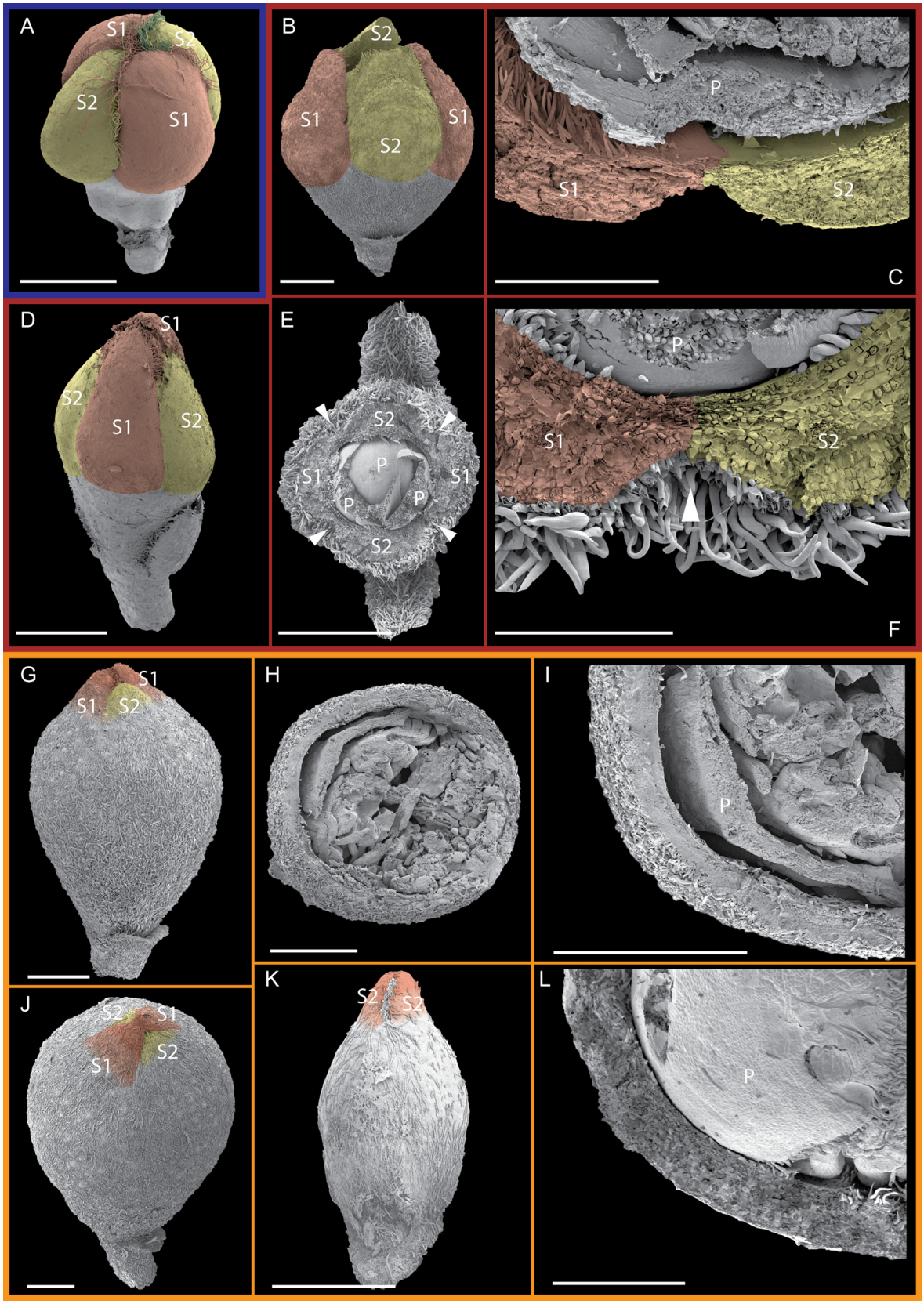

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Fig. 2. Comparative development patterns of the calyx in *Eugenia*. (A – blue box) Standard condition of free calyx lobes in *Eugenia uniflora*. (B-F – dark-red box) *Heterosepalous* pattern. *E. brevistyla* (B) and *E. subterminalis* (D) with calyx lobes partially fused and free at the apex; transverse section of *E. acutata* (E) in a fused portion of the bud showing the heterogeneous thickness of the calyx tissue caused by tapering between each sepal lobe indicated by arrows; detail of the transverse section of *E. brevistyla* (C) and *E. acutata* (F). (G-L – orange box) *Homosepalous* pattern. *E. neoriedeliana* (G) and *E. vattimoana* (J) with calyx lobes nearly closed except at the apex where the remains of the four vestigial lobes are free; in *E. guanabarina* (K) only the two external lobes can be seen at this stage; transverse section of *E. neoriedeliana* (H) in a fused portion of the bud showing the homogeneous thickness of the calyx tissue; detail of the transverse section of *E. neoriedeliana* (I) and *E. guanabarina* (L). Scale bars = 1 mm. Color coding: external sepals (S1), red; internal sepals (S2), yellow; petal (P). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

There are four free petals, each positioned between the bases of the sepals. The stamens are straight in bud, attached to the tissue between the corolla and style ('hypanthium' *sensu* Vasconcelos et al., 2018).

3.2.2. Homosepalous pattern

Buds that follow this pattern are nearly closed except at the apex where the remains of four vestigial lobes are free (Fig. 2G, J and K). Again, the presence of vestigial lobes indicates early fusion of calyx lobes. In this arrangement, the line of fusion is also along the edge of each calyx lobe but results in a homogeneous tissue without evidence of sepalar seams (Fig. 2H, I and L). Anthesis follows two possible opening patterns that can vary between accessions of the same observed species: the fused calyx (1) tears resulting in two to four irregular lobes; (2) opens transversely via a tear at the calyx base resulting in a structure that resembles a calyptra (Fig. 4B). The four petals are free, each positioned between the bases of the sepals. Occasionally, the corolla is reduced to one petal (e.g. *Eugenia guanabarina*). The stamens are straight in bud, attached to the tissue between the corolla and style.

3.2.3. Membranisepalous pattern

Flowers that follow this pattern have four calyx lobes visible in the bud that are partially fused in the lower third of the bud (Fig. 3A and B). The fully developed lobes suggest later fusion during the developmental process. The line of fusion between the external and internal sepals occurs along the boundary of the external sepals but well within the edge on the dorsal face of the internal sepals leaving free, membranous tissue beneath the seam (Fig. 3C and D). At anthesis tearing occurs in this lower third of the calyx and the membranous tissue is evident in open flowers (Fig. 4D). In some cases, e.g. *Eugenia fasciculiflora*, the fusion can appear complete to the apex of the bud (Fig. 3C). However, the membranous parts of the free lobes are so fragile that rather than opening freely, internal pressures cause these membranous regions to split unevenly. This latter condition and the degree (length) of splitting, varies within an individual accession. In the *membranisepalous* pattern, the four petals are free, and each petal is positioned between the bases of the sepals. The stamens are straight in bud and are attached to the tissue between the corolla and style.

3.2.4. Petaloid pattern

Flowers that develop according to this pattern have buds that are nearly closed except at the apex where the remains of two vestigial lobes persist (Fig. 3E and H). These vestigial lobes suggest early fusion of the two external sepals during development. In this arrangement, the line of fusion is along the edge of the external pair of calyx lobes and results in a homogeneous or heterogeneous thickening of the calyx. Anthesis follows three possible opening patterns, rarely varying between accessions of observed species: the fused calyx (1) tears irregularly in two to six lobes; (2) splits into two regular lobes; (3) opens transversely via a tear at the calyx base resulting in a structure that resembles a calyptra. Two internal sepals remain free and always in the same orientation as the bracteoles (Fig. 3F and I). Internal sepals are white and petal-like but differ from the petals in their greater size and thickness. The four petals are free, each positioned between the bases of the sepals (Fig. 3G and I). Occasionally, the corolla is increased to five or more petals (e.g. *Calyptrogenia grandiflora*). Stamens are straight in the bud, attached to the tissue between the corolla and style. At anthesis the floral display commonly gives the impression that the flower has six petals, i.e. four petals and two petaloid-sepals (Fig. 4C).

3.2.5. Longohypanthium pattern

Flowers that follow this unusual pattern have nearly closed buds with the remains of four, thick vestigial lobes surrounding an apical pore. The tissue between the corolla and style lengthens into a tubular hypanthium that extends up to two-thirds of the bud, internally covered in staminal whorls (Fig. 3J). The line of fusion of the calyx lobes is along their edges resulting in a homogeneous tissue. The line of concrescence of the calyx lobes and hypanthium is indistinguishable (Figs. 3J, 4E) but the extending tissue with staminal scars can be used as a proxy of the hypanthium (Fig. 4F). At anthesis, the fused calyx and staminal whorls tear into three or four irregular lobes. Four petals, each positioned between the bases of the sepals are attached at the hypanthium summit. Again, rare in *Eugenia*, the stamens are strongly incurved in the bud. From outside, two-thirds of the bud are the visible hypanthium; at anthesis the irregular lobes reflex, exhibiting the attached stamens. The exhibition of the stamens, the showiest part of the *Eugenia* display, is particularly striking in this arrangement; the petals further increase the diameter of the flower.

3.3. Ancestral character reconstruction of development patterns

The ancestral state of *Eugenia* is free calyx lobes (Fig. 5). Transitions treating calyx fusion in a binary fashion (i.e. fused or free) resulted in approximately 16 changes among states on average according to reconstructed simulations. However, when different modes of calyx fusion revealed by this study on flower development are analysed separately, a more complex evolutionary history is uncovered with 21 changes on average. The most recurrent changes occurred from free lobes to fused calyx, while reversions were seldom (Fig. 6). The fused calyx condition is homoplastic and evolved independently, several times in *Eugenia,* as did the different development patterns. The only exception is *membranisepaly* that is exclusive to the '*Eugenia feijoi group*' (Fig. 1, node A). *Heterosepaly* has evolved at least five times and is the most common arrangement in *Eugenia* sect. *Schizocalomyrtus*. *Eugenia* sect. *Schizocalomyrtus* also includes species opening following the *longohypanthium* pattern that has been recorded so far only once in neotropical *Eugenia*. *Homosepaly* appears five times and is the most common pattern by which fusion is achieved. The *petaloid* pattern arose independently four times and is exclusive to node B (Fig. 1) of *Eugenia* sect. *Umbellatae*.

4. Discussion

Independent evolutionary specializations are important in angiosperm diversification (Soltis et al., 2005). However, careful investigation of the floral whorls is required to prevent misinterpretation of morphological variation. For instance, petals positioned between sepals are consistently found in tribe Myrteae (Belsham and Orlovich, 2002, 2003) as well as decussate aestivation of the calyx in *Eugenia,* with very few exceptions (Vasconcelos et al., 2018). Thus, interpretations here adopted rely on the tetramerous floral ground-plan rather than the less common exceptions of pentamerous or hexamerous flowers.

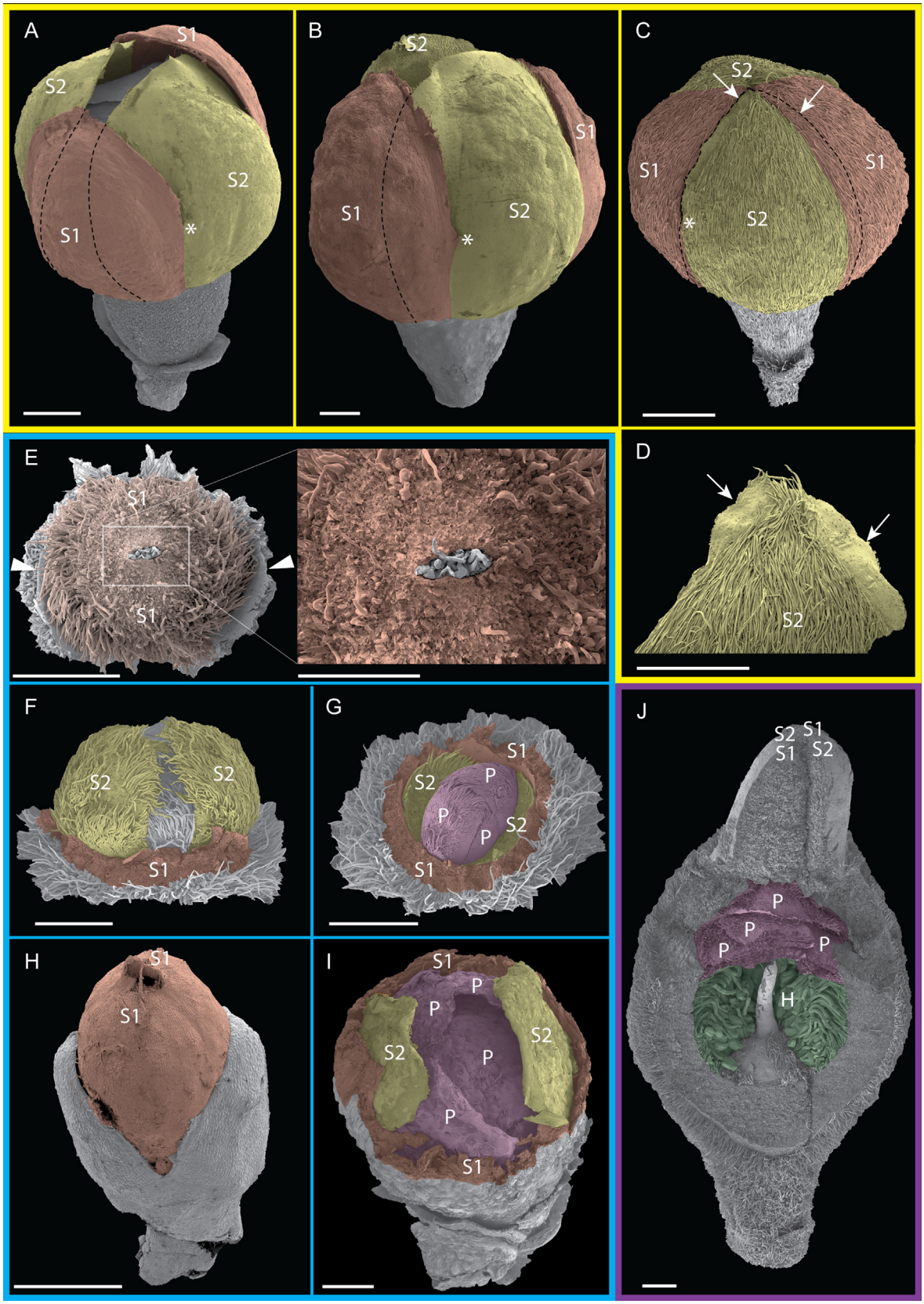

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Fig. 3. Comparative development patterns of the calyx in *Eugenia*. (A–D - yellow box) *Membranisepalous* pattern displaying calyx lobes partially fused in the lower third of the bud by the line of fusion between the S1 and S2 (indicated by *) occurs along the boundary of the S1 but well within the edge on the dorsal face of the S2 leaving free, membranous tissue beneath the seam (dashed-line) in *Eugenia joseramosii* (A), *E. pisonis* (B) and *E. fasciculiflora* (C); detail of the membranous tissue indicated by the arrows in *E. fasciculiflora* (C–D). (E–I - light-blue box) *Petaloid* pattern with bud nearly closed except at the apex where the remains of the two vestigial lobes persist in *Eugenia neograndifolia* (E); arrow heads indicate bracteole position; successively, both fused S1 were removed revealing two free and petal-like S2 (F); and both S2 were removed showing the three of the four petals underneath (G); bud nearly closed except by the remain of the two lobes of S1 at the apex of *Hottea neibensis* (H); fused S1 removed revealing two free, petal-like S2 and four petals of *Calyptrogenia cuspidata* (I). (J – purple box) *Longohypanthium* pattern displayed by a longitudinal section of a bud showing the extending hypanthium covered in staminal whorls which supports strongly incurved stamens of *Eugenia longohypanthiata* (J). Scales bars = 1 mm. Color coding: external sepals (S1), red; internal sepals (S2), yellow; petal (P), purple; stamens attached to the inner wall of the hypanthium (H), green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.1. Fused calices in the context of Myrtaceae

Five morphological development patterns leading to calyx fusion are reported here, plus the ancestral condition with free lobes. Calyx lobes are free in *Myrcianthes* (sister to *Eugenia*) and in some early diverging *Eugenia* lineages. Although *Eugenia* with fused calices have traditionally been treated as *Calycorectes* or *Catinga* (Amshoff, 1951; McVaugh, 1968), results presented here clearly show that characters of the calyx alone cannot delimit genera. Morphological argument for a more inclusive arrangement had already been made (McVaugh, 1969; Landrum and Kawasaki, 1997; Sobral, 2003) and as a consequence, many traditional *Calycorectes* species have already been transferred to *Eugenia* (see Mattos, 2005; Giaretta et al., 2018).

The mode of bud tearing during anthesis in *Eugenia* is convergent, arising from independent modes of development that result in closed flowering buds, and is therefore not a useful character for systematics

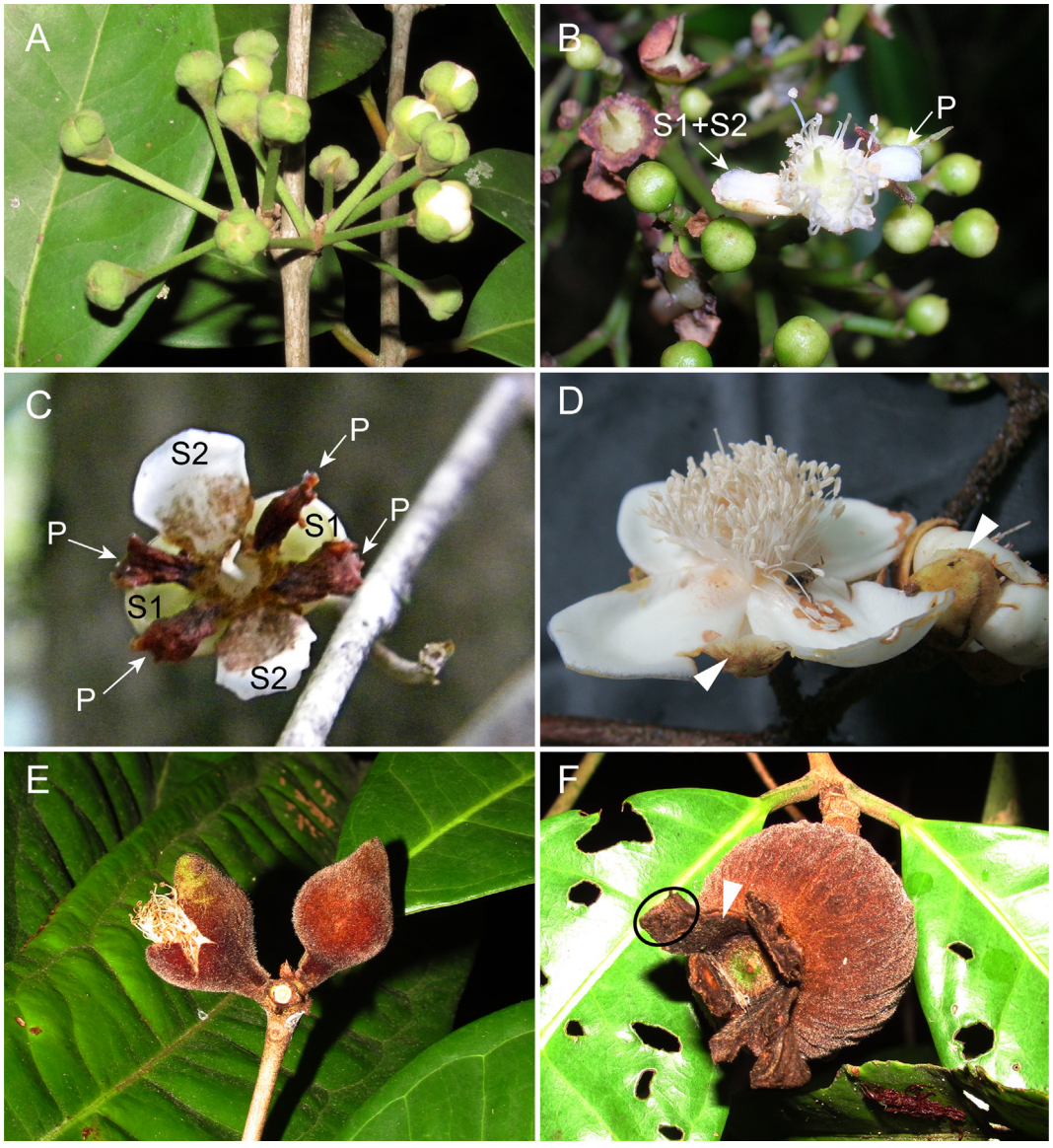

Fig. 4. Field pictures of flowers showing the different patterns of calyx fusion in *Eugenia*. (A) *Heterosepalous* pattern in *Eugenia acutata*. (B) *Homosepalous* pattern in *E. guanabarina* opening transversely resulting in a structure calyptra-like. (C) *Petaloid* pattern in *E. petaloidea*. (D) *Membranisepalous* pattern in *E. abunan*; arrowheads indicate the membranous tissue. (E) flower bud and flower at anthesis of *E. longohypanthiata*. (F) Remnant of the calyx (circled) and hypanthium (arrowhead) in the fruit of *E. longohypanthiata*. External sepals (S1); internal sepal (S2); petal (P). Pictures by B. Amorim (C), M. Simon (D) and A. Giaretta (all besides C and D).

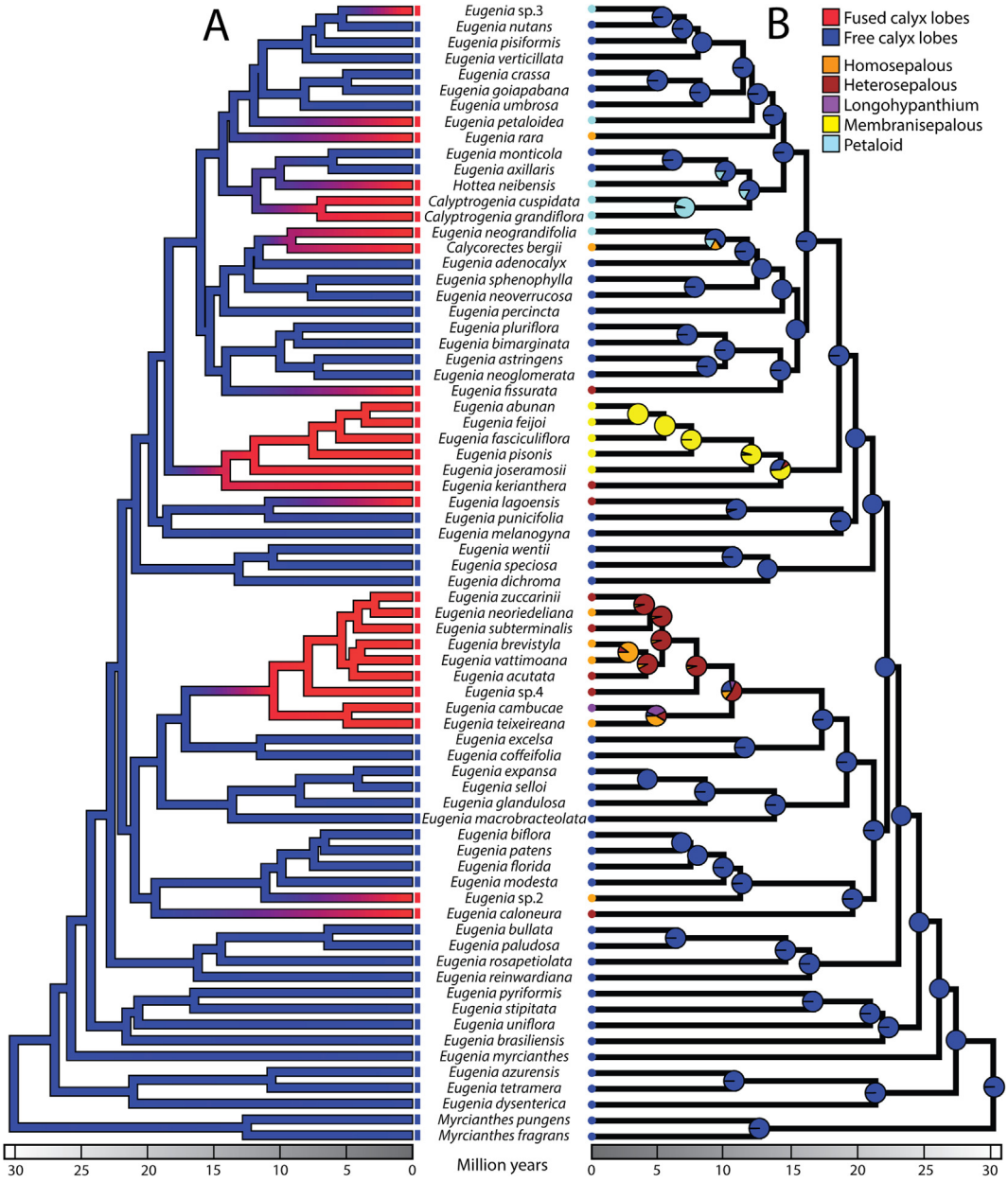

Fig. 5. Time-calibrated phylogeny of development patterns of calyx fusion in *Eugenia* as result of aggregation of 10,000 stochastic character maps. (A) Fused calyx scored as a single character state with two states, i.e. free lobes in blue and fused calyx in red; the degree of color tones indicate relative frequency across stochastic mapping (posterior probability). (B) Fused calyx scored according to the patterns herein described; pies provide posterior probabilities. Colors correspond to the colored boxes of Figs. 2 and 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

when used alone. This variation has misled taxonomy in other Myrtaceae genera such as *Angophora* and *Eucalyptus* (Drinnan and Ladiges, 1988; Ladiges et al., 1995; Bayly, 2016) in which bud opening is achieved via differing development pathways. Fused calyx anthesis in other Neotropical genera such as *Myrcia* s.l. (Lucas et al., 2011; Wilson et al., 2016) is also morphologically and developmentally homoplastic, with low systematic value at the generic level (Vasconcelos et al., 2017a). The complexity of perianth development in Myrtaceae is further aggravated by the origin of the tissue that undergoes fusion or adherence. This tissue can have calycine origin, as in *Eugenia* (this study) and *Myrcia* (Vasconcelos et al., 2017a), or a combination of both calycine and coralline origins as in most eucalypts (Drinnan and Ladiges, 1989; 1991).

Heterosepalous and *homosepalous* development patterns were often recovered arising at least five times each in *Eugenia*. Transition rates of *homosepalous* pattern seems most prone to occur in *Eugenia* originating from free lobes and from *heterosepaly* as shown in the heatmap (Fig. 6). Calyx fusion with apparent *homosepaly* is observed in other neotropical genera such as *Campomanesia guazumifolia*, *Psidium brownianum* and *Plinia brachybotria* (see descriptions in Legrand and Klein (1971); Giaretta and Peixoto (2015)). Such species have often experienced unstable circumscription (e.g. Berg, 1856, 1857; Kuntze, 1891) as a result of the misleading fusion of the calyx lobes.

Heterosepaly and *homosepaly* development patterns are recurrent but appear phylogenetically linked as they are mostly concentrated in *Eugenia* sect. *Schizocalomyrtus*. In contrast, *membranisepaly* is more systematically informative, appearing just once (Fig. 1, node A), as the result of a single transition in the evolution of the genus. Traits in Myrtaceae are commonly highly homoplastic (i.e. fleshy versus dry fruits (Biffin et al., 2010). Thus, the constant presence of *membranisepaly* in a single clade is unusual in Myrtaceae and has strong taxonomic and systematic value.

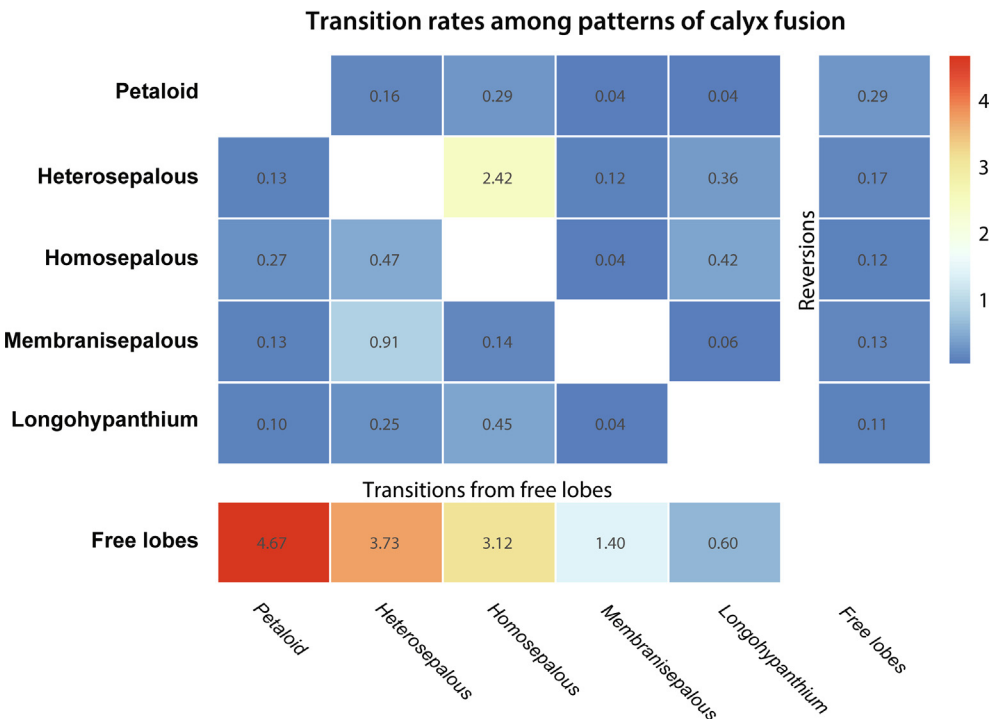

Fig. 6. Heatmap of transition rates among calyx fusion patterns in *Eugenia*. Vertical and horizontal data variables correspond to patterns of calyx fusion, read must follow from left to right. Transitions from free lobes to the respective patterns are indicated in the right-hand column; reversions to the ancestral free lobes condition are indicated in the bottom row.

Eugenia sect. *Umbellatae* is the section that contains most species, embracing approximately 700 species of *Eugenia* (Mazine et al., 2018). As a result, a wider variation in floral morphology is expected. All but three of the 12 identified occurrences of calyx fusion and four of the development patterns described here are found in this section. This expressive diversity may be linked to accelerated diversification rates and recent speciation reported in *Eugenia* sect. *Umbellatae* (Vasconcelos et al., 2017b). Despite its great diversity of development patterns, some of the fusion modes described here are also shown to have a strong link with particular clades within the section. The *petaloid* pattern, for instance, arose independently four times and has apparently fixed in only one clade that includes *Calyptrogenia* and *Hottea*, both nested in the Caribbean clade (Fig. 1, node D). Thus, it is likely that larger phylogenetic frameworks of *Eugenia* sect. *Umbellatae* may identify clades in which species with fused calices are common or exclusive.

It remains remarkable that *Eugenia* sect. *Schizocalomyrtus* with only 15 species (Mazine et al., 2018) encompasses three development patterns including the rare exception *longohypanthium* pattern. This mode of development with elongation of the hypanthium and the extended tissue bearing whorls of stamens, however, can possibly be found in other sections of the genus if a broader phylogenetic context is taken in consideration. Species of the genus *Monimiastrum* and *Stereocaryum* recently synonymized to *Eugenia* and placed in sect. *Jossinia* also seem to present this mode of development (e.g. Van Der Merwe et al. (2005); *E. alletiana*, Baider and Florens (2013); *E. ovigera*, Snow et al. (2016)). Further analyses can demonstrate that even this seemly stable mode can be homoplastic in the genus.

4.2. Parallelism and dead-ends in Eugenia

Although parallelism and convergence are indistinguishable at the level of patterns recovered (Wake et al., 2011), shared basic developmental mechanisms can be used to indicate underlying common inheritance. Parallelism (or parallel evolution) is the recurrence of structural or developmental pattern following the expression of shared genetic background among related lineages (Scotland, 2011) found throughout angiosperms (e.g. inflorescence in grasses, Bess et al. (2005); traits in *Prunus*, Bortiri et al. (2006); the inflorescence in Leguminosae, Sokoloff et al. (2007); stamens in *Miconia*, Goldenberg et al. (2008)). Calyx fusion and hypanthium extension synchrony in *Eugenia* has resulted in the five development patterns described here that share morphological/developmental similarities with three development pathways in *Myrcia* s.l., i.e. *aposepalous*, *gamosepalous* and hyper-hypanthium (Vasconcelos et al., 2017a). Such similarity reinforces the idea of a latent genetic mechanism regulating the flower phenotype in *Eugenia* and *Myrcia*, re-expressed in unrelated lineages for unknown reasons, widely discussed in the literature on homology (reviewed by Scotland (2010); for underlying synapomorphy see Saether (1979), and for latent homology see de Beer (1971)).

Floral evolution has been labile but directional in *Eugenia*. Transitions among development patterns are homogeneously distributed as depicted in the heatmap (Fig. 6) except by the recurrence of approximately 13 transitions from free lobes to a fused pattern. This implies that transition to the fused calyx and extended hypanthium in *Eugenia* are signatures that may not revert to the plesiomorphic condition, suggested by low reversion rates. Evolutionary dead-ends are reported in many angiosperms (Kay et al., 2005; Pérez et al., 2006; Whittall and Hodges, 2007) and are often associated with pollination systems (Tripp and Manos, 2008; Barrett, 2013), however, there is no evidence that the fused calyx in *Eugenia* is related to mechanisms of reproductive isolation or adaptation resulting from interactions with pollinators. Studies examining the functional role of fused calyx in *Eugenia* are highly desirable.

4.3. Flower traits and evolution

Similar floral displays were recovered by different opening patterns of the calyx. For instance, species that tear into irregular lobes can result from three patterns (*petaloid*, *longohypanthium* and *homosepalous*) whereas species that open via a calyptra-like structures can result from two patterns (*petaloid* and *homosepalous*). The systematic inconsistency attributed to the mode of anthesis reflects the hypothesis that this trait may not affect negatively the survival of the lineage (Futuyma, 2009) resulting in a morphological recurrence with little or no clear relation to the environment. In this sense, it is still unclear what is the evolutionary significance of the re-appearance of these different modes of

Table 1
Summary of the diagnostic characters for *Eugenia* sect. *Schizocalomyrtus*, and two informal groups with fused calices within *Eugenia* sect. *Umbellatae*.

	Eugenia sect. *Schizocalomyrtus*	"*Calycorectes* group"	"*Eugenia feijoi* group"
Calyx fusion	Two-thirds of the bud to completely closed	Calyx lobes nearly closed leaving two or four vestigial lobes at the tip	Four calyx lobes visible in the bud that are partially fused in the lower third of the bud leaving a membranous tissue beneath the seam
Development patterns	Mostly *heterosepalous*, less often *homosepaly*, rarely *longohypanthium*	*Homosepalous* and *petaloid*	*Membranisepalous*
Distribution	Mainly Atlantic forest extending to the gallery forests of the Brazilian savanna	Mostly in the lowland forests of the Guiana Shield	Mainly found in Amazon lowland forest extending to the Guiana Shield and gallery forests of the Brazilian savanna

development in the phylogenetic hypothesis of *Eugenia*.

However, some possible ecological advantages related to changing modes of development can be hypothesized. The tetramerous flower is the basic arrangement of most *Eugenia* species, but *Eugenia neograndifolia*, for instance, is unusual in having an apparently hexamerous corolla (Amshoff, 1951; Lemée, 1953; McVaugh, 1969). Results presented here newly interpret this condition as the *petaloid* pattern, exclusive to node B (Fig. 1) within *Eugenia* sect. *Umbellatae*, where the two external fused calyx lobes cover the bud while the two internal calyx lobes are free and petaloid, giving the impression of six petals. A similar condition is found in *Eugenia petaloidea* (Fig. 4C) in which the effective display of the flower contrasts with the common *Eugenia* display of four petals. In this case, two external fused calyx lobes open by regular splitting while an internal par of white, free and thicker lobes significantly intensify the floral display (Giaretta et al., 2019) affecting floral size and visibility by pollinators (Kettle et al., 2011). In the Myrtales, flower diameter is highly labile indicating that traits quickly respond to selective pressures by the environment (Vasconcelos and Proença, 2015). The flower of *Eugenia petaloidea* is also slightly bilateral (Fig. 4C), a trait associated with higher diversification in angiosperms (O'Meara et al., 2016) and possibly a consequence of changes in relationship with pollinators (Fenster et al., 2004).

4.4. Morphology and diagnosis of clades

As demonstrated here, the fused calyx, associated with several floral development mechanisms can be found throughout *Eugenia.* However, fused calyx species are spread in the phylogeny or were recovered in three strongly supported clades. One of these is *Eugenia* sect. *Schizocalomyrtus*, the two others fall within *Eugenia* sect. *Umbellatae* (Table 1).

Eugenia sect. *Schizocalomyrtus* has calyx lobes fused for two-thirds of the bud to completely closed, usually leaving a 1–3 mm diameter pore surrounded by four vestigial lobes. Calyx fusion is mostly *heterosepalous* but *homosepaly* and the unusual *longohypanthium* pattern are also found in this clade. The only apparent instance of reversal to free lobes was observed in *Eugenia zuccarinii.* However, this species is scored as *heterosepalous* here due to a small degree of fusion (ca. 2 mm long) at the base of the lobes. *Eugenia* sect. *Schizocalomyrtus* occurs principally in the Atlantic Forest of eastern Brazil.

The *"Calycorectes* group*"* includes the type species *Calycorectes grandifolius* (currently *E. neograndifolia*), and *Calycorectes bergii*; it has calyx lobes that are nearly closed leaving two or four vestigial lobes. Calyx fusion in this group follows the *homosepalous* and *petaloid* patterns. The *"Calycorectes group"* mostly occurs in the lowland forests of the Guiana Shield.

The *"Eugenia feijoi* group*"* consistently follows the *membranisepalous* development pattern, with *Eugenia kerianthera*, sister to the rest, exhibiting *heterosepaly*. The *"Eugenia feijoi group"* is mainly found in Amazon lowland forest extending to the Guiana Shield and gallery forests of the Brazilian *savanna* in the case of the widespread *Eugenia feijoi* complex (McVaugh, 1969).

Stamen position in the bud has systematic implication in *Myrteae* (Vasconcelos et al., 2015) and taxonomic relevance for *Eugenia*. This is a useful character when only flowering material is available, allowing distinction of e.g. fused calyx *Eugenia* from *Plinia* by the presence of a flat disc and straight stamens in the bud in the former and a prolonged cup-shaped hypanthium with strongly curved stamens in the latter. The character of straight stamens in the bud is almost ubiquitous in *Eugenia* and sister clade *Myrcianthes* making it likely the ancestral condition in *Eugenia*. However, in an exception to neotropical *Eugenia*, flowers of the *longohypanthium* pattern have strongly curved stamens as found in *Eugenia longohypanthiata* (Fig. 3J). These exceptional stamens are due to extension of the hypanthium in a similar pattern as found in *Marlierea* ("hyper-hypanthium" in Vasconcelos et al. (2017a)). As result, the hypanthium tears to expose the stamens and produces a display with increases flower diameter.

5. Conclusions

Results presented here show that free vestigial lobes in fused *Eugenia* calices are ubiquitous and that a non-fused calyx represent the plesiomorphic condition in the genus. This finding is reinforced by the character reconstruction that indicates fused calyx species to evolve from free calyx lobed flowers where two or four lobes fuse in different ways and to different degrees. Thus, systematic incongruence resulting from multiple, independent origins of the fused calyx in *Eugenia* is further aggravated by an inability to distinguish parallelism and convergence within the recovered patterns. This situation is an example of how detailed morphological survey of homoplastic traits using a phylogenetic framework reveals complexity rather than simplification. This complexity should be considered in future classifications as part of more integrative taxonomy that incorporates evidence from multiple sources. In *Eugenia,* the parallel evolution of the calyx fusion provides more evidence that sets of characters should be used to diagnose taxa instead of the pursuit of single traits. Due to high species diversity in *Eugenia*, this study is not an exhaustive survey of fused calyx species. Homoplasy may further increase as the number of taxa studied increases. However, results presented here have important implications for better interpreting patterns of floral evolution and systematics in one of the largest genera of angiosperms. Future directions include investigation of functional and ecological factors driving flower morphology and diversifications rates, particularly in the mega-diverse *Eugenia* sect. *Umbellatae*. Evaluation of the mechanisms that shape diversity in *Eugenia* is likely to contribute significantly to the understanding of the diversification of the Neotropical flora.

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Appendix A. Molecular sampling list, collection locality, DNA bank number (those starting with "KEW") and genbank accession codes for the species used in the phylogenetic analysis. Blank space indicates missing data. Accessions with en-dash (–) indicate DNA aliquots that are not available from the DNA bank.

Species	Collector	Number	Herbarium	ITS	psbA-trnH	rpl16	rpl32-trnL	trnQ-rps16	Country
Algrizea macrochlamys (DC.) Proença & Nic Lugh.	A. Giulietti	1648	BHCB	KEW16833	KEW16833	KEW16833	KEW16833	KEW16833	Brazil
Calycorectes bergii Sandwith	A. Giaretta	1587	K, SPF	KEW46506	KEW46506	KEW46506	KEW46506	KEW46506	French Guiana
Calyptrogenia cuspidata Alain	T. Vasconcelos	593	K	MF954023	MF954280	MF954321	MF954207	MF954087	Dominican Republic
Calyptrogenia grandiflora Burret	T. Vasconcelos	588	K	MF954024	MF954281	MF954322	MF954208	MF954088	Dominican Republic
Campomanesia ilhoensis Mattos	M. Ibrahim	122	K	KEW34650	KEW34650	KEW34650	KEW34650	KEW34650	Brazil
Eugenia abunan Sobral	G. Pereira-Silva	16487	CEN	KEW46519	KEW46519	KEW46519	KEW46519	KEW46519	Brazil
Eugenia acutata Miq.	T. Vasconcelos	506	K	MF954031	MF954288	MF954331	MF954216	MF954095	Brazil
Eugenia adenocalyx DC.	A. Giaretta	1441	K	MF954042	MF954299	MF954342	MF954219	MF954105	Brazil
Eugenia astringens Cambess.	F. Mazine	782	ESA, K	KJ187606	KJ469655	KEW20843	KEW20843	KEW20843	Brazil
Eugenia axillaris (Sw.) Willd.	M. Hamilton	553	K	KJ187607	KJ469656	KEW30702	KEW30702	KEW30702	Turks & Caicos
Eugenia azuruensis O.Berg	J. Faria	4186	UB	MF954033	MF954290	MF954333	MF954423		Brazil
Eugenia biflora (L.) DC.	F. Mazine	1075	ESA	KJ187610	KJ469659	KEW20687	KEW20687	KEW20687	Brazil
Eugenia bimarginata DC.	F. Mazine	469	ESA, K	KJ187611	KJ469660	KEW20830	KEW20830	KEW20830	Brazil
Eugenia brasiliensis Lam.	E. Lucas	126	K	KEW20949	KEW20949	KEW20949	KEW20949	KEW20949	Brazil
Eugenia brevistyla D.Legrand 1	A. Giaretta	1493	K, SPF	–	–	–	–	–	Brazil
Eugenia brevistyla D.Legrand 2	F. Mazine	993	ESA, K	KJ187614	KJ469663	KEW20683	KEW20683	KEW20683	Brazil
Eugenia bullata Pancher ex Guillaumin	T. Vasconcelos	608	K	MF954034	MF954291	MF954334	MF954424	MF954097	New Caledon
Eugenia caloneura Sobral & Rigueira	E. Lucas	1160	K	KEW46494	KEW46494	KEW46494	KEW46494	KEW46494	Brazil
Eugenia coffeifolia DC.	B. Holst	9516	SEL	KEW36243	KEW36243	KEW36243	KEW36243	KEW36243	Brazil
Eugenia crassa Sobral	L. Giacomin	1860	BHCB	KX789269	KX789296	KX789321	KX789350	KX910671	Brazil
Eugenia dichroma O.Berg	T. Vasconcelos	466	K	MF954041	MF954298	MF954341	MF954218	MF954104	Brazil
Eugenia dodonaeifolia Cambess.	E. Lucas	257	ESA, K	KJ187644	KJ469693	KEW45793	KEW45793	KEW45793	Brazil
Eugenia dysenterica DC.	F. Mazine	466	ESA, K	KJ187620	KEW20844	KJ469669	KEW20844	KEW20844	Brazil
Eugenia excelsa O.Berg	E. Lucas	125	ESA, K	KJ187621	KJ469670	KEW20950	KEW20950	KEW20950	Brazil
Eugenia expansa (O.Berg) Nied.	M. Bünger	634	BHCB, K	KX789279	KX789297	KX789322	KX789351	KX910672	Brazil
Eugenia fasciculiflora O.Berg	M. Simon	2032	CEN	KEW46515	KEW46515	KEW46515	KEW46515	KEW46515	Brazil
Eugenia fissurata Mattos 1	A. Giaretta	1639	K	KEW46510	KEW46510	KEW46510	KEW46510	KEW46510	Brazil
Eugenia fissurata Mattos 2	A. Giaretta	1640	K	KEW46511	KEW46511	KEW46511	KEW46511	KEW46511	Brazil
Eugenia florida DC.	F. Mazine	965	ESA, K	KJ187622	KJ469671	KEW20841	KEW20841	KEW20841	Brazil
Eugenia glandulosa Cambess.	J. Faria	3019	BHCB	KX789277	KX789299	KX789324	KX789353	KX910674	Brazil
Eugenia goiapabana Sobral & Mazine	M. Bünger	s/n	BHCB	KX789270	KX789300	KX789325	KX789354	KX910675	Brazil
Eugenia guanabarina (Mattos & D.Legrand) Giaretta & M.C.Souza	A. Giaretta	1629	K, SPF	KEW46509	KEW46509	KEW46509	KEW46509	KEW46509	Brazil
Eugenia joseramosii M.A.D. Souza & Scudeller 1	A. Giaretta	1651	SPF, K	KEW46513	KEW46513	KEW46513	KEW46513	KEW46513	Brazil
Eugenia joseramosii M.A.D. Souza & Scudeller 2	A. Giaretta	1655	SPF, K	KEW46514	KEW46514	KEW46514	KEW46514	KEW46514	Brazil
Eugenia kerianthera M.A.D.Souza	A. Giaretta	1517	SPF	KEW46504	KEW46504	KEW46504	KEW46504	KEW46504	Brazil
Eugenia lagoensis Kiaersk.	C. Fraga	2436	K	–	–	–	–	–	Brazil
Eugenia longohypanthiata Giaretta	A. Giaretta	1500	SPF, K	–	–	–	–	–	Brazil
Eugenia macrobracteolata Mattos	J. Faria	3050	UB	KX789283	KX789303	KX789328	KX789357	KX910678	Brazil
Eugenia melanogyna (D.Legrand) Sobral	F. Mazine	969	ESA, K	KJ187624	KJ469673	KEW20694	KEW20694	KEW20694	Brazil
Eugenia modesta DC.	F. Mazine	854	ESA, K	KJ187625	KEW20832	KEW20832	KEW20832	KEW20832	Brazil
Eugenia monticola (Sw.) DC.	T. Vasconcelos	566	K	MF954037	MF954294	MF954337	MF954427	MF954100	Dominican Republic
Eugenia feijoi O.Berg	M. Simon	971	CEN	KEW46492	KEW46492	KEW46492	KEW46492	KEW46492	Brazil
Eugenia costata O.Berg	A. Giaretta	1514	SPF	KEW46503	KEW46503	KEW46503	KEW46503	KEW46503	Brazil
Eugenia myrcianthes Nied. 1	J. Faria	2850	UB	KEW44019	KEW44019	KEW44019	KEW44019	KEW44019	Brazil
Eugenia myrcianthes Nied. 2	A. Giaretta	s/n	K	–	–	–	–	–	Brazil
Eugenia neoglomerata Sobral	F. Mazine	461	ESA, K	KJ187626	KJ469674	KEW20939	KEW20939	KEW20939	Brazil
Eugenia neograndifolia Mattos 1	A. Giaretta	1615	SPF	KEW46507	KEW46507	KEW46507	KEW46507	KEW46507	French Guiana
Eugenia neograndifolia Mattos 2	A. Giaretta	1616	SPF	KEW46508	KEW46508	KEW46508	KEW46508	KEW46508	French Guiana
Eugenia neoriedeliana M.C.Souza & Giaretta	A. Giaretta	1489	SPF, K	KEW46500	KEW46500	KEW46500	KEW46500	KEW46500	Brazil
Eugenia neoverrucosa Sobral	E. Lucas	118	ESA, K	KJ187628	KJ469676	KEW20951	KEW20951	KEW20951	Brazil
Eugenia nutans O.Berg	E. Lucas	281	ESA, K	KJ187629	KJ469677	KEW20829	KEW20829	KEW20829	Brazil
Eugenia paludosa Pancher ex Brongn. & Gris	T. Vasconcelos	646	K	MF954038	MF954295	MF954338	MF954428	MF954101	New Caledon
Eugenia patens Poir.	E. Lucas	104	ESA, K	KJ187633	K20947	KJ469681	KX789361	KX910681	French Guiana
Eugenia percincta McVaugh	M. Simon	1158	CEN	KEW46493	KEW46493	KEW46493	KEW46493	KEW46493	Brazil
Eugenia petaloidea 1 Giaretta & B.S.Amorim	B. Amorim	1765[4]	UFP	–	–	–	–	–	Brazil
Eugenia petaloidea 2 Giaretta & B.S.Amorim	B. Amorim	1765[23]	UFP	–	–	–	–	–	Brazil
Eugenia pisiformis Cambess.	E. Lucas	232	ESA, K	KJ187634	KJ469682	KEW20948	KEW20948	KEW20948	Brazil

Eugenia pisonis O.Berg	A. Giaretta	1419	SPF	KEW46495	KEW46495	KEW46495	KEW46495	KEW46495	Brazil
Eugenia pluriflora DC.	F. Mazine	961	ESA, K	KJ187636	KJ469684	KEW20831	KEW20831	KEW20831	Brazil
Eugenia punicifolia Kunth (DC.)	F. Mazine	1065	ESA, K	KJ187638	KJ469686	KEW20691	KEW20691	KEW20691	Brazil
Eugenia pyriformis Cambess.	F. Mazine	1028	ESA, K	KJ187639	KJ469687	KEW20944	KEW20944	KEW20944	Brazil
Eugenia rara Rigueira & Sobral	A. Giaretta	1646	SPF, K	KEW46512	KEW46512	KEW46512	KEW46512	KEW46512	Brazil
Eugenia reinwardtiana (Blume) DC.	E. Biffin	9245	Cultivated QRS	AY487301		AY463131			Queensland
Eugenia roseopetiolata N.Snow & Cable	T. Vasconcelos	s/n	Cultivated Kew	MF954040	MF954297	MF954340	MF954430	MF954103	Brazil
Eugenia selloi B.D.Jacks	M. Bünger	566	BHCB, RB	KX789278	KX789308	KX789334	KX789363	KX910684	Brazil
Eugenia sp.2	A. Brandão	283	RBR	KEW46488	KEW46488	KEW46488	KEW46488	KEW46488	Brazil
Eugenia sp.3	A. Brandão	305	RBR	KEW46489	KEW46489	KEW46489	KEW46489	KEW46489	Brazil
Eugenia sp.4	P. Fiaschi	3141	SPF	KEW46518	KEW46518	KEW46518	KEW46518	KEW46518	Brazil
Eugenia speciosa Cambess.	M. Bünger	585	BHCB	KX789274	KX789310	KX789336	KX789365	KX910686	Brazil
Eugenia stipitata McVaugh	T. Vasconcelos	677	K	MF954043	MF954300	MF954343	MF954220		Brazil
Eugenia subterminalis DC.	F. Mazine	s/n	K	KEW35910	KEW35910	KEW35910	KEW35910	KEW35910	Brazil
Eugenia tetramera (McVaugh) M.L.Kawasaki & B.K.Holst	B. Holst	9422	SEL	KJ187648	KJ469698	KEW35647	KEW35647	KEW35647	French Guiana
Eugenia umbrosa O.Berg	A. Giaretta	1498	SPF, K	KEW46502	KEW46502	KEW46502	KEW46502	KEW46502	Brazil
Eugenia uniflora L.	E. Lucas	207	Cultivated K	AM234088	AM489828	AF215627*		KP722202	Brazil
Eugenia vattimoana Mattos 1	A. Giaretta	1465	K, SPF	KEW46496	KEW46496	KEW46496	KEW46496	KEW46496	Brazil
Eugenia vattimoana Mattos 2	A. Giaretta	1487	K, SPF	KEW46499	KEW46499	KEW46499	KEW46499	KEW46499	Brazil
Eugenia verticillata (Vell.) Angely	Duarte	s/n (ESA85678)	ESA, K	KJ187650	KJ469700	KEW45805	KEW45805	KEW45805	Brazil
Eugenia wentii Amshoff	B. Holst	9421	K	KJ187651	K35646	KJ469701	KX789368	KX910689	French Guiana
Eugenia zuccarini O.Berg	A. Brandão	159	RBR	KEW46487	KEW46487	KEW46487	KEW46487	KEW46487	Brazil
Hotea neibensis Alain	T. Vasconcelos	590	K	MF954046	MF954303	MF954347	MF954224	MF954109	Dominican Republic
Myrceugenia alpigena (DC.) L.R. Landrum	E. Lucas	167	K	KX789289	KX789313	KEW19066	KX789370	KEW19066	Brazil
Myrcia tomentosa (Aubl.) DC.	Savassi	s/n (ESA85681)	ESA	KEW20697	KEW20697	KEW20697	KEW20697	KEW20697	Brazil
Myrcianthes fragrans (Sw.) McVaugh	M. Hamilton	552	K	KEW30701	KEW30701	KEW30701	KEW30701	KEW30701	Turks & Caicos
Myrcianthes pungens (O.Berg) D.Legrand	J.E.Q. Faria	2759	UB	KEW43970	KEW43970	KEW43970	KEW43970	KEW43970	Brazil
Myrtus communis L.	E. Lucas	211	Cultivated K	AM234149	AM489872	KEW10347	KEW10347	KEW10347	Unknown
Plinia cordifolia (D.Legrand) Sobral	F. Mazine	957	ESA	KX789291	KX789315	KEW20679	KX789372	KEW20679	Brazil

Appendix B. Species analysed using the scanning electron microscopy (SEM)

Collector	Number	Herbarium	Species	Section/group	Collection locality	Survey	Patterns
E. Lucas	1125	K	*Calyptrogenia cuspidata* Alain	*Umbellatae*	Dominican Republic	Herbarium specimen	*Petaloid*
T.N. Vasconcelos	593	K	*Calyptrogenia cuspidata* Alain	*Umbellatae*	Dominican Republic	Herbarium specimen	*Petaloid*
T.N. Vasconcelos	506	K	*Eugenia acutata* Miq.	*Schizocalomyrtus*	Brazil	Spirit collection	*Heterosepalous*
F.F. Mazine	1009	K	*Eugenia brevistyla* D.Legrand	*Schizocalomyrtus*	Brazil	Herbarium specimen	*Heterosepalous*
F.F. Mazine	993	K	*Eugenia brevistyla* D.Legrand	*Schizocalomyrtus*	Brazil	Herbarium specimen	*Heterosepalous*
E. Lucas	1160	K	*Eugenia caloneura* Sobral & Rigueira	*uncertain*	Brazil	Herbarium specimen	*Homosepalous*
Oldman	B3581	K	*Eugenia fasciculiflora* O.Berg	*Umbellatae*	French Guiana	Herbarium specimen	*Membranisepalous*
A. Giaretta	1629	K	*Eugenia guanabarina* (Mattos & Legrand) Giaretta & M.C.Souza	*Schizocalomyrtus*	Brazil	Spirit collection	*Homosepalous*
A. Giaretta	1630	K	*Eugenia guanabarina* (Mattos & Legrand) Giaretta & M.C.Souza	*Schizocalomyrtus*	Brazil	Spirit collection	*Homosepalous*
J.E.L.S. Ribeiro	1767	INPA	*Eugenia joseramosii* M.A.D. Souza & Scudell.	*Umbellatae*	Brazil	Spirit collection	*Membranisepalous*
s.c.	s.n.	INPA	*Eugenia kerianthera* M.A.D.Souza	*Umbellatae*	Brazil	Spirit collection	*Membranisepalous*
A. Giaretta	1500	K	*Eugenia longohypanthiata* Giaretta	*Schizocalomyrtus*	Brazil	Spirit collection	*Longohypanthium*
A. Giaretta	1616	K	*Eugenia neograndifolia* (O.Berg) Mattos	*Umbellatae*	French Guiana	Spirit collection	*Petaloid*
H.C. Lima	2244	K	*Eugenia neoriedeliana* M.C.Souza & Giaretta	*Schizocalomyrtus*	Brazil	Herbarium specimen	*Homosepalous*
A. Giaretta	1419	K	*Eugenia pisonis* O.Berg	*Umbellatae*	Brazil	Spirit collection	*Membranisepalous*
Zardini	3616	K	*Eugenia subterminalis* DC.	*Schizocalomyrtus*	Paraguay	Herbarium specimen	*Heterosepalous*
T.N. Vasconcelos	s.n.	K	*Eugenia uniflora* L.	*Eugenia*	RBG Kew	Spirit collection	Free lobes
J.E.Q. Faria	6294	K	*Eugenia uniflora* L.	*Eugenia*	Brazil	Spirit collection	Free lobes
Angeli	597	K	*Eugenia vattimoana* Mattos	*Schizocalomyrtus*	Brazil	Herbarium specimen	*Homosepalous*
Angeli	191	K	*Eugenia vattimoana* Mattos	*Schizocalomyrtus*	Brazil	Herbarium specimen	*Homosepalous*
T.N. Vasconcelos	590	K	*Hottea neibensis* Alain	*Umbellatae*	Dominican Republic	Spirit collection	*Petaloid*
T.N. Vasconcelos	535	K	*Myrcianthes fragrans* (Sw.) McVaugh	*Myrcianthes*	Costa Rica	Spirit collection	Free lobes

J.E.Q. Faria	4277	K	*Myrcianthes pungens* (O.Berg) D.Legrand	*Myrcianthes*	Brazil	Spirit collection	Free lobes
T.N. Vasconcelos	s.n.	K	*Myrtus communis* L.	*Myrtus*	RBG Kew	Spirit collection	Free lobes
A. Giaretta	s.n.	K	*Myrtus communis* L.	*Myrtus*	RBG Kew	Spirit collection	Free lobes

Appendix C. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.106553.

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