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Polysporangiate anthers described in *Eugenia* (Myrtaceae) with notes on evolutionary patterns

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Abstract

Shifts towards compartmentalisation resulting in multilocular anthers have been described in some families of Myrtales but to date, not in Myrtaceae. In these other families sporogeneous tissue is partitioned by transverse or longitudinal septa, fitting the definition of the 'polysporangiate anther'. Polysporangiate anther in *Eugenia*, a Neotropical genus of Myrtaceae is here discussed, with a detailed description of their morphology and in the context of known phylogenetic relationships within *Eugenia*. Anthers of *Eugenia joseramosii*, *E. kerianthera* and *E. petaloidea* were examined using scanning electron microscopy; *E. multilocellata* and *E. quilombola* were imaged with light microscope. Molecular sequences of five regions were used to place these species in a representative phylogenetic framework for character reconstruction analysis. Analysed anthers share dithecal organization in which each anther theca is divided in dorsal and ventral rows of sporangia. Polysporangiate anthers in *Eugenia* appear to have evolved independently on at least two occasions in *Eugenia* sect. *Umbellatae*. *Eugenia* flowers with polysporangiate anthers fit a generalist pollination pattern but *Eugenia petaloidea* demonstrates some unusual features that may be linked to pollination by vibration. Anthers in *Eugenia* is a rare condition with low systematic value. Resources should be invested to survey Myrtaceae for more species with polysporangiate anthers, to improve understanding of their evolutionary relevance and to record pollinators and modes of pollination.

Key words: Myrtales, Myrteae, multilocular anther, polysporangiate anther, sporangium, stamen

Introduction

Morphological shifts towards compartmentalisation of anthers are found repeatedly throughout angiosperms with examples of independent appearance of multilocular anthers in at least 31 families of angiosperms (Endress & Stumpf 1990; Endress 1996, 1998; González & Rudall 2010; Suaza-Gaviria *et al.* 2016). In Myrtales, compartmentalisation resulting in anthers with several pollen sacs have been described in Penaeaceae (Tobe & Raven 1984), Onagraceae (Tobe & Raven 1986), and Melastomataceae (Baumgratz *et al.* 1996; Lima *et al.* 2019). In these families the sporogenous tissue is partitioned by transversal or longitudinal septa, fitting the definition of polysporangiate anthers (Tobe & Raven 1986; Endress 1998). All species of Neotropical Myrtaceae but one, the southern South American *Metrosideros stipularis* (Hook. & Arn. 1833: 316) Hook.f. (1846: 275), are recognised as belonging to tribe Myrteae.

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Myrteae encompasses ca. 2,500 species (Vasconcelos *et al.* 2017) and is characterised by fleshy berries consumed by birds, mammals and generalists and usually white flowers that provide pollen instead of nectar as a reward to a variety of bees (Gressler *et al.* 2006; Vasconcelos *et al.* 2019). Despite an abundance of ca. 5,500 species distributed mainly in tropical and subtropical regions (Wilson 2011; POWO 2024), polysporangiate anthers are not detailed described in Myrtaceae. Tetrasporangiate anthers releasing pollen by longitudinal slits is the standard condition in Myrtaceae and Myrteae (Wilson 2011), with most exceptions found in extra-Neotropical lineages. For instance, trisporangiate anthers were recorded in the Australasian genus *Corynanthera* J.W.Green (1979: 368) while uni or bisporangiate anthers occur in *Malleostemon* J.W.Green (1983: 296) (Rye 2016). In addition to the standard condition of longitudinal dehiscence, dehiscence may also occur via pores or transverse/oblique slits, particularly common in subtribe Melaleuceae (including previously segregated genera; Wilson 2011). Further extraordinary anther morphology is recorded in Myrteae, in *Myrcia* sect. *Gomidesia* (O.Berg 1854: 6) B.S. Amorim & E. Lucas (Lucas *et al.* 2018: 6) where anthers have slightly dislocated pollen sacs that in combination with incomplete wall retraction during dehiscence result in anthers speculated to be functionally poricidal (Vasconcelos *et al.* 2019) and associated with pollination by vibration (Proença 1992; Nic Lughadha 1998), also known as buzz pollination (De Luca & Vallejo-Marín 2013). Buzz pollination recurs sporadically in Myrtaceae and may be related to morphological floral traits (Fidalgo & Kleinert 2009). The prolongation of the hypanthium above the summit of the ovary, as found in subtribes Myrtiinae and Pliniinae (Lucas *et al.* 2019; Vasconcelos *et al.* 2019) is believed to assist the attachment of the pollinating bee to the flower and its vibration upon it (Proença 1992).

Flowers of the large Myrteae genus, *Eugenia* P.Micheli ex Linnaeus (1753: 470) are characterised by flat staminal discs with the hypanthium not or slightly prolonged above the summit of the ovary. These features, in combination with a tetramerous flower, polyandrous androecium and inferior ovary, result in a general ‘ground-plan’ shared by most species of the genus (Landrum & Kawasaki 1997). Despite superficial morphological homogeneity, flexibility in *Eugenia* floral development is reported to result from changes related to reproductive strategies such as anthers maturing at different phases (Souza 1996; Vasconcelos *et al.* 2018) and this flexibility may be related to the reproductive success of *Eugenia*, one of the largest Angiosperm genera with nearly 1,200 species (POWO 2024) and the second most diverse tree genus in the world (Beech *et al.* 2017). Buzz pollination has been reported in *Eugenia* (Souza 1996; Fidalgo & Kleinert 2009), despite flowers having flat staminal discs and the hypanthium not or little elongated above the summit of the ovary.

To investigate reports of apparent polysporangiate anthers in Myrtaceae, anthers of five species of *Eugenia* observed to potentially possess this feature (Fig. 1) are examined in detail. All species examined are members of *Eugenia* sect. *Umbellatae*, and are the only species of the genus believed to display this trait. Molecular studies have included three of these species, showing *Eugenia joseramosii* M.A.D. Souza & Scud. (Souza 2017: 1) and *E. kerianthera* M.A.D. Souza (2015: 90) emerged in a strongly supported clade informally known as the “*Eugenia feijoi* group” (Mazine *et al.* 2018; Giaretta *et al.* 2019b; Giaretta *et al.* 2022) and *Eugenia petaloidea* Giaretta & B.S. Amorim (2019a: 67), elsewhere in *Eugenia* sect. *Umbellatae* (Giaretta *et al.* 2019a). *Eugenia multilocellata* Sobral & M.A.D. Souza (Souza *et al.* 2022: 205) and *E. quilombola* B.S. Amorim, M.A.D. Souza & Giaretta (2022: 32) are placed in this section as a result of their morphologies (Amorim *et al.* 2022; Souza *et al.* 2022). The aims of this study are 1) to provide a detailed description of the morphology of these unusual anthers and 2) to examine the feature in the context of known phylogenetic relationships in *Eugenia* with consideration of whether polysporangiate anthers are correlated to any other floral traits or pollination mode.

Material and Methods

Morphological assessment

Anthers of *Eugenia joseramosii* (*M.A.D Souza et al. 155*—INPA 181920; *A.Vicentini et al. 1141*—INPA 182028), *E. kerianthera* (*M.A.D. Souza et al. 485*—INPA 191849; *W. Rodrigues 5522*—INPA 14195) and *E. petaloidea* (*B.S. Amorim 1765*—UFP 73275) were studied in detail using scanning electron microscopy (SEM) in the Jodrell Laboratory, Royal Botanic Gardens, Kew, UK. Selected material was collected from herbarium specimens and rehydrated by boiling for 10 minutes and left to soak overnight. Samples were cut into radial, longitudinal and transversal sections by hand. The material was then processed through an alcohol series, culminating in 100% alcohol before dehydration in an Autosamdri-815B (Tousimis Research, Rockville, Maryland, USA) critical-point dryer. Dried material was mounted on aluminium stubs, coated for 45 seconds with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinsted, UK) and examined with a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan). Herbarium material of *Eugenia multilocellata* and *E. quilombola* was imaged with a light microscope as material was not available for SEM analysis.

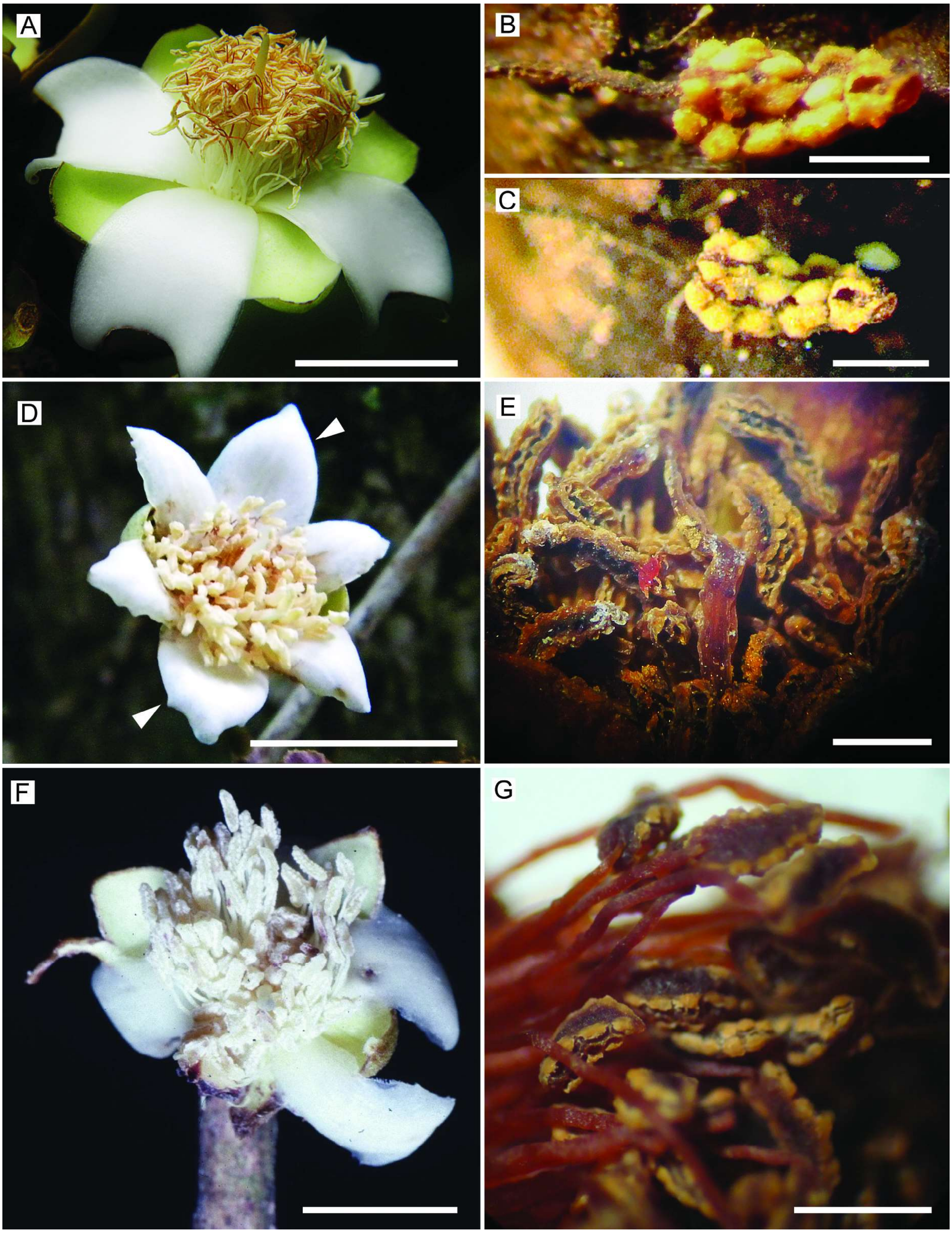

FIGURE 1. Multilocular anthers in *Eugenia*. (A) Flower anthesis of *Eugenia quilombola* with showy corrugated-like anthers. (B–C) Anthers of *Eugenia multilocellata* showing yellowish sporangia when dry. (D) Flower anthesis of *Eugenia petaloidea* with two petaloid sepals indicated by arrowheads. (E) Detail of opened sporangia of *E. petaloidea* when dry. (F) Flower anthesis of *Eugenia kerianthera*. (G) Anthers of *Eugenia joseramosii* with showy corrugated-like anthers. Scale bars = 1 cm (A, D, F); 1 mm (E); 0.5 mm (B, C, G). (Photos A, D, E by B. Amorim; photos B, C e G by f M.A.D Souza; photo F by PFRD 1999).

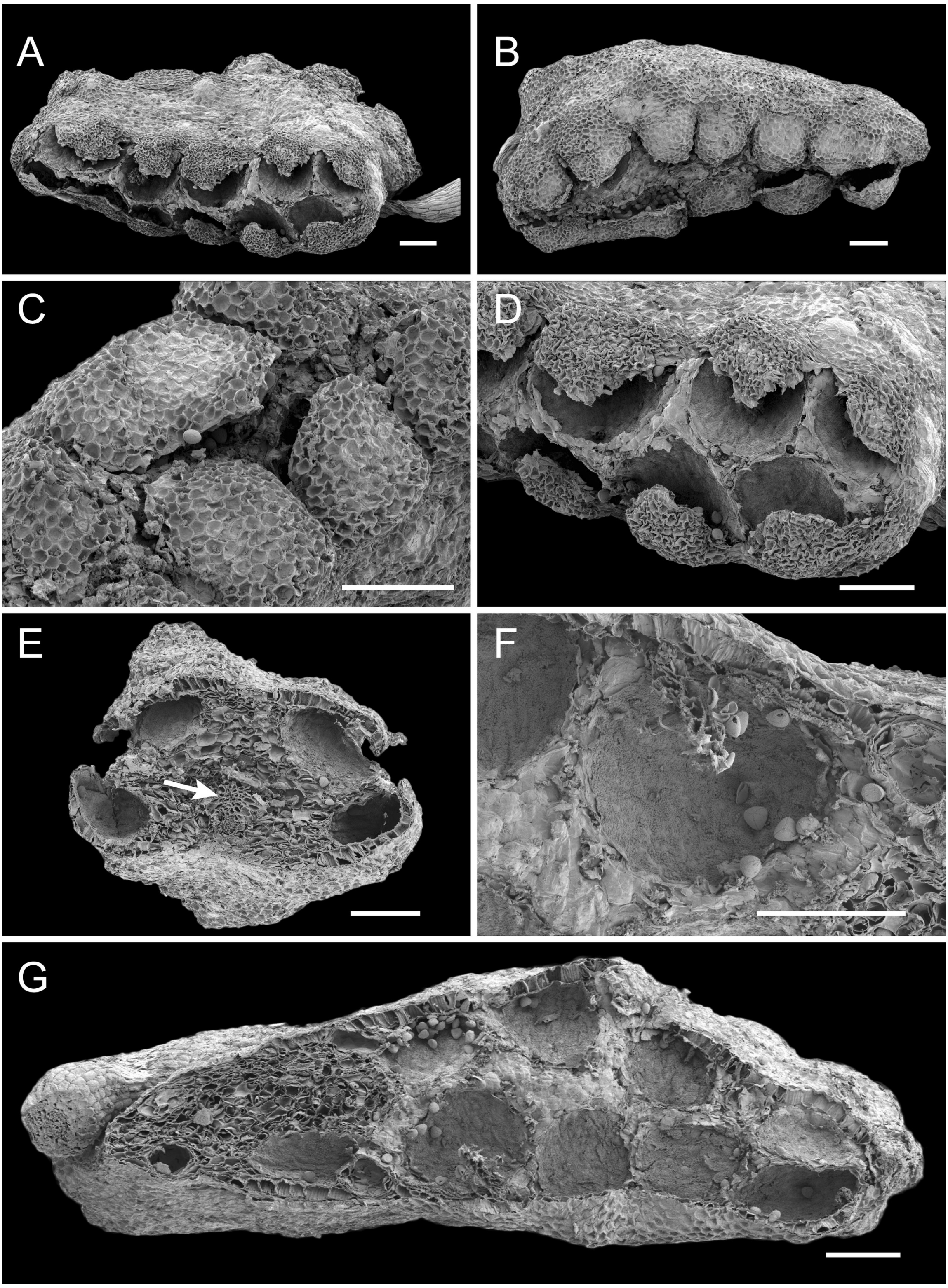

FIGURE 2. Anthers of *Eugenia joseramosii*. (A) Ventral-lateral surface. (B) Dorsal-lateral surface. (C) Detail of the sporangia. (D) sporangia with collapsed lids showing the concave portion. (E) Transverse section with the ventral surface facing down, arrow indicates the connective. (F) Concave portion of the sporangium where pollen grains are retained until release. (G) Longitudinal section with sporangium lids removed. Scale bars = 0.1 mm (A–G).

Ancestral character reconstruction analysis

The dated phylogenetic tree of *Eugenia* (Giaretta *et al.* 2019b) was used as a framework for ancestral character reconstruction. That time-calibrated phylogenetic analysis includes a broad and representative sample of the 11 currently known sections of *Eugenia*, including the three species here analysed with SEM. Giaretta *et al.* (2019b) based their phylogenetic reconstruction on information from the nuclear spacer region ITS and four plastidial regions (*psb*A-*trn*H, *rpl*16, *trn*L-*rpl*32 and *trn*Q5′-*rps*16; Lucas *et al.* 2007; Mazine *et al.* 2014, 2018; Bünger *et al.* 2016; Vasconcelos *et al.* 2017; Giaretta *et al.* 2019a, b). For the character reconstructions presented here, outgroups and taxa with more than one accession were pruned from the original phylogenetic tree to prevent bias during estimation of ancestral states, resulting in 72 species of subtribe Eugeniinae O.Berg, including *Myrcianthes fragrans* (Swartz 1788: 79) McVaugh (1963: 485) and *M. pungens* (O.Berg 1857: 224) Legrand (1968: 52) used as outgroups (Appendix S1). At least two specimens of each species sampled in the phylogeny held in the K, SPF and RB herbaria were consulted using stereomicroscopy to code anther as tetrasporangiate or polysporangiate. Ancestral states were reconstructed with Bayesian stochastic mapping (Huelsenbeck *et al.* 2003) using the model 'equal rates' with the function 'make.simmap' available in *phytools* v0.6-99 (Revell 2012) implemented in R (R Core Team 2022; RStudio Team 2019). Probabilities were calculated using 10,000 simulations.

Results

Anther morphology

The stamens of the five *Eugenia* species surveyed in this study share introrse, dorsifixed, versatile anthers due to a narrow filament tip towards the transition to the anther are dorsi-ventrally flattened. As expected in dithecal organization, a thin portion of connective tissue separates the thecae. Each theca is then further divided, resulting in two collateral rows of pollen sacs. The sporogeneous tissue that would normally be expected to continuously cover the interior of the pollen sac, is divided into two rows of five to twelve sporangia, one row occupying the place of the dorsal and ventral thecae, respectively. Sporangia are rounded or oblong chambers, separated by septa, and aligned transversally, interlocking to form a median 'longitudinal zigzag groove' (Figs. 2A–D; 3A–B; 4A–F). The alignment of the collateral rows of sporangia gives the lateral surface of the two thecae a distinctive corrugated appearance to the anther that is obvious in both fresh and dry material. The number of sporangia appears related to the length of the anther while its diameter may be related to the position of a given sporangium within the anther, as those located towards anther tips tend to be smaller than those in the middle (Fig. 5).

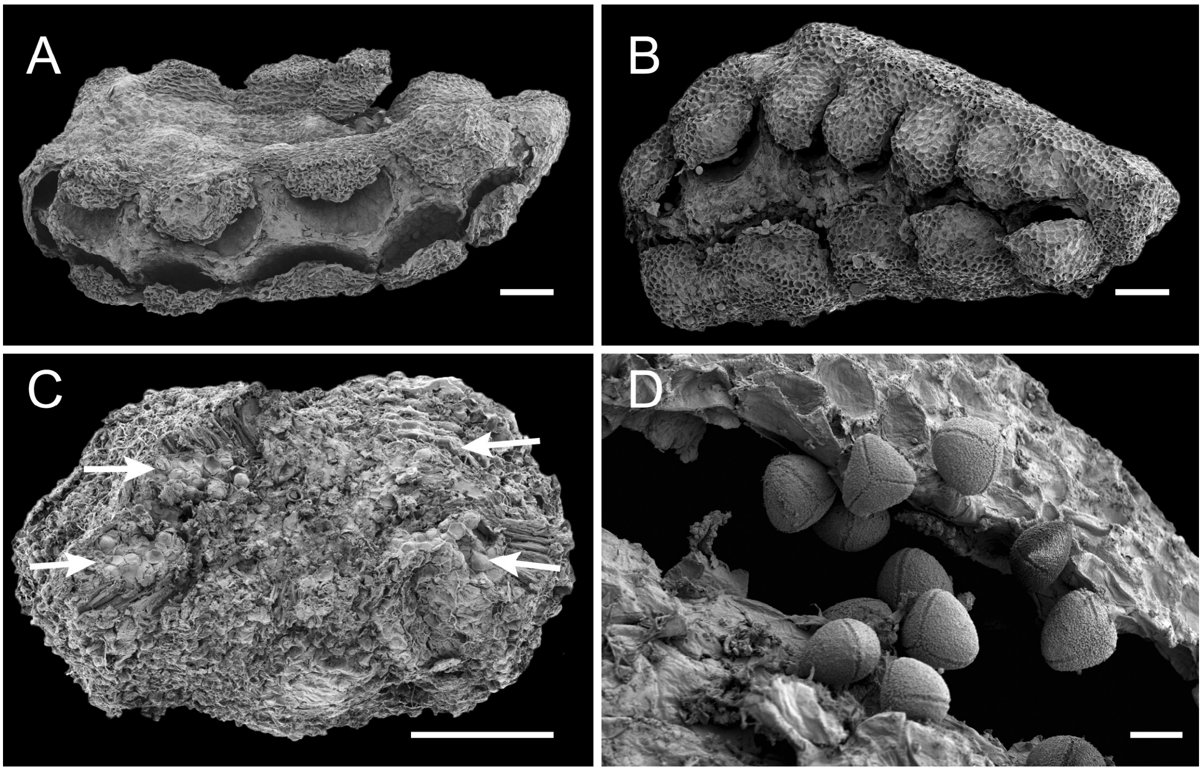

FIGURE 3. Anthers of *Eugenia kerianthera*. (A) Ventral-lateral surface. (B) Dorsal-lateral surface. (C) Transverse section at an early stage with arrows indicating sporangia. (D) Pollen grains attached to the sporangium opening. Scale bars = 0.1 mm (A–C); 0.01 mm (D).

Each sporangium opens via a hemispherical stomium (Figs. 2B, C; 3B; 4C, D). Sporangium opening is followed by release of pollen grains from inside concave depressions (Figs. 2D, F; 3D; 4F). Transverse sections of anthers from mature (Figs. 2E; 4G) and developing (Fig. 3C) buds indicate secondary septa separating sporangia. In longitudinal sections of the anther the sporangia are visibly disconnected (Figs. 2G; 4E, F); individual sporangia are visible as rounded polygons (Figs. 2D, F; 3A; 4A–D). Oil glands are visible as swollen protuberances on both surfaces of the anthers.

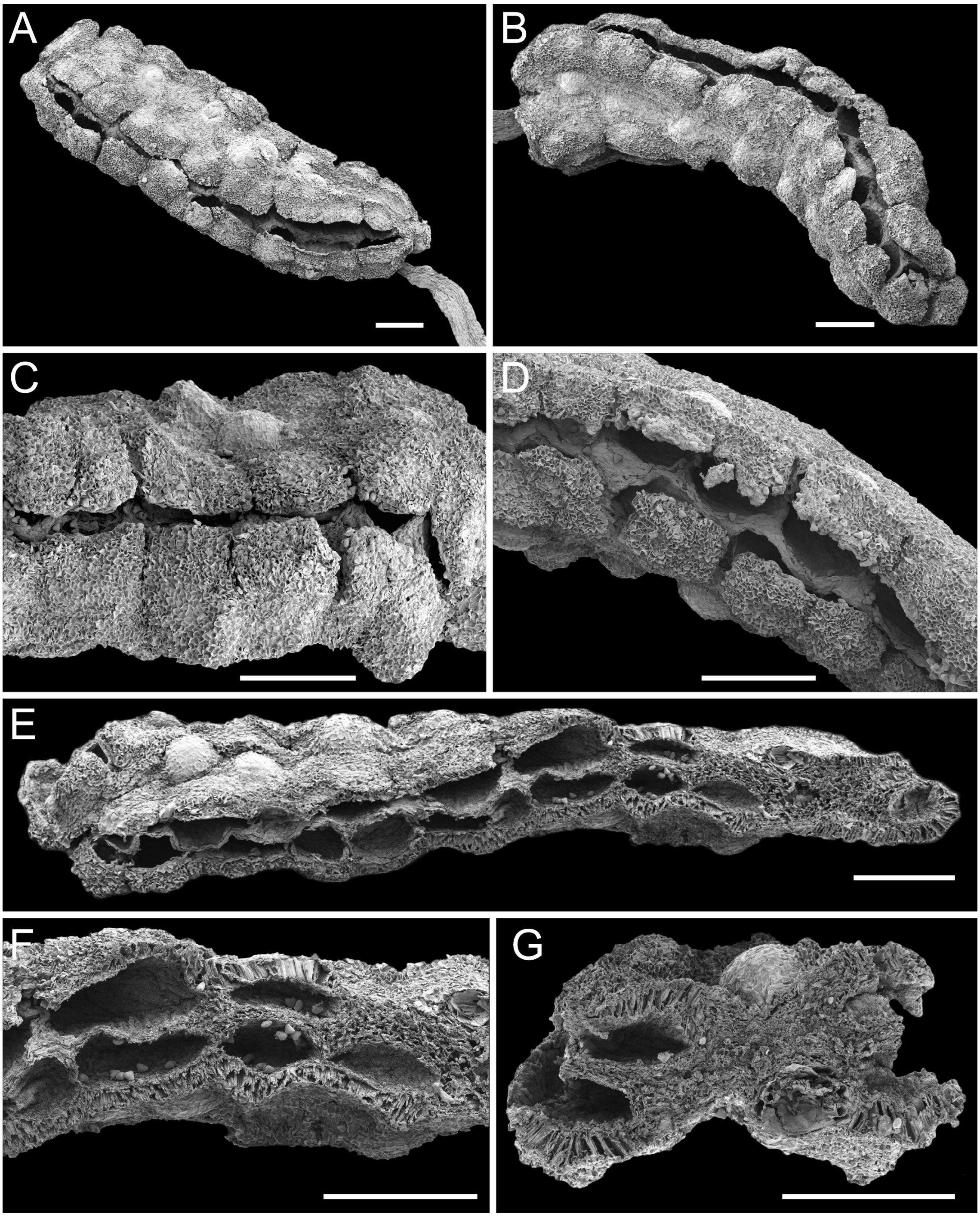

FIGURE 4. Anthers of *Eugenia petaloidea*. (A) Ventral-lateral surface. (B) Dorsal-lateral surface. (C) Detail of the sporangia. (D) Detail of the sporangia and concave portions. (E) Longitudinal section with sporangium lids removed. (F) Detail of the longitudinal section showing the concave portions with pollen grains. (G) Transverse section with the ventral surface downwards. Scale bars = 0.2 mm (A–G).

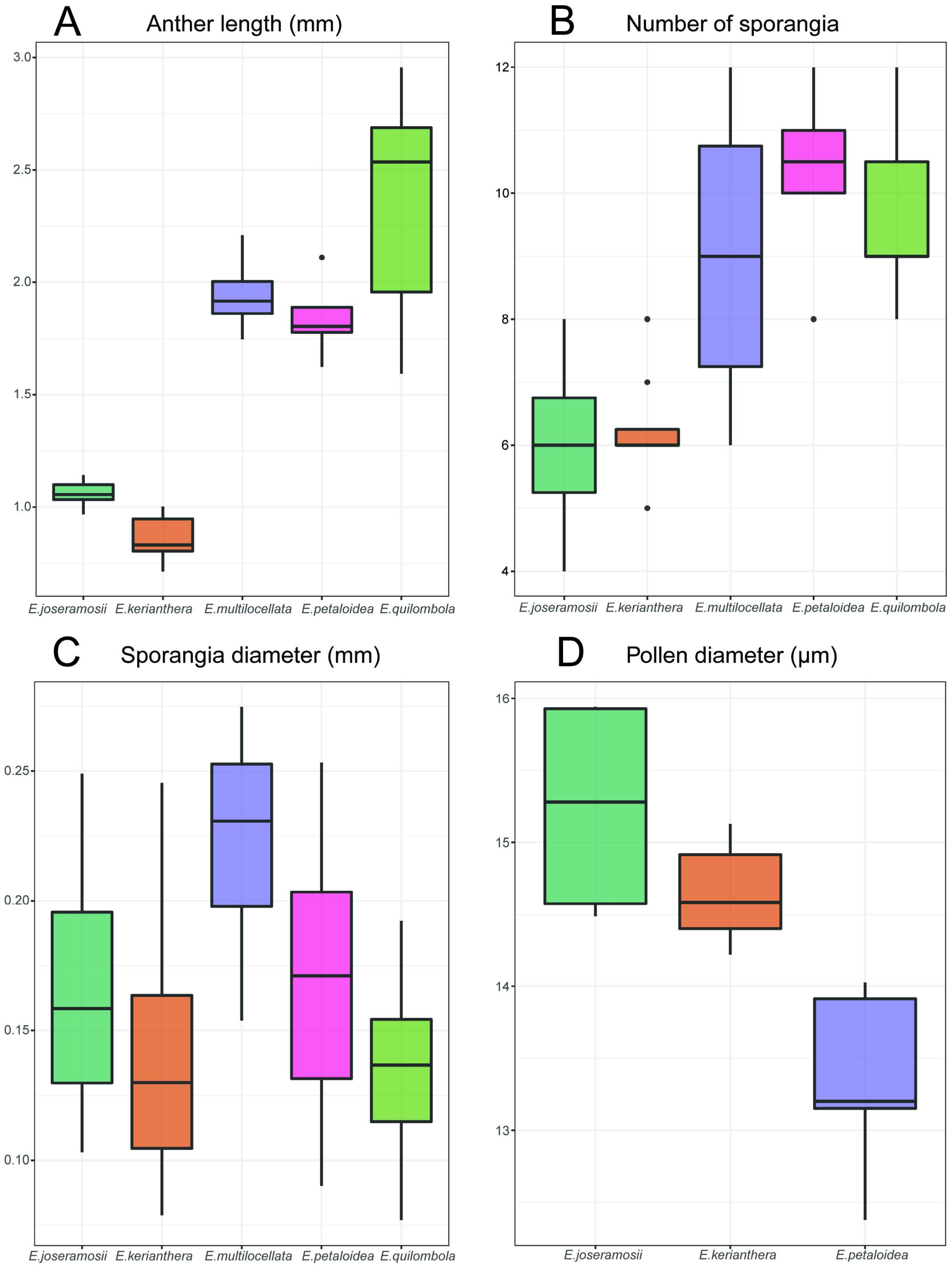

FIGURE 5. Morphological traits of the multilocular anthers in three species of *Eugenia*. (A) Anther length. (B) Number of sporangium in each side of the anther. (C) Diameter of the sporangia. (D) Pollen grain diameter.

Anthers in *Eugenia joseramosii* range from 0.9 to 1.1 mm in length (1.05 mm on average) with 5 to 8 sporangia in each row; *Eugenia kerianthera* range from 0.7 to 1 mm in length (0.86 mm on average) (Fig. 5A) with 4 to 8 sporangia in each dorsal and ventral row (Fig. 5B); *E. multilocellata* range from 1.8 to 2.2 mm in length (1.9 mm on average) with 6 to 12 sporangia; *E. petaloidea* anthers are larger and range from 1.6 to 2.1 mm (1.84 mm on average) with 8 to 12 sporangia while *E. quilombola* range from 1.5 to 2.9 mm (2.3 mm on average) with 8 to 12 sporangia. Individual sporangia diameter is similar between species (Fig. 5C). Pollen grains range from 14.4 to 15.9 µm in diameter in *Eugenia joseramosii*, 14.2 to 15.1 µm in *E. kerianthera* while in *E. petaloidea* anthers range from 12.3 to 14 µm (Fig. 5D). Anther and pollen measures are provided in Appendix S2.

Character reconstruction

The ancestral anther of *Eugenia* was recovered to be tetrasporangiate. This state was widely recovered with high probability along the backbone of the tree as well as at internal nodes, even at the ancestral nodes of polysporangiate anthers species (Fig. 6; Appendix S3). Transitions from tetrasporangiate to polysporangiate anthers condition resulted in 2.95 changes between states on average while few reversals are apparent, with 0.18 transitions on average. According to the dated phylogenetic tree, polysporangiate anthers arose on at least in two occasions and independently in *Eugenia* sect. *Umbellatae,* between 12 and 14 million years ago (Mya).

Discussion

Most species of tribe Myrteae exhibit tetrasporangiate anthers (Vasconcelos *et al.* 2019). This is mostly the case in subtribe Eugeniinae except by the species presented here. The division of the sporogeneous tissues by septa is recorded in anthers characterised as polysporangiate in other Angiosperm families (Endress & Stumpf 1990; Endress 1996, 1998) including those in the order Myrtales (Tobe & Raven 1984, 1986; Baumgratz *et al.* 1996; Almeda & Martins 2012; Lima *et al.* 2019). In Melastomataceae, this is especially common, with at least 74 species in three genera displaying the trait (Caetano *et al.* 2020), suggested to improve microspore nutrition by increasing contact between the sporogeneous tissue and the tapetum (see Lima *et al.* 2019). However, until now polysporangiate anthers have not been detailed described in Myrtaceae. Instead, citations of unusual anther morphology exist, such as is seen for *Psidium hians* Mart. ex DC. ("*antherae longissimae ... margine crenata*" in Berg (1857: 394)) and protologues of species here assessed (Souza *et al.* 2015, 2016; Giaretta *et al.* 2019a; Amorim *et al.* 2022). After consideration of the results, the anthers reported here for the five assessed species of *Eugenia* are polysporangiate.

Morphology of polysporangiate anthers

Organization of polysporangiate anthers in Angiosperms has been classified as thecal or athecal (Endress 1996, 1998). Athecal anthers are extreme forms in which the several sporangia converge into a single pore as seen in *Rafflesia* R.Br. (Rafflesiaceae), or each sporangium can open individually as in *Polyporandra* Becc. (Icacinaceae) (Endress & Stumpf 1990) and *Clusia* L. (Clusiaceae) (Amaral *et al.* 2017). Although monothecal anthers can occur (e.g. *Jacaranda* Juss., Ragsac *et al.* 2019), a dithecal arrangement, with two thecae and usually four sporangia, is the standard organization observed in Angiosperms (Endress 2011). Within the family Melastomataceae, polysporangiate anthers with transverse tissue forming septa that divide the pollen sacs is the most common arrangement (Endress 1996, 1998). Polysporangiate anthers in *Eugenia* conserve dithecal organization but with a dorsal and a ventral row of sporangia in each theca that opens individually, with the lines of dehiscence forming a median zig-zagging groove along the length of the anther. Further study is required to establish whether the ventral and dorsal sporangia open synchronously or asynchronously.

Dehiscence by longitudinal slits is the standard condition in Angiosperms (Endress 1998). However, polysporangiate anthers can cause pollen release in atypical ways, for instance, opening via an apical pore is often associated with specialized systems such as buzz pollination, observed in Melastomataceae (Brito *et al.* 2016; Caetano *et al.* 2020), or *Clusia* where pollen is associated with resin as a reward (Amaral *et al.* 2017). Dehiscence observed here in polysporangiate *Eugenia* anthers is via longitudinal slits as seen in Annonaceae (Tsou & Johnson 2003), Metteniusaceae (González & Rudall 2010; Dickison & Bittrich 2016), Onagraceae (Wagner *et al.* 2007), and Rubiaceae (Kirkbride 1985; Oliveira *et al.* 2011; Judkevich *et al.* 2020). In the case of the *Eugenia* species studied here, it appears that each row of sporangia is homologous to a sporangium of a tetrasporangiate anther, divided by septa into multiple, independent secondary sporangia.

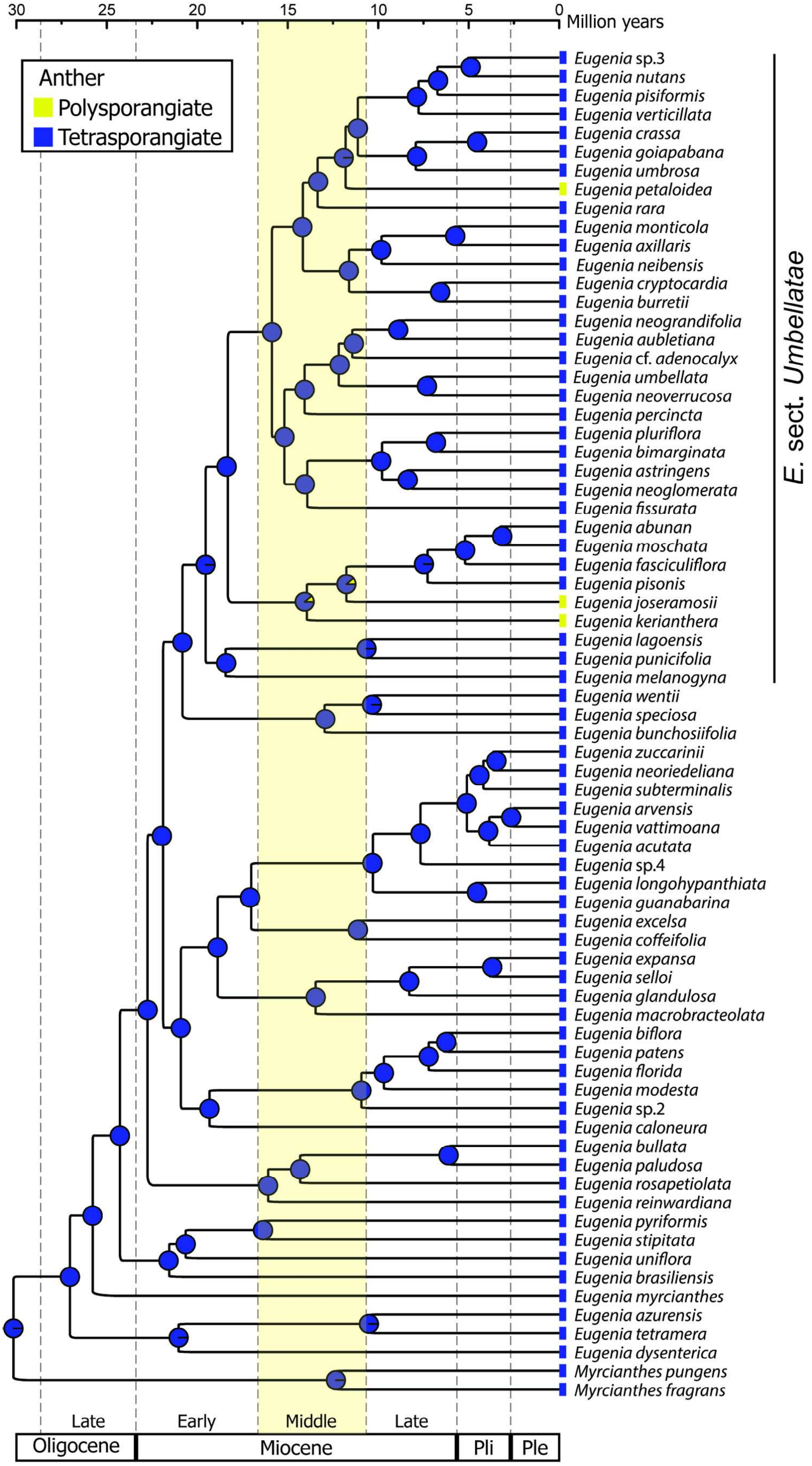

FIGURE 6. Time calibrated phylogeny of Eugeniinae and reconstruction of anther dehiscence traits resulting from aggregation of 10,000 stochastic character maps. Middle Miocene when the lineages with multilocular anthers arose is highlighted. The dated phylogeny is from Giaretta *et al*. (2019b).

Evolution of polysporangiate anthers in Eugenia

Optimatization of polysporangiate anthers in *Eugenia* sect. *Umbellatae* indicates that this arrangement has arisen independently at least twice (Fig. 6) and it is a relatively unusual state, currently known in just a few species of the genus. This rarity limits the use of polysporangiate anthers in *Eugenia* systematics. *Eugenia* flowers typically have free calyx lobes but are fused in some unusual lineages (Giaretta *et al.* 2019a, b). It is worth noting that species assessed here, with polysporangiate anthers, all have fused calyces which lineages have crown node between 10 and 14 Mya (Giaretta *et al.* 2019b), except *Eugenia multilocellata* with free calyx lobes. According to the dated phylogenetic reconstruction, lineages that gave rise to polysporangiate *Eugenia* arose between 12 and 14 Mya. So far there is no evidence that fused calyces and polysporangiate anthers are correlated or related to a particular pollination strategy in *Eugenia*.

Functional significance

Versatile attachment of anthers, as seen in all species of *Eugenia*, has not generally been associated with pollination by vibration (Endress 1996) where tubular, rigid anthers are often more common. Movement of thecae during pollination by vibration may be less effective, with pollinating insect vectors more easily grasping and manipulating a more solid structure. Lack of evidence of a distinct pollination syndrome associated with polysporangiate anthers in *Eugenia* favours consideration of alternative hypotheses for the origin of this trait in the genus. Sporangia divided into small compartments may increase pollen contact with tapetum cells and improve nutrition to the developing microspores (Pacini *et al.* 1985; Lima *et al.* 2019). This arrangement has been observed in other families of Myrtales (Tobe & Raven, 1986; Lima *et al.* 2019) and is presumably associated with optimising the surface contact ratio between the tapetum and sporogeneous tissue (Baumgratz *et al.* 1996). A second hypothesis suggests that polysporangiate anthers are an evolutionary intermediate state towards a reduction in anther size (Lersten 1971). The current data available does not support this hypothesis as polysporangiate *Eugenia* anthers are mostly ca. 1 mm long, as observed in other Neotropical *Eugenia* sections (see Sobral *et al.* 2012; Lourenço *et al.* 2013; Coutinho *et al.* 2015; Souza *et al.* 2015, 2016; Valdemarin *et al.* 2019; Giaretta *et al.* 2021). Furthermore, *Eugenia petaloidea* and *E. quilombola* have longer anthers, up to 2 and 3 mm, respectively. A third hypothesis may be that division of the sporangium by septa allows regulation of pollen release, ensuring more pollinators benefit and/or are attracted over time (Baumgratz *et al.* 1996). However, this hypothesis has not been supported by septa that fully degenerate during anthesis in poricidal polysporangiate anthers (see Lima *et al.* 2019). However, the condition of pollen sac divided into secondary sporangia with pollen release by longitudinal dehiscence was not scrutinised. Further studies of the reproductive biology of *Eugenia* species with polysporangiate anthers are needed to provide more evidence to examine these hypotheses.

Flowers in the few *Eugenia* species known to have polysporangiate anthers do not appear to deviate from the generalist pollination pattern noted in the genus. However, *Eugenia petaloidea* flowers demonstrate unusual features even in comparison to species whose floral ground-plans fit the standard arrangement of *Eugenia. Eugenia petaloidea* stamens have especially short filaments (3–4 mm) and long anthers (ca. 2 mm long) that are congested at the centre of the flower (Fig. 1D), traits associated with flowers pollinated by vibration (Endress 1998; De Luca & Vallejo-Marín 2013). Additionally, *Eugenia petaloidea* has an apparent “six-petal” display as result of the modification of an internal sepal pair (Giaretta *et al.* 2019a) also an uncommon arrangement (Giaretta *et al.* 2019b). Although a series of floral morphologies are linked to buzz pollination (De Luca & Vallejo-Marín 2013), species with alternative combinations of traits are also known to be targeted for pollen by bees using vibration (Proença 1992; Vallejo-Marín *et al.* 2010). Alternatively, could be that the unusual polysporangiate anthers in *Eugenia* are the result of loss of buzz pollination, or at least the typical syndromes buzzed anthers display (i.e. pores). Buzz-pollination has been documented in Neotropical Myrtaceae species with generalist floral ground-plans, in subtribes Eugeniinae, Myrciinae and Pliniinae (Proença 1992; Souza 1996; Fidalgo & Kleinert 2009).

Conclusions

Analyses reported here show polysporangiate anthers in *Eugenia* to be a rare condition with low systematic value. Resources should be invested to survey for other species with polysporangiate anthers in the genus and so to further assess their evolutionary relevance, relationship to pollinators and modes of pollination. Further studies to gather data on reproductive biology patterns in *Eugenia*, with emphasis on species with polysporangiate anthers is desirable, to provide evidence of the functional role and evolutionary drivers of this trait. Further directions of future study include investigation of anther developmental anatomy to confirm the origin of the sporogeneous tissue and better assess its biological significance.

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Author Contributions

AG, MADS and BSA designed the study. AG conducted the survey and imaging while under supervision of EL, carried out the analyses, and wrote the original draft that was discussed and improved with the input of all authors who approved the final version.

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Supplementary Materials

The following supporting information can be downloaded at the DOI landing page of this paper

APPENDIX S1. Voucher specimens and GenBank accessions of samples used in the phylogenetic reconstructions. Index Herbariorum abbreviations follow Thiers (2024). Missing sequences are blank spaces.

APPENDIX S2. Time-calibrated phylogenetic tree resulted from BEAST analysis of Giaretta *et al*., (2019b). Black dots indicate nodes receiving posterior probabilities greater than 0.95. Maximum likelihood (ML) bootstrap percentages equal or greater than 50 are shown close to the nodes. * Indicate nodes not recovered by the ML analysis.

APPENDIX S3. Measures data of anther length, sporangium diameter, number of sporangia, and pollen diameter.