



Systematic and evolutionary implications of stamen position in Myrteae (Myrtaceae)

THAIS N. C. VASCONCELOS^{1,2,3*}, GERHARD PRENNER², MARIANA O. BÜNGER⁴,
PLAUTO S. DE-CARVALHO⁵, ASTRID WINGLER³ and EVE J. LUCAS¹

¹Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK

²Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

³Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK

⁴Laboratório de Sistemática Vegetal, Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, MG, Brazil

⁵Universidade Estadual de Goiás, 76.190-00 Palmeiras de Goiás, GO, Brazil

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The taxonomy of Myrteae, the most species-rich tribe of Myrtaceae, is notoriously difficult. Although the understanding of the phylogeny has improved, the morphological characteristics that support its cladistic configuration are still unknown. The present study evaluates stamen position and anthesis type as characters of systematic and evolutionary relevance. Sixty-nine species from 41 genera across the tribe were checked using herbarium material and spirit collections. The results recognize three patterns of stamen position in the pre-anthetic bud: straight, semi-curved and strongly incurved. The three patterns of stamen position correspond to the phylogenetic structure of the tribe, supporting the topology of the clades. Incurving of stamens across the evolutionary history of Myrteae appears to be linked to hypanthium extension and leads to different anthesis types that may be related to shifts in pollination strategy. The accessibility of stamen position and its systematic consistency make this character a useful tool for field and herbarium identification, and allow the inference of relationships for taxa not yet sampled in molecular analyses. © 2015 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2015, **179**, 388–402.

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INTRODUCTION

Myrtaceae (c. 5500 species; Wilson, 2011) is one of the ten most species-rich angiosperm families (Stevens, 2001 onwards; Wilson, 2011). Members of Myrtaceae are particularly diverse in the tropical biomes of America and Asia and throughout Australia, always contributing to a significant proportion of species composition (Govaerts *et al.*, 2008). Many of these areas of highest diversity of Myrtaceae are also home to some of the highest biodiversity on Earth and are under high environmental threat from deforestation

(Achard *et al.*, 2002; Geist & Lambin, 2002). Molecular phylogenetic studies in Myrtaceae classified the family into two subfamilies: Psiloxylloideae, with two monospecific tribes, and Myrtoideae, with 15 tribes (Wilson *et al.*, 2005). Among these tribes, Myrteae is the richest in terms of species (c. 2500) and genera (52), representing more than half of the family diversity (Wilson, 2011). With the exception of the monospecific genus *Tepualia* Griseb. (tribe Metrosidereae), Myrteae is also the only tribe in the family that naturally occurs in the New World (Wilson *et al.*, 2005; Lucas *et al.*, 2007; Wilson, 2011).

The taxonomy of Myrteae is notoriously difficult, resulting in routine misidentification or lack of

*Corresponding author. E-mail: t.vasconcelos@kew.org

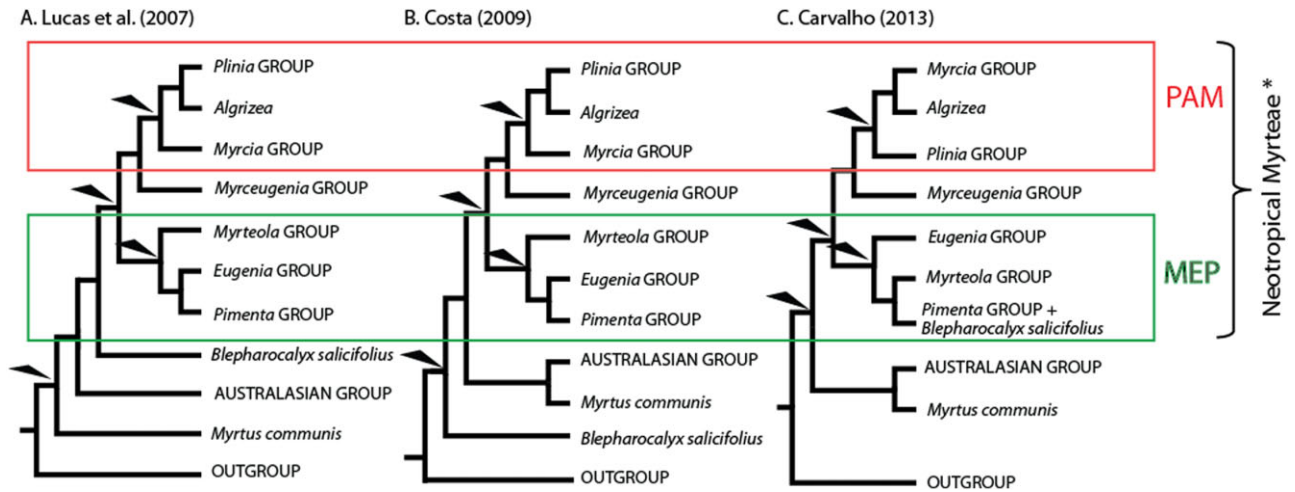


Figure 1. Comparison of three molecular phylogenetic analyses of Myrteae, all three using data from nuclear and plastid sequences. Arrows indicate Bayesian probabilities > 0.95. *With exception of *c.* 10% of the species in *Eugenia* and the New Zealand genera *Neomyrtus* and *Lophomyrtus* (*Myrteola* group).

identification of species in floristic inventories that often underpin conservation initiatives (McVaugh, 1968; Kawasaki, 1989; Barroso, 1994; e.g. Carvalho & Braga, 2007, in Atlantic Rainforest; Moro *et al.*, 2014, in caatinga). The consequences of this problem are exacerbated, given that Myrteae represents 10–15% of tree species diversity in Brazilian savannas and Atlantic forests (Sobral *et al.*, 2014), which are habitats under most acute pressure from deforestation (Mori *et al.*, 1983; Oliveira-Filho & Fontes, 2000).

Prior to the first DNA-based phylogenetic analyses, the tribe Myrteae was classified into three subtribes on the basis of characters of the embryo (Berg, 1855–56, 1857–59). Preliminary molecular phylogenetic analyses demonstrated that these subtribes were not monophyletic and that characters of the embryo were not congruent with the subtribal classification (Lucas *et al.*, 2005). Lucas *et al.* (2007) recovered seven morphologically cohesive clades in Myrteae and informally named them the *Plinia*, *Myrcia*, *Myrceugenia*, *Myrteola*, *Eugenia*, *Pimenta* and Australasian groups. Three species remained ungrouped: *Algrizea macrochlamys* (DC.) Proença & NicLugh., *Blepharocalyx salicifolius* (Kunth) O.Berg and *Myrtus communis* L. This initial study has been revisited by Costa (2009) and De-Carvalho (2013), who added more molecular information (i.e. DNA regions and taxa) and recognized, overall, a similar phylogenetic structure, with two main clades for the Neotropical lineages consistent throughout the studies: one clade formed by the *Plinia* group, *Algrizea macrochlamys* and the *Myrcia* group (henceforward PAM clade), which appears as sister to the *Myrceugenia* group in all studies, and the other formed by the *Myrteola*, *Eugenia* and *Pimenta* groups

(henceforward MEP clade) (Fig. 1). The most significant changes between the three studies relate to the relationships inside the PAM and MEP clades, the position of *Blepharocalyx salicifolius* in the tribe (discussed by De-Carvalho, 2013) and the position of *Myrtus communis* as sister to the Australasian group.

The suprageneric groups of Myrteae are poorly understood from an evolutionary point of view and only a few morphological characters support the phylogenetic structures. Recent studies on evolution in the tribe have struggled to score morphological characters in homologous states for phylogenetic reconstruction and character optimization (e.g. Lucas *et al.*, 2007, 2011); the results demonstrate low phylogenetic signal from these characters and high levels of homoplasy.

To understand the tribal evolution and relationships, it is crucial to revisit the morphological aspects. A better understanding of the evolutionary aspects of Myrteae may contribute to ecological studies in Neotropical biomes in which they are most diverse. Such a framework can then be used in conjunction with dating, historical biogeography and the identification of diversification rate shifts to provide an insight into the origins of the biomes in which they are found.

In flowers of Myrtales, the hypanthium often extends into a cup which can influence the development of the androecium, i.e. stamens, filaments and anthers (Decraene & Smets, 1991). Descriptive studies on the development of the hypanthium and stamen behaviour in the bud show differences in these characters in various species in Myrteae (Proença & Gibbs, 1994; Belsham & Orlovich, 2002, 2003). Proença & Gibbs (1994) also observed differences among different genera in Myrteae, possibly as a consequence of the

different arrangement of the stamen in the bud. However, these studies were produced before any molecular phylogenetic analysis was available and they were based on few species without detailed systematic and evolutionary discussion.

The pattern of stamen position in the bud and the anthesis type are easy characters to access in the field or herbarium material. Incurved stamens in the bud are considered to be a synapomorphy in the angiosperm order Myrtales (Stevens, 2001 onwards), with exceptions recorded in Vochysiaceae, Onagraceae and the Myrtaceae subfamily Psiloxylloideae (Dahlgren & Thorne, 1984; Johnson & Briggs, 1984). However, this character has never before been considered for Myrteae as a feature of systematic importance. The aims of this study were to investigate patterns of stamen position in the buds of the main clades of Myrteae, correlate this character with the anthesis type in Myrteae and associate it with the most recent phylogenetic hypotheses and evolution in Myrteae.

MATERIAL AND METHODS

SAMPLING

At least one species from 41 genera in Myrteae was sampled. *Heteropyxis natalensis* Harv. (Psiloxylloideae) was also sampled to represent the exceptional character of straight stamens in Myrtales. Buds were sampled from herbarium material, spirit collections and from collections (more details in the following sections). Buds were analysed pre-anthesis, i.e. mature buds in the final stage before flower opening. This standardized the observations and maximized the sampling because most herbarium material was found to be at this stage. When available, younger buds were also studied. Species were analysed using a stereomicroscope, scanning electron microscope and field photography. The species-rich genera *Eugenia* (c. 1100 species) *sensu* Mazine *et al.* (2014), including *Hexachlamys* O.Berg and *Calycorectes* O.Berg, and *Myrcia* (c. 700 spp.) *sensu* Lucas *et al.* (2011), including *Gomidesia* O.Berg, *Marlierea* Cambess. and *Calyptranthes* Sw., were represented by at least one species per subgeneric clade reported in these studies. We also included species from genera that had not yet been sampled in any phylogenetic analyses of the tribe: *Calypstrogenia cuspidata* Alain, *Calycolpus goetheanus* (Mart. ex DC.) O.Berg, *Hottea ekmanii* (Urb.) Borhidi, *Uromyrtus archboldiana* (Merr. & L.M.Perry) A.J.Scott, *Chamguava schippii* (Standl.) Landrum and *Mitranthes ottonis* O.Berg. These taxa were sampled from herbarium material only and organized according to the informal groups of Wilson (2011). In total, 69 species were analysed (for species and voucher lists see Appendix).

HERBARIUM MATERIAL

Samples were preferentially taken from the vouchers from the phylogenetic analysis of Lucas *et al.* (2007). When these vouchers did not have buds, or when they were unavailable, material identified by specialists was used. Buds from each sample were rehydrated in boiling water and dissected using fine tweezers, a razor blade and a dissecting microscope. Buds were analysed in two ways: (1) a frontal view from above after removing the sepals and petals; and (2) a longitudinal cut of the whole bud. Aims when dissecting were: (1) to verify the visibility of anthers in top view within the bud; and (2) to determine the nature of the filaments (straight or incurved). Digital images were taken with a Nikon coolpix 4500 digital camera mounted on a Leica WILD M3Z binocular microscope. Images were used as the basis for schematic drawings employing Adobe Illustrator CS5. The analysed buds were returned to the herbarium voucher. All vouchers, apart from *Faria J.E.Q.* collections, are deposited at the Royal Botanic Gardens, Kew (K). *Faria J.E.Q.* collections are deposited at the Universidade de Brasilia herbarium (UB).

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSES

SEM analysis of alcohol-preserved material was carried out in parallel to the study on herbarium samples. At least one species of each informal group of Myrteae *sensu* Lucas *et al.* (2007), the still not firmly placed *Blepharocalyx salicifolius* (here considered as a member of the *Pimenta* group following the most up-to-date phylogenetic analysis of De-Carvalho, 2013) and the ungrouped *Myrtus communis* and *Algrizea minor* Sobral, Faria & Proença (a recently described species sister to *A. macrochlamys*, Sobral, Faria & Proença, 2010) were included. The SEM images of pre-anthesis buds were analysed in the same way as the herbarium dissections. Material was taken from the spirit collections in the herbaria K, MO, NY and US. Additional material was added from the living collection at the Royal Botanic Gardens, Kew, and field collections made in Brazil. Vouchers for field collections are deposited at K and UB herbaria.

Material was dissected in 70% ethanol, dehydrated through an alcohol series to absolute ethanol and critical point dried using an Autosamdri-815B critical point dryer (Tousimis Research, Rockville, MD, USA). Dried material was further dissected and mounted onto specimen stubs using nail polish, coated with platinum using a Quorum Q-150-T sputter coater (Quorum Technologies, East Grinstead, UK) and examined with a Hitachi cold field emission SEM S-4700-II (Hitachi High Technologies, Tokyo, Japan).

ANTHESIS TYPE OBSERVATION

The anthesis pattern of different genera was observed and photographed during fieldwork in Brazil between September and November 2014. Fieldwork was carried out in the Brazilian Amazon (Amazonas and Roraima states), caatinga (Bahia state), cerrado (DF, Goias, Bahia and Minas Gerais states) and Atlantic rainforest (Bahia, Espirito Santo, Minas Gerais and Sao Paulo states). Flowers at anthesis were selected and photographed using a digital Nikon D200 camera with a 60-mm macrolens.

RESULTS

STAMEN POSITION

Results show different stamen positions in pre-anthetic buds for different Myrteae groups in both herbarium and SEM analysis. In all analysed species of the Australasian Group [*Archirhodomyrtus beckleri* (F.Muell.) A.J.Scott, *Decaspermum parviflorum* (Lam.) A.J.Scott, *Gossia bidwillii* (Benth.) N.Snow & Guymmer, *Octamyrtus arfakensis* Kaneh. & Hatus. ex C.T.White, *Pilidiostigma tropicum* L.S.Sm., *Rhodamnia dumetorum* (DC.) Merr. & L.M.Perry, *Rhodomyrtus tomentosa* (Aiton) Hassk.; see *Rhodomyrtus* sp. and *Octamyrtus* sp.; Fig. 2A, B] and in *Myrtus communis* (Fig. 2C, E), straight filaments with anthers visible from the top in pre-anthetic buds were recorded. The same pattern was found in the *Eugenia* group and was consistent in all analysed species of *Eugenia* s.l. [*Eugenia coffeifolia* DC. (Fig. 2D), *E. florida* DC., *E. involucrata* DC., *E. klotzschiana* O.Berg, *E. pluriflora* DC., *E. pyriformis* Cambess., *E. uniflora* L. (Fig. 2F), *Hexachlamys edulis* (O.Berg) Kausel & D.Legrand and *Calycorectes bergii* Sandwith] and in *Myrcianthes fragrans* (Sw.) McVaugh. Straight stamens were also found consistently in all samples of the *Myrteola* group [*Ugni candollei* (Barnéoud) O.Berg (Fig. 2G), *Lophomyrtus obcordata* (Raoul) Burret, *Neomyrtus pedunculata* (Hook.f.) Allan and *Myrteola nummularia* (Lam.) O.Berg]. Most species of the *Pimenta* group also showed straight pre-anthesis filaments with anthers visible from the top. However, the pattern was not consistent in this group. Stamens were completely straight in *Acca sellowiana* (O.Berg) Burret (Fig. 3A, B) and in all analysed species of *Psidium* L. (see *P. guineense* Sw., Fig. 3C, D) and *Campomanesia* Ruiz & Pav. However, there was variation in the position of stamens in *Pimenta* Lindl. and *Blepharocalyx salicifolius*. *Pimenta racemosa* (Mill.) J.W.Moore and *P. dioica* (L.) Merr. (herbarium material only) showed semi-curved stamens, similar to those in the *Myrceugenia* group (discussed in the next paragraph), whereas *P. pseudocaryophyllus* (Gomes) Landrum

(Fig. 3E) and *Blepharocalyx salicifolius* (Fig. 3F) showed strongly incurved stamens in the pre-anthesis bud, with anthers touching the bottom of the floral disc. In these species, the filaments developed from the rim of the hypanthium cup formed by the hypanthium extension.

A semi-curved pattern was found consistently in the *Myrceugenia* group [*Luma apiculata* (DC.) Burret (Fig. 3G), *Myrceugenia alpigena* (DC.) Landrum, *M. planipes* (Hook. & Arn.) O.Berg (Fig. 3H), *M. bananalensis* Gomes-Bezerra & Landrum (Fig. 3I)]. Species with this pattern showed straight to slightly incurved outer filaments. The hypanthium was also extended in these species and the inner filaments, on the other hand, were strongly incurved. The anthers remained facing downwards during development, touching the bottom of the hypanthial cup. On removal of the calyx and corolla, only anthers from the outer whorls were visible from above. *Blepharocalyx cruckshanksii* (Hook. & Arn.) Nied., also in the *Myrceugenia* group, was only available as herbarium material and was difficult to interpret. Filaments were clearly incurved, but it was not clear whether the stamens were strongly curved, as in *Blepharocalyx salicifolius*, or semi-curved, as in other taxa of the *Myrceugenia* group.

In all samples of the *Plinia* group [*Plinia cauliflora* (Mart.) Kausel (Fig. 4A), *Myrciaria floribunda* (H.West ex Willd.) O.Berg (Fig. 4B), *Siphoneugena densiflora* O.Berg, *Neomitranthes obscura* (DC.) N.Silveira and *N. cordifolia* (D.Legrand) D.Legrand], filaments were strongly incurved in the bud, a characteristic that was visible even to the naked eye. *Plinia cauliflora* was exceptional, in that it had small buds and, although the filaments were strongly incurved, the anthers were sometimes visible from above because they did not physically fit in the bud and they were therefore pushed outside (Fig. 4A). In all samples of the *Myrcia* group [*Calypttranthes* aff. *blanchetiana* O.Berg (Fig. 4F), *Gomidesia* sp. (Fig. 4D), *Myrcia amplexicaulis* (Vell.) Hook.f., *M.* aff. *eriopus* DC., *M. laxiflora* Cambess., *M. pubipetala* Miq., *M. splendens* (Sw.) DC., *M. trimera* Sobral *in press.*, *M. truncada* Sobral, *M. spectabilis* DC. (Fig. 4E, H), *M. sylvatica* (G.Mey.) DC. (Fig. 4C)] and in *Algrizea minor* (Fig. 4G), the stamens were also strongly incurved in the pre-anthetic bud, and the anthers were never visible from above. In the *Plinia* and *Myrcia* groups and in *Algrizea minor* and *A. macrochlamys*, the hypanthium was often extended, forming a hypanthial cup. In all samples analysed from buds in early stages, the filaments remained strongly incurved throughout the development of the bud, forcing the anthers downwards to the bottom of the hypanthial cup (Fig. 4H).

Genera that have not yet been included in the phylogenetic analyses were recorded as follows:

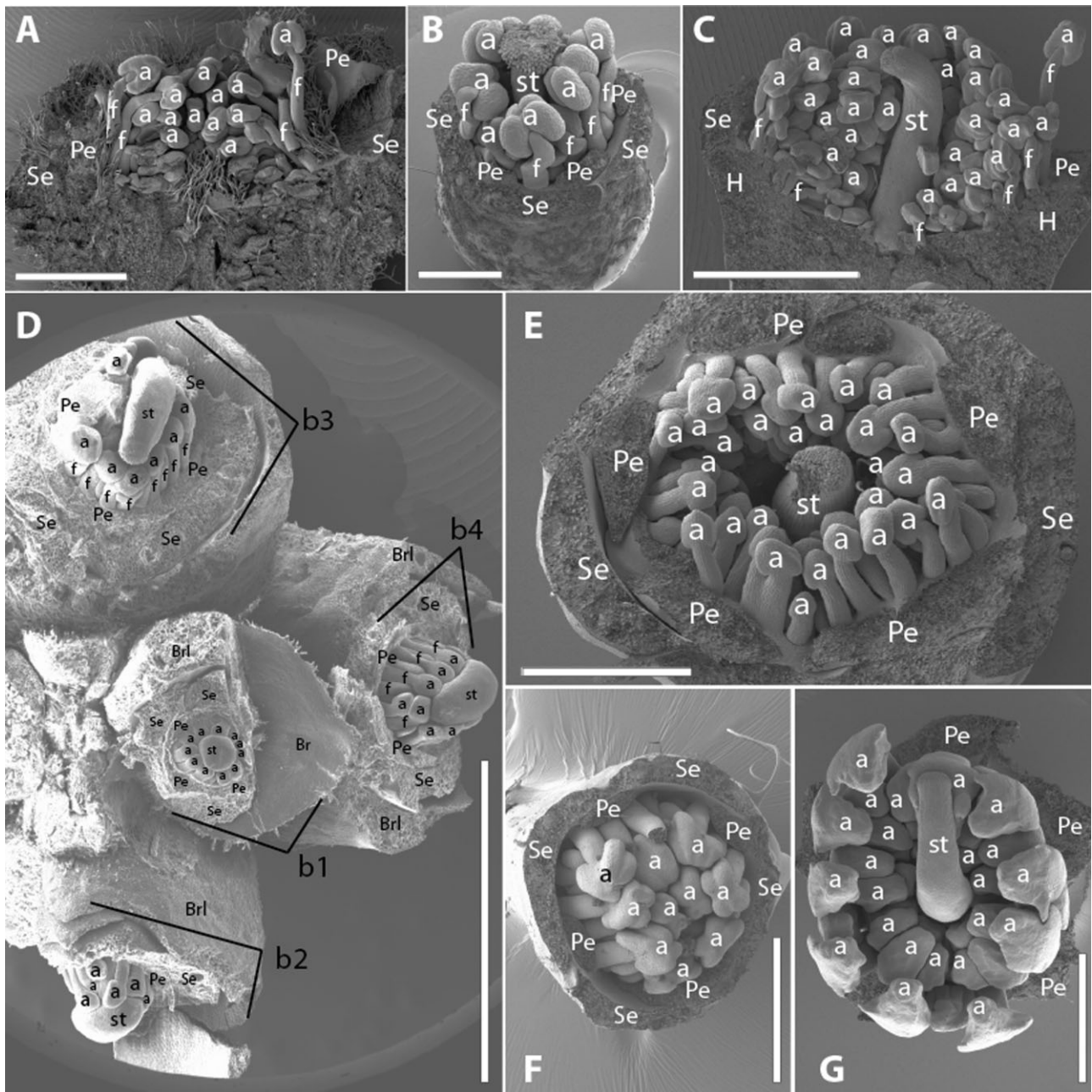


Figure 2. Scanning electron microscopy (SEM) images of buds of species from the Australasian group, *Eugenia* group and *Myrtus communis*. Perianth removed in all. A, *Rhodomyrtus* sp. (Australasian group) showing straight filaments with anthers facing upwards. B, *Octamyrtus* sp. (Australasian group) showing anthers visible from above in the bud. C, E, *Myrtus communis* at two different developmental stages. C, A nearly pre-anthetic bud with straight filaments growing from a slightly extended hypanthia and anthers facing upwards. E, Anthers already growing upwards in a young bud. D, Inflorescence of *Eugenia coffeifolia* (*Eugenia* group) with buds in different developmental stages showing filaments always straight and anthers visible from above. F, *Eugenia uniflora* (*Eugenia* group) showing anthers visible from above. G, *Ugni candollei* (*Myrteola* group) in pre-anthetic stage, showing anthers visible from above. a, anther; b1–b4, flower buds from the youngest to oldest; Br, bracts; Brl, bracteoles; f, filament; H, hypanthium; Pe, petal scar; Se, sepal scar; st, style. Scale bars: 250 μ m (B), 500 μ m (E, F), 1 mm (A, C, G), 2 mm (D).

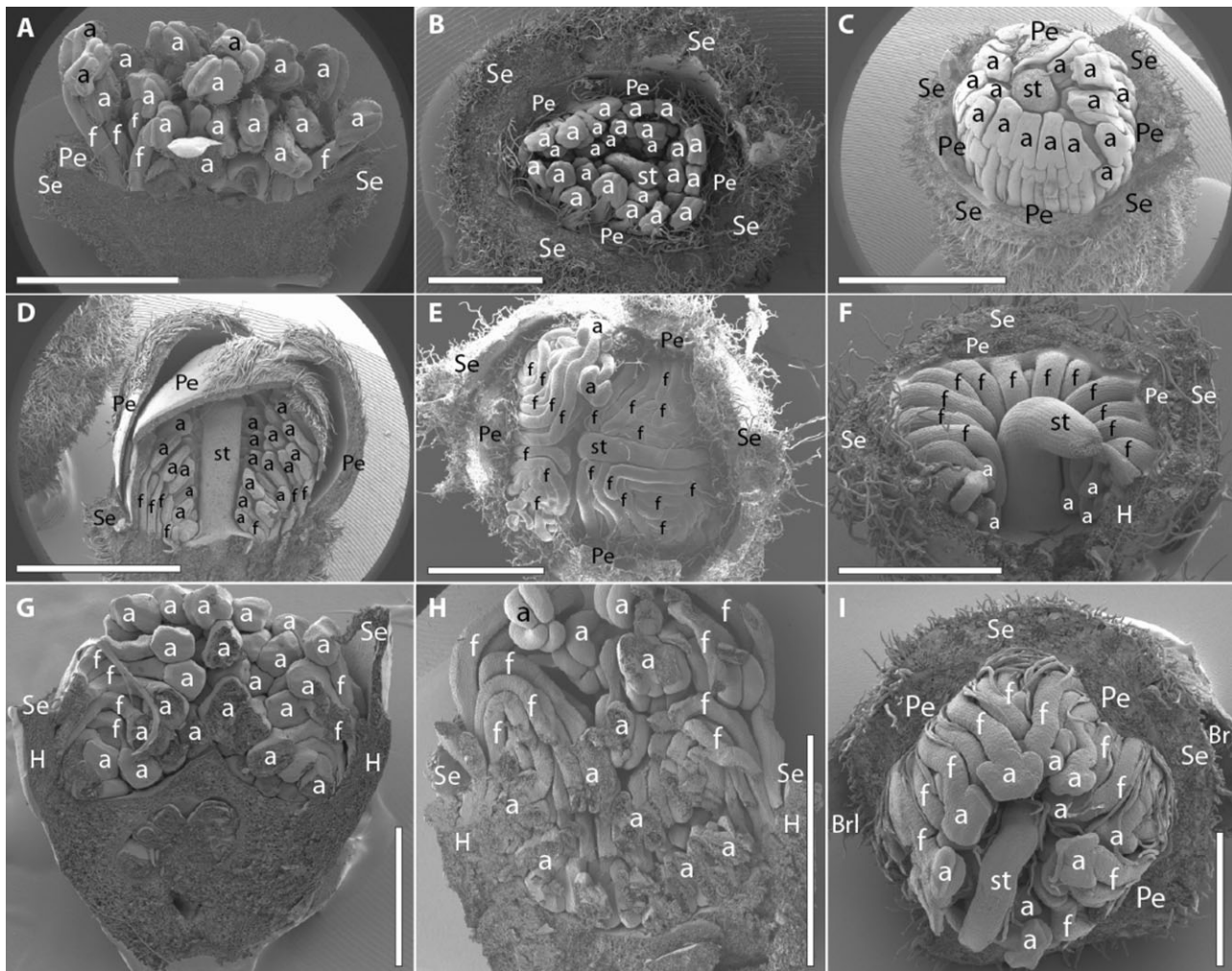


Figure 3. Scanning electron microscopy (SEM) images of buds of *Pimenta* group and *Myrceugenia* group species. Perianth completely or partly removed in all. A, B, *Acca sellowiana* (*Pimenta* group). A, Bud in a nearly pre-anthetic stage, with straight filaments and anthers upwards. B, Bud in an earlier developmental stage, with anthers already visible from above. C, D, *Psidium guineense* (*Pimenta* group) buds in pre-anthetic stage. C, Anthers visible from above. D, Straight filaments with anthers touching inner surface of petals. E, *Pimenta pseudocaryophyllus* (*Pimenta* group). Exceptional incurved filaments in the pre-anthetic bud with most anthers not visible from above. F, *Blepharocalyx salicifolius* (*Pimenta* group) also showing the exceptional incurved filaments, with anthers touching the bottom of the hypanthia cup formed by the hypanthial extension in a longitudinal view. G, *Luma apiculata* (*Myrceugenia* group) with inner filament whorls curved and outer whorls straight. Only the anthers from the inner whorls touch the bottom of the hypanthia cup. H, *Myrceugenia planipes* (*Myrceugenia* group) in a pre-anthetic stage, showing outer filaments straight and inner filaments curved, with anthers from the latter touching the bottom of the hypanthial cup. I, *Myrceugenia bananalensis* (*Myrceugenia* group) showing only anthers from the outer staminal whorls visible from above. a, anther; Br, bracts; Brl, bracteoles; f, filament; H, hypanthium; Pe, petal scar; Se, sepal scar; st, style. Scale bars: 500 µm (F, I), 1 mm (B, E, G, H), 2 mm (A, C, D).

straight stamens in the bud, similar to those in the Australasian, *Eugenia* and *Myrteola* groups and most of the *Pimenta* group, occurred in *Calycolpus goetheanus* (ungrouped, *sensu* Wilson, 2011), *Calyptronia cuspidata*, *Hottea ekmanii* (*Plinia* group, *sensu* Wilson, 2011), *Uromyrtus archboldiana* (Australasian group, *sensu* Wilson, 2011) and *Chamguava schippii*

(*Pimenta* group, *sensu* Wilson, 2011). Strongly incurved stamens occurred in *Mitranthes ottonis* (*Myrcia* group, *sensu* Wilson, 2011).

ANTHESIS TYPE

Species of different genera in the *Eugenia* group, *Pimenta* group (MEP clade), *Myrcia* group and *Plinia*

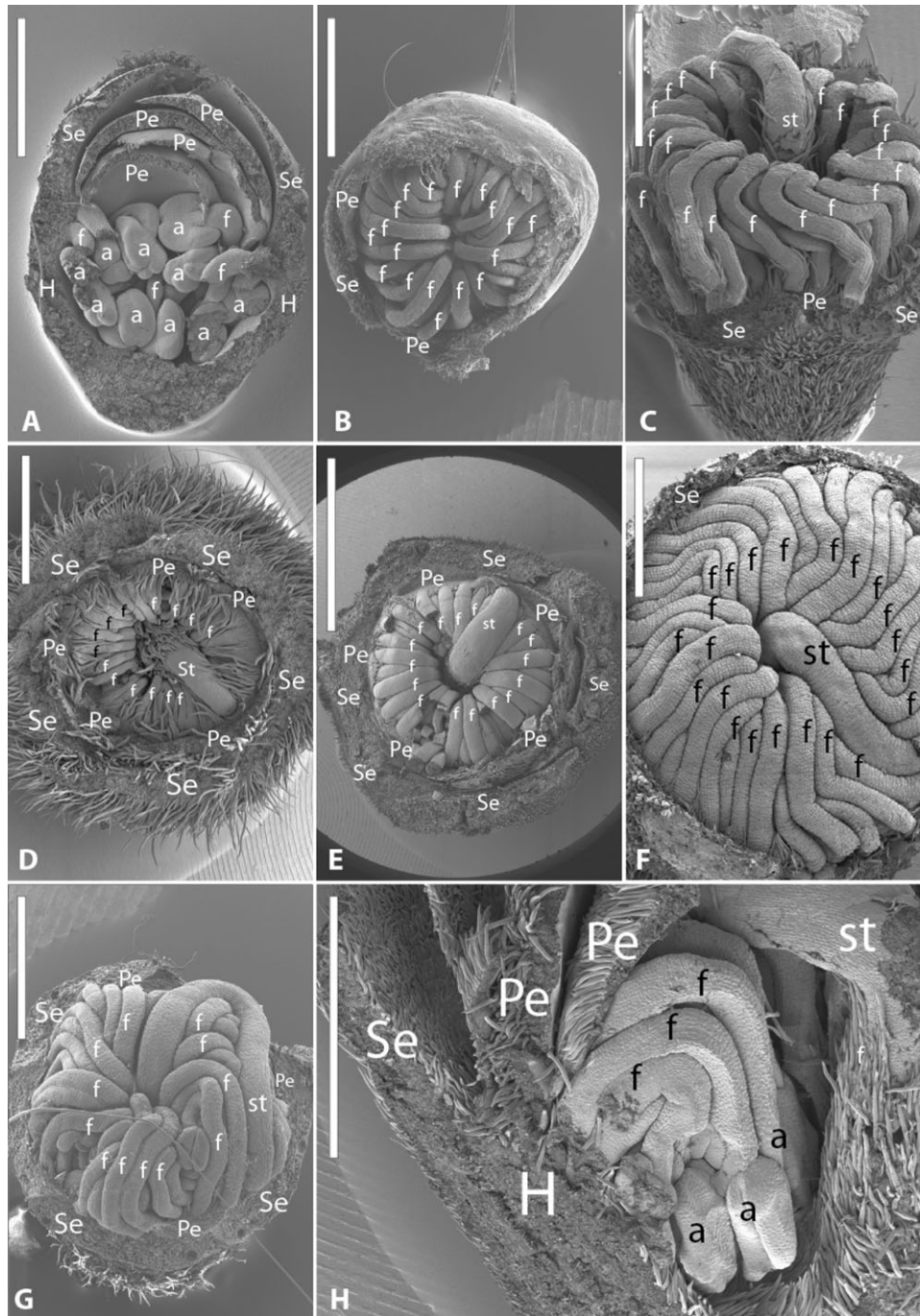


Figure 4. Scanning electron microscopy (SEM) images of buds of species from the *Plinia* and *Myrcia* groups and in *Algrizea minor*. Perianth completely or partly removed in all. A, *Plinia cauliflora* (*Plinia* group). Longitudinal section of nearly pre-anthetic bud, showing strongly incurved filaments and anthers facing downwards. Some of them do not fit inside the hypanthial cup as a result of the reduced size of the bud. Petals and sepals were not removed. B, *Myrciaria floribunda* (*Plinia* group). Pre-anthetic bud from above, with strongly incurved filaments and anthers not visible. C, *Myrcia sylvatica*. D, *Gomidesia* sp. E, *Myrcia spectabilis*. F, *Calypttranthes* aff. *blanchetiana* (all *Myrcia* group). G, *Algrizea minor* (ungrouped, sister to *A. macrochlamys*). All in pre-anthetic stage, showing filaments strongly incurved with anthers not visible from above. H, Detail of strongly incurved stamens in mid-development in *Myrcia spectabilis* (*Myrcia* group). Filaments are strongly incurved and force the anthers to the bottom of the hypanthial cup, which is formed by the extended hypanthium. a, anther; f, filament; H, hypanthium; Pe, petal scar; Se, sepal scar; st, style. Scale bars = 500 μ m (A, C), 1 mm (B, F, G, H), 2 mm (D, E).



Figure 5. Different anthesis types in Myrteae. Taxa with straight stamens in the bud and anthers which emerge first from the bud, found in *Eugenia cristaensis* (*Eugenia* group) (A), *E. stictosepala* (*Eugenia* group) (B), *E. coffeifolia* (*Eugenia* group) (C), *E. involucrata* (*Eugenia* group) (D), *Psidium guajava* (*Pimenta* group) (E) and *P. acutangulum* (*Pimenta* group) (F). Taxa with incurved stamens in which the filaments have to straighten first before the anthers face outwards, found in *Calyptanthes brasiliensis* (*Myrcia* group) (G), *Myrcia* sect. *Aulomyrcia* (*Myrcia* group) (H), *M. splendens* (*Myrcia* group) (I), *M. subavenia* (*Myrcia* group) (J), *Myrciaria floribunda* (*Plinia* group) (K) and *Blepharocalyx eggersii* (*Pimenta* group) (L). [Photographs taken by: E. J. Lucas (B, G, J) and T. N. C. Vasconcelos (A, C, D, E, F, H, I, K, L)].

group (PAM clade) were found with anthetic buds and photographed in the field. All species of *Eugenia* and *Myrcianthes* (*Eugenia* group) and *Acca*, *Campomanesia* and *Psidium* (*Pimenta* group) showed a similar type of anthesis. In these species, the anthers were the first organs to appear after anthesis, and their filaments seemed to have a continuous growth during and after bud opening (Fig. 5A–F). However, all species of *Myrcia* s.l. (*Myrcia* group), *Myrciaria*, *Plinia* (*Plinia* group) and *Blepharocalyx salicifolius* and *B. eggersii* (*Pimenta* group) showed a different type of anthesis. In these species, the filaments (which were strongly incurved before anthesis) were

the first part of the androecium to appear, and had to be unfolded during anthesis to expose the anthers for pollination (Fig. 5G–L).

DISCUSSION

SYSTEMATIC IMPLICATIONS OF STAMEN POSITION AND ANTHESIS TYPE IN MYRTEAE

There are three clear patterns of pre-anthetic stamen position in different genera of Myrteae (Fig. 6).

1. Straight stamens: species with this pattern show straight to slightly curved filaments. Removal of

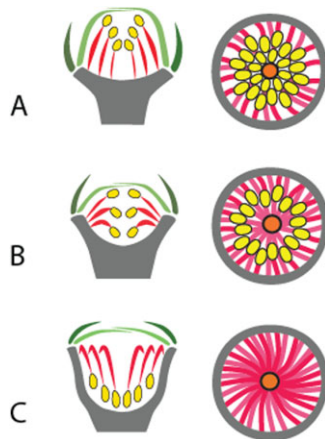


Figure 6. Variation in stamen position in Myrteae. Position of filaments and anthers in longitudinal section (left) and anther visibility from above in the bud after removing petals and sepals (right). A, Straight stamen pattern. B, Semi-curved stamen pattern. C, Strongly incurved stamen pattern. Colours indicate: grey, hypanthium; red, filaments; yellow, anthers; light green, petals; dark green, sepals; orange, gynoecium.

the calyx and corolla reveals the anthers of almost all staminal whorls visible from above. Pre-anthesis anthers touch the inner surface of the corolla (Fig. 6A). This pattern is found in *Myrtus communis* and the Australasian, *Eugenia*, *Myrteola* and *Pimenta* groups.

2. Semi-curved stamens: species with this pattern show straight to slightly incurved outer filaments, whereas the inner filaments show strongly incurved stamens and anthers facing downwards. On removal of the calyx and corolla, only anthers from the outer whorls are visible from above (Fig. 6B). This pattern is found consistently in the *Myrceugenia* group (*Luma apiculata*, *Myrceugenia alpigena*, *M. planipes*, *M. bananalensis* and *Blepharocalyx cruckshanksii*).
3. Strongly incurved stamens: species with this pattern show strongly incurved pre-anthetic stamens. Here, all filaments are acutely curved down towards the centre of the bud and all anthers touch the bottom of the hypanthial cup. After removal of the calyx and corolla, anthers are obscured by filament tissue on the view from above (Fig. 6C).

With regard to the type of anthesis, the two different extremes of stamen position, straight stamens and strongly incurved stamens, also seem to be related to differences in anthesis types. In the first type, anthers are presented first and the latter filaments have to unfold for anther exposure. Proença & Gibbs (1994) have reported differences in stamen behaviour during

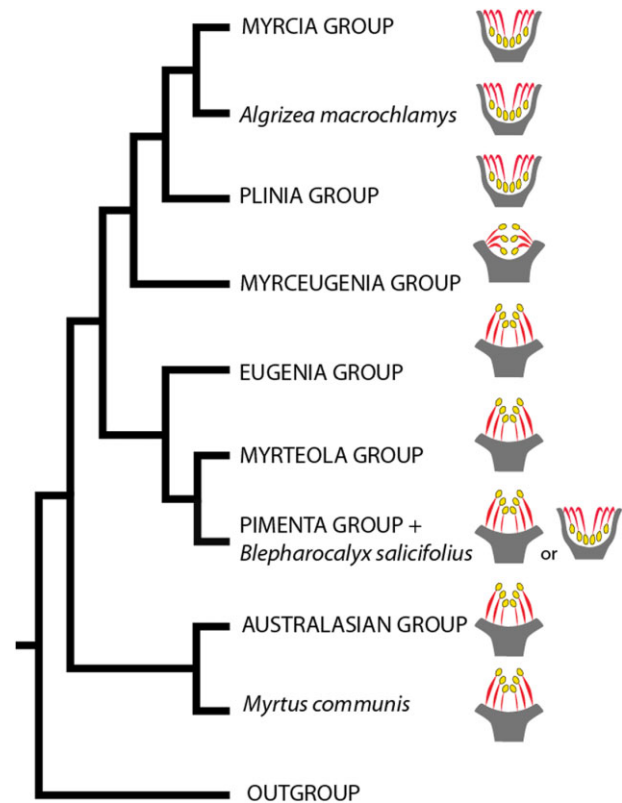


Figure 7. Summarized phylogenetic tree of Myrteae (based on De-Carvalho, 2013) with stamen patterns per clade. Colours indicate the same structures as in Figure 6.

anthesis in different species of Myrteae. These authors classified species in which anthers were upright during this phase as ‘*Psidium* like’ (corresponding to ‘straight stamens’ as defined here), and those in which filaments unfolded at anthesis to expose the anthers (corresponding to ‘strongly incurved stamens’ as defined here) were classified as ‘*Myrcia* like’.

An examination of pre-anthesis stamen position and, consequently, anthesis type against a summary of the most current phylogenetic hypothesis for Myrteae (De-Carvalho, 2013) demonstrates that these characters are congruent with the phylogenetic topology (Fig. 7). Straight pre-anthesis stamens with *Psidium*-like anthesis appear to be a plesiomorphic state in Myrteae. This character is found in the *Myrtus communis* + Australasian group clade (although the anthesis type was not checked) at the base of the tribe and in most of the MEP clade, except for *Pimenta* and *Blepharocalyx salicifolius*. The incurved stamens and *Myrcia*-like anthesis of the PAM clade, and in *Pimenta* and *Blepharocalyx salicifolius*, may have a secondary evolutionary origin. The PAM clade is recovered in all phylogenetic studies with high Bayesian and bootstrap support (Lucas *et al.*, 2007; Costa, 2009; De-Carvalho, 2013) and incurved stamens appear to

be synapomorphic for this clade. The *Myrceugenia* group at the base of the PAM clade presents semi-curved stamens, and it is tempting to interpret this as an intermediate stage between the straight stamens of the MEP clade and the strongly incurved stamens of the PAM clade. Strongly incurved stamens occur in *Blepharocalyx salicifolius* and in *Pimenta* (both *Pimenta* group) providing support for the recent placement of *B. salicifolius* in the *Pimenta* group (P. S. De-Carvalho, pers. comm.).

The consistency of these results across the phylogenetic tree of Myrteae potentially allows them to be used diagnostically to place as yet unsampled genera. For example, *Calypptrogenia* and *Hottea* were interpreted as belonging to the *Plinia* group by Wilson (2011). However, members of the *Plinia* group consistently have strongly incurved stamens, casting doubt on this placement of these two genera. The analysis of pre-anthetic stamen and geographical distributions suggests that both genera are more likely to be related to *Psidium* or *Eugenia*. In addition, *Mitranthes*, another Caribbean genus distributed in Jamaica and Cuba, was suggested in the same work to be part of the *Myrcia* group, and this is supported by the presence of incurved stamens in the bud. In *Uromyrtus*, straight stamens in the bud, together with the geographical distribution, support its placement in the Australasian group (Wilson, 2011). Further phylogenetic and morphological analyses are required to verify the precise position of these genera within the tribe.

RELATIONSHIP BETWEEN STAMEN POSITION AND HYPANTHIUM EXTENSION

As inferred by previous studies (Proença, 1992; Belsham & Orlovich, 2002, 2003), our study demonstrates a relationship between pre-anthesis stamen position and hypanthium extension. In general, species with an extended hypanthium have incurved stamens developing just below the hypanthial rim, suggesting that the hypanthial extension has ‘carried’ the stamens upwards (also suggested by C. E. B. Proença, pers. comm.). However, species with no hypanthial extension (i.e. a flat floral base) generally have straight stamens, and those with a short hypanthial extension have a semi-curved pattern.

Nevertheless, exceptions exist to these rules. Belsham & Orlovich (2002, 2003) studied androecium and hypanthium ontogeny in the *Myrteola* group. These studies found *Lophomyrtus* and *Neomyrtus*, two closely related New Zealand genera, to have initially a short hypanthium cup (exceptional in genera with straight stamens) with laterally and slightly incurved stamens resembling *Luma apiculata* (*Myrceugenia* group). In pre-anthesis bud, however,

Lophomyrtus and *Neomyrtus* stamens assume the straight position as in other species of the *Myrteola* group. This also seems to occur in *Campomanesia* (*Pimenta* group), in which the stamens are straight but the hypanthial extension is also variable.

In the *Myrcia* group, where strongly incurved stamens occur consistently, one clade (*Myrcia* section *Myrcia*, sensu Lucas *et al.*, 2011) does not have an extended hypanthium. Results for this group also show strongly incurved pre-anthesis stamens despite the non-extended hypanthium (Fig. 4C). This suggests that the flat hypanthium may be a secondary condition that arose from the extended hypanthium state with the remaining curved stamens a ‘relictual’ arrangement. This hypothesis is congruent with the younger age of *Myrcia* section *Myrcia* relative to other clades of *Myrcia s.l.* (Staggemeier *et al.*, 2015).

EVOLUTIONARY IMPLICATIONS OF HYPANTHIAL EXTENSION, STAMEN POSITION IN THE BUD AND FLORAL ECOLOGY

The present study concludes that floral evolution played a role in the divergence of Myrteae, with other aspects associated with dispersal, such as embryo type (Landrum & Kawasaki, 1997). Stamens are recognized to be a variable condition in Myrtales (Decraene & Smets, 1991), and the evolution of these characteristics in Myrteae may have been a driving force in its diversification, especially in the Neotropical lineages, in which stamen position is a character that separates the two large clades (i.e. the MEP from the PAM + *Myrceugenia* group).

Differences in hypanthium extension (leading to stamen position in the bud) and anthesis type may be linked to shifts in pollination strategy. Proença (1992) noted that Myrteae species with extended hypanthia often show pollen collection via ‘buzz-pollination’, and the hypanthium extension may assist the bees in gripping flowers during vibration. Species of *Myrcia s.l.* (*Myrcia* group) and *Siphoneugenia* (*Plinia* group) with extended hypanthia attract bees that use buzz strategies, whereas, in *Campomanesia*, *Psidium* (*Pimenta* group) and *Eugenia* (*Eugenia* group), species with flat hypanthia, buzzing was not reported (Proença, 1992). Exceptionally, *Myrcia linearifolia*, a species without extended hypanthia (as *Myrcia sylvatica*; Fig. 4C), was the only *Myrcia* sp. that did not attract buzz-pollinators. This provides further support for the suggestion that hypanthia are implicated in buzz-pollination. However, Fidalgo & Kleinert (2009) also observed buzz-pollination in species of Myrteae without hypanthial extension. Further field and experimental studies are required to clarify the function of the hypanthium in connection with pollen-collecting bees.

An alternative hypothesis of the adaptive advantage of hypanthial extension is linked to the transition from pollen to nectar as the main floral reward. Hypanthial extension ultimately leads to the formation of a hypanthial cup which is frequently linked to nectar production (Harder & Cruzan, 1990). Myrteae flowers are known to be almost exclusively pollen-reward flowers (Nic Lughadha & Proença, 1996). Nevertheless, there are records of nectar presence in flowers of *Plinia* (*Plinia* group; Malerbo, Toledo & Couto, 1991; Pirani & Cortopassi-Laurino, 1993), a genus with a hypanthial cup, and also in flowers with hypanthial cups in other tribes of Myrtaceae, such as Syzygieae (Lack & Kevan, 1984; Crome & Irvine, 1986; Abe, 2006) and Eucalypteae (Bond & Brown, 1979), suggesting that these two characteristics may be linked in the family.

Adaptive advantages in having a flat hypanthium and straight stamens can also be hypothesized. *Eugenia* and *Psidium*, two genera with straight stamens and 'Psidium-like' anthesis, have filaments and styles that grow continuously after anthesis (Proença & Gibbs, 1994; Silva & Pinheiro, 2007). In this scenario, pollen is immediately available at anthesis (Silva & Pinheiro, 2007), which may lead to a higher degree of pollination success. In addition, Silva & Pinheiro (2007) analysed the reproductive biology of six *Eugenia* spp. and noted that the straight, continuously growing style might help with self-pollination by making contact with mature anthers during growth. Furthermore, Fidalgo & Kleinert (2009) compared *Eugenia speciosa* with five other species from other genera of Myrtaceae and found that it was the only species with significant fruit production when pollinators were excluded. Proença & Gibbs (1994) also found *Eugenia dysenterica* and *Psidium firmum* to be completely self-compatible, with *P. firmum* having an even higher percentage of fruit set in self-pollinated flowers.

SYSTEMATIC IMPLICATION FOR STRAIGHT STAMENS IN MYRTEALES

Classic works (Dahlgren & Thorne, 1984; Johnson & Briggs, 1984) cited incurved stamens in the bud as a synapomorphy of Myrtales with exceptions in Onagraceae, Vochysiaceae, *Heteropyxis* and *Psyloxylon*. The present study extends the exception to most taxa of Myrteae. Buds of *Heteropyxis* were also checked to understand these authors' definition of 'straight stamens'. Despite *Heteropyxis* having only five stamens, these stamens strongly resemble the straight stamens recorded here in polystemonous flowers in Myrteae.

Molecular phylogenetic studies in Myrtales, in conjunction with character evolution interpretations,

showed incurved stamens as the likely plesiomorphic state in Myrtales, with straight stamens evolving independently in Onagraceae, Vochysiaceae, *Heteropyxis* and *Psiloxylon* (Johnson & Briggs, 1984; Conti *et al.*, 1997). Conti *et al.* (1997) further hypothesized that straight stamens are the plesiomorphic condition for the Vochysiaceae + Myrtaceae clade and that this character is lost in subfamily Myrtoideae. Our results challenge this hypothesis by suggesting that straight stamens are plesiomorphic in tribe Myrteae, even though, in other tribes of Myrtaceae, pre-anthesis stamens are mostly strongly incurved (Drinnan & Ladiges, 1991; Orlovich, Drinnan & Ladiges, 1999; Bohte & Drinnan, 2005; Drinnan & Carrucan, 2005). Further character reconstruction studies are required in order to better understand the evolution of this character across Myrtales.

CONCLUSIONS

This study reveals a previously undetected morphological pattern in Myrteae that consolidates the taxonomic understanding in the tribe and provides a means for specimen identification to genus level. Pre-anthesis buds are the most common phase found in herbarium specimens (T. N. C. Vasconcelos, pers. observ.) and can be easily manipulated to verify whether anthers are visible from above when the perianth is removed. This, aligned with other traditional characteristics, can be used as a complementary identification tool in the field and herbarium. At tribal level, the stamen pattern is more consistent than the inflorescence, embryo, placentation, number of locules per ovary, number of sepals and other characteristics that have previously been used in systematics of Myrteae. Congruent characters are rare in Myrteae, although recent work on the development of the gynoecium (Pimentel *et al.*, 2014) has found other positively correlated characters, indicating that characters of flower development might be important in understanding evolution in Myrteae.

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APPENDIX

Species analysed using herbarium or spirit collection organized by clade (following Lucas *et al.*, 2007, 2011; Costa, 2009; Snow *et al.*, 2011; De-Carvalho, 2013; Mazine *et al.*, 2014). All specimens are deposited in Kew, with the exception of *J.E.Q. Faria* collections, which are deposited in UB. Herbaria acronyms: E, Royal Botanic Garden Edinburgh; K, Royal Botanic Gardens, Kew; UB, Universidade de Brasilia; US, Smithsonian Institution.

| Group | Species | Voucher |
|-------------------------------------|------------------------------------------------------------|-------------------------------------------------|
| Australasian group | <i>Archirhodomyrtus beckleri</i> (F.Muell.) A.J.Scott | Gray B 1548 |
| | <i>Decaspermum parviflorum</i> (Lam.) A.J.Scott | FRI 1232 |
| | <i>Gossia bidwillii</i> (Benth.) N.Snow & Guymmer | Smith LS 4516a |
| | <i>Octamyrtus arfancensis</i> Kaneh. & Hatus. ex C.T.White | Van Royen P 7925 |
| | <i>Octamyrtus</i> sp. | Johns 9885 (from K spirit collection) |
| | <i>Pilidiostigma tropicum</i> L.S.Sm. | Kajewski SF 1265 |
| | <i>Rhodamnia dumetorum</i> (DC.) Merr. & L.M.Perry | Schanzer I 148c |
| | <i>Rhodomyrtus tomentosa</i> (Aiton) Hassk. | Amin SAN 116159 |
| | <i>Rhodomyrtus</i> sp. | s.n. (from US spirit collection) |
| | <i>Eugenia</i> group | <i>Calycorectes acutatus</i> (Miq.) Toledo |
| <i>Calycorectes bergii</i> Sandwith | | Myers JG 5955 |
| <i>Eugenia coffeifolia</i> DC. | | Giaretta A 1441 |
| <i>Eugenia cristaensis</i> O.Berg | | Vasconcelos TNC 284 |
| <i>Eugenia florida</i> DC. | | Ratter JA R7568 |
| <i>Eugenia involucrata</i> DC. | | Reitz 7261 |
| | | <i>Faria</i> JEQ 4047 |
| | | Vasconcelos TNC 256 |
| | | <i>Eugenia klotzschiana</i> O.Berg |
| | | Heringer 1975 |
| | <i>Eugenia pluriflora</i> DC. | |
| | Hatschbach 19022 | |
| | <i>Eugenia pyriformis</i> Cambess. | |
| | Reitz 11341 | |
| | <i>Eugenia stictosepala</i> Kiaersk. | |
| | <i>Faria</i> JEQ 4269 | |
| | Vasconcelos TNC s.n. (from K living collection) | |
| | Wooleton A 1022 | |
| | <i>Hexachlamys edulis</i> (O.Berg) Kausel & D.Legrand | |
| | Pedersen TM 2756 | |
| | <i>Myrcianthes fragrans</i> (Sw.) McVaugh | |
| | Chaco R 350 | |
| | <i>Myrcianthes pungens</i> (O.Berg) D.Legrand | |
| | <i>Faria</i> JEQ 4277 | |
| <i>Myrceugenia</i> group | <i>Blepharocalyx cruckshanksii</i> (Hook. & Arn.) Nied. | Gardner MF 4193 |
| | <i>Luma apiculata</i> (DC.) Burret | Vasconcelos TNC s.n. (from K living collection) |
| | <i>Myrceugenia planipes</i> (Hook. & Arn.) O.Berg | Lucas E J s.n. (from E living collection) |
| | <i>Myrceugenia bananalensis</i> Gomes-Bezerra & Landrum | <i>Faria</i> JEQ 4049 |
| <i>Myrcia</i> group | <i>Calyptranthes</i> aff. <i>blanchetiana</i> O.Berg | Lucas EJ 1208 |
| | <i>Myrcia fenziiana</i> O.Berg | Nic-Lughada E H50637 |
| | <i>Myrcia pubipetala</i> Miq. | Lucas EJ 477 |
| | <i>Myrcia splendens</i> (Sw.) DC. | Herrera GC 9932 |
| | <i>Myrcia spectabilis</i> DC. | Lucas EJ 1214 |
| | | Lucas EJ 1210 |
| | | Lucas EJ 1207 |
| | | Lucas EJ 1221 |
| | | Lucas EJ 1205 |
| | | Lucas EJ 1219 |
| | Lucas EJ 1216 | |
| | Lucas EJ 1222 | |
| <i>Myrteola</i> group | <i>Lophomyrtus obcordata</i> (Raoul) Burret | Cult Lord Headfort (from K spirit collection) |
| | <i>Myrteola nummularia</i> (Lam.) O.Berg | Gardner MF 3579 |
| | <i>Neomyrtus pedunculata</i> (Hook.f.) Allan | Macmillan BH 76/102 |
| | <i>Ugni candollei</i> (Barnéoud) O.Berg | Vasconcelos TNC s.n. (from K living collection) |

APPENDIX *Continued*

| Group | Species | Voucher | |
|----------------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| <i>Pimenta</i> group | <i>Acca sellowiana</i> (O.Berg) Burret | <i>Spirit collection 14462</i> <i>Vasconcelos TNC s.n.</i> (from K living collection) | |
| | <i>Capomanesia adamantium</i> (Cambess.) O.Berg | <i>Vasconcelos TNC 273</i> | |
| | <i>Campomanesia guazumifolia</i> (Cambess.) O.Berg | <i>Lobao A 1372</i> | |
| | <i>Pimenta pseudocaryophyllus</i> (Gomes) Landrum | <i>Irwin HS 19844</i> <i>Duarte AP 8722</i> <i>de Lima HC 3453</i> <i>Lucas EJ 193</i> <i>Vasconcelos TNC 403</i> | |
| | <i>Pimenta racemosa</i> (Mill.) J.W.Moore | <i>Axelrod F 7796</i> | |
| | <i>Psidium oligospermum</i> Mart. ex DC. | <i>Franca F 5431</i> | |
| | <i>Psidium guajava</i> L. | <i>Vasconcelos TNC 389</i> | |
| | <i>Psidium guineense</i> Sw. | <i>Lucas EJ 1213</i> | |
| | <i>Mosiera longipes</i> (O.Berg) Small | <i>Hamilton MA 630</i> | |
| | <i>Myrrhinium atropurpureum</i> Schott | <i>Hatchbachi G 61056</i> | |
| | <i>Plinia</i> group | <i>Myrciaria floribunda</i> (H.West ex Willd.) O.Berg | <i>Harley RM 54895</i> |
| | | <i>Neomitranthes cordifolia</i> (D.Legrand) D.Legrand | <i>Souza MC 550</i> |
| | | <i>Neomitranthes obscura</i> (DC.) N.Silveira | <i>Carvalho AM 816</i> |
| | | <i>Plinia cauliflora</i> (Mart.) Kausel | <i>Vasconcelos TNC 388</i> |
| Ungrouped | <i>Siphoneugena densiflora</i> O.Berg | <i>G Martinelii11939</i> | |
| | <i>Algrizea macrochlamys</i> (DC.) Proença & NicLugh. | <i>Melo E 4496</i> | |
| | <i>Algrizea minor</i> Sobral, Faria & Proença | <i>Faria JEQ 4157</i> | |
| | <i>Blepharocalyx salicifolius</i> (Kunth) O.Berg | <i>Azevedo MLM 337</i> <i>Ratter JA 5984</i> <i>Silva TRS 13494</i> <i>Faria JEQ 4050</i> <i>Lucas EJ 211</i> <i>Vasconcelos TNC s.n.</i> (from K living collection) | |
| | <i>Myrtus communis</i> L. | <i>Vasconcelos TNC 485</i> | |
| | <i>Accara elegans</i> (DC.) Landrum | | |
| | | | |
| Group (based on Wilson, 2011) | Species | Voucher | |
| Species not yet in the phylogenetic analyses | | | |
| | Ungrouped | <i>Blepharocalyx eggersii</i> (Kiaersk.) Landrum | <i>Nelson BW 923</i> <i>Sugden AM 1230</i> <i>Vasconcelos TNC 458</i> |
| <i>Plinia</i> group | <i>Calycolpus goetheanus</i> (Mart. ex DC.) O.Berg | <i>Vasconcelos TNC 332</i> | |
| | <i>Calyptrogenia cuspidata</i> Alain | <i>Lucas EJ 1125</i> | |
| <i>Pimenta</i> group | <i>Chamguava schippii</i> (Standl.) Landrum | <i>Gentle PH 8354</i> | |
| <i>Plinia</i> group | <i>Hottea ekmanii</i> (Urb.) Borhidi | <i>Ekman EL 2502c</i> | |
| <i>Myrcia</i> group | <i>Mitranthes ottonis</i> O.Berg | <i>Otto E 272</i> | |
| Australasian group | <i>Uromyrtus archboldiana</i> (Merr. & L.M.Perry) A.J.Scott | <i>Puradyatmika P 7425</i> | |
| Other (non-Myrteae) | | | |
| Psyloxyloideae | <i>Heteropyxis natalensis</i> Harv. | <i>Correia MF 594</i> | |