

Convergent evolution in calyptrate flowers of Syzygieae (Myrtaceae)

THAIS N. C. VASCONCELOS^{1,2,*}, EVE J. LUCAS¹, MARIA CONEJERO³,
AUGUSTO GIARETTA² and GERHARD PRENNER¹

¹Comparative Plant and Fungal Biology Department, Royal Botanic Gardens Kew, TW9 3DS, UK

²Laboratório de Sistemática Vegetal, Instituto de Biociências, Departamento de Botânica, Universidade de São Paulo, 05508900, São Paulo, SP, Brazil

³Analytical Methods Team, Royal Botanic Gardens Kew, TW9 3DS, UK

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Shedding a fused perianth as a calyptra at anthesis is a trait that has evolved independently multiple times in angiosperm evolutionary history. However, calyptras do not correspond to homologous structures in all cases. Here, we describe calyptra development in the evolutionary context of Myrtaceae tribe Syzygieae. We use scanning electron and light microscopy to contrast calyptra development in calyptrate and non-calyptrate species in the group. Results show that calyptras in Syzygieae are not all homologous, but correspond to two ontogenetically distinct structures involving different perianth whorls that resemble each other by convergence: a calycine structure, in which the sepals are fused; and a pseudocalyptra, in which petals fall as a single unit but are not fused. Presence of non-calyptrate flowers is the ancestral state in the tribe, and both calyptra types appeared multiple times in the evolution of the group, with infrequent reversals from the calyptrate to the non-calyptrate state. Results highlight the fact that similar terminology in non-homologous structures can affect even relatively restricted lineages. The recurrent evolution of the calyptra in Syzygieae, with little evidence for reversal, shows that these structures may be advantageous in certain conditions but also correspond to an evolutionary dead-end in the group.

ADDITIONAL KEYWORDS: calyx – convergence – corolla – ontogeny – perianth – underlying homology.

INTRODUCTION

In Werberling's (1989) definition of a perfect flower, the perianth corresponds to the outer rings of floral appendages, formed by two whorls of leaf-like organs. The external whorl (the calyx) is formed by sepals, and the internal whorl (the corolla) is formed by petals, which, despite their leaf-like appearance, are sometimes regarded as evolutionarily closer to the androecium (Ronse De Craene, 2007). Across angiosperms, the calyx most commonly has a protective role, covering fragile reproductive organs during flower ontogeny. The corolla, frequently showy, is usually linked to pollinator attraction (Endress, 1994). Nevertheless, variations of these functions are frequently observed. A perianth that appears partially or completely fused in the bud, for instance, is a common trend in some angiosperm families (e.g.

Euphorbiaceae: Esser, 1999; Solanaceae: D'Arcy, 1986), and specific terminology exists to describe behaviour of these structures during anthesis. 'Calyptrate' or 'operculate' flowers are designated as such when a perianth appears completely fused in bud, detaching at the base and falling off as a single 'cap-like' structure during anthesis (e.g. McVaugh, 1956; Fig. 1).

Calyptrate flowers are observed in many lineages of angiosperms, including Vitaceae (Soejima & Wen, 2006), Eupomatiaceae (Endress, 2003) and Myrtales (e.g. Goldenberg & Meirelles, 2011; Kriebel *et al.*, 2015). In Myrtales, calyptrate flowers are an especially common trend in Myrtaceae, appearing in at least 17 of the 144 genera, mainly distributed in tribes Eucalypteae, Myrteae and Syzygieae (Wilson, 2011; Fig. 1). Drinnan & Ladiges (1988, 1989, 1991) provided a detailed description of this structure in Eucalypteae; and Vasconcelos *et al.* (2017) and Giaretta *et al.* (2019) provided descriptions for the structure for the two largest genera of Myrteae (*Myrcia* DC. and *Eugenia* L.,

*Corresponding author. E-mail: thais.nogales@gmail.com

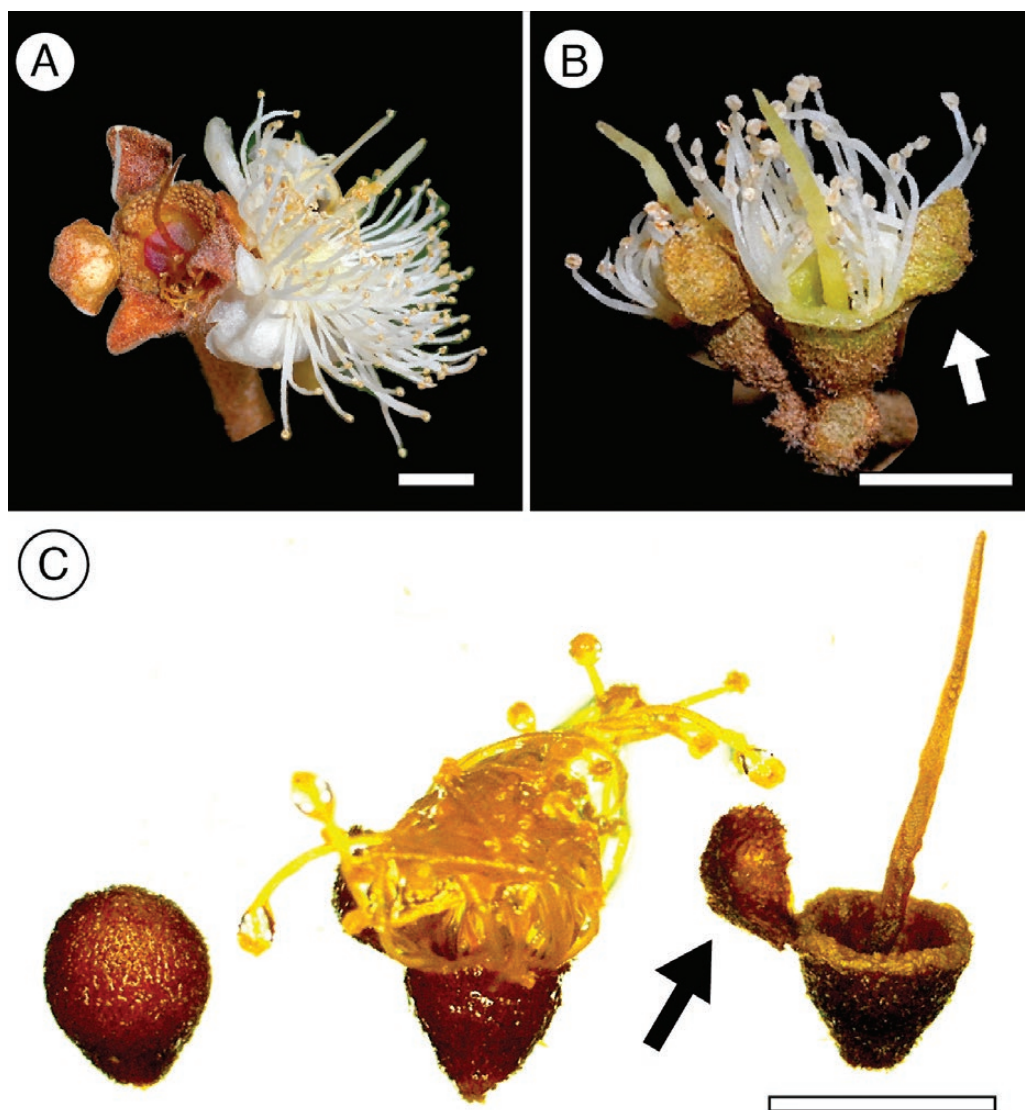


Figure 1. Calyprate and non-calyprate flowers in Myrtaceae, as shown in *Myrcia* (for more information, see Vasconcelos *et al.*, 2017). A and B show, respectively, flowers of the closely related *Myrcia* and *Calyptranthes*, the former with four free sepals and petals and the latter with a calyprate perianth (arrow). C, Anthesis sequence in *Calyptranthes brasiliensis*, showing, in order, closed bud, open flower and old flower, with adherent calyprate (arrow). Scale bars: 2 mm.

respectively). However, the development and evolution of this structure in the second largest tribe in the family, Syzygieae, remains to be described.

Syzygieae comprise > 1000 species and are mainly distributed across South-East Asia, with species also occurring in Africa, Australia and the Pacific region. The presence of calyprate flowers in Syzygieae has been considered of strong systematic relevance, supporting the circumscription of two genera, *Cleistocalyx* Blume and *Piliocalyx* Brongn. & Gris, both with calyprate flowers. Molecular systematic studies, however, showed that this diagnostic trait does not define natural groups, and nomenclatural

arrangements were required to move *Acmena* DC., *Cleistocalyx* and *Piliocalyx* to *Syzygium* Gaertn. (Craven & Biffin, 2010). The appearance of the same trait multiple times in a phylogenetic tree may lower its potential as a diagnostic character for higher taxonomic ranks, but it is still important to clarify structural homologies before assuming that it has low phylogenetic signal. Despite its clear homoplasy, this character has not yet been examined in light of the phylogenetic history of the group.

To better understand the morphological diversity of this structure, we describe perianth development in a sample of three species with calyprate and one

with non-calyptrate flowers in Syzygieae. Given the macromorphological nature of this character, as soon as homologies are understood these can be observed and, in most cases, easily scrutinized from herbarium material. In this sense, and given that evolutionary patterns in Syzygieae are still relatively unknown (Ahmad *et al.*, 2016), we use this information to expand trait coding and consider calyptra evolution in the framework provided by the current phylogenetic hypothesis for the tribe, highlighting evolutionary convergences, parallelisms and reversals.

MATERIAL AND METHODS

STUDY GROUP AND SAMPLING APPROACH

Buds in different developmental stages were collected in 70% alcohol during field expeditions in Brazil [cultivated *Syzygium cumini* (L.) Skeels, voucher *T. Vasconcelos* 298] and New Caledonia (*Piliocalyx* sp., voucher *T. Vasconcelos* 651) or retrieved from the living collection at the Singapore Botanic Gardens (*S. nervosum* A.Cunn. ex DC., *RB1999-0250*) and the Royal Botanic Gardens, Kew (*S. paniculatum* Gaertn., voucher *T. Vasconcelos* s.n.) (see Appendix I). *Syzygium cumini*, *S. nervosum* and *Piliocalyx* sp. are described as having calyptrate flowers in the literature, whereas *S. paniculatum* is described as non-calyptrate (Linnaeus, 1753; Gaertner, 1788; De Candolle, 1828; Wilson, 2011). These species were selected due to ease of access and because their calyptras represent different calyptra morphologies. *Syzygium cumini* has a calyptra type described in the literature as 'petaline opercular structures [that] consist of the imbricate petals that cohere and fall as a unit' (Wilson, 2011: 216), whereas *S. nervosum* and *Piliocalyx* have calyptras made from the outer perianth whorl (i.e. the calyx). This is a small sample of species, but illustrates the diversity of this structure in the tribe.

ONTOGENETIC AND ANATOMICAL SURVEY

For ontogenetic analyses, floral buds and flowers in different developmental stages were dissected in 70% ethanol, dehydrated through an alcohol series to 100% ethanol and critical-point dried using an Autosamdri-815B critical-point dryer (Tousimis Research, Rockville, Maryland, USA). Dried material was mounted onto specimen stubs, 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 scanning electron microscope (SEM) (Hitachi High Technologies, Tokyo, Japan). Key stages of perianth development, such as organ initiation, point of fusion and degree of

fusion at anthesis, were noted and described. Distinct types of calyptra were described and classified based on the nature of perianth parts at these stages (see Results).

Additional anatomical analyses of the perianth were performed for *S. nervosum* and *S. cumini*. Samples of pre-anthetic buds passed through a series of alcohol to histoclear (100%) and then embedded in wax (paraplast 100%). Sectioning was performed using a microtome (Leica RM2155) and slides were stained with safranin red and Alcian blue. These stains colour lignified tissues in red and cellulose in blue. Slides were analysed under light microscopy and are accessible in the slide collection of Royal Botanic Gardens, Kew.

PHYLOGENETIC ASSESSMENT

The most up-to-date phylogenetic hypothesis for Syzygieae, extracted as a subset from Thornhill *et al.* (2015), was used to analyse ancestral reconstruction of the calyptra as a trait. The original phylogenetic tree is based on three molecular markers (plastid *ndhF* and *matK* and nuclear ITS) and comprises 199 species of Myrtaceae, representing all tribes in the family and including 63 species of Syzygieae. This is the broadest phylogenetic hypothesis for Myrtaceae to date and is also exceptional as 12 fossils were used in its calibration, improving branch length estimations. Discussing of divergence times in Syzygieae is not among our main objectives, but sound estimations of branch length are important for robust reconstructions of trait evolution (Harmon, 2019).

CHARACTER CODING AND RE-CODING

Once calyptra categories were established (see Results), these are straightforward to recognize in dried herbarium material. Following the ontogenetic survey of a selected sample, calyptra categories were assigned to all tips of the phylogenetic tree to examine evolution of the trait. Some *Syzygium* spp., particularly those with small buds, were difficult to code due to their fragile perianth post-herborization. As a result, these species [*Syzygium anisatum* (Vickery) Craven & Biffin, *S. laxeracemosum* (Guillaumin) J.W.Dawson, *Syzygium mackinnonianum* (B.Hyland) Craven & Biffin, *Syzygium purpureum* (L.M.Perry) A.C.Sm.)] were excluded because no calyptra category could be confidently assigned. These species were trimmed from the original phylogenetic tree using function *drop.tip* from package *ape* (Paradis *et al.*, 2004).

For all analyses, traits were coded into simple binary states (presence or absence), first for all calyptrate taxa without distinction of calyptra categories and then re-coded using distinct homologous calyptrate states (trait coding matrix is available in the Supplementary

Material). In species with combinations of calyptrate categories, each category was coded separately.

TRAIT RECONSTRUCTION

Characters were reconstructed on the phylogenetic hypothesis using stochastic character mapping implemented in the function *make.simmap* from package *phytools* in R (Revell, 2012; R core team, 2018), used as an empirical Bayesian method to map appearance and reversal of the calyptrate flower state in the evolution of Syzygieae. Traits were reconstructed using the equal rates ('ER') model, the model that fit best according to AIC criteria, and 1000 simulations. The analysis was run three times, once for each distinct coding configuration of calyptrate flowers in the tips of the phylogenetic tree (i.e. calyptrate vs. non-calyptrate; calycine calyptra vs. all other floral types and pseudocalyptra vs. all other floral types).

RESULTS

CALYPTRA HOMOLOGY

Ontogenetic analyses of selected species confirm that the structure named as the calyptra in Syzygieae cannot always be treated as a homologous structure. Closed perianths occur via at least two distinct developmental pathways that involve distinct organs (calyx or corolla) and types of fusion. These are here named as calycine calyptra and pseudocalyptra and are described in the following sections. Perianth SEM images of a species with free perianth (*S. paniculatum*) are provided for comparison (Fig. 2) and show four sepals developing decussately (two older and two younger; Fig. 2A, B) and four petals developing almost simultaneously (Fig. 2C). In species with a fully free perianth, sepals and petals are never shed as a single unit and can be easily identified at anthesis (Fig. 2A–F).

THE CALYCINE CALYPTRA

In *S. nervosum* and *Piliocalyx* sp., the calyx (i.e. the outermost floral whorl) forms the calycine calyptra. Four sepals initiate free following a decussate pattern (Fig. 3A). After a short period of elongation, the base of the four sepals fuse into a homogeneous calycine tissue (i.e. a late-congenital fusion; Fig. 3C, E, F, L, M). During this process, the free sepal tips meet or overlap slightly at the top of the bud (Fig. 3E, L). The now mostly gamosepalous structure continues its development until pre-anthetic stage (Fig. 3G, H). At this point, the four initially free calyx lobes remain as inconspicuous scars at the top of the bud, characterizing this developmental mode (Fig. 3F, M).

During anthesis, pressure from within the bud tears the calycine tissue at the weakest point, frequently at the base, resulting in a 'cap-like' structure that often remains attached to the side of the flower forming a calyptra (Fig. 3I, K). Petals remain free during the whole flower development in both species (Fig. 3B, D, G, J, K, N).

THE PSEUDOCALYPTRA

Calyx and corolla development of the pseudocalyptrate *S. cumini* is identical to that of species with a free perianth. The four sepals initiate in a decussate fashion followed by four petals that initiate simultaneously or almost simultaneously in the radius of sepals and soon overlap (Fig. 4A–C) forming a complex of four layers of petals (Fig. 4D). Both petals and sepals elongate and develop with no fusion until anthesis (Fig. 4E–H). However, sepals either stop developing early or elongate at a slower rate when the bud is mature, so that at pre-anthesis the four sepals are barely visible (Fig. 4G, H). The surfaces of the four still free, tightly packed petals adhere strongly to each other, but no mechanism of post-genital fusion was observed in anatomical sections (Fig. 4J). The whole corolla sheds as a single unit at anthesis, remaining attached to the flower in the same way as the calycine calyptra (Fig. 4I, white arrow).

RE-CODING CALYPTRAS IN SYZYGIEAE

Clarification of distinct modes of development in calyptrate flowers shows that calyptras are not homologous structures in Syzygieae. They can be formed by the outer perianth whorl (i.e. the calyx in a calycine calyptra) or by adhering, but not fused, petals that shed as a single unit in a misleading anthesis behaviour that resembles tissue fusion (pseudocalyptra). In this sense, these cannot be seen as different states of the same character (i.e. as in 'calyptra: present or absent'), but rather as two different characters of two states each (i.e. as in 'calycine calyptra: present or absent' and 'pseudocalyptra: present or absent') (Fig. 5).

When the two non-homologous modes of calyptra development are clarified, they are easily distinguishable in herbarium material, and the nature of the calyptra in different species can be assessed and analysed against the phylogenetic tree. When analysed together as a single character ('calyptrate flower: absent/present'), 19 out of 63 species of Syzygieae sampled across the phylogenetic tree for the tribe were scored as calyptrate (Fig. 5A). Non-calyptrate flowers (i.e. a free perianth) were recovered as the ancestral state in the tribe and calyptras are shown to have evolved c. 14 times (14.46 – numbers are not

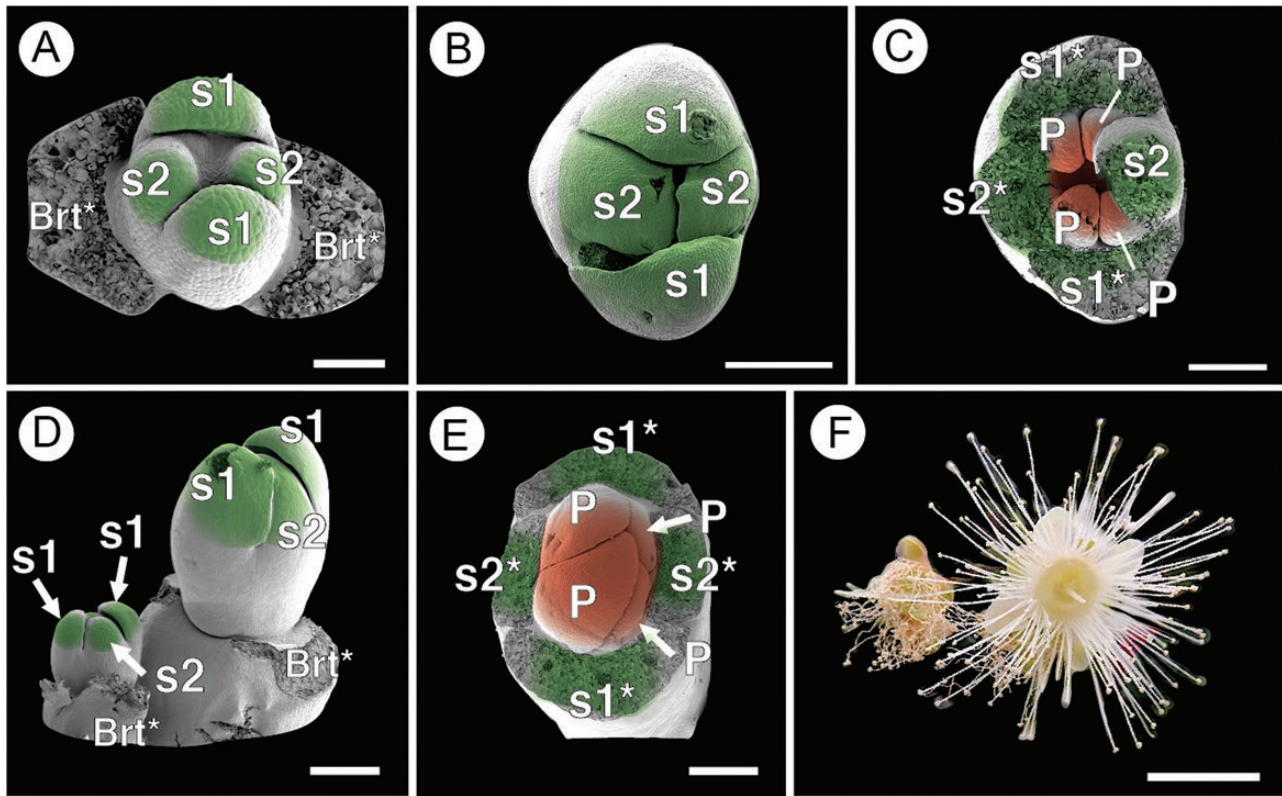


Figure 2. Free perianth development in *Syzygium paniculatum*. A, B, D, Early development of floral buds, showing the four sepals developing in decussate fashion. C, E, Buds at early stages of development with dissected calyx, showing corolla developing as four free petals. F, Flowers at anthesis, with all four sepals and petals free and non-calyptrate. Brt: bracteoles; S: sepals; P: petals; *: dissected structure. Scale bars: 100 μ m (A, C), 250 μ m, (B, D, E) and 5 mm (F). Colour code: green: calyx, red: corolla.

exact because they represent a summary of 1000 simulations) times independently, with approximately seven (6.814) reversions to the non-calyptrate state.

This scenario changes when the two non-homologous modes of calyptra development are analysed separately as two different characters against the phylogenetic tree. From the 19 species previously coded as calyptrate, only seven are shown to have calycine calyptras (i.e. true tissue fusion). In this way, transitions from non-calyptrate to calyptrate drop to approximately six (5.60) with only one (1.14) evidence of reversal to the non-calyptrate state (in *S. concinnum*) (Fig. 5B). When pseudocalyptras are coded separately, 13 species are shown to have this trait state; approximately eight (8.033) independent transitions from non-calyptrate to calyptrate are observed with approximately three (2.867) reversals (Fig. 5C). In all cases, transitions from the non-calyptrate to calyptrate state were observed in the last 10 Myr.

The numbers of species with calycine calyptra (seven) and pseudocalyptra (13) do not add up to the 19 species of the first analyses (Fig. 5A) because one

species, *S. nervosum*, appears to possess both calycine calyptras and pseudocalyptras. This shows that both calyptra modes can appear in conjunction (i.e. because they affect different perianth whorls, the presence of one does not exclude the other), emphasizing the calyptra types as two distinct characters and not distinct states of the same character. More species could potentially be coded as having both types if further studies were to improve sampling.

DISCUSSION

CALYPTRATE FLOWERS IN SYZYGIEAE

The structure traditionally referred to as a calyptra in Syzygieae corresponds to at least two distinct, non-homologous structures. This distinction could be inferred by previous studies that separated calyptrate species in *Syzygium* into groups with either calycine or coralline calyptras (e.g. Schmid, 1972; Ashton, 2011), the latter here referred to as pseudocalyptras. In *S. cumini*, the pseudocalyptra is formed by the

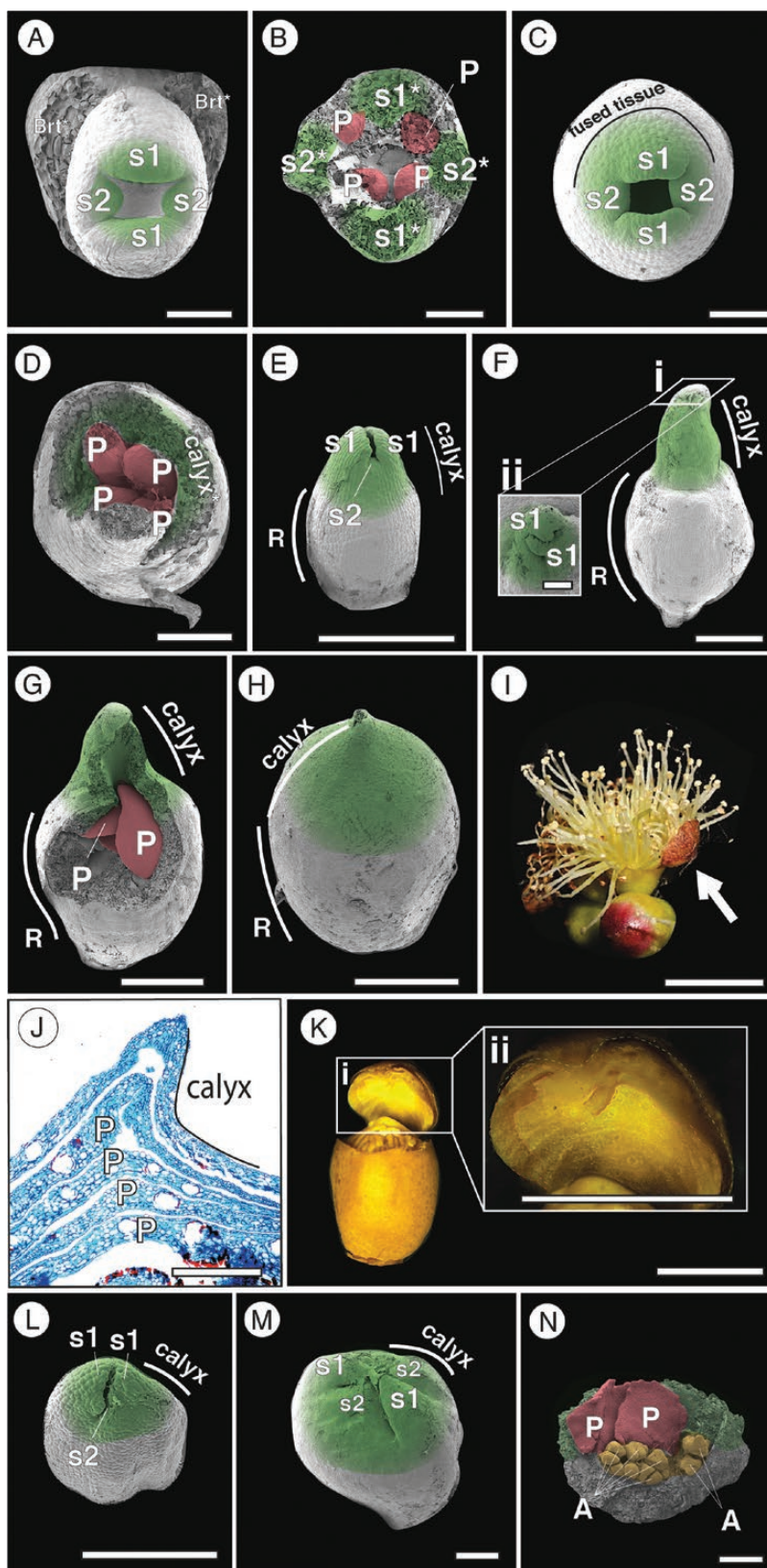


Figure 3. A–K, Calycine calyptra development in *Syzygium nervosum* and L–N, *Piliocalyx* sp. A, Decussate development of four free sepals followed by B, simultaneous initiation of four free petals in the same radius as the sepals. C, Calyx

adherence of petal surfaces without any type of tissue fusion (Fig. 4J), but this may not be the rule for all Syzygieae with pseudocalyptras. In the New Caledonian *S. multipetalum* Pancher ex Brongn. & Gris, for instance, the pseudocalyptra is frequently fleshy and petals are not always distinguishable (T. N. C. Vasconcelos, pers. obs.), appearing more homogeneous than in *S. cumini*. Investigation of corolla morphology in a larger sample of *Syzygium* spp. will probably reveal different types of petal adherence in pseudocalyptras.

Descriptions of species with calyptrate flowers in Syzygieae are common; e.g. Henderson (1949) reported that half of the *Syzygium* spp. listed for Singapore are calyptrate. Calyptrate species seem to follow little biogeographical trend (i.e. they are recorded from different areas in the distribution of the tribe, from New Caledonia (Biffin *et al.*, 2005) to Australia (Craven, 2003) and India (Ramana & Venu, 2014), or phylogenetic pattern, as shown here. Nevertheless, having a calyptra, of any type, is frequently used to distinguish and circumscribe closely related or sympatric species in Syzygieae (as observed in Ramana & Venu, 2014). A better understanding of this trait may help in future studies aiming to stabilize the complex taxonomy in Syzygieae (Ahmad *et al.*, 2016).

Besides Syzygieae, calyptrate flowers are also commonly found in other tribes of Myrtaceae, particularly Eucalypteae and Myrteae (Wilson, 2011). These three tribes are not directly related in the phylogenetic tree for the family (Thornhill *et al.*, 2015), but calyptra development in these tribes is remarkably similar. Calycine calyptras, as described for Syzygieae (i.e. initially free calyx lobes with late-congenital fusion), are also found in Eucalypteae and Myrteae (Drinnan & Ladiges, 1989a; Giaretta *et al.*, 2019). Pseudocalyptras are not observed in Myrteae, but occur in at least one species of Eucalypteae, *Eucalyptus curtisii* Blakely & C.T.White (Drinnan & Ladiges, 1991), for which the authors state that ‘the corolla [...] clearly consists of free, imbricate parts that closely adhere by their cuticles’ (Drinnan & Ladiges, 1991: 539). Eucalypteae are exceptional in frequently presenting late-congenital fusion of both calyx and corolla (e.g. Drinnan & Ladiges, 1989b), often adhering into a single structure referred to as an ‘operculum’ (Drinnan & Ladiges, 1989a), a pattern not observed in

any other tribe of Myrtaceae. The repeated evolution of similar modes of calyptra development in non-related tribes shows that underlying homology (i.e. parallelism; Scotland, 2011), is widespread in Myrtaceae.

Despite the large number of species, Syzygieae are still one of the least understood lineages of Myrtaceae (Ahmad *et al.*, 2016). In our analyses, 19 species were coded as calyptrate (c. 30% of the sampled species), but this probably reflects sampling bias in this small subset from the phylogenetic tree for Syzygieae, since the tribe has > 1000 species (WCSP, 2019). At this moment, trait state transitions and reversals can only be estimated from this small sample, but a broader sample of both species and molecular markers in the phylogenetic inference may reveal different scenarios. In this sense, our results and discussion represent a starting point for understanding floral evolution in the mega-diverse but still poorly studied Syzygieae.

A MATTER OF SEMANTICS: STANDARDIZING TERMINOLOGY FOR CALYPTRATE STRUCTURES IN MYRTACEAE

Pseudocalyptra (as that observed in *S. cumini*) and calycine calyptras (as those observed in *S. nervosum* and *Piliocalyx* sp.) have similar behaviour at anthesis resulting from evolutionary convergence rather than true developmental homology. The same terminology has been applied to the two different structures as a result of the similarity of their overall macromorphology, but close investigation reveals they do not result from the same ontogenetic pathway. This highlights a mismatch between terminology and trait homology that can affect systematics in Myrtaceae and also broader studies aiming to automatically collect data from the literature (e.g. by machine learning) (Walls *et al.*, 2012).

The challenge of standardizing calyptra terminology in Myrtaceae began as part of the original description of *Eucalyptus* L'Hér. (L'Héritier, 1788); however, terminological consensus for structural variation of the calyptra in the family still does not exist. As discussed, studies of floral development in *Eucalyptus* describe the combination of fully fused calyx and corolla as an ‘operculum’ (Drinnan & Ladiges, 1989), whereas similar structures (not fully fused) can be referred to as ‘calyptrate calyx and corolla’ in Syzygieae (Ashton,

undergoing late-congenital fusion while D, petals remain free and overlap. E, M, Sepal tips meet and overlap leaving F, L, remnant free lobes on the top of the bud. G, H, N, Continuous development of the bud with fused calyx and free petals. I, Anthesis; arrow indicates the calycine calyptra that remains attached to the flower. J, Anatomical cross-section of perianth, showing a homogeneous calyx and four layers of free petals. K, Detail of anthetic bud, showing a combination of calycine calyptra and pseudocalyptra. R: cup-shaped receptacle; Brt: bracteole; S: sepals; P: petals; A: androecium; *: dissected structure. Scale bars: 50 µm (A, B, C), 100 µm (D, F(ii)), 250 µm (E, Fi, G, J, L, M, N), 1 mm (H), 5 mm (K) and 10 mm (I). Photograph in I: courtesy of A. Lambrianides. Colour code: green: calyx, red: corolla, yellow: androecium.

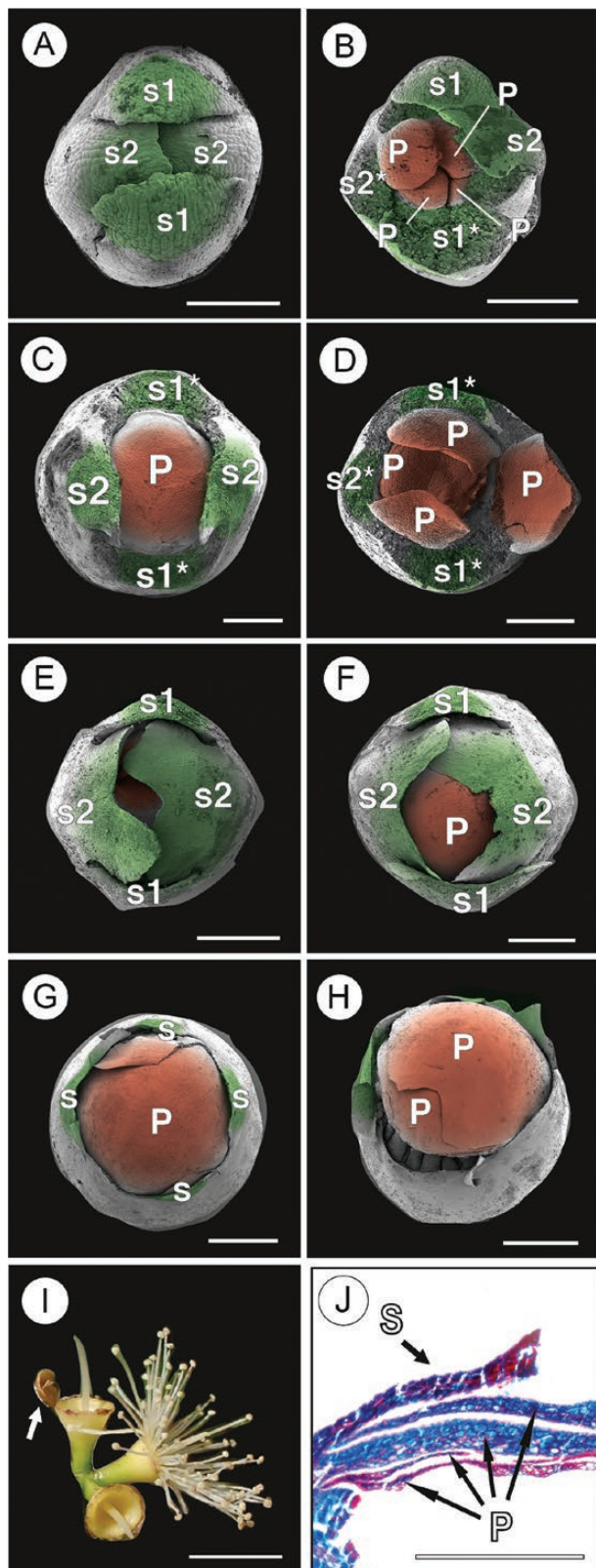


Figure 4. Pseudocalyptra development in *S. cumini*. A, Early development of four free sepals in a decussate

2011). Pseudocalyptras of Syzygieae can be referred to as such (e.g. Ramana & Venu, 2014) or as coralline calyptras (Schmid, 1972), depending on the author. Given that calyptrate flowers recur throughout Myrtaceae (Wilson *et al.*, 2011, Vasconcelos *et al.*, 2017, Giaretta *et al.*, 2019), it is highly desirable to standardize a coherent terminology.

We propose that a calyptra *s.s.* consists of ontogenetically uniform tissue formed by late-congenital fusion of sepals (as observed sporadically in Myrteae, Eucalypteae and Syzygieae and in a single genus of Xanthostemoneae, *Pleurocalyptus* Brongn. & Gris) and/or petals (observed only in Eucalypteae). Thus, Syzygieae with pseudocalyptras should not be described as calyptrate, because their perianth shares the same developmental pattern as species with a free perianth. Their anthetic condition, in which imbricate petals fall as a unit can still be used as a trait of taxonomic value if treated as a 'pseudocalyptra', but should not be treated as homologous to 'true' calyptras. This proposed terminology may or may not be valid for calyptrate species outside Myrtaceae, such as those found in Eupomatiaceae (Endress, 2003) or Melastomataceae (Goldenberg & Meirelles, 2011). In these groups, other organs can be involved (e.g. bracts in Eupomotiaceae, Endress, 2003), so further comparative studies will need to be performed before a stable terminology that encompasses all the diversity of calyptras in angiosperms can be established.

REPEATED EVOLUTION OF CALYPTRATE FLOWERS

Recurrence and persistence of traits in evolution is usually related to two factors: (1) the trait increases fitness (i.e. positive effect on survival and reproductive success rate) for the lineage in a given niche; and/

fashion. B, As in A, but further dissected to show the four petals in early stages of development. C, Slightly older bud with both S1 dissected, showing already overlapping petals. D, Same as C, but all sepals dissected and petals forced open to highlight their free condition. E–G, Sequential bud development, showing that free sepal parts stop developing and are very reduced in the mature bud. H, Bud opening, highlighting petals detaching from the base of the bud as a single unit. I, Old inflorescence indicating the pseudocalyptra formed by the four layers of petals (arrow). J, Anatomical cross-section of corolla, showing no evidence for fusion between petal surfaces; note that the apparent distinct thickness of each petal in the cross-section results from how they are organised in the flower and over each other. (Brt: bracteoles; S: sepals; P: petals; *: dissected structure. 100 μ m (A, F), 200 μ m (B), 250 μ m (C, D), 500 μ m (E, F), 1 mm (G, H, J), 5 mm (I). Photograph in I: courtesy of J. U. Germer. Colour code: green: calyx, red: corolla.

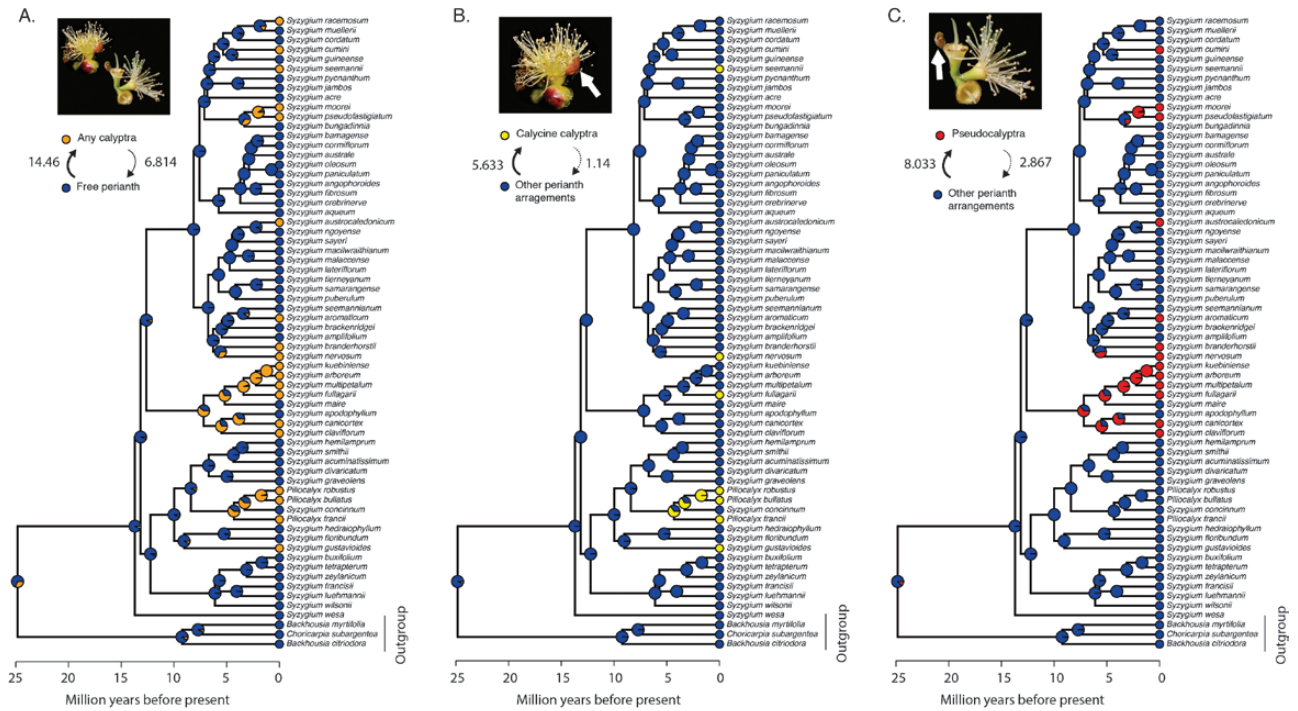


Figure 5. Ancestral state reconstruction of calyptrate flowers in Syzygieae. A, Calyptrate flowers appear 14.46 times independently in the tribe, with 6.814 reversals to non-calyptrate state. B, When only calycine calyptras are reconstructed, independent event of calyptra evolution are dropped to 5.633 times, with only 1.14 reversals. C, Similarly, when only pseudocalyptras are reconstructed, they are shown to appear 8.033 times, with 2.867 reversals to non-calyptrate flowers.

or (2) the trait does not bring any negative effect that would lead to a higher extinction rates for the lineage in a given niche. If calyptrate flowers appear multiple times and result from different ontogenies in a convergent basis, as in Syzygieae, assumptions are that certain external pressures are positively selecting this structure multiple times in the evolution of the group.

As previously described, sepals and petals protect the flower (mainly sepals) and enhance pollination (mainly petals) (Endress, 1994). An extra resistant layer that completely covers the floral bud until anthesis can be intuitively associated with protection; as in, e.g. *Rosmarinus* L. (Bottega & Corsi, 2000) and *Chrysothemis* Decne. (Carlson & Harms, 2007).

Nevertheless, evidence that calyptrate species appeared at distinct times and environments during the evolution of Myrtaceae suggests that protection conferred by this structure is not necessarily related to the same environmental conditions in all cases. In *Eucalyptus*, for example, calyptras are present in the fossil record of the genus since the Palaeocene (Gandolfo *et al.*, 2011), and the group is today diverse in dry Australian forests (WCSP, 2019). A strongly lignified bud coverage may have been key to their long survival in dry and fire-predisposed environments of Australia (Crisp *et al.*, 2011), especially due to their

particularly extensive flowering period (Birchnell & Gibson, 2006). This hypothesis is also supported by the restricted distribution of *Angophora* Cav., a relative species-poor, non-calyptrate genus sister to *Eucalyptus*, native to more humid environments of eastern Australia (Ladiges *et al.*, 2003). On the other hand, Syzygieae is a group of a more recent origin, in which species diversification occurred principally in the tropical forests of South-East Asia. In this sense, the presence of an extra layer of an organ could be associated with protection against herbivory rather than just harsh environments. Empirically, calyptrate species in Neotropical Myrteae are often associated with more humid environments, suggesting this trait may facilitate survival or reproduction in these conditions. Although this hypothesis has not been explicitly tested, it has been widely noted by taxonomists working with Myrteae (e.g. P. Ashton, A. Giaretta, E. J. Lucas, M. F. Santos and T. N. C. Vasconcelos, pers. obs.).

However, if a calyptrate perianth has different advantages and evolves frequently in different lineages (i.e. without phylogenetic constraint), why do most species of Myrtaceae still have an open, non-fused perianth? The answer may be related to the fact that calyptrate flowers often have reduced petals (e.g. *Myrcia*, Vasconcelos *et al.*, 2017) or lose the corolla completely at anthesis (as in pseudocalyptras, e.g.

S. nervosum Fig. 3K). In many cases, attractiveness of flowers of Myrtaceae to pollinators relies on the brush-blossom in which the polyandrous androecium is the main showy structure (Johnson & Briggs, 1984). This system eases selective pressure for pollinator attraction to the perianth, making the corolla somewhat dispensable from pollinator attraction and thus better used for protection (in a ‘transference of function’, *sensu* Corner, 1958). In this way, a shift to a calyptrate perianth may be favourable and thus more common.

This strategy may be constraining, since the acquisition of a calyptra may restrict a lineage from occupying ecological niches where pollinator attraction is perianth-dependent (e.g. bird-pollinated *Myrrhinium* Schott; Roitman *et al.*, 1997). In this sense, the calyptra may represent a trade-off between protection and pollinator attraction. Furthermore, the uneven transition rates between the non-calyptrate to calyptrate state (shown by low number of reversals) indicate that the calyptra is repeatedly an evolutionary dead-end; i.e. once this trait appears, the lineage can only thrive or go extinct (Barrett, 2013). Further analyses of geographical distribution and trait evolution with broader samples are necessary to corroborate these points.

CONCLUSIONS

Identification of underlying homologies in superficially homoplastic characters clarifies terminology and evolutionary interpretation of individual lineages. Reassessing morphology also enables the generation of new hypotheses for the relationship of a given lineage to its environment. This is only possible when phylogenetic trees are available so that structural changes can be inserted in the evolutionary context of a lineage and independent origins for a trait state can be tracked. In Syzygiaceae, evolutionary convergence is responsible for similar but non-homologous structures that involve different perianth whorls in the flower. The recurrence of a perianth that is completely closed in the bud and ‘disposable’ at anthesis may be linked to selective pressures for protection and reliance on brush blossoms with the androecium as the main floral display. Why structures that may confer adaptive advantage appear to correspond to an evolutionary dead-end in the group is a question that still persists.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

SM1 Trait coding matrix

SM2 Syzygieae phylogenetic inference (extracted from [Thornhill et al., 2015](#))

APPENDIX I. VOUCHERS USED IN ONTOGENETIC AND ANATOMICAL ANALYSES OF CALYPTRATE FLOWERS OF SYZYGIEAE. ALL DEPOSITED AT THE HERBARIUM OF RBG KEW. SPECIES NAMES FOLLOW [WCSP \(2019\)](#).

Tribe	Species	Voucher	Collection locality
Syzygieae	<i>Piliocalyx</i> sp.	<i>T. Vasconcelos</i> 651	New Caledonia
Syzygieae	<i>Syzygium cumini</i> (L.) Skeels	<i>T. Vasconcelos</i> 296	Brazil (cultivated, native to South-East Asia)
Syzygieae	<i>Syzygium paniculatum</i> Gaertn.	<i>T. Vasconcelos</i> s.n.	RBG Kew Living Collection (native to Australia)
Syzygieae	<i>Syzygium nervosum</i> A.Cunn. ex DC.	<i>RB1999-0250</i>	Singapore (cultivated, native to South-East Asia)