

Floral development and vasculature in *Eriocaulon* (Eriocaulaceae) provide insights into the evolution of Poales

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- **Background and Aims** Floral developmental studies are crucial for understanding the evolution of floral structures and sexual systems in angiosperms. Within the monocot order Poales, both subfamilies of Eriocaulaceae have unisexual flowers bearing unusual nectaries. Few previous studies have investigated floral development in subfamily Eriocauloideae, which includes the large, diverse and widespread genus *Eriocaulon*. To understand floral variation and the evolution of the androecium, gynoecium and floral nectaries of Eriocaulaceae, we analysed floral development and vasculature in *Eriocaulon* and compared it with that of subfamily Paepalanthoideae and the related family Xyridaceae in a phylogenetic context.
- **Methods** Thirteen species of *Eriocaulon* were studied. Developmental analysis was carried out using scanning electron microscopy, and vasculature analysis was carried out using light microscopy. Fresh material was also analysed using scanning electron microscopy with a cryo function. Character evolution was reconstructed over well-resolved phylogenies.
- **Key Results** Perianth reductions can occur due to delayed development that can also result in loss of the vascular bundles of the median sepals. Nectariferous petal glands cease development and remain vestigial in some species. In staminate flowers, the inner stamens can emerge before the outer ones, and carpels are transformed into nectariferous carpellobes. In pistillate flowers, stamens are reduced to staminodes and the gynoecium has dorsal stigmas.
- **Conclusions** Floral morphology is highly diverse in *Eriocaulon*, as a result of fusion, reduction or loss of perianth parts. The nectariferous carpellobes of staminate flowers originated first in the ancestor of Eriocaulaceae; petal glands and nectariferous branches of pistillate flowers originated independently in Eriocaulaceae through transfer of function. We present a hypothesis of floral evolution for the family, illustrating a shift from bisexuality to unisexuality and the evolution of nectaries in a complex monocot family, which can contribute to future studies on reproductive biology and floral evolution in other groups.

Key words: Dimery, *Eriocaulon*, Eriocaulaceae, Eriocauloideae, floral anatomy, floral development, floral evolution, floral ontogeny, floral vasculature, nectary, Poales, xyrids.

INTRODUCTION

Floral developmental studies help us to understand the ontogenetic bases that have led to key innovations in different groups of angiosperms (e.g. Endress, 2006, 2011; Remizowa *et al.*, 2010; Sokoloff *et al.*, 2018). The floral bauplan is genetically controlled and under strong developmental constraints, but other factors can also influence development, such as available space in floral buds, size of organ primordia, timing of organ initiation and rate of organ growth (Rudall, 2010; Ronse De Craene, 2016, 2018). The size of the floral meristem can affect the number, size and arrangement of floral organs, and mechanical pressure from outer floral organs can modify the growth

and development of subsequent organs (Bull-Hereñu *et al.*, 2016, 2018; Dos Santos and Ronse De Craene, 2016). The diversity of floral morphology found in angiosperms also reflects their distinctive reproductive strategies (e.g. Bull-Hereñu *et al.*, 2016; Leite *et al.*, 2018; Vasconcelos *et al.*, 2018). Thus, developmental studies on groups with complex floral morphology can help to elucidate the developmental processes that have influenced floral evolution.

Poales is a highly diverse order of approx. 20 000 species, representing more than a third of all monocots (Givnish *et al.*, 2010). Within the order, Eriocaulaceae usually emerge as the sister group of Xyridaceae in phylogenetic analyses

(Bouchenak-Khelladi *et al.*, 2014; Givnish *et al.*, 2018; Hochbach *et al.*, 2018), and both families share some floral characters such as the presence of staminodes and unusual floral nectaries (Stützel, 1990; Oriani and Scatena, 2012; Sajo *et al.*, 2017). Some earlier phylogenetic studies placed Eriocaulaceae as sister to Mayacaceae (Givnish *et al.*, 2010; Darshetkar *et al.*, 2019) and, even with increased phylogenomic data, precise relationships among Eriocaulaceae, Mayacaceae and Xyridaceae have <100 % support (Givnish *et al.*, 2018).

Eriocaulaceae (approx. 1200 species) are characterized by their compact bisexual capituliform inflorescences with generally unisexual flowers. They are classified into two subfamilies strongly supported by molecular phylogenetic studies (Andrade *et al.*, 2010): Eriocauloideae and Paepalanthoideae (Stützel, 1998). Eriocauloideae includes two genera: *Eriocaulon* L., approx. 470 species in North and South America, Africa, Asia and Europe; and *Mesanthemum* Körn., 16 species endemic to Africa (Giulietti and Hensold, 1990; Liang *et al.*, 2020; WCSP, 2021). Paepalanthoideae includes nine genera, with most species occurring in America (Giulietti and Hensold, 1990; Stützel, 1998). The main floral differences between the two subfamilies are presented in Table 1.

Previous developmental and anatomical studies on Eriocaulaceae have mostly centred on Paepalanthoideae, so further comparative studies of Eriocauloideae are needed to clarify floral evolution in the family. An inner androecial whorl is present in both Eriocauloideae and Paepalanthoideae, but an outer androecial whorl is present only in Eriocauloideae (Stützel, 1990; Rosa and Scatena, 2003; Silva *et al.*, 2021). In Paepalanthoideae, outer staminodes and functional inner fertile stamens have been described in staminate flowers, suggesting that the ancestor of the subfamily had a two-whorled androecium (Rosa and Scatena, 2003, 2007). However, these outer staminodes were later reinterpreted as late-developing petals (Silva *et al.*, 2016). Sajo *et al.* (2017) studied floral development and vasculature of Xyridaceae to reconstruct androecial evolution in the xyrids; they pointed out the need for further comparative studies on Eriocaulaceae to elucidate this question.

Anatomical and developmental studies have addressed the floral homologies of Eriocaulaceae and Xyridaceae, as well as those of the two subfamilies of Eriocaulaceae, with a focus on the number of androecium whorls and the presence

and position of nectaries (Stützel, 1990; Rosa and Scatena, 2003, 2007; Remizowa *et al.*, 2012; Sajo *et al.*, 2017; Silva *et al.*, 2021). Eriocaulaceae and Xyridaceae include genera with a single- or two-whorled androecium, and some species have similar gynoeceal nectaries that arise in a dorsal position. However, the gynoeceum of Eriocaulaceae has puzzling characteristics that make it difficult to reconstruct floral evolution in the family. In subfamily Paepalanthoideae, the gynoeceum has nectariferous branches that were considered homologous to the nectariferous appendages found in some Xyridaceae; both structures have a dorsal position, similar ontogenetic stages and are vascularized by a dorsal carpellary bundle (Stützel, 1990; Rosa and Scatena, 2003; Oriani and Scatena, 2012). However, differences in both development and vasculature of the gynoeceum indicate that they may have originated independently (Sajo *et al.*, 2017). Nectariferous perianth glands are frequently present on the adaxial epidermal surface of the petals in subfamily Eriocauloideae, but they are apical, sub-apical or even absent in some species of *Eriocaulon* (Ruhland, 1903; Oliveira and Bove, 2015). In *Eriocaulon megapotamicum* Malme, these glands appear on the petal apex in the floral primordia and change position during flower development (Stützel, 1984). However, the evolutionary significance of these characters remains controversial because the ancestral floral characteristics of the two families are unresolved.

In Eriocauloideae, the stigmas have a dorsal position and receive the dorsal carpellary bundle, like the nectariferous branches of Paepalanthoideae, leading Rosa and Scatena (2007) to suggest that the nectariferous branches originated from an evolutionary shift in stigma function. The stigmatic branches of Paepalanthoideae have a commissural position and are formed through the rise and fusion of adjacent carpel margins (Stützel, 1990). In staminate flowers of Eriocaulaceae, the gynoeceum is sterile and has nectariferous carpelodes. In Paepalanthoideae, the carpelodes are in the same position as the nectariferous branches of their pistillate flowers and are vascularized by the dorsal carpellary bundle (Rosa and Scatena 2003, 2007); the two structures share developmental, morphological and anatomical characteristics, at least in the species studied so far (Silva *et al.*, 2016), indicating a single evolutionary origin. However, the presence of carpelodes in staminate flowers of Eriocauloideae and the lack of nectariferous branches in their

TABLE 1. Main floral characters compared between Eriocauloideae and Paepalanthoideae (Eriocaulaceae) based on Stützel (1998) and Rosa and Scatena (2003, 2007).

	Eriocauloideae	Paepalanthoideae
Sexuality	Unisexual or bisexual	Unisexual or bisexual
Merism	Trimerous or dimerous	Trimerous or dimerous
Staminate flower		
Perianth	Two-whorled; petals with or without nectariferous glands	Two-whorled; petals without nectariferous glands
Androecium	Fertile; two-whorled, with inner and outer stamens	Fertile; single-whorled, with inner stamens
Gynoeceum	Sterile; with carpels modified into nectariferous carpelodes	Sterile; with carpels modified into nectariferous carpelodes
Pistillate flower		
Perianth	Two-whorled; with or without petal glands	Two-whorled; without petal glands
Androecium	Sterile; two-whorled, with inner and outer stamens modified into staminodes	Sterile; single-whorled, with inner stamens modified into staminodes
Gynoeceum	Fertile; with dorsal stigmas	Fertile; with dorsal nectariferous branches and commissural stigmatic branches

pistillate flowers suggest that nectariferous branches were absent in the ancestor of Eriocaulaceae and appeared only in Paepalanthoideae. The floral anatomy of *Mesanthemum radicans* shows that the carpellobes are in the same position as those of Paepalanthoideae (Silva et al., 2021). Despite that, further developmental data on the carpellobes of Eriocauloideae may help to clarify the relationship between these structures and the nectariferous branches of Eriocaulaceae.

Floral developmental studies on *Eriocaulon* have included relatively few species and focused on early development, the origin of the petal glands of *E. megapotamicum* and perianth reduction of some Indian species (Smith, 1910; Stützel, 1984, 1990; Sokoloff et al., 2020). *Eriocaulon* has highly variable floral morphology, and recent phylogenetic studies including several species have indicated synapomorphies among infrageneric groupings, though the results showed little congruence with the existing morphology-based infrageneric classification (Larridon et al., 2019; Darshetkar et al., 2021). In the present work, we study the floral development of *Eriocaulon* in a phylogenetic context to address floral morphological variation in the genus, the possible ancestral characters of Eriocaulaceae, and the evolution of the androecium, gynoecium and the nectariferous structures in the family.

MATERIALS AND METHODS

For this study, we selected 13 species of *Eriocaulon*. Part of the material was collected during scientific expeditions in Brazil, and vouchers were deposited in the Herbário Rioclarense (HRCB). The North American species were obtained from the *ex situ* collection of the Botanical Garden of the Ruhr-Universität Bochum (RUB) in Germany. The African species and the single Asian species were obtained from the spirit collection of the Royal Botanic Gardens Kew, UK. Details of the collections and data analysed are listed in Table 2.

Inflorescences at distinct developmental stages were collected in the field or in the Botanical Garden of the RUB, fixed in FAA (formaldehyde:ethanol:acetic acid) 50 (Johansen, 1940) and stored in 70 % ethanol. Material obtained from the Kew spirit collection was already fixed in FAA 50. For morphological and developmental analyses, flowers and inflorescences

were dehydrated in formaldehyde dimethyl acetal (FDA) and critical-point dried. The material was mounted on metallic stubs with adhesive tape and coated with gold for observation using a scanning electron microscope (Sigma VP, Zeiss, Oberkochen, Germany). For detection of secretory activity, fresh inflorescences of *E. decangulare* and *E. compressum* were collected from the Botanical Garden of the RUB. The flowers were dissected under a stereomicroscope, mounted on metallic stubs and rapidly cooled with liquid nitrogen. The samples were analysed using a scanning electron microscope (Sigma VP). Images were taken using the software SmartSEM (Zeiss, Munich, Germany). The floral diagrams were generated using the Floral Diagram Generator (Kebert, 2020) and edited using the software program Inkscape®.

For anatomical analysis, mature flowers were dehydrated in an *n*-butyl alcohol series and embedded in histological resin (Leica Histo-resin, Leica Biosystems, Nussloch, Germany). The material was serial cross-sectioned with disposable steel knives using a rotary microtome at 8–10 µm (DM2245, Leica Biosystems, Nussloch, Germany), and the sections obtained were stained with periodic acid–Schiff reagent (PAS reaction) and Toluidine Blue (Feder and O'Brien, 1968). Permanent slides were mounted in Entellan, and photomicrographs were taken using a light microscope (DM4000, Leica Microsystems, Wetzlar, Germany) with a coupled camera. The image plates were made using the software program Inkscape®.

The characters floral sexuality, floral merism, outer androecium whorl, carpellobes carpels and nectariferous appendages, and their respective states were established on a binary matrix of primary homologies using Mesquite 3.61 (Maddison and Maddison, 2019) according to topographical and ontogenetic principles. All characters were mapped over a most comprehensive consensus tree from recent phylogenies (Andrade et al., 2010; Trovó et al., 2013; Bouchenak-Khelladi et al., 2014; Echernacht et al., 2014; Givnish et al., 2018; Andriano et al., 2021) applying the Trace Character History' option implemented in Mesquite 3.61 (Maddison and Maddison, 2019). All characters were considered as unordered and the optimization follows accelerated transformations. The morphological matrix with characters and states for each species is available in Supplementary data Appendix 1.

RESULTS

Floral diagrams of the *Eriocaulon* species studied are presented in Fig. 1. Most species possess pentacyclic trimerous flowers (Fig. 1A–D, G–J). However, *E. decangulare* (Fig. 1E, K) and *E. guyanense* (Fig. 1F, L) have dimerous flowers.

Organography of staminate flowers

The staminate flowers of *Eriocaulon* have sepals that are free (Fig. 1A, D–F) or fused (Fig. 1B, C) at their median region. The sepals are entirely free in *E. decangulare* (Fig. 1E). They are almost entirely free in *E. kunthii*, *E. sellowianum*, *E. teusczii* and *E. twaitheii* (Figs 1A, D, 2A), but are fused into a short tube at their basalmost part (noticeable only under light microscopy). In *E. twaitheii* (Fig. 1D), the median sepal is reduced. The sepals

TABLE 2. List of species of *Eriocaulon* selected for the study and their respective collection data.

Species	Collector number	Local of origin
1. <i>Eriocaulon abyssinicum</i> Hochst.	Wood 446	Uganda
2. <i>E. aquatile</i> Körn.	Coan et al. 100	Brazil
3. <i>E. cinereum</i> R. Br.	Silva et al. 52	Brazil
4. <i>E. compressum</i> Lam.	Cultivated (RUB)	USA
5. <i>E. decangulare</i> L.	Cultivated (RUB)	USA
6. <i>E. guyanense</i> Körn.	Silva et al. 54	Brazil
7. <i>E. kunthii</i> Körn.	Scatena et al. 199	Brazil
8. <i>E. modestum</i> Kunth	Silva et al. 59, 61, 67	Brazil
9. <i>E. nigericum</i> Meikle	Jones 20718	Nigeria
10. <i>E. sellowianum</i> Kunth	Silva et al. 62	Brazil
11. <i>E. teusczii</i> Engl. & Ruhland	Taylor 9163	Tanzania
12. <i>E. twaitheii</i> Körn.	Barnes 89	India
13. <i>E. xeranthemoides</i> Bong.	Stanfield 117	Nigeria

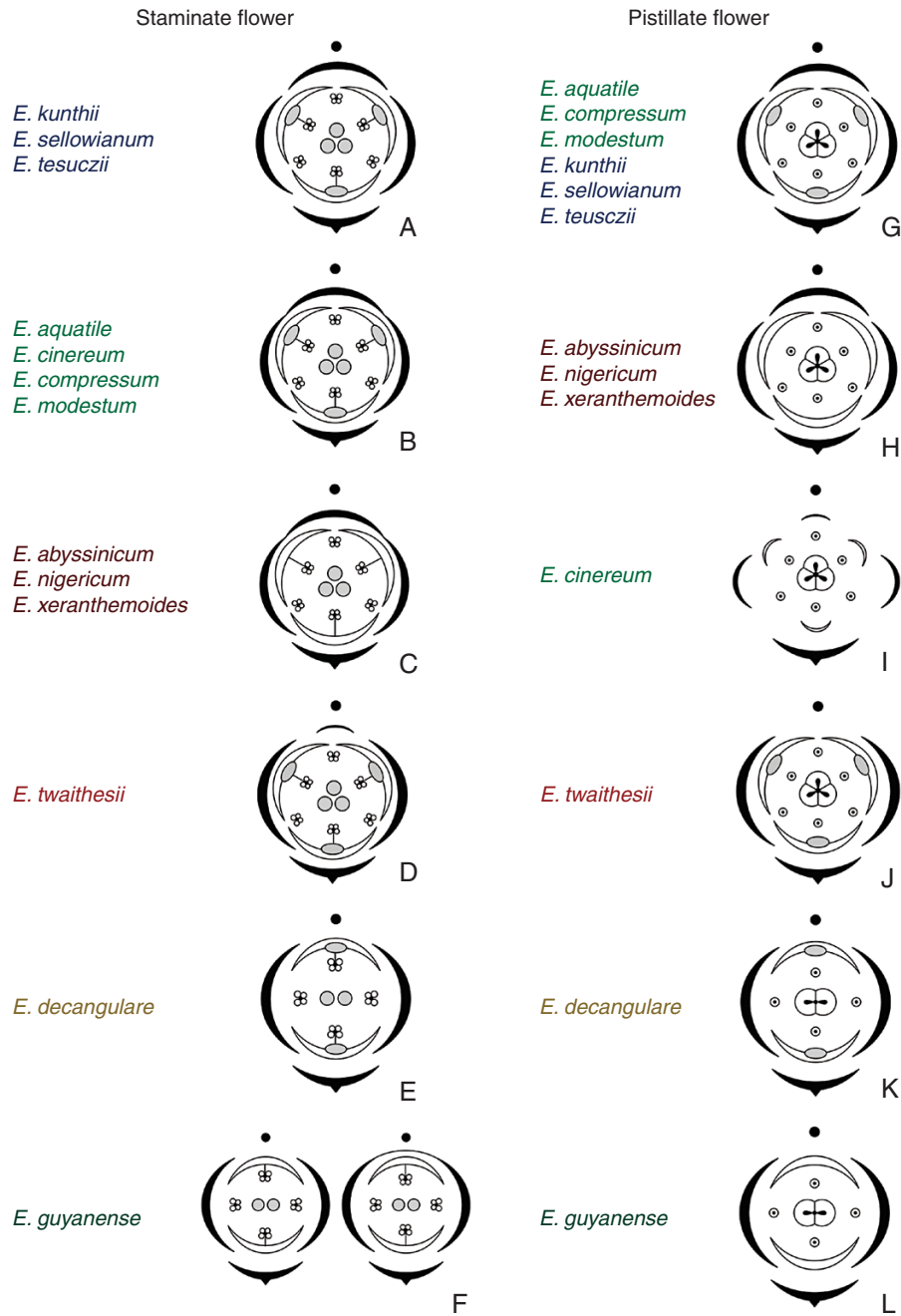


FIG. 1. Floral diagrams of staminate and pistillate flowers of *Eriocaulon*: transverse sections of the middle region. Similar colours indicate species with similar floral morphology. (A–D) Trimerous staminate flowers. (E, F) Dimerous staminate flowers. (G, H) Trimerous pistillate flowers. (I, J) Heteromerous pistillate flowers. (K, L) Dimerous pistillate flowers.

are fused into a spatheaceous calyx in *E. cinereum* (Fig. 2B), *E. modestum* (Fig. 1B) and *E. nigericum* (Fig. 1C). In *E. guyanense*, the sepals are free or fused up to their median region (Fig. 1F) and bear an abaxial midrib keel (Fig. 2C). In *E. abyssinicum*, *E. aquatile*, *E. compressum* and *E. xeranthemoides*, the sepals are fused up to their median region (Fig. 1B, C). Sepals have many trichomes at their apex, on the margin and dorsal side (Fig. 2A), except in *E. abyssinicum*, *E. cinereum* (Fig. 2B), *E. guyanense*, *E. nigericum* and *E. xeranthemoides*.

The internode between the sepals and petals is elongated; we refer to it as an anthophore (Fig. 2A, B), as usually described for Eriocaulaceae (Stützel, 1998). Petals are free and similar in size in most species (Fig. 2B), but the median petal is larger than the lateral ones in *E. modestum* (Fig. 2D), *E. sellowianum* and *E. tesuczii*. Petals bear a nectariferous gland on their upper adaxial surface (Fig. 2E); this gland is vestigial in *E. abyssinicum*, *E. guyanense* (Fig. 2F), *E. nigericum* and *E. xeranthemoides*. Trichomes occur at the

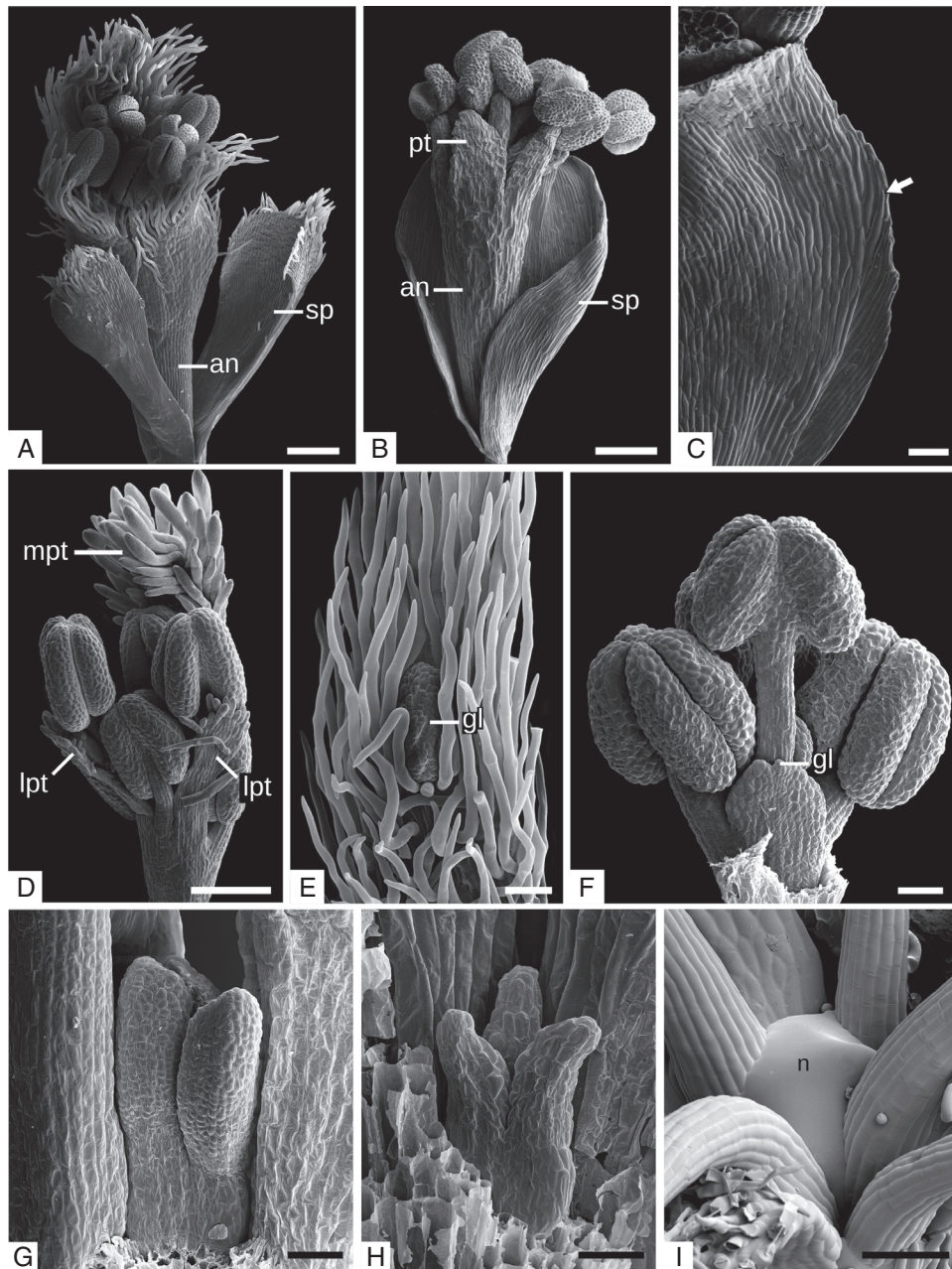


FIG. 2. Organography of staminate flowers of *Eriocaulon*. (A, B) General aspects of *E. sellowianum* (A) and *E. cinereum* (B). (C) Lateral view of a sepal of *E. guyanense* showing the midrib keel (arrow). (D) A young flower of *E. modestum* with sepals removed and showing petals with different sizes. (E) Detail of a petal apex of *E. modestum* with a gland. (F) A young flower of *E. guyanense*, indicating a petal with an aborted gland. (G) Detail of a sterile gynoecium of *E. kunthii*. (H, I) Nectariferous carpelodes of *E. compressum* without (F) and with (G) nectar drop covering it completely. Arrow, midrib keel; an, anthophore; gl, petal gland; lpt, lateral petal; mpt, median petal; n, nectar drop; pt, petal; sp, sepal. Scale bars: (A) 400 μm ; (B, D, I) 200 μm ; (C, E, F, G) 100 μm ; (H) 80 μm .

petal apex (Fig. 2D, E), except in *E. abyssinicum*, *E. cinereum* (Fig. 2B), *E. guyanense* (Fig. 2F), *E. nigericum* and *E. xeranthemoides*.

The androecium is two whorled (Fig. 1A–F). The outer stamens are shorter than the inner ones (Fig. 2D), and the anthers are bithecate (Fig. 2F). The gynoecium is stalked and sterile, with three carpelodes in the trimerous species (Figs 1A–D, 2G, H) and two carpelodes in the dimerous species (Fig. 1E, F); the carpelodes are nectariferous (Fig. 2I).

Organography of pistillate flowers

All species studied of *Eriocaulon* have pistillate flowers with free sepals at their median region (Figs 1G–L, 3A–D), but sepals are fused into a tube at their basalmost part in *E. kunthii* (noticeable only under light microscopy). In the other species, sepal basal fusion was not observed. Sepals are keeled only in *E. guyanense* (Fig. 3B). They have trichomes at their apex (Fig. 3A, C), except in *E. abyssinicum*, *E. cinereum*,

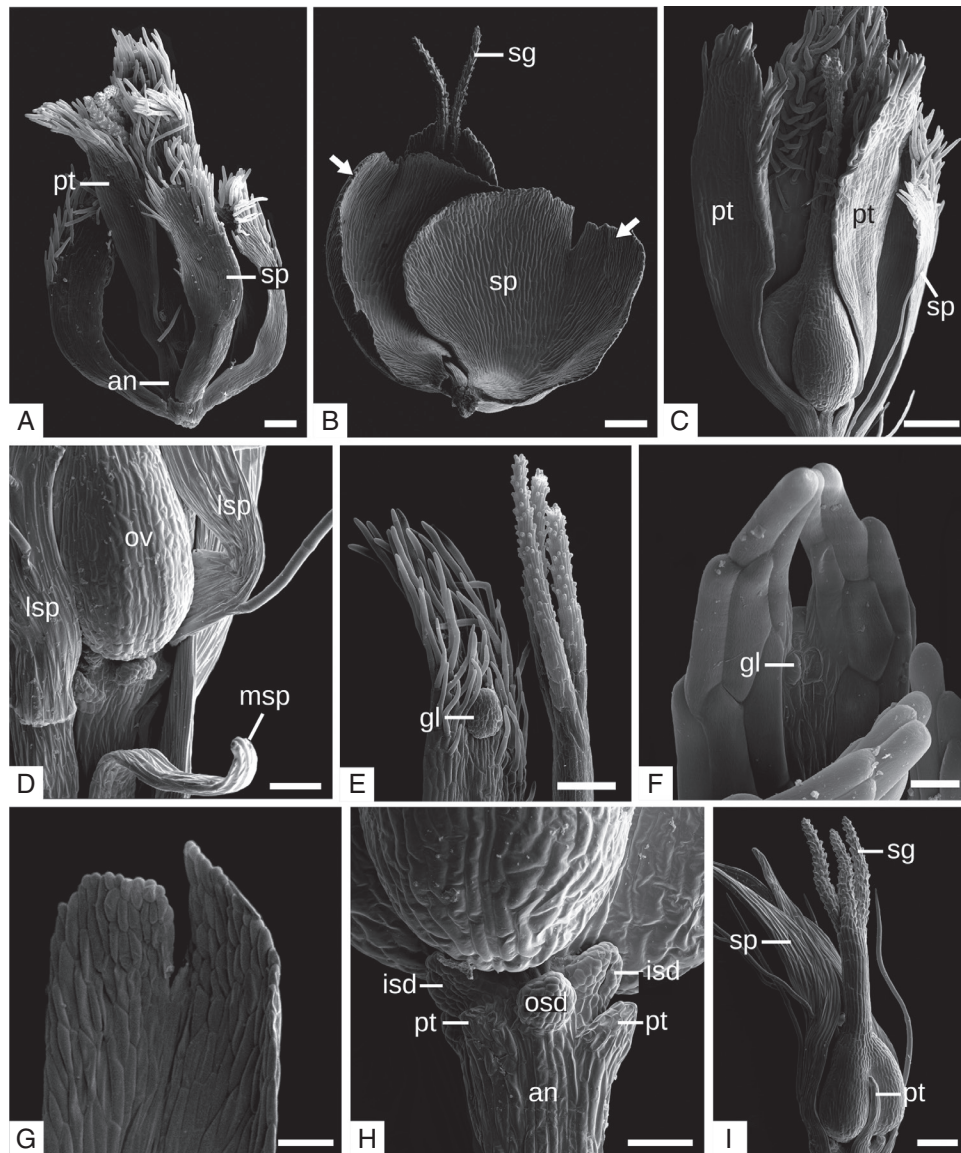


FIG. 3. Organography of pistillate flowers of *Eriocaulon*. (A–C) General aspects of *E. teusczii* (A), *E. guyanense* (B) and *E. modestum* (C). (D) Flower base of *E. cinereum* showing the reduced median sepal. (E) Detail of a petal apex of *E. teusczii*, indicating a nectariferous gland. (F) Detail of a vestigial gland on a petal apex of *E. abyssinicum*. (G) Detail of the petal apex of *E. guyanense* lacking the petal gland. (H) Detail of the ovary base of *E. cinereum* indicating vestigial petals and staminodes from the inner and outer whorls. (I) A flower of *E. cinereum* indicating a linear petal. Arrow, sepal keel; gl, petal gland; isd, inner staminode; lsp, lateral sepal; msp, median sepal; osd, outer staminode; ov, ovary; pt, petal; sg, stigma; sp, sepal. Scale bars: (A–C, E) 200 μm ; (D, G, I) 100 μm ; (F) 20 μm ; (H) 40 μm .

E. guyanense (Fig. 3B), *E. nigericum* and *E. xeranthemoides*. In *E. cinereum*, the median sepal is reduced (Figs 1I, 3D), while in *E. twaitesii* it is absent (Fig. 1J).

An elongated anthophore is present in *E. aquatile*, *E. cinereum*, *E. compressum*, *E. guyanense*, *E. modestum* and *E. teusczii* (Fig. 3A). Petals are free (Fig. 3C) and present a nectariferous gland like those of staminate flowers (Fig. 3E). This gland is vestigial in *E. abyssinicum* (Fig. 3F), and absent in *E. cinereum*, *E. guyanense* (Fig. 3G), *E. nigericum* and *E. xeranthemoides*. In *E. cinereum*, the petals are reduced and scale like (Fig. 3H) or linear (Fig. 3I). Petals have trichomes at their apex (Fig. 3A, C, E), except in *E. abyssinicum* (Fig. 3F), *E. cinereum* (Fig. 3I), *E. guyanense* (Fig. 3G), *E. nigericum* and *E. xeranthemoides*.

The sterile androecium consists of two whorls of scale-like staminodes located at the ovary base (Figs 1G–L, 3H). The outer staminodes are inserted opposite the sepals, while the inner staminodes are opposite the petals (Fig. 3H). The gynoecium consists of a superior ovary and a style with three filiform stigmas in trimerous species (Fig. 3I) and two in dimerous species (Fig. 3B). In all species, stigmas are papillose and in a dorsal position (Fig. 3I).

Ontogeny – early development

Staminate and pistillate flowers of *Eriocaulon* are indistinguishable at early developmental stages (Fig. 4A–F). The flowers develop centripetally in the inflorescence and are each subtended

by a floral bract (Fig. 4A). The sepals emerge almost simultaneously (Fig. 4B). The two lateral sepals emerge at an 180° angle in both trimerous and dimerous flowers; the median sepal of the trimerous flowers emerges on the adaxial position (Fig. 4B). The stamen primordia emerge almost simultaneously (Fig. 4C, D), but in some flowers the inner stamens emerge shortly before the outer ones (Fig. 4C). The carpel primordia emerge around the floral apex (Fig. 4E–H). Petals have late development and emerge by division of the common petal–stamen primordia (Fig. 4E–G).

At this developmental stage, the lateral sepals are similar in size in trimerous flowers, while the median adaxial sepal is smaller (Fig. 4G). In dimerous flowers, the two lateral sepals are the same size (Fig. 4H).

Ontogeny of staminate flowers

In staminate flowers, the sepals elongate and cover the young flowers (Fig. 5A, B). In species with a fused calyx, the sepals

fuse through late congenital fusion (Fig. 5A). In *E. cinereum* (Fig. 5B), *E. modestum* and *E. nigericum*, sepals fuse into a spatheous calyx but remain free and distinguishable at their uppermost parts. In *E. twaitesii*, the two lateral sepals develop completely (Fig. 5C, D), but development of the median sepal is arrested at an early stage (Fig. 5D). In *E. guyanense*, sepals grow free (Fig. 5E) or fused (Fig. 5F). In this species, each sepal develops a keel on its abaxial surface (Fig. 5E, F). The anthophore starts elongating, and the stamen primordia differentiate into a filament and anther (Fig. 5B–D). The apex of each petal is modified into a prominence (Fig. 6A), which will later become the petal gland. The apical petal margins develop into two lateral prominences that fuse abaxially through late congenital fusion (Fig. 6B, C), dislocating the central apical prominence to the adaxial petal surface (Fig. 6C). However, in *E. abyssinicum*, *E. guyanense* (Fig. 6D), *E. nigericum* and *E. xeranthemoides*, the petal apex stops developing, resulting in an aborted gland.

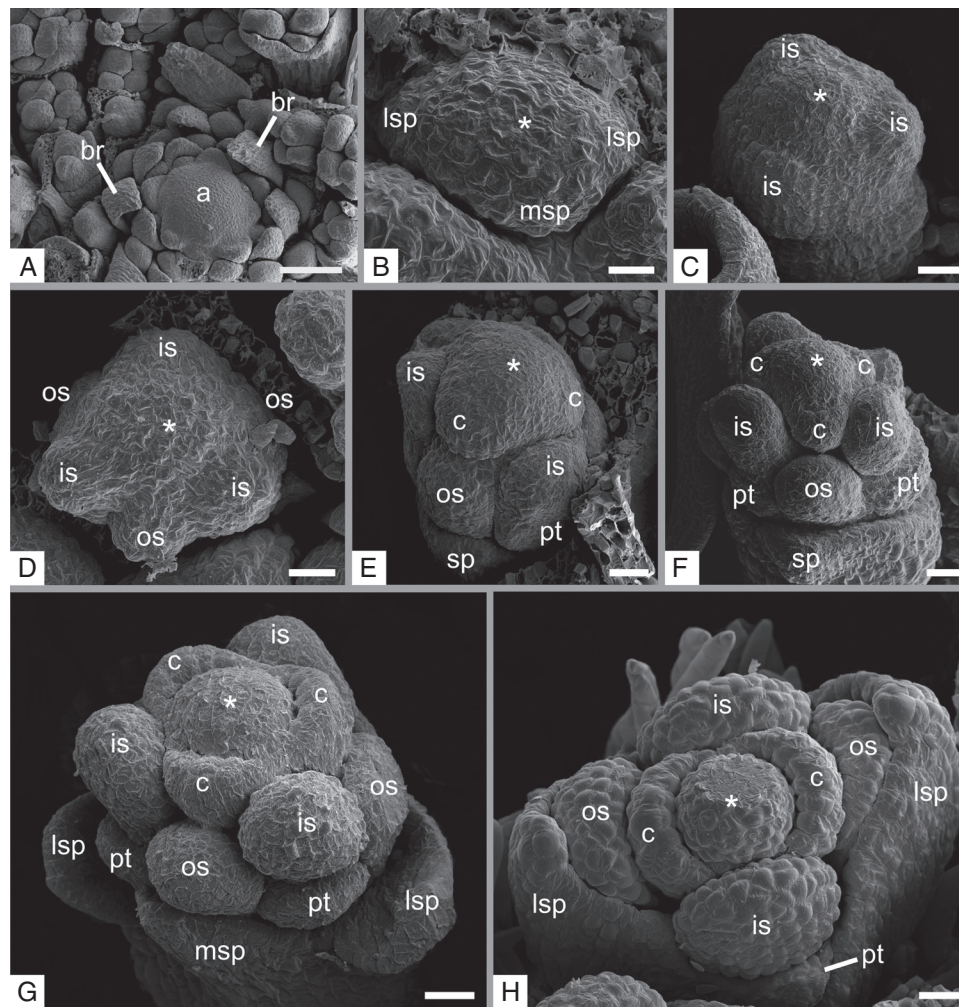


FIG. 4. Early developmental stages of staminate and pistillate flowers of *Eriocaulon*. (A) Inflorescence apex of *E. aquatile*, indicating the centripetal development of flowers. (B) Floral primordium of *E. aquatile* indicating the sepals' inception (abaxial view). (C) Floral primordium of *E. compressum*, indicating primordia of the inner stamens and the gynoecium. (D) Floral primordium of *E. modestum*, showing the initiation of the outer stamens (abaxial view). (E, F) Floral primordia of *E. kunthii* (E) and *E. compressum* (F) showing the initiation of the petals. (G, H) Floral primordia of *E. compressum* (G) and *E. decangulare* (H) showing the initiation of the carpels. a, inflorescence apex; asterisk, floral apex; br, floral bract; c, carpel; is, inner stamen; os, outer stamen; pt, petal; sp, sepal. Scale bars: (A) 100 μ m; (B–H) = 20 μ m.

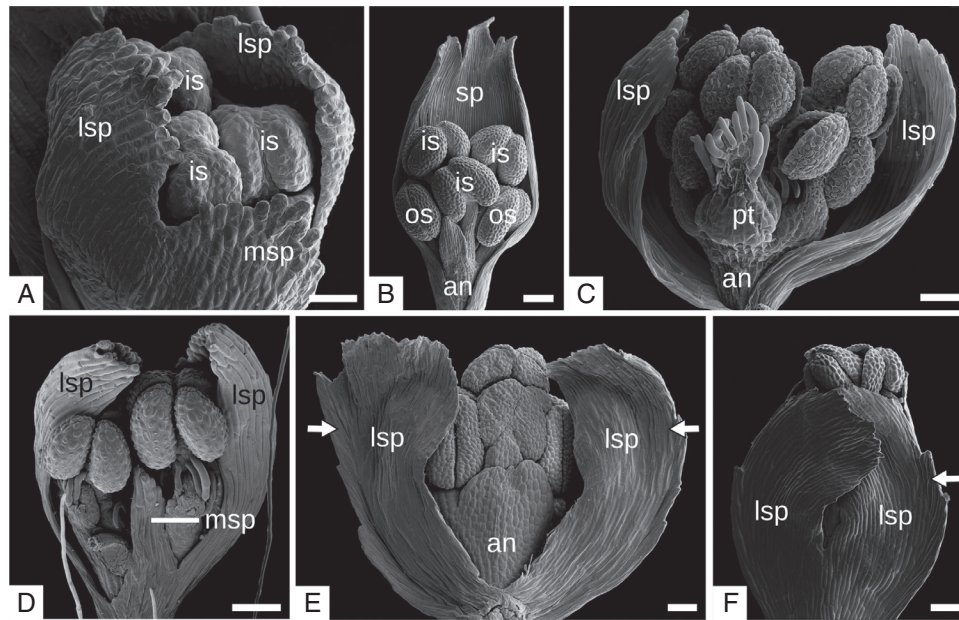


FIG. 5. Development of staminate flowers of *Eriocaulon*. (A) A young flower of *E. aquatile* showing fusion of sepals. (B) A young flower of *E. cinereum* showing developing anthophore and spatheous sepals. (C, D) Flowers of *E. twaitesii* showing developing anthophore, lateral sepals (C, D) and reduced median sepal (D). (E, F) Flowers of *E. guyanense* showing developing gibbose sepals. an, anthophore; arrow, sepal protrusion; is, inner stamen; os, outer stamen; lsp, lateral sepal; msp, median sepal; pt, petal; sp, sepal. Scale bars: (A) 40 μ m; (B–F) 100 μ m.

In all species, the inner stamens become longer than the outer ones (Fig. 5A, B). Carpels start closing around the floral apex (Fig. 6E), but they develop into carpelodes shortly afterwards (Fig. 6F–H). During their development, the carpelodes fold slightly inwards, forming a median slit, which may be more prominent (Fig. 6G) or less prominent (Fig. 6H).

Ontogeny of pistillate flowers

In pistillate flowers, the sepals grow and remain free in all species (Fig. 7A–C). In *E. guyanense*, sepals develop a dorsal keel, as in staminate flowers (Figs 2B, 5E, F). In *E. cinereum*, the lateral sepals develop completely, but development of the median sepal is arrested at an early developmental stage and it is reduced in the mature flower (Figs 1I, 7D). We were unable to analyse sepal development in *E. twaitesii* due to the lack of pistillate flowers at younger developmental stages, but it seems that the median sepal aborts during early development and is thus no longer distinguishable.

In species that possess an anthophore, this structure starts elongating after all floral parts are differentiated, with a higher growth rate immediately before anthesis (Fig. 7C). In most species, the apex of each petal is modified into a prominence that will later become the nectariferous gland (Fig. 8A), and the apical petal margins are raised and fuse abaxially, dislocating the central prominence to the adaxial petal surface (Fig. 8B, C); this process is similar to that in staminate flowers. In *E. cinereum*, petal development ceases at an early stage, forming scale-like (Fig. 3F, 7D) or linear structures (Fig. 3G). In this species, nectariferous glands do not appear at any time. In *E. abyssinicum* (Fig. 3F) and *E. guyanense* (Figs 3G, 8D–F), the petal apex does not develop into a gland, but the apical petal margins grow normally, which results in an emarginate petal.

In all species examined, the stamen primordia cease development and become scale-like staminodes at the ovary base (Figs 3H, 7D, 8D). The outer staminodes are inserted opposite the ovary locules, while the inner staminodes are opposite the ovary septa (Figs 3H, 8F–H). In the gynoecium, the carpels close into an ovary (Fig. 7A, B, D) and stigmas differentiate at their apices in a dorsal position (Fig. 8A, D). The style is formed by zonal growth (Fig. 9F–H) and the stigmas elongate and become filiform (Fig. 8G, H). None of the species displays vestigial structures in the commissural position.

Vasculature of staminate flowers

The pedicel of the staminate flower has a central vascular cylinder from which diverge the floral vascular traces (Fig. 9A). Each sepal receives a single vascular trace (Fig. 9B) but, in *E. cinereum* (Fig. 9C), *E. decangulare*, *E. guyanense* and *E. twaitesii*, two lateral sepal traces diverge from the vascular cylinder with no remnant of a median sepal trace. In all species, each sepal is vascularized by a single vascular bundle (Fig. 9D, E), except for *E. cinereum* (Fig. 9F) and *E. twaitesii*, in which the median sepal lacks vasculature. In *E. guyanense*, the sepal keel is formed solely by the epidermis and is opposite the sepal vascular bundle (Fig. 9E). In *E. cinereum*, the sepals are fused into a spatheous calyx with only two vascular bundles, corresponding to the lateral sepal bundles (Fig. 9F).

At the anthophore, the vascular cylinder divides into six vascular traces in trimerous species (Fig. 10A) and four traces in dimerous ones (Fig. 10B). The traces opposite the sepals enter the outer stamens (Fig. 10C, D); the traces alternating with the sepals divide radially into petal and inner stamen traces (Fig. 10C, E, F). The petals are each vascularized by a single vascular bundle that reaches the base of the

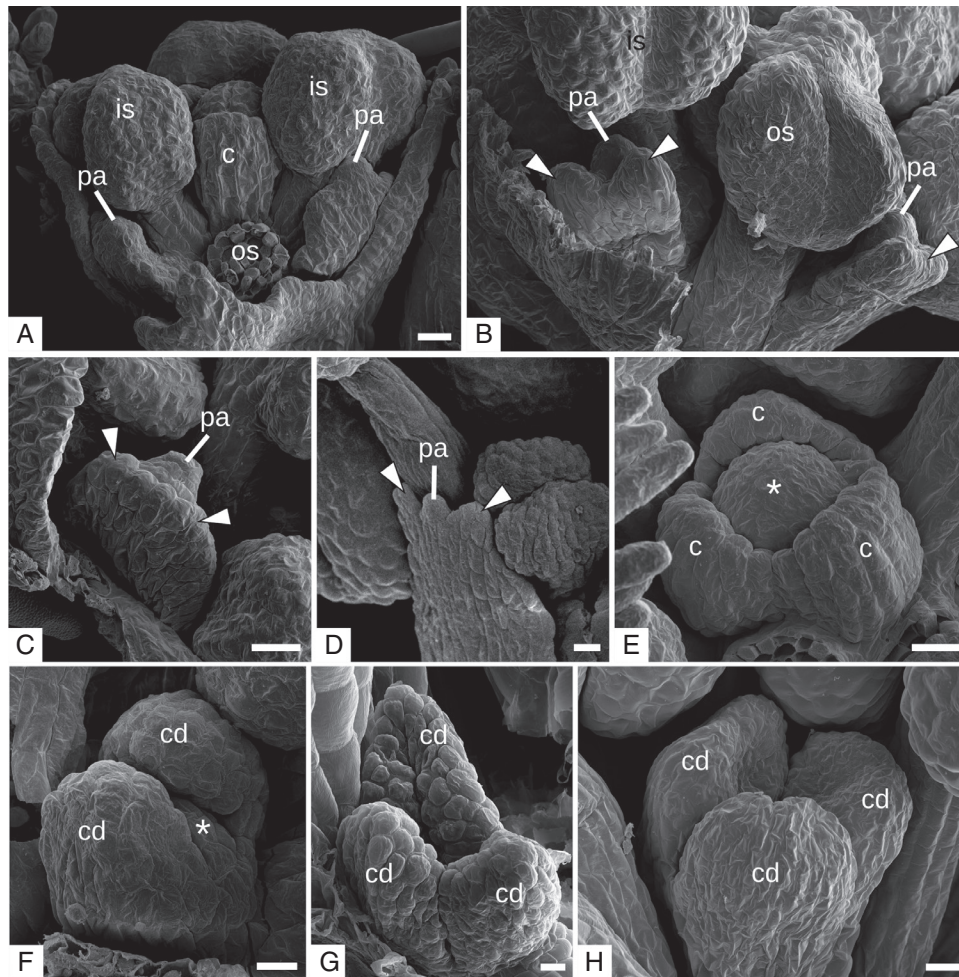


FIG. 6. Development of staminate flowers of *Eriocaulon*. (A) A young flower of *E. aquatile* with developing petals and showing the modifying petal apex. (B) A young flower of *E. cinereum* with developing petals and showing the petal apex and raising petal margins (arrowheads). (C) Detail of a developing petal of *E. aquatile* showing the dislocated petal apex and fused petal margins. (D) Detail of a developing petal of *E. guyanense* showing the aborted petal apex and the petal margins. (E) Detail of the developing gynoecium of *E. modestum* with carpels closing around the floral apex. (F) Detail of a developing sterile gynoecium of *E. guyanense* with three carpellodes and the floral apex. (G) Detail of developing ascidiate carpellodes of *E. sellowianum*. (H) Detail of the developing carpellodes of *E. aquatile*. Arrowhead, petal margins; asterisk, floral apex; c, carpel; cd, carpellode; gl, petal gland; is, inner stamen; os, outer stamen; pa, petal apex. Scale bars: (A, C–E, G, H) 20 μm ; (B, F) 10 μm .

petal gland (Figs 10F, 11A). In *E. xeranthemoides*, the vascular bundle does not reach the gland, which is vestigial (Fig. 11B). In *E. abyssinicum*, *E. nigericum* and *E. guyanense*, the vestigial glands are indistinguishable under light microscopy. The stamens are vascularized by a single vascular bundle that reaches the connective (Fig. 11C, D). The remaining vascular cylinder divides into the vascular bundles that enter the carpellodes (Fig. 11D, E). The ventral carpelary bundles are absent, so the remaining floral apex lacks vasculature (Fig. 11D, F).

Vasculature of pistillate flowers

The pedicel of the pistillate flower has a central vascular cylinder, from which diverge the traces of all floral parts (Fig. 12A). As in the staminate flowers, each sepal receives a single vascular trace (Fig. 12B). In *E. decangulare*, *E. guyanense*, *E. cinereum* (Fig. 12C, D) and *E. twaitesii*, the lateral sepals

receive vascular traces, but there is no remnant of a median sepal trace. In *E. guyanense*, the sepals of the pistillate flower have a keel like that of the staminate flower (Fig. 9E).

In *E. aquatile*, *E. cinereum*, *E. compressum*, *E. guyanense*, *E. modestum* and *E. teuszii*, the vascular cylinder does not divide at the anthophore (Fig. 12D). Divergence of the petal traces from the vascular cylinder alternates with the sepals (Fig. 12E, F). The petals are each vascularized by a single vascular bundle that reaches the base of the petal glands (Fig. 13A, B). In species that lack petal glands, no differentiated tissue was observed (Fig. 13C). In *E. aquatile*, *E. kunthii*, *E. modestum* and *E. nigericum* (Fig. 13D), a vascular trace alternating with the petals reaches the base of each outer staminode. In these species, a small vascular trace diverges from the petal trace and reaches the base of each inner staminode (Fig. 13E, F).

At the ovary base, dorsal carpelary traces diverge from the vascular cylinder (Fig. 14A, B). More distally, the remaining vascular cylinder divides into ventral vascular bundles, which

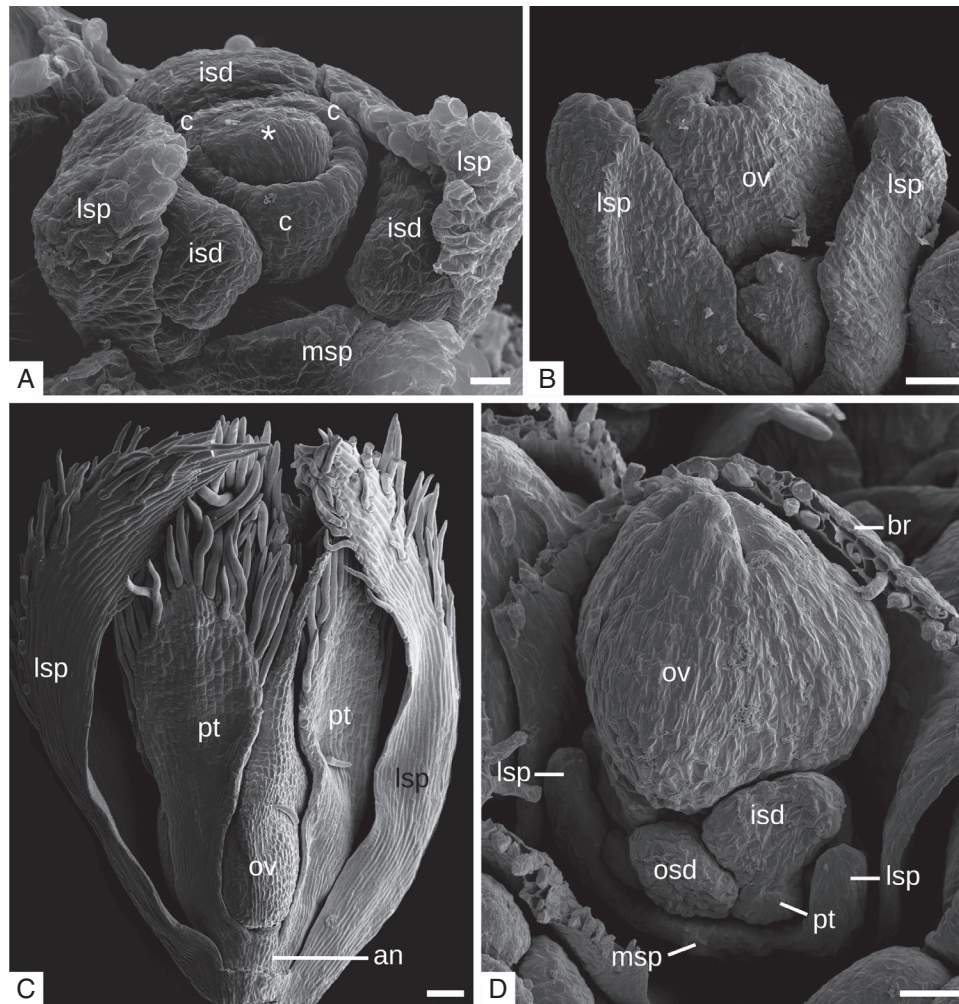


Fig. 7. Development of pistillate flowers of *Eriocaulon*. (A, B) Young flowers of *E. teuszii* (A) and *E. decangulare* (B) showing developing sepals and carpels closing into the ovary. (C) Young flower of *E. modestum* showing the free sepals. (D) Young flower of *E. cinereum* showing developing lateral sepals, the aborted median sepal, an aborted petal and staminodes. Asterisk, floral apex; c, carpel; isd, inner staminode; lsp, lateral sepal; msp, median sepal; osd, outer staminode; ov, ovary; pt, petal. Scale bars: (A, D) 20 μ m; (B) 40 μ m; (C) 100 μ m.

are in the commissural position (Fig. 14C, D). Overall, the gynoeceum vasculature consists of two or three dorsal carpellary bundles and two or three synlateral ventral bundles (Fig. 14B–D), with the number depending on the number of carpels. In *E. abyssinicum*, *E. cinereum*, *E. nigericum*, and *E. xeranthemoides*, the remaining vascular cylinder usually does not divide into ventral bundles but remains in the central region of the ovary (Fig. 14E). The ventral bundles, or the remaining vascular cylinder, reach the placenta and vascularize the ovules (Fig. 14D). The dorsal carpellary bundles vascularize the style in all species studied (Fig. 14F). In some flowers, the dorsal bundles are reduced, resulting in a non-vascularized style (Fig. 14G). The stigmas usually lack vasculature in all species (Fig. 13B, C), but the dorsal carpellary bundles reach their base in some flowers (Fig. 14H).

Character optimization

The character optimization was inconclusive about the flower sexuality in the ancestral of the xyrids, but it indicates

that the ancestor of Eriocaulaceae had unisexual flowers and that bisexual flowers evolved independently in *Eriocaulon*, *Rondonanthus* and *Syngonanthus* (Fig. 15A). The results indicate that trimerous flowers are a plesiomorphic state in the xyrids and that dimerous flowers evolved independently at least six times in Eriocaulaceae (Fig. 15B). The results were inconclusive about the presence of the outer androecium whorl in the ancestral of the xyrids and the Eriocaulaceae, but they show that the outer whorl was present in the ancestor of Eriocauloideae and absent in the ancestor of Paepalanthoideae (Fig. 15C).

The optimization shows that carpelloides were absent in the ancestor of Xyridaceae but present in the ancestor of the Eriocaulaceae (Fig. 15D). The results also indicate that the ancestor of the xyrids had carpels with a stigmatic apex and undifferentiated margins, while carpels with a nectariferous apex and stigmatic margins appeared in the ancestor of Paepalanthoideae (Fig. 15E). Species with an aborted carpel apex and stigmatic margins appeared independently in Paepalanthoideae (Fig. 15E). Finally, the results show that the nectariferous appendages on the gynoeceum appeared only once and are a synapomorphy

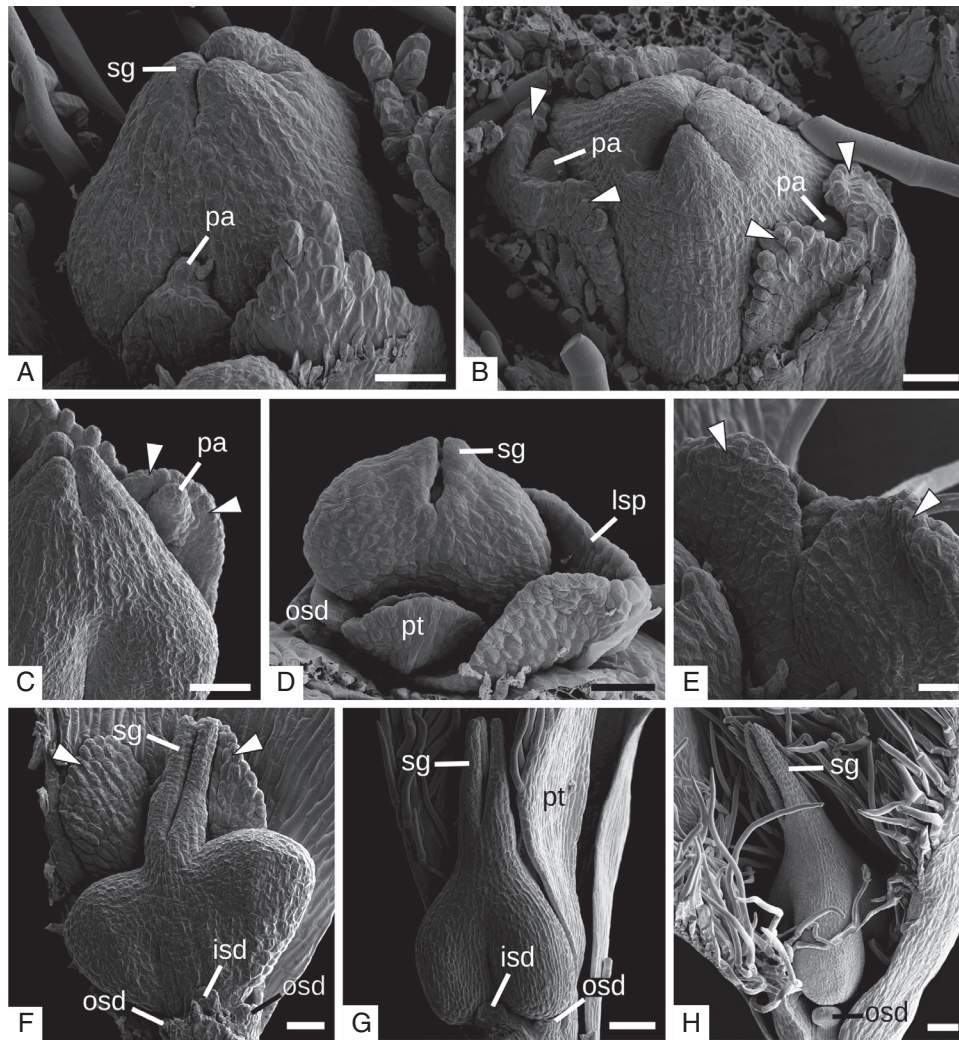


FIG. 8. Development of pistillate flowers of *Eriocaulon*. (A–C) Young flowers of *E. aquatile* (A), *E. kunthii* (B) and *E. modestum* (C) showing successive development of petals and young stigmas. (D) A young flower of *E. guyanense* showing developing petal and stigma. (E) Detail of a developing petal of *E. guyanense* in frontal view. (F) A young flower of *E. guyanense* showing an emarginate petal, staminodes at the base of the ovary and developing stigmas. (G, H) Young flowers of *E. modestum* (G) and *E. kunthii* (H) showing staminodes at the base of the ovary and developing stigmas. Arrow, petal margin; isd, inner staminode; lsp, lateral sepal; osd, outer staminode; pa, petal apex; pt, petal; sg, stigma. Scale bars: (A, B) 40 μm ; (C, F) 50 μm ; (D, E) 20 μm ; (G, H) 100 μm .

of *Abolboda*, *Aratitiopea* and *Orectanthe*, the more recently diversified genera of Xyridaceae.

DISCUSSION

Floral diversity and reduction in Eriocaulon

Early floral development in *Eriocaulon* resembles that observed in other Eriocaulaceae (Stützel, 1990; Stützel and Gansser, 1995; Silva et al., 2016; Sokoloff et al., 2020). The sepals are the first floral organs to emerge on the floral primordium, followed by the stamens and the gynoecium; the petals differentiate from the division of common petal–stamen primordia (Stützel, 1990; Silva et al., 2016; Sokoloff et al., 2020). Despite this relatively stable general pattern in *Eriocaulon*, there is variation in floral merism, size and number of perianth parts, and presence of petal glands. Our comparative analysis of

floral development and vasculature in several species provides clues to possible evolutionary processes.

Eriocaulon decangulare and *E. guyanense* differ from the other species examined by their dimerous flowers. In both species, the median sepal does not emerge, and the two equal lateral sepals resemble the lateral sepals of trimerous species in morphology and position. In a developmental study of dimerous *Paepalanthus* (Paepalanthoideae), Silva et al. (2016) hypothesized that dimery evolved in this genus from the loss of an entire floral sector, including the median sepal, so that the floral parts shifted position, and the flowers became zygomorphic. In *Eriocaulon*, trimerous flowers are slightly zygomorphic due to the position of the lateral sepals, but the other floral parts are not dislocated, as also observed by Sokoloff et al. (2020). However, in dimerous flowers, all floral parts and their vascular bundles have shifted position so that the flowers are zygomorphic. Therefore, it is likely that dimery in *Eriocaulon* also evolved from the loss of a median sepal, as in *Paepalanthus*.

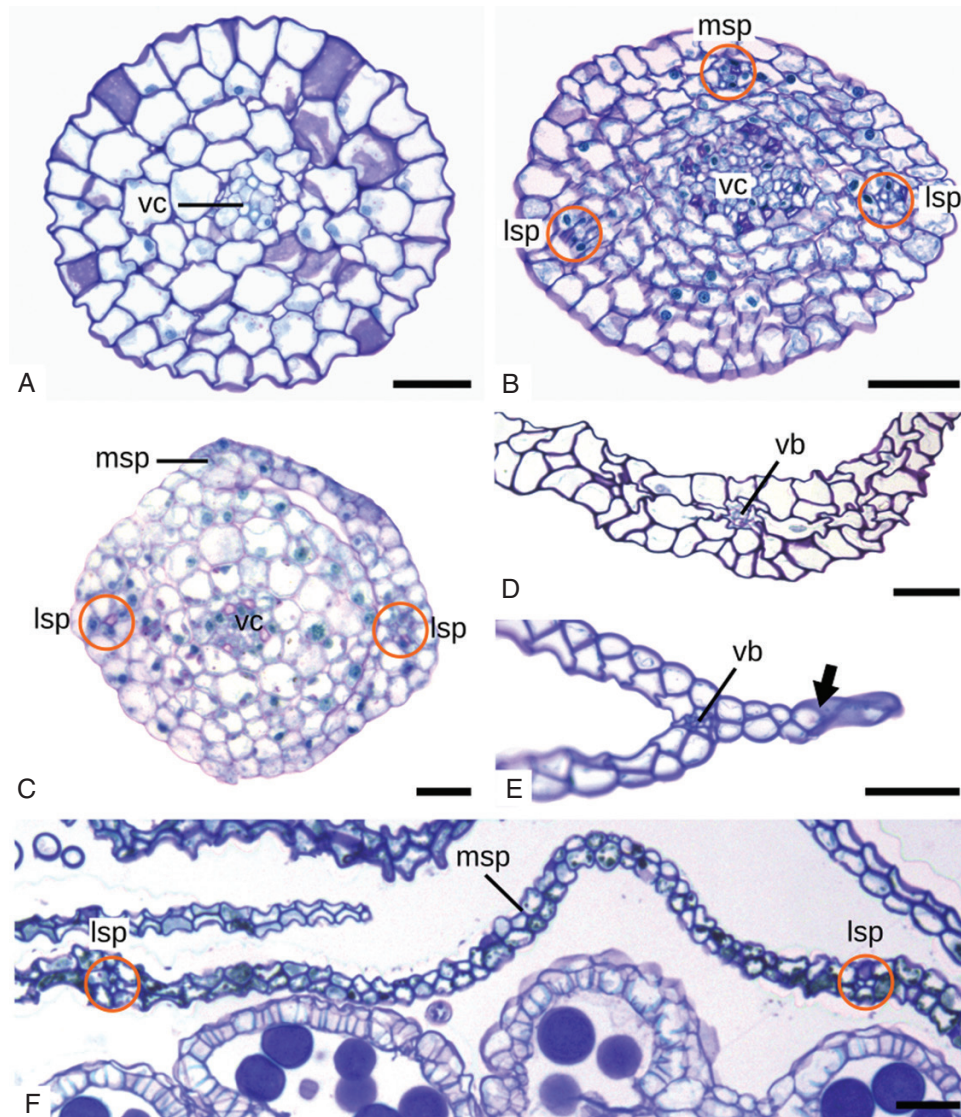


FIG. 9. Vasculature of staminate flowers of *Eriocaulon*: transverse sections. (A) Pedicel of *E. compressum*. (B) Sepal traces of *E. modestum*. (C) Sepal traces of *E. cinereum*. (D) Sepal of *E. decangulare*. (E) Sepal of *E. guyanense*. (F) Spathaceous calyx of *E. cinereum*. Arrow, sepal protrusion; lsp, lateral sepal; msp, median sepal; vb, vascular bundle; vc, vascular cylinder. Scale bars: (A, B, D–F) 50 μm ; (C) 25 μm .

In *Eriocaulon*, sepals are free at earlier developmental stages of the staminate flowers. The sepals may remain free or become fused through late congenital fusion at later stages, resulting in considerable diversity in calyx morphology. The synorganization of the perianth in mature flowers may differ in staminate and pistillate flowers. The tendency is that pistillate flowers have mostly free sepals but, in staminate flowers, different degrees of fusion occur. *Eriocaulon guyanense* has staminate flowers with either free or fused sepals, and the sepal keel may be more or less conspicuous. Similar morphological variations were also observed in an African dimerous species of *Eriocaulon* and are apparently related to distinct zones in the capitulum (S. Phillips, Kew Gardens, UK, pers. comm.). This variation is novel to Brazilian species of Eriocaulaceae, so we emphasize the importance of studying floral morphological variation in other species to address morphological and taxonomic problems in the family.

In *E. cinereum* and *E. twaitesii*, late development differs considerably from that of the other species studied and indicates distinct degrees of perianth reduction. In *E. cinereum*, the calyx is spathaceous in staminate flowers and has only two lateral vascular bundles, which correspond to the vascular bundles of lateral sepals. The median sepal lacks vasculature, but the tripartite tip of the spathaceous calyx shows that the median sepal develops to some degree and becomes incorporated in the calyx.

In pistillate flowers of *E. cinereum*, the median sepal has an arrested development and is reduced and non-vascularized. The petals emerge in the same way as those of other species, but they are reduced and lack vasculature. This pattern of perianth reduction also occurs in *E. redactum* (Sokoloff *et al.*, 2020), a species closely related to *E. cinereum*. Although flowers of both species develop similarly, *E. redactum* differs by its smaller sepals with trichomes at their apices (Sokoloff *et al.*, 2020). Sokoloff *et al.* (2020) also noted the

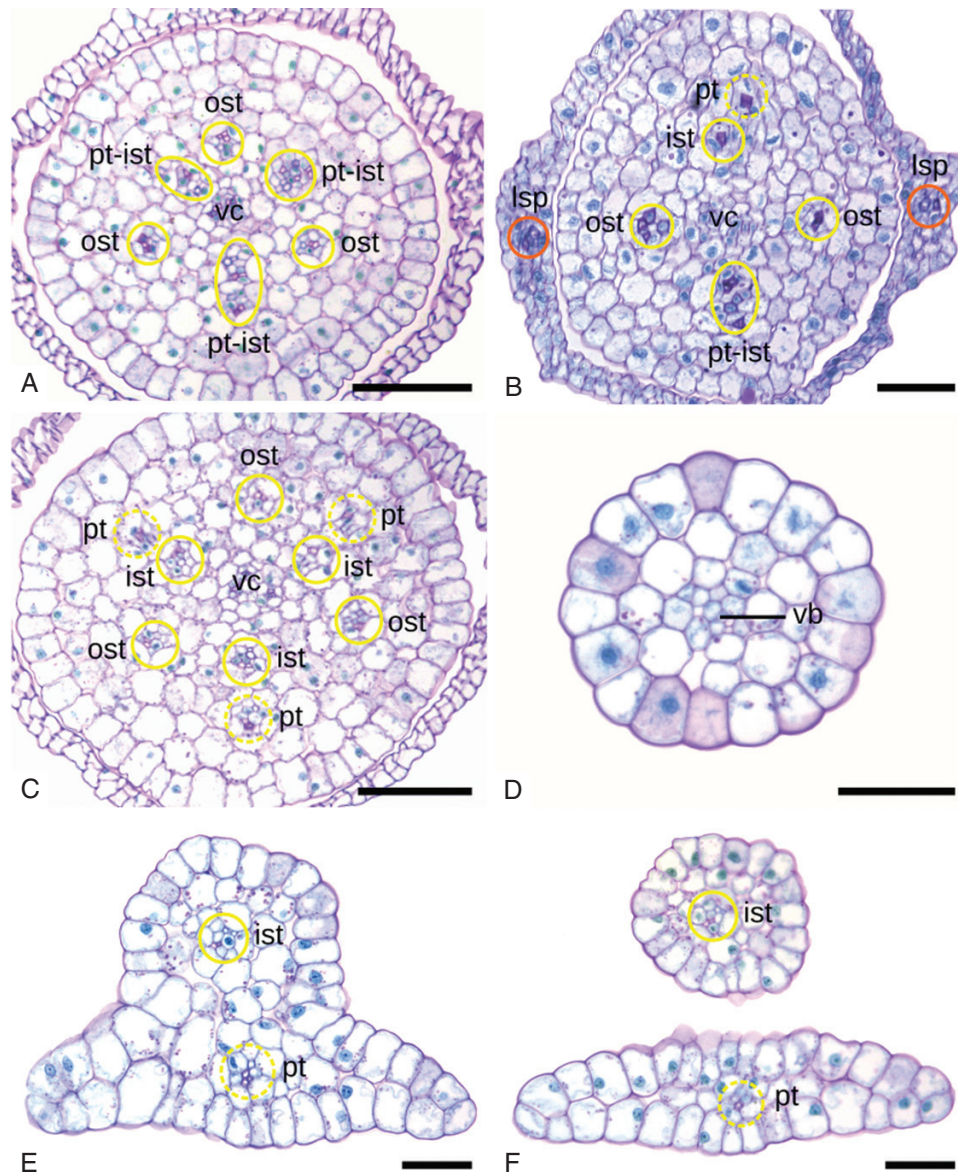


FIG. 10. Vasculature of staminate flowers of *Eriocaulon*: transverse sections. (A, B) Anthophore of *E. teusczii* (A) and *E. decangulare* (B) showing the outer stamen traces and common petal–inner stamen traces. (C) Anthophore of *E. teusczii* showing the outer stamens traces and separate traces of petals and inner stamens. (D) Detail of the filament of an outer stamen of *E. teusczii* showing the vascular bundle. (E, F) Inner stamen of *E. compressum* with the base of a filament adnate to a petal (E) and the same filament free at its median region (F). ist, inner stamen; ost, outer stamen; pt, petal; pt-ist, common trace of a petal and an inner stamen; vb, vascular bundle; vc, vascular cylinder. Scale bars: (A–F) 50 µm.

importance of studying other species that belong to the *E. cinereum* group to understand its morphological variation.

In *E. twaithesii*, the median sepal is reduced in comparison with the lateral ones, and lacks vasculature. The reduced median sepal is noticeable in staminate flowers of this species because sepals are free at their median region. In pistillate flowers, the median sepal is superficially absent in the mature flower, but a complete development series is still missing. Based on data from species with dimerous and apparently heteromerous flowers, there seems to be a tendency that, when outer perianth reduction occurs in Eriocaulaceae, the median sepal is reduced or lost. Furthermore, reduction in perianth parts is frequently accompanied by complete loss of their vasculature.

The presence of petal glands is a variable character state in *Eriocaulon*. They are commonly present in the genus but are absent in five out of the 13 species studied here. Development of the petal glands is quite uniform in *Eriocaulon* and is in accordance with what was previously reported for the genus (Stützel, 1984). However, glands are lacking in both staminate and pistillate flowers of *E. abyssinicum*, *E. guyanense*, *E. nigericum* and *E. xeranthemoides*, and in pistillate flowers of *E. cinereum*. A recent study including several Indian species of *Eriocaulon* optimized floral morphological characters on a phylogeny and concluded that the presence of petal glands is an ancestral character in the genus and that such glands were lost secondarily several times (Darshetkar et al., 2021). In *E. cinereum*, the lack of

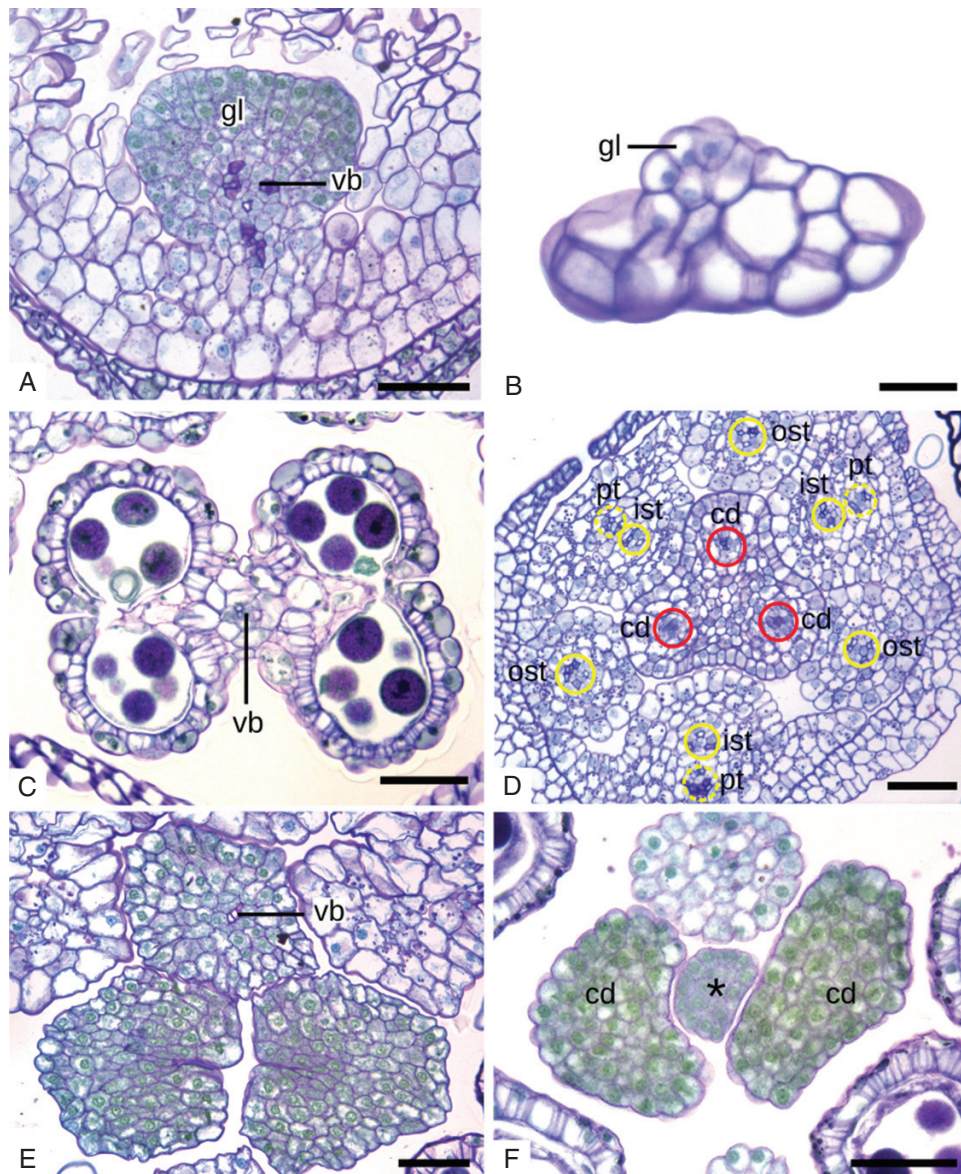


FIG. 11. Vasculature of staminate flowers of *Eriocaulon*: transverse sections. (A) Detail of a petal gland of *E. kunthii* showing the vascular bundle. (B) Detail of a petal of *E. xeranthemoides* showing a vestigial petal gland without vasculature. (C) Detail of an anther of *E. nigericum* showing the vascular bundle in the connective. (D) Region above the anthophore in *E. kunthii*, showing vascular bundles of stamens and vascular bundles of the carpellodes at the stalk of the sterile gynoecium. (E) Detail of a sterile gynoecium of *E. modestum* showing the median carpelloid with a vascular bundle and lateral carpelloids folded inwards. (F) Detail of a sterile gynoecium of *E. guyanense* showing the two carpelloids and the remaining floral apex. Asterisk, floral apex; cd, carpelloid; gl, petal gland; ist, inner stamen; ost, outer stamen; pt, petal; vb, vascular bundle. Scale bars: (A, C–F) 50 μ m; (B) 25 μ m.

petal glands in pistillate flowers is likely to be related to petal reduction, since these glands develop fully in staminate flowers. In the remaining species, vestigial glands are found either in staminate or in pistillate flowers, corroborating that petal glands were secondarily lost. In *E. abyssinicum* and *E. guyanense*, the apical petal margins are raised but do not fuse together in the pistillate flowers, so petals become emarginate as a result of gland abortion.

Vasculature of the androecium and gynoecium

Androecium and gynoecium morphology are quite uniform in the species studied here, with some variation in

their vasculature. Stamines are generally vascularized in Eriocaulaceae, at least to some degree. In Paepalanthoideae and in *Mesanthemum* (Eriocauloideae), vascular bundles reach the base of staminodes (Rosa and Scatena, 2007; Silva et al., 2016, 2021). In *Eriocaulon*, staminodes are vascularized in *E. aquatile*, *E. kunthii*, *E. modestum* and *E. nigericum*, but not in the other species studied, indicating that the presence of staminodial vasculature is variable in the genus, and its absence displays a higher degree of stamen reduction.

The gynoecium of Eriocaulaceae is generally vascularized by both dorsal and ventral carpillary bundles (Rosa and Scatena 2003, 2007; Silva et al., 2016, 2021). In *Mesanthemum*, the dorsal carpillary bundles reach the style, but the stigmas are

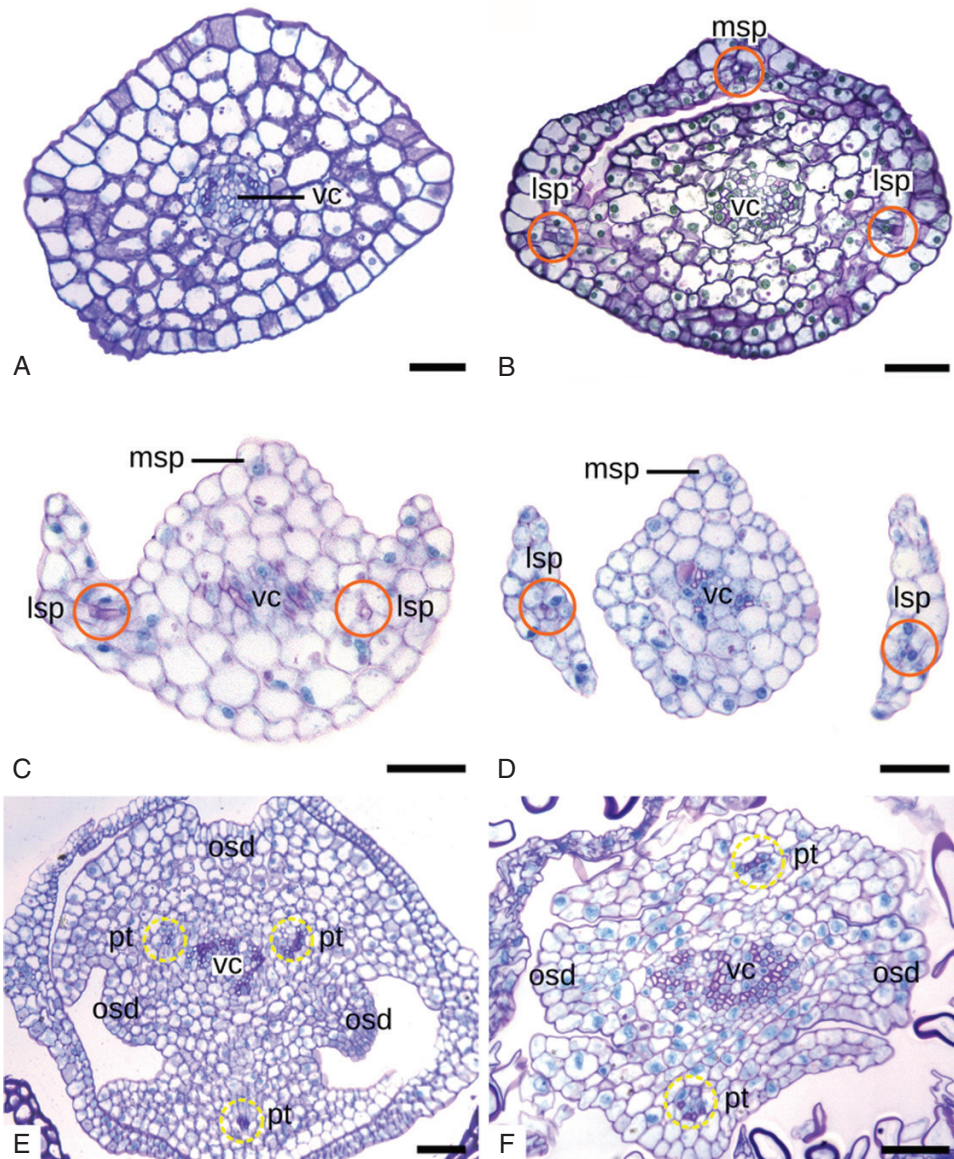


FIG. 12. Vasculature of pistillate flowers of *Eriocaulon*: transverse sections. (A) Pedicel of *E. kunthii*. (B) Sepal traces of *E. aquatile*. (C) Sepal traces of *E. cinereum*. (D) Anthophore *E. cinereum* with a vascular cylinder. (E, F) Flower base of *E. kunthii* (E) and *E. decangulare* (F) showing the vascular traces of petals and the outer staminodes. lsp, lateral sepal; msp, median sepal; osd, outer staminode; pt, petal; vc, vascular cylinder. Scale bars: (A, E) 100 μ m; (B) 200 μ m; (C, D) 25 μ m; (F) 50 μ m.

non-vascularized, probably because of reduction of vasculature (Silva et al., 2021). Similarly, in *Eriocaulon*, the dorsal vascular bundles reach the style but rarely the stigmas. The lack of vasculature in stigmas of Eriocauloideae indicates that the dorsal vascular bundle is reduced and is probably related to the presence of filiform stigmas, which do not require large vascular supply.

The remaining central vascular cylinder divides into the ventral vascular bundles in the ovary of *Eriocaulon* and lies in a commissural position. However, in *E. abyssinicum*, *E. cinereum*, *E. nigericum* and *E. xeranthemoides*, the central vascular cylinder does not divide, and ventral vascular bundles are absent probably because of vascular reduction. In *Mesanthemum*, the ventral vascular bundles are opposite the locules, but they shift position along

the ovary regions and become commissural, indicating that distinct types of carpel fusion occur in this genus (Silva et al., 2021). The comparative analysis of Eriocauloideae flowers shows that the gynoeceum vasculature varies in the subfamily and, although morphological reductions are apparently lacking in the gynoeceum, vascular reductions occur frequently.

Evolutionary implications for the xyrids

Our study of floral development of *Eriocaulon* allows us to discuss in greater detail the floral homologies between Eriocaulaceae and Xyridaceae. Character optimization also improves our understanding of these structures in Eriocauloideae and Paepalanthoideae.

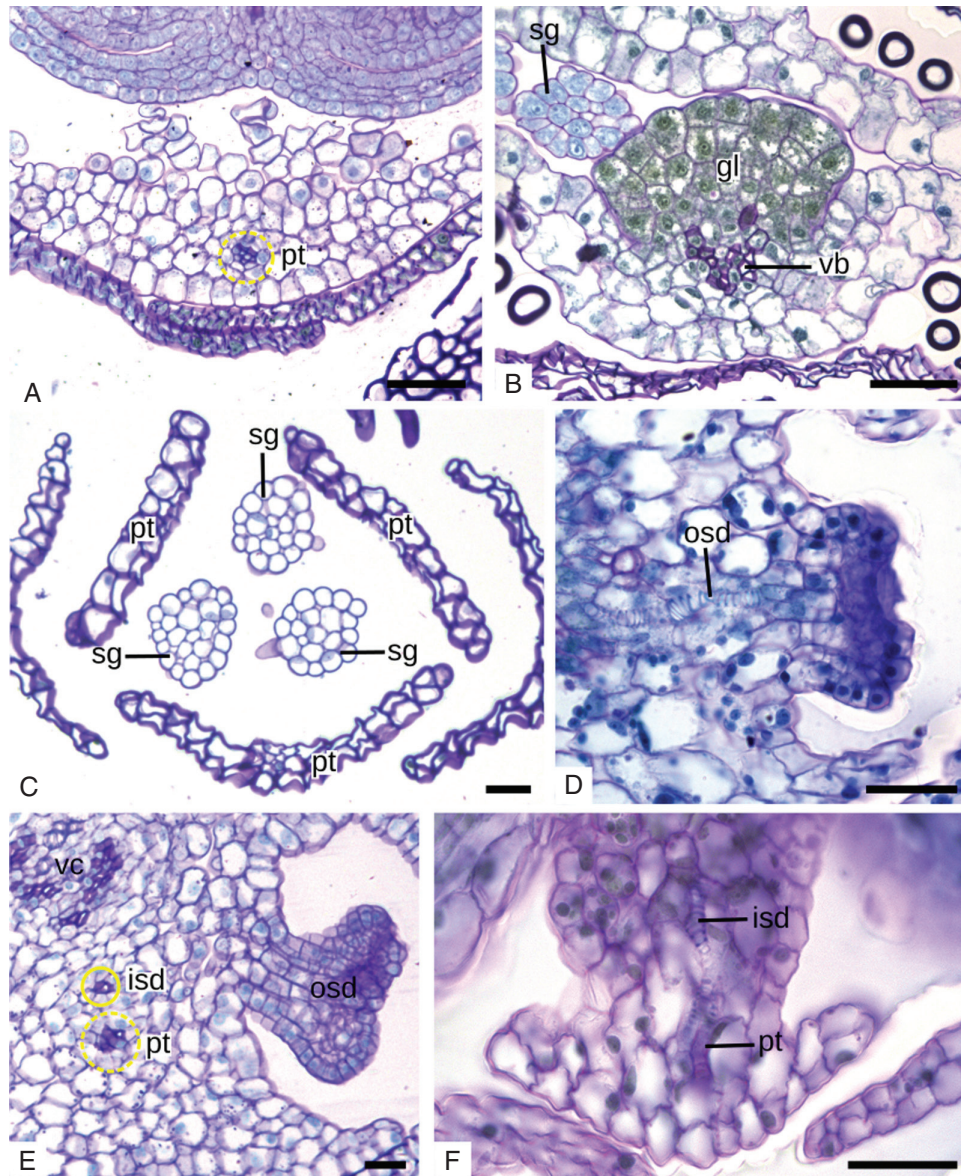


FIG. 13. Vasculature of pistillate flowers of *Eriocaulon*: transverse sections. (A) Detail of a petal of *E. kunthii* showing the vascular bundle. (B) Detail of a petal gland of *E. decangulare* showing the vascular bundle. (C) Apical region of a flower of *E. xeranthemoides* showing petals without glands and stigmas. (D) Detail of a vascular trace reaching the base of an outer staminode in *E. nigericum*. (E) Detail of the flower base of *E. kunthii* showing a petal trace and an inner staminode trace. (F) Detail of an inner staminode trace diverging from a petal trace in *E. nigericum*. gl, petal gland; isd, inner staminode; osd, outer staminode; pt, petal; sg, stigmal vb, vascular bundle; vc, vascular cylinder. Scale bars: (A, B) 200 μm ; (C, E) 50 μm ; (D, F) 25 μm .

Although most species of Eriocaulaceae are unisexual, flowers of all species are bisexual at early developmental stages and become unisexual as a result of carpel or stamen abortion (Smith, 1910; Stützel, 1990; Stützel and Gansser, 1995; Silva et al., 2016; this study). A few species with bisexual flowers occur in *Syngonanthus* (Watanabe et al., 2015), *Rondonanthus* (Hensold and Giulietti, 1991) and *Eriocaulon* (Prajaksood et al., 2017), but our analysis indicates that bisexuality is a reversal in Eriocaulaceae that appeared multiple times independently (Fig. 15A). Therefore, we assume that the ancestor of the xyrids had bisexual flowers and that the shift to unisexuality occurred in the ancestor of Eriocaulaceae. The adaptive significance of this shift is not clear, but could be related partly to

mechanical constraints due to the small flowers and compact inflorescence, and partly to pollinator preference in the family; further studies on the reproductive biology of the family are needed to elucidate this question.

Most species of Eriocaulaceae are trimerous, while Xyridaceae are exclusively trimerous. In Xyridaceae, the lateral sepals emerge before the median adaxial sepal, in contrast to *Eriocaulon*. Our character optimization shows that dimery has evolved at least six times in Eriocaulaceae (Fig. 15B). In *Paepalanthus* and *Eriocaulon*, dimery has evolved from the loss of an entire floral sector, without vestigial remnants of floral parts, and is associated with a shift in floral symmetry (Silva et al., 2016). We predict that further analyses of dimerous

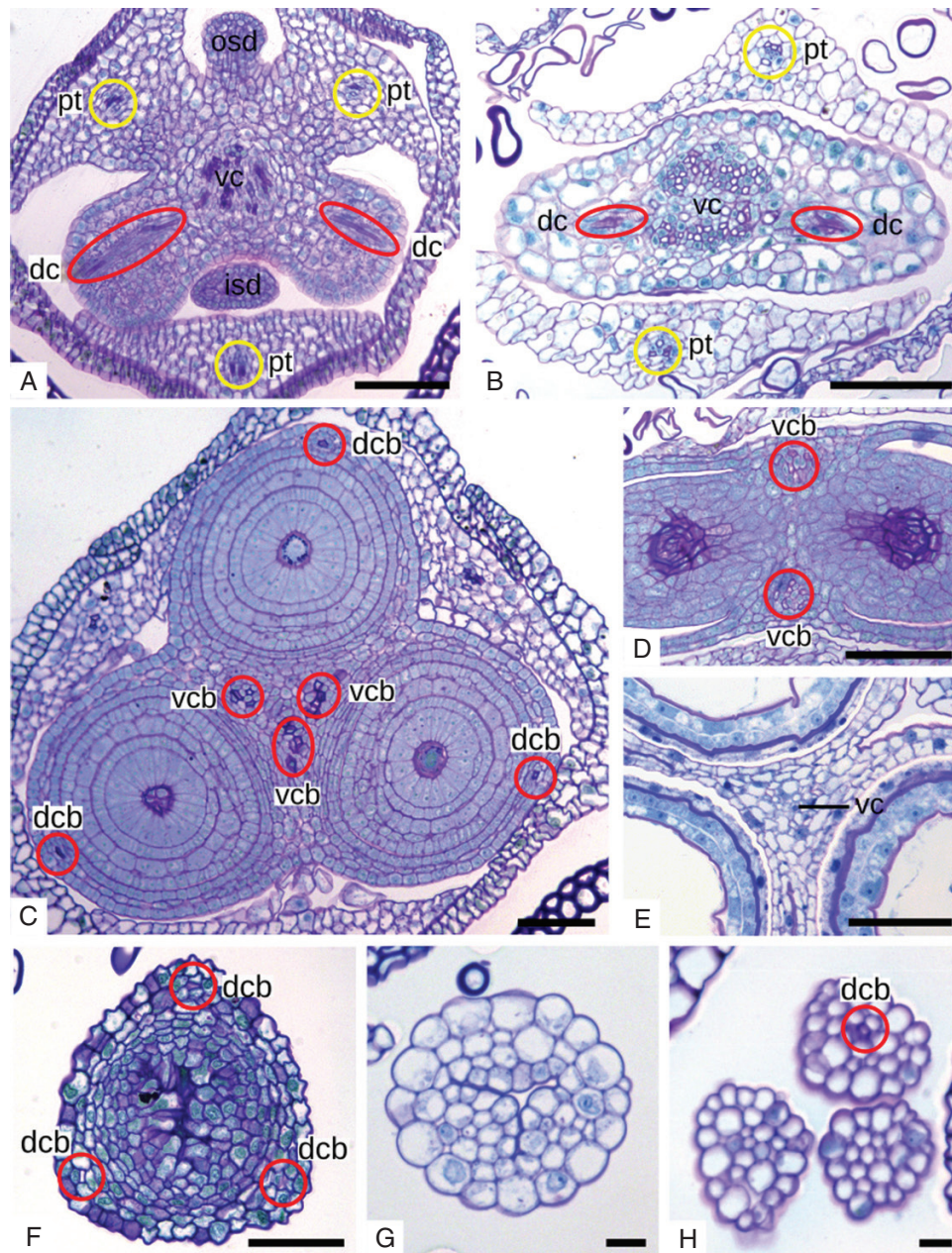


FIG. 14. Vasculature of pistillate flowers of *Eriocaulon*: transverse sections. (A) Ovary base of *E. kunthii* showing two out of three dorsal carpellary traces alternate to the petals (oblique section). (B) Ovary base of *E. decangulare* showing the two dorsal carpellary traces alternate to the petals. (C) Middle region of the ovary of *E. kunthii* showing the dorsal carpellary bundles and vascular cylinder dividing into ventral carpellary bundles. (D) Distal region of the ovary of *E. decangulare* showing the two ventral carpellary bundles in the commissural position. (E) Middle region of the ovary of *E. teusczii* showing the undivided central vascular cylinder. (F) Style of *E. modestum* showing dorsal carpellary bundles. (G) Style of *E. cinereum* without vasculature. (H) Stigmas of *E. xeranthmoides* showing a single vascular bundle. dc, dorsal carpellary trace; dcb, dorsal carpellary bundle; isd, inner staminode; osd, outer staminode; pt, petal; vc, central vascular cylinder; vcb, ventral carpellary bundle. Scale bars: (A, C) 200 μ m; (B, D–H) 50 μ m; (E) 100 μ m.

Syngonanthus and *Comanthera* will indicate a similar process within these genera.

The presence of common petal–stamen primordia was observed in all species of *Eriocaulon* studied here, and seems to be a common feature in Eriocaulaceae (Stützel, 1990; Silva et al., 2016; Sokoloff et al., 2020). Common petal–stamen primordia are linked with a delay in petal initiation in eudicots and may lead to petal reduction or loss (Ronse De Craene, 2018). Probably, this is also true for Eriocaulaceae (Sokoloff

et al., 2020) and may have contributed to the reduction of petals in some *Eriocaulon* (e.g. *E. cinereum*), as well as for the loss of petals in *Lachnocaulon* (Paepalanthoideae). However, common petal–stamen primordia also occur in Xyridaceae, and petals are not delayed in this family (Sajo et al., 2017), which implies that common petal–stamen primordia are not always related to petal delay in Poales.

Staminodes are sometimes present in both Eriocaulaceae and Xyridaceae, but their origin differs between the two families.

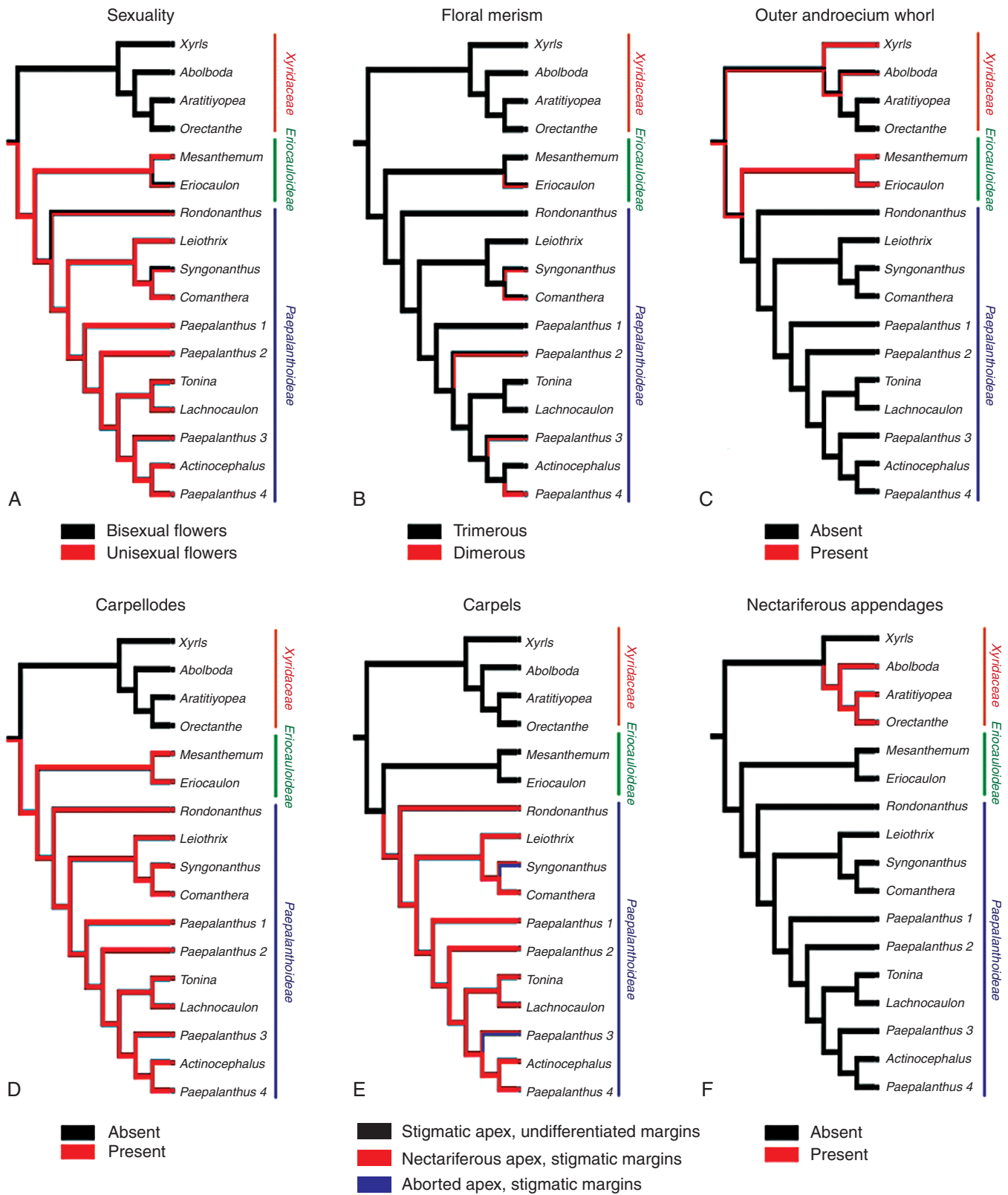


FIG. 15. Optimization of six floral characters onto a combined phylogenetic tree of the xyrids. (A) Sexuality. (B) Floral merism. (C) Outer androecium whorl. (D) Carpelloides. (E) Carpels. (F) Nectariferous appendages.

In Eriocaulaceae, the presence of staminodes is associated with a shift to unisexuality, as stamen development is arrested only in pistillate flowers; in Paepalanthoideae, a whorl is lost entirely. In contrast, flowers of Xyridaceae are bisexual; when staminodes are present, the fertile stamens correspond to the inner androecium whorl and staminodes to the outer whorl.

Our character optimization was inconclusive regarding the evolution of the outer androecium whorl in the xyrids (Fig. 15C), but floral development of Eriocaulaceae and Xyridaceae indicates possible interpretations. Across angiosperms, whorled staminodes tend to occur in more recently diversified groups whose ancestors possessed two-whorled stamens (Walker-Larsen and Harder, 2000). In Xyridaceae, the outer androecial whorl is staminodial in *Xyris* and some species of *Abolboda*, and tends to be lost in more recently diversified genera (Fig. 15C), which also suggests that the ancestor of the family had a two-whorled androecium. The complete loss of whorled staminodes seems to be irreversible (Walker-Larsen and Harder, 2000), implying that flowers with a two-whorled androecium could not be derived from flowers such as those of Paepalanthoideae (Eriocaulaceae), *Arattitiopea*, *Orectanthe* and some species of *Abolboda* (Xyridaceae), in which the outer androecium whorl is lost from inception (Stützel, 1990; Stützel and Gansser, 1995; Silva et al., 2016; Sajo et al., 2017). Therefore, we assume that the presence of two androecial whorls is a plesiomorphic character state in both Eriocaulaceae and Xyridaceae.

In *Eriocaulon*, stamens are arranged in two whorls that emerge simultaneously in the floral primordia (Stützel, 1990), but we show that the outer stamens emerge slightly later than the common petal–inner stamen in a few flowers. In all species of *Eriocaulon*, the inner stamens are longer than the outer ones throughout floral development. In contrast, in Paepalanthoideae, the inner stamens are the same height and outer stamens are completely lost (Stützel, 1990; Stützel and Gansser, 1995; Rosa and Scatena, 2007; Silva et al., 2016). Delayed development of the outer androecial whorl was also observed in *Mesanthemum* and is probably related to lack of space for the stamens to develop side by side, and could represent an ancestral state that has led to their loss in Paepalanthoideae (Silva et al., 2021).

Early gynoecium development of *Eriocaulon* is similar to that observed in other genera of Eriocaulaceae (Stützel, 1990; Stützel and Gansser, 1995; Silva et al., 2016), in which the floral centre is a convex bulge around which the carpel primordia emerge. In *Paepalanthus* (Paepalanthoideae) and *Mesanthemum* (Eriocauloideae), this central bulge was interpreted as an undeveloped septum, since it raises the placenta and the ovules during development of the gynoecium in pistillate flowers of Eriocaulaceae (Coan et al., 2010). Sokoloff et al. (2020) stated that the nature of the central bulge is problematic, as it has had different interpretations. Comparison with other angiosperms, including eudicots (Endress, 2006; Ronse De Craene, 2020), suggests that the central bulge in Eriocaulaceae could be a floral apex that becomes enclosed in the ovary during the development of the pistillate flowers.

In Poales, the presence of a floral apex with surrounding carpel primordia is also found in Restionaceae (including Anarthriaceae and Centrolepidaceae) (Ronse De Craene et al., 2001, 2002; Fomichev et al., 2019). Sokoloff et al. (2020) considered that the similarity between the early gynoecium

development of *Eriocaulon* and Restionaceae, along with the presence of pendulous ovules and dorsal fruit dehiscence, may support the position of Eriocaulaceae as sister to the restiids + graminids. Nevertheless, the fruit of Xyridaceae is also a capsule with dorsal dehiscence (Oriani and Scatena, 2014, 2017; Nardi et al., 2015), and a prominent convex floral apex was recently reported in some species of *Xyris* (Xyridaceae) (Nardi et al., 2021), which may also corroborate the close relationship between Eriocaulaceae and Xyridaceae.

In staminate flowers of *Eriocaulon*, carpels start to close at early developmental stages, as observed in Indian species of *Eriocaulon* (Sokoloff et al., 2020), but the fused part of the carpels becomes almost unnoticeable at maturity. Therefore, carpels develop into mostly free nectariferous carpellodes. A similar developmental pattern was described in *Paepalanthus* (Silva et al., 2016), but also occurs in other genera of Paepalanthoideae (A. L. Silva, UNESP, Brazil, unpubl. res.). In *Paepalanthus*, carpels do not close during development of the sterile gynoecium (Silva et al., 2016), but this difference is related to the point when the carpels start to differentiate. Our character reconstruction was inconclusive about the plesiomorphic state in the xyrids (Fig. 15D). However, the occurrence of a sterile gynoecium with nectariferous carpellodes in both Eriocauloideae and Paepalanthoideae indicates that these structures appeared only once, in the Eriocaulaceae ancestor, and is clearly associated with the shift to unisexuality.

In pistillate flowers of *Eriocaulon*, the stigmas are in a dorsal position and emerge as an apical bulge shortly after carpel closure, leading to ovary formation. In *Eriocaulon*, commissural structures do not emerge, and the overall gynoecium structure resembles that of many other Poales, consisting of ovary, style and dorsal stigmas, as in Cyperaceae (Vrijdaghs et al., 2009), Juncaceae (Oriani et al., 2012), Restionaceae (Ronse De Craene et al., 2002), Thurniaceae (Silva et al., 2020) and Xyridaceae (Remizowa et al., 2012; Sajo et al., 2017). In Paepalanthoideae, the nectariferous branches are in a dorsal position and follow the same developmental pattern as the stigmas of *Eriocaulon*, while stigmatic branches arise in a commissural position by fusion of adjacent carpel margins (Stützel, 1990; Stützel and Gansser, 1995; Rosa and Scatena, 2003; Silva et al., 2016). However, in *Syngonanthus* and *Paepalanthus*, species without nectariferous branches may occur because of abortion of the carpel apex (Stützel and Gansser, 1995; Silva et al., 2016). This gynoecium complexity observed in Eriocaulaceae shows that evolutionary interpretations should consider modifications of the different carpellary regions.

In Xyridaceae, the nectariferous appendages emerge later, only after style formation (Sajo et al., 2017). Previous studies have reported that the nectariferous branches of Paepalanthoideae and the stigmas of Eriocauloideae are vascularized by the dorsal carpel bundle (Rosa and Scatena, 2003, 2007; Silva et al., 2016), while the nectariferous appendages of Xyridaceae are vascularized by the nectariferous of the dorsal carpel bundles (Sajo et al., 2017). Data on vasculature should be discussed with caution, as reductions occur frequently in Eriocaulaceae, as shown in the present work. In any case, comparative analysis of flowers of *Eriocaulon* shows that gynoecium development in Eriocauloideae is more similar to that of Xyridaceae than that of Paepalanthoideae. Comparative

gynoecium development also supports the suggestion that the nectaries of Xyridaceae and Paepalanthoideae are not homologous (Sajo *et al.*, 2017).

Our character optimization considering gynoecium development (Fig. 15E) agrees with the hypothesis of Rosa and Scatena (2007) that the nectariferous branches of Paepalanthoideae are derived by morphological and functional modification of the dorsal region of the carpel, and that species of Paepalanthoideae lacking nectariferous branches have lost them secondarily. Like the nectariferous appendages of Paepalanthoideae, commissural stigmas appeared only once in this subfamily. Moreover, character optimization confirms that the nectaries of Eriocaulaceae and Xyridaceae had independent origins (Fig. 15F), as suggested by Sajo *et al.* (2017).

In previous morphological studies, the gynoecial nectaries of Eriocaulaceae and Xyridaceae are commonly referred to as ‘nectariferous appendices’ (Rosa and Scatena, 2003, 2007), ‘nectariferous appendages’ (Oriani and Scatena, 2012; Sajo *et al.*, 2017) or ‘nectariferous branches’ (Silva *et al.*, 2016, 2021; this study), but we note the importance of using distinct and standardized terminologies in these families since the structures are not homologous. In Xyridaceae, the term ‘nectariferous appendages’ is commonly used (Stützel, 1990; Oriani and Scatena, 2012; Sajo *et al.*, 2017), and we agree it should be maintained. In Eriocaulaceae (Paepalanthoideae), we propose the usage of the terms ‘nectariferous branches’ and ‘stigmatic branches’ for the nectariferous and stigmatic portions of the style, respectively. These terms have been used in taxonomic descriptions by several authors and in several genera, e.g. in *Actinocephalus* (Echternacht *et al.*, 2011), *Comanthera* (Echternacht *et al.*, 2015), *Leiothrix* (Giulietti and Silva, 2016; Giulietti, 2020), *Paepalanthus* (Sano *et al.*, 2015; Trovó *et al.*, 2015) and *Syngonanthus* (Watanabe *et al.*, 2015). In addition, in Eriocauloideae, we propose the usage of the term ‘stigmas’, as commonly used (Prajaksood and Chantaranonthai, 2002; Phillips and Masterházy, 2015; Prajaksood *et al.*, 2017; Sokoloff *et al.*, 2020), to make clear that they have a different origin from the stigmatic branches of Paepalanthoideae.

Finally, species of Eriocauloideae usually have petals with glands in both staminate and pistillate flowers. As previously discussed, although these glands are absent in some *Eriocaulon*, they are a synapomorphy of Eriocauloideae and were secondarily lost in the genus. In Paepalanthoideae, petal glands are also absent, but, in contrast to *Eriocaulon*, they do not emerge at any stage of development (Silva *et al.*, 2016).

Hypothesis of floral evolution of Eriocaulaceae

The ancestor of Eriocaulaceae probably had trimerous, bisexual flowers with a two-whorled androecium (Fig. 16A). Its gynoecium resembled the gynoecium of Eriocauloideae and many other Poales, which is syncarpous and has dorsal stigmas (Fig. 16A). The first and key evolutionary step toward the floral types of Eriocaulaceae was a shift from bisexual to functionally unisexual flowers (Fig. 16B, C). In staminate flowers, the carpels became sterile, changed their function and became nectariferous (Fig. 16A). In pistillate flowers, the stamens became staminodes (Fig. 16C).

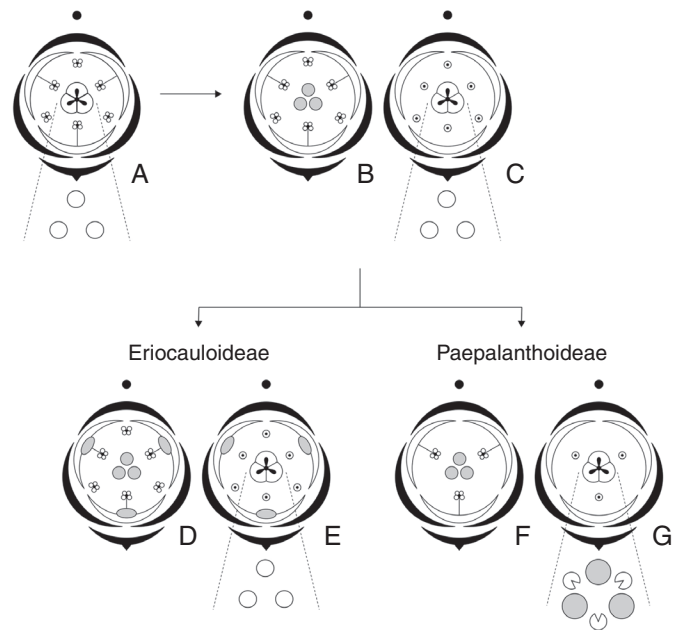


FIG. 16. Floral diagrams illustrating a hypothesis of floral evolution in Eriocaulaceae. (A) Ancestor of Eriocaulaceae with bisexual flowers. (B, C) Staminate (B) and pistillate (C) flowers of an intermediate ancestor. (D, E) Staminate (D) and pistillate (E) flowers of Eriocauloideae. (F, G) Staminate (F) and pistillate (G) flowers of Paepalanthoideae.

The current patterns found in Eriocauloideae and Paepalanthoideae were both derived from an intermediate ancestor with unisexual flowers and nectariferous carpelodes.

(1) In Eriocauloideae, the androecium remained two whorled (Fig. 16D, E) and the gynoecium retained its dorsal stigmas (Fig. 16E). However, Eriocauloideae developed petal glands in both staminate and pistillate flowers (Fig. 16D, E), originating from modification of the petal apex (Fig. 16B). As carpelodes and petal glands have a distinct position in the flower and distinct development, they are clearly not homologous. Instead, a transference of function could have occurred from carpelodes to petal glands. This hypothesis could also explain the morphological and anatomical similarities between these structures. The appearance of petal glands in Eriocauloideae seems to have been a key change in the subfamily, as they are apical and make nectar more readily available to floral visitors than the carpelodes.

(2) In Paepalanthoideae, the androecium became single whorled, as a result of complete loss of the outer stamen whorl (Fig. 16F, G). In pistillate flowers, the stigmas arise in the commissural region of adjacent carpels, while the nectariferous branches originate in the dorsal region by differentiation of the carpel apex (Fig. 16G). Although nectariferous carpelodes and nectariferous branches have a similar position and originate from the gynoecium primordium, they cannot be considered homologous, since their development and evolutionary timing are distinct. Nectariferous carpelodes originate from entire carpels and were already present in the Eriocaulaceae ancestor, while nectariferous branches originate from the carpel apex and appear only in the Paepalanthoideae lineage. The stigmatic branches probably originated concurrently with the nectariferous branches.

As the carpel apex became nectariferous in the gynoecium of Paepalanthoideae, the absence of stigmatic function would prevent the plant from reproducing. Thus, the apical carpel margins took over the stigmatic function to compensate the appearance of a dorsal nectary (Fig. 16C).

Conclusions

The floral morphology of *Eriocaulon* is highly diverse, with different degrees of fusion and reduction of floral parts. Our study provides clues to some of the developmental processes underlying this floral variation. Further developmental and anatomical studies including more species of *Eriocaulon*, combined with a robust phylogenetic analysis, will allow greater understanding of the evolution of floral characters within the genus.

Comparative floral development of *Eriocaulon* has also added important information about the floral structure of Eriocauloideae, allowing us to infer homologies between Eriocaulaceae and Xyridaceae, and helping us to reconstruct the evolutionary history of some floral characters in these families. The major evolutionary steps towards the flowers of Eriocaulaceae encompassed both a sexuality shift, and morphological and functional changes in the carpels or parts of the carpels in both pistillate and staminate flowers. Finally, our study helps us to place floral evolution of Eriocaulaceae in a broader context within the order Poales and emphasizes the importance of comparative morphological and developmental studies to comprehend floral evolution.

SUPPLEMENTARY DATA

Supplementary data are available at <https://academic.oup.com/aob> and consist of Appendix 1: morphological matrix with morphological characters and their respective states. (1) Sexuality: 0 bisexual flowers, unisexual flowers; (2) floral merism: 0 trimerous, 1 dimerous; (3) outer androecium whorl: 0 absent, 1 present; (4) carpellobes: 0 absent, 1 present; (5) carpels: 0 stigmatic apex and undifferentiated margins, 1 nectariferous apex and stigmatic margins, 2 aborted apex and stigmatic margins; (6) nectariferous appendages: 0 absent, 1 present.

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