Floral anatomy points to autogamy as the evolutionary path in Elatinaceae (Malpighiales)

Stéphani Karoline Vasconcelos Bonifácio (✉ skvbonifacio@gmail.com)
Universidade Federal de Minas Gerais

André M. Amorim
Universidade Estadual de Santa Cruz

Denise Maria Trombert Oliveira
Universidade Federal de Minas Gerais

Abstract

Since being established, the phylogenetic position of Elatinaceae has been controversial, but recent phylogenetic studies place it in Malpighiales as the sister group of Malpighiaceae. Molecular data support this relationship, but some morphological aspects of it are still poorly understood, especially floral evolution in Elatinaceae and its relationship with close lineages. Based on representatives of the two accepted genera in Elatinaceae, *Bergia*, and *Elatine*, we describe the floral anatomy of the family, compare it to that of the phylogenetically closest families, and discuss the relevance of anatomical floral characters. The presence of a glandular connective and hypostase are possible synapomorphies of the Elatinaceae + Malpighiaceae clade. The calyx glands present in Elatinaceae comprise cells with phenolic content without secretory activity. Some characters are autapomorphies for Elatinaceae, such as a reduction in the number of microsporangia and a thin-walled endothecium. However, other characteristics, such as a gamosepalous calyx, non-functional calyx glands, axile placentation, pendulous ovules, and intercarpellary vascular complexes, also occur in other families in the Malpighiales. The autapomorphies of Elatinaceae are evidence of structural adaptations of the flower in favor of autogamy, which could explain numerous morphological reversions. This aspect constitutes a marked difference in the Elatinaceae + Malpighiaceae clade since the latter is allogamous and Elatinaceae is autogamous, processes that are relevant to the evolutionary history of the two lineages.

Introduction

Although monophyletic, the order Malpighiales contains highly morphologically diverse lineages, and some interfamilial relationships are still not clearly understood (Chase et al. 1993; Soltis et al. 2000; Davis et al. 2005; Wurdack and Davis 2009; Xi et al. 2012). Phylogenetically, one of the surprising changes to the topology of the order was positioning Elatinaceae as the sister group of Malpighiaceae (Davis and Chase 2004). The position of Elatinaceae had been controversial for a long time, as seen by the following: initially placed in Caryophyllales (Adanson 1763); related to Clusiaceae s.l. (Cambessédes 1829; Gray 1849) but without a clear position concerning any order; related to Frankeniaceae and Tamaricaceae (Niedenzu 1925; von Wettstein 1935); in Theales (Cronquist 1968); and, more recently, in Malpighiales but closest to Phyllanthaceae (Savolainen et al. 2000; Davis and Chase 2004).

Elatinaceae currently comprises two genera, *Bergia* L. and *Elatine* L.; each genus has around 25 species, and *Bergia* has the most plesiomorphic characters (Tucker 1986; Davis and Chase 2004; Kubitzki 2014a; Razifard et al. 2017a,b; Hassemer 2020). The family has a cosmopolitan distribution with small, annual or perennial species, terrestrial, aquatic, or amphibious herbs (Tucker 1986; Kubitzki 2014a). Despite being distributed on many continents, the family has never been the subject of a recent comparative anatomy study, especially considering its phylogenetic position. Further, the Elatinaceae + Malpighiaceae clade is known for its lack of morphological synapomorphies due to differences among its representatives.

Comparative floral anatomy studies of Malpighiales clades have revealed evolutionary characteristics of some families in the order: Chrysobalanaceae, Dichapetalaceae, Euphroniaceae, Trigoniaceae (Matthews and Endress 2008), Caryocaraceae, Ctenolophonaceae, Erythroxylaceae, Irvingiaceae, Linaceae, Rhizophoraceae (Matthews and Endress 2011), Medusagynaceae, Ochnaceae, Quiinaceae (Matthews et al. 2012), Lophopyxidaceae, and Putranjivaceae (Matthews and Endress 2013). However, there is still no floral structure data that could be used to help recognize synapomorphies of the Elatinaceae + Malpighiaceae clade and plesiomorphies in Malpighiaceae. Thus, this work aimed to search for anatomical synapomorphies of the Elatinaceae + Malpighiaceae clade and, consequently, possible plesiomorphies in Malpighiaceae.

Materials And Methods

We selected floral buds and flowers at the anthesis of species of the two genera of Elatinaceae. The botanical material was taken from herbarium specimens, *Bergia perennis* F.Muell. (SP 174868) and *Elatine gratioloides* A.Cunn. (SP 169666), and field collections (Fig. 1a−b), *Elatine lindbergii* Rohrb. (BHCB 208077) and *Elatine triandra* Schkuhr (BHCB 208076). The species were chosen based on our ability to collect them and what was available in herbarium collections and in good condition. Young fruits were used to confirm the vasculature when only small procambial strands were found in the flowers.

Fresh material was fixed in FAA (Johansen 1940) or Karnovsky fixative (Karnovsky 1965); the samples of the latter were partially dehydrated, and all the samples were stored in 50% ethanol. The samples from herbarium specimens were boiled in water, treated following the method Mello et al. (2019) modified from Smith and Smith (1942), dehydrated in an increasing ethyl alcohol series, and stored in 50% ethanol.

Subsequently, all the material was embedded in (2-hydroxyethyl)-methacrylate Leica (Paiva et al. 2011) and sectioned (4−6 µm thick) in transversal and longitudinal series using a Zeiss Hyrax M40 rotary microtome. The sections were stained with 0.05% toluidine blue in an acetate buffer at pH 4.7 (O'Brien et al. 1964 modified) and mounted in synthetic resin (Entellan).

Part of the material was submitted to the following histochemical tests: ferric chloride for total phenolic compounds, lugol for starch, ruthenium red for pectins (Johansen 1940), Sudan red B for lipids (Brundrett et al. 1991 modified), and Ponceau xylidine for proteins (Vidal 1970).

To compare the floral anatomy of Elatinaceae and Malpighiaceae, we listed the main characteristics in the literature of the closest phylogenetically related families based on the most recent topology for Malpighiales (Xi et al. 2012).

The images were taken using an LC20 camera coupled to an Olympus CX41 microscope. The figures were edited, and the plates were made using CorelDraw Graphics Suite 2020.

Results

Bergia perennis

Floral Vasculature:

A median longitudinal section of a floral bud is shown in Fig. 2a and is the reference to the height of the images described below. The pedicel has a vascular cylinder that is a eustele (Fig. 2b). In the receptacle, the vascular cylinder is formed by inconspicuous bundles, from which a trace is emitted to each of the five sepals (Fig. 2c). Above this, traces to each of the five petals are emitted, and alternate to these there is a trace to each of the five antisepalous stamens (Fig. 2d–e). Each bundle divides into three bundles in the middle of the sepals (Fig. 2a, f). Acropetally, the carpellary bundles can be seen; initially, a dorsal bundle is emitted to each of the five carpels (Fig. 2g). In the central portion of the ovary, five intercarpellary complexes differentiate (Fig. 2h). Each ventral complex supplies the ovules of adjacent carpels through projections of the placenta (Fig. 2i); each complex emits one ventral trace the supplies two adjacent carpels (Fig. 2j). At this height, the ventral bundle supplies the placenta, which is axile and projects into the locule with ovules on its margin (Fig. 2j–k).

Floral anatomy

The calyx is formed by five free sepals (Fig. 2e) that have tector trichomes on the abaxial surface (Fig. 3a). In the mesophyll of the sepals, there are crystals in the subepidermal layer and phenolic idioblasts (Fig. 3a). The petals have idioblasts with druses in the subepidermal layer of the adaxial surface; the abaxial surface is characterized by the presence of phenolic content, as well as idioblasts in the mesophyll (Fig. 3b). The stamens, at the height of the filaments, are characterized by a unistratified epidermis that surrounds the parenchymatous tissue (Fig. 3c).

In the middle region of the ovary, the wall of each carpel is formed by an outer epidermis with cells with phenolic content, parenchymatous mesophyll with the most inner layer with crystalliferous content (Fig. 3c), and a unistratified inner epidermis (Fig. 3d). In the apical region of the ovary, the ovary walls are not fused, evidencing the compitum (Fig. 3d). The compitum is followed by the stylar canal, delimited by the five fused styles, which are vascularized by the respective dorsal bundles (Fig. 3e) and separate further up the flower, as evidenced by the five papillose stigmas (Fig. 3f).

The anthers are introrse and tetrasporangiate (Fig. 3e). In the connectives, the epidermis has idioblasts with phenolic content (Fig. 3g). Each microsporangium is covered by epidermis and endothecium with filiform thickness, which border partially collapsed parietal layers (Fig. 3h); the pollen grains do not exhibit apparent ornamentation (Fig. 3h). The ovules are anatropous, bitegmic, and have a zigzag micropyle (Fig. 3i).

Elatine **spp.**

Floral vasculature

Median longitudinal sections of floral buds of *Elatine gratioloides*, *E. lindbergii*, and *E. triandra* are shown in Fig. 4a–c and reference the heights of images described below. The vascular cylinder in the pedicel has few tracheary elements and is a siphonostele (Fig. 4d–f). In all the *Elatine* species studied, a single trace is emitted to each sepal, totaling four traces in *E. gratioloides* and three in *E. lindbergii* and *E. triandra* (Fig. 4g–i). Each petal has one vascular bundle; it has the same origin as the antipetalous stamens in *E. gratioloides* (Fig. 4j), resulting from a petal-antipetalous stamen complex, or is directly from the vascular cylinder in *E. lindbergii* and *E. triandra* (Fig. 4k–l). In all species, each stamen receives one trace, which is shared with the adjacent petal in *E. gratioloides* (Fig. 4m) or directly from the vascular cylinder in the other species (Fig. 4n–o). In *E. gratioloides*, the antipetalous stamens share their origin with the carpels, specifically with the dorsal bundles, constituting antisepalous stamen-carpel complexes (Fig. 5a). At the same height, in the other *Elatine* species, dorsal bundles are emitted; however, in the central portion, ventral bundles differentiate in *E. lindbergii* (Fig. 5b, f) and ventral intercarpellary complexes differentiate in *E. triandra* (Fig. 5c, g). In *E. gratioloides*, the ventral bundles are organized in ventral complexes corresponding to the fusion of ventral bundles of adjacent carpels (Fig. 5d).

The extension of the gamosepalous calyx varies in the species studied. It extends to the height of the ovary locules in *Elatine gratioloides* (Fig. 5d), to the height of the emission of the carpellary bundles in *E. lindbergii* (Fig. 5b), and to the emission of the stamen traces in *E. triandra* (Fig. 4o). Acropetally, in *E. gratioloides* the dorsal bundles and antisepalous stamens are individualized (Fig. 5e). Each ventral complex reconfigures and supplies one carpel, emitting traces that project through the placenta towards the ovules (Fig. 5h–j).

Floral anatomy

The epidermal sepal surfaces are the same and have (Fig. 6a) or lack (Fig. 6b–c) phenolic idioblasts. The mesophyll of the sepals comprises a few cell layers that are not continuous throughout the sepals (Fig. 6c). The petals are similar to the sepals in the structure of the epidermis and mesophyll (Fig. 6d-f). The stamens, at the height of the filaments, are characterized by a unistratified epidermis surrounding parenchymatous tissue (Fig. 6g-i).

In the median region of the ovary, the wall of each carpel is formed by a unistratified outer epidermis that has (Fig. 6j–k) or lacks (Fig. 6f) cells with phenolic content, parenchymatous mesophyll (Fig. 6f, j–k), and a unistratified inner epidermis (Fig. 6f, j–k). In the apical region of the ovary, the ovary walls are not fused in *Elatine lindbergii* and *E. triandra*, revealing the compitum (Fig. 6l–m). Acropetally, the ovary walls that delimit the locules fuse (Fig. 6n) and are followed by three distinct styles (Fig. 6o) with papillose stigmas at the apex (Fig. 6p). In *E. gratioloides*, a compitum does not form (Fig. 6q) and, atypically, the styles of the carpel are fused to the anthers of the antisepalous stamens (Fig. 6r).

In all the species, the ovules form on projections of the placenta, which can be apical or basal. They are anatropous with a zigzag micropyle and distinct curvature in the chalazal region with a hypostase. The trace emitted by the ventral bundle reaches the ovule through a short funiculus (Fig. 7a−c).

The anthers are bi- (Fig. 7d−e) or trisporangiate (Fig. 7f), with each microsporangium covered by a unistratified epidermis, endothecium with filiform (Fig. 7g−h) or thin-walled (Fig. 6i) thickness and other parietal layers; the pollen grains are tricellular at the time of dispersion (Fig. 7i). In *Elatine lindbergii*, the epidermis of the anther in the region of the connective and near the line of dehiscence has idioblasts (Fig. 7j); in this species, we registered pollen grains that germinate inside the anthers (Fig. 7k).

Secretory Structures

Sections of the sepals revealed that *Bergia perennis* has gland-like marginal structures (Fig. 8a), and *Elatine gratioloides* (Fig. 8b) and *E. lindbergii* have apical glands. These apical glands can only be differentiated by their idioblasts with dense cytoplasm and xylem vasculature (Fig. 8c−e). *Elatine triandra* has no sepal modifications or vasculature in the apical portion (Fig. 8f). We did not observe secretory activity or exudate from the apical sepal glands. The histochemical tests had a positive reaction for total phenolic compounds (Fig. 8g), cuticle on the epidermis (Fig. 8h), pectic cell walls (Fig. 8i), and protein bodies in the idioblasts (Fig. 8j).

In addition to these structures, we also observed idioblasts in the epidermis of the anther of *Elatine lindbergii* (Fig. 8k) with content similar to the apical cells of the sepals. During the histochemical analyses, we observed the presence of pectic substances in the cell walls and inside the pollen grains (Fig. 8l); the pollen grains also reacted positively to protein (Fig. 8m). Concerning the content in the idioblasts, there was only a slight reaction that indicated the presence of phenolic compounds (Fig. 8n).

Discussion

Floral anatomy of Elatinaceae in a Malpighiales context

Compared to Malpighiaceae, the floral anatomy of Elatinaceae exhibits the following character reversions: absence of fused lateral sepal traces, lack of calyx and corolla adnation, and absence of a hypanthium (Table 1). However, a connate calyx, axile placentation, and pendulous ovules are present in Elatinaceae, Caryocaraceae, Lophopyxidaceae, Malpighiaceae, and Putranjivaceae.

Table 1

– Main floral anatomy characters observed in Elatinaceae, compared to those of the phylogenetically close families in Malpighiales (sensu Xi et al. 2012): Malpighiaceae, Centroplacaceae, Caryocaraceae, Putranjivaceae and Lophopyxidaceae. (?) indicates unknown data; (-) indicates lack of literature; (*) our personal observation; it indicates that characters described in the literature were referred applying the terminology adopted in this work.

Flower region	Characters	Elatinaceae	Malpighiaceae	Centroplacaceae	Caryocaraceae	Putranjivaceae	Lophopyxidaceae
Pedicel	1. Vascular cylinder organization	Siphonostele[1]	Siphonostele or eustele[24]	?	Siphonostele or eustele[11,14]	Siphonostele or eustele[15]	Eustele[15]
Calyx	2. Gamosepalous at some extesion	Present[1,17]	Present[3,5]	?	Present[11,14]	Present[15]	Present (pistillate flowers) [15]
	3. Fusion of the lateral sepal traces	Not applicable[1]	Present[9,24]	?	Present[11,14]	Present[15]	Absent[15]
	4. Traces per sepal	1[1]	3[9,16,24]	?	3-many[11,14]	1 or 3[15]	3[15]
	5. Origin of vasculature	Sepal traces[1]	Sepal traces and sepal-petal complexes[9,16,24]	?	*Sepal traces and sepal-petal complexes[11,14]	Sepal traces[15]	Sepal traces and sepal-petal complexes? [15]
	6. Calyx glands on abaxial face of sepals	Absent[1]	Present[3,4]	?	Present[14]	Absent[15]	Absent[15]
	7. Type of gland	Vestigial[1]	Elaiophores or nectaries or mixed secretion[6,12,18,27,28]	?	?	Not applicable[15]	Not applicable[15]
	8. Fusion with the corolla	Absent[1]	Present (ground and vascular tissues) [9,16,24]	?	Present (vascular complexes)[11,14]	Not applicable[15]	Present (ground and vascular tissues) [15]
	9. Vasculature in the external region of the receptacle produced by basipetal sepal traces	Absent[1]	Present[9,16,24]	?	Present[11,14]	Absent (?)[15]	Absent (?)[15]
Corolla	10. Connate petals	Absent[1,17]	Absent[3,5]	?	Absent[11,14]	Not applicable[15]	Absent[15]
	11. Traces per petal	1[1]	1 (3) [9,16,24]	?	3-many[11,14]	Not applicable[15]	1[15]
	12. Adnation to androecium	Present (rare)[1]	Present (vascular complexes)[9]	?	Present[11,14]	Not applicable[15]	Absent[15]
Androecium	13. Fusion degree among stamens	Free[1]	Free or connate[3,5]	?	Connate[11,14]	Free or connate[15]	Free[15]
	14. Traces per stamen	1[1]	1[9,16,24]	?	1[11,14]	1[15]	1[15]
	15. Anther glandular epidermis	Present (restricted to land species)[1]	Present[3,7,8,18]	?	?	?[15]	?[15]
	16. Occurrence of staminodes	Absent[1]	Present[3,5]	Present in pistillate flowers[13]	Present[11,14]	Absent[15]	Absent[15]
Hypanthium	17. Occurrence of the hypanthium (floral tube)	Absent[1]	Present[9]	?	Present?[11,14]	Present[15]	Present[15]

Data sources: (1) This study; (2) Aliscioni et al. 2019; (3) Anderson 1979; (4) Anderson 1990; (5) Anderson et al. 2006; (6) Araújo and Meira 2016; (7) Arévalo-Rodrigues et al. 2020; (8) Avalos et al. 2020; (9) Bonifácio et al. 2021; (10) Dathan and Singh 1971; (11) Dickison 1990; (12) Guesdon et al. 2019; (13) Kubitzki 2014; (14) Matthews and Endress 2011; (15) Matthews and Endress 2013; (16) Mello 2017; (17) Niedenzu 1925; (18) Possobom et al. 2015; (19) Ramayya and Rajagopal 1971; (20) Salisbury 1967; (21) Souto and Oliveira 2005; (22) Souto and Oliveira 2008; (23) Souto 2011; (24) Souto and Oliveira 2013; (25) Souto and Oliveira 2014; (26) Souto and Oliveira 2020; (27) Vogel 1974; (28) Vogel 1990.

Flower region	Characters	Elatinaceae	Malpighiaceae	Centroplacaceae	Caryocaraceae	Putranjivaceae	Lophopyxidaceae
	18. If hypanthium is present, whorls involved	Not applicable[1]	Perianth + androecium[9]	?	Corolla + androecium + gynoecium[11,14]	Calyx + androecium or calyx + gynophore[15]	Perianth (pistillate flowers) or perianth + androecium (staminate flowers)[15]
Gynoecium	19. Number of carpels	3–5 (rarely 2)[1,10,20]	3[3,5]	?	3-many[11,14]	1-many[15]	4-5[15]
	20. Styles fusion	Styles are free or fused[1]	Styles are free or fused[3,5]	?	Free styles[11,14]	Fused to the mid or apical region of the style[15]	Fused to the mid or apical region of the style[15]
	21. Differentiated carpel dorsal bundle	Present[1]	Absent[16,24,25,26]	?	Absent[11,14]	Present[15]	Present[15]
	22. Ventral bundle per carpel	1[1]	1[9,16,24]	?	2[11,14]	1-2[15]	2?[15]
	23. Fusion of ventral bundles between adjacent carpels	Present[1]	Absent[9,16,24]	?	Absent[11,14]	Absent?[15]	Absent?[15]
	24. Styles vascularized by adjacent carpels	Absent[1]	Absent[9,16,24]	?	Present[11,14]	Absent?[15]	Absent?[15]
	25. Occurrence of compitum	Present[1]	Present[2]		Present[14]	Present[15]	Present[15]
	26. Insertion of ovules	Basal or pendulum[1]	Pendulum[21,25]	?	Pendulum[14]	Pendulum[15]	Pendulum[15]
	27. Type of placentation	Axile[1,17,19]	Axile[26]	?	Axile[11,14]	Axile[15]	Axile[15]
	28. Placenta invading locules	Present[1]	Absent[26]	?	Absent[11,14]	Present[15]	Absent?[15]
	29. Occurrence of hypostase	Present[1]	Present[21,22,25]	?	Absent[11,14]	Present[15]	Absent?[15]
Secretory structures	30. Occurrence of nectaries	Absent[1]	Absent (in neotropical species)[4] Present (in paleotropical species)[12]	Present[13]	Present[11,14]	Present (Absent in *Putranjiva*)[15]	Present[15]
	31. If nectaries are present, position	Not applicable[1]	Abaxial face of sepals[12]	Receptacular?[13]	Staminodes[11,14]	Around the gynoecium[15]	Inner surface of the hypanthium[15]
	32. If nectaries are present, origin of vasculature	Not applicable[1]	Calyx?[12]	?	Androecium[11,14]	Gynoecium[15]	Corolla[15]

Data sources: (1) This study; (2) Aliscioni et al. 2019; (3) Anderson 1979; (4) Anderson 1990; (5) Anderson et al. 2006; (6) Araújo and Meira 2016; (7) Arévalo-Rodrigues et al. 2020; (8) Avalos et al. 2020; (9) Bonifácio et al. 2021; (10) Dathan and Singh 1971; (11) Dickison 1990; (12) Guesdon et al. 2019; (13) Kubitzki 2014; (14) Matthews and Endress 2011; (15) Matthews and Endress 2013; (16) Mello 2017; (17) Niedenzu 1925; (18) Possobom et al. 2015; (19) Ramayya and Rajagopal 1971; (20) Salisbury 1967; (21) Souto and Oliveira 2005; (22) Souto and Oliveira 2008; (23) Souto 2011; (24) Souto and Oliveira 2013; (25) Souto and Oliveira 2014; (26) Souto and Oliveira 2020; (27) Vogel 1974; (28) Vogel 1990.

A basally connate calyx, here observed only in *Elatine*, has also been reported for Caryocaraceae (Matthews and Endress 2011), Lophopyxidaceae (Matthews and Endress 2013), and Malpighiaceae (Anderson 1979). In these families, the calyx and corolla share a vascular complex (Dickison 1990; Matthews and Endress 2011, 2013; Souto and Oliveira 2013; Bonifácio et al. 2021), which represents the adnation of these whorls. This characteristic was

lost in Elatinaceae and Putranjivaceae (Matthews and Endress 2013), and the latter lacks petals. Similarly, a hypanthium was lost in Caryocaraceae (Dickison 1990; Matthews and Endress 2011) and Elatinaceae (our data).

In the clades phylogenetically closest to the malpighioid clade (Xi et al. 2012), the presence of glands on the sepals of Malpighiaceae (Anderson 1979, 1990; Vogel 1990) and Caryocaraceae (Matthews and Endress 2011) is notable. In Malpighiaceae, the calyx glands are commonly elaiophores, or nectaries in some paleotropical lineages, which are between adjacent sepals (Anderson 1979, 1990; Vogel 1990; Souto and Oliveira 2013; Mello 2017; Bonifácio et al. 2021). We found that the sepal glands in Elatinaceae are vestigial and located only at the apex, with no distinction between the adaxial and abaxial surfaces. At the same time, the literature reports the presence of these glands only on the abaxial surface of the sepals (Endress et al. 2013). In Caryocaraceae, these glands are on the abaxial surface near the apex of the sepals. They are structurally similar to the elaiophores in Malpighiaceae, but their secretory nature is not known (Matthews and Endress 2011). The presence of floral nectaries, independent of the whorl they are on, occurs in all the close families in Malpighiales except in Elatinaceae (in this work): Caryocaraceae (Dickison 1990; Matthews and Endress 2011), Centroplacaceae (Kubitzki 2014b), Malpighiaceae (Guesdon et al. 2019), Lophopyxidaceae, and Putranjivaceae (Matthews and Endress 2013). The absence of nectaries is evidence that Elatinaceae diverged in the direction of autogamy and was independent of biotic pollination agents.

Evolutionary analyses of anatomical flower characters of Malpighiales revealed the relevance of the gynoecium (Endress et al. 2013), notably the presence of pendulous ovules, a characteristic of the COM clade (Celastrales, Oxalidales, and Malpighiales) (Endress 2011; Endress et al. 2013), and axile placentation, a well-established character in the malpighioide clade (Endress et al. 2013).

The fused ventral bundles observed in Elatinaceae have also been reported for Malpighiaceae (Souto and Oliveira 2013, 2020; Mello 2017). However, unlike Malpighiaceae, in Elatinaceae, intercarpellary complexes vascularize two carpels simultaneously (as recorded here in *Bergia perennis* and *Elatine lindbergii*), indicating lateral fusion between carpels. The presence of intercarpellary complexes has been recorded in other families of Malpighiales, such as Euphorbiaceae (our observation in Fig. 4i of De-Paula et al. 2011), Linaceae (our observation in Fig. 24l of Matthews and Endress 2011), Putranjivaceae (our observation in Fig. 13e of Matthews and Endress 2013), Rhizophoraceae (our observation in Fig. 5n of Matthews and Endress 2011), and Dichapetalaceae and Trigoniaceae (our observation in Figs. 13p and 17j, respectively, of Matthews and Endress 2008). However, sharing ventral bundles between adjacent carpels is not commonly reported; it has been recorded in *Anthodiscus* (Caryocaraceae), where each style receives one bundle from adjacent carpels (Dickison 1990). The presence of intercarpellary complexes could be an interesting character in investigating the lateral fusion and the numeric variation of carpels, which are very recurrent in Malpighiales (Endress et al. 2013).

Potential Synapomorphies Between Elatinaceae And Malpighiaceae

The flower characters shared between Elatinaceae and Malpighiaceae observed in this study are the presence of a glandular connective and hypostase. The presence of a glandular connective has never been reported for Elatinaceae. It is variable among species since it was not observed in the plants with an aquatic habit (*Elatine gratioloides* and *E. triandra*). The glandular connective is possibly related to a terrestrial habit because it is present in species that occupy this type of environment (*Bergia perennis* [Leach 1989] and the amphibious *E. lindbergii* [Bittrich 2002]). Considering that the aquatic lineage of Elatinaceae diverged more recently, in contrast to the terrestrial lineages with secondary growth (Davis and Chase 2004), the presence of a glandular connective is plesiomorphic for the family.

For Malpighiaceae, the presence of secretory structures on the anther connective has been known for some time (Gates 1982; Anderson 1990) and repeatedly reported in the literature (Possobom et al. 2015; Arévalo-Rodrigues et al. 2020; Avalos et al. 2020). These glands are composed of globose epidermal cells with phenolic content in the vacuole and secrete oil (Arévalo-Rodrigues et al. 2020) or are glands that secrete a mixture of oil and sugars (Possobom et al. 2015).

In relation to the ovules, the presence of a hypostase has also been reported for Malpighiaceae in *Byrsonima* (Souto and Oliveira 2005), *Banisteriopsis* and *Diplopterys* (Souto and Oliveira 2008), and *Janusia*, *Mascagnia*, and *Tetrapterys* (Souto and Oliveira 2014). Further, a hypostase has been reported for other families of Malpighiales, such as Euphorbiaceae s.l. (Landes 1946; Carmichael and Selbo 1999) and Chrysobalanaceae (Tobe and Raven 1984). Since ovule characters are significant in superfamilial analyses in Malpighiales (Endress et al. 2013), future studies comparing Malpighiaceae and Elatinaceae and including more clades could provide more information about the importance and constancy of these characters. In the same way, characters associated with seeds are conserved in *Elatine*, unlike its vegetative organs that exhibit high phenotypic plasticity when submitted to environmental variations (Molnár et al. 2015).

The gynoecium characters that differ the most between Elatinaceae and Malpighiaceae, such as more carpels and numerous ovules in Elatinaceae, are related to greater investment in the female flowers associated with attracting pollinators in Malpighiaceae. The evolution of Malpighiaceae was especially influenced by a mutualistic relationship with oil-collecting bees, resulting in allogamy and dependence on pollinators (Sigrist and Sazima 2004). In contrast, Elatinaceae tend to not depend on pollinators and instead rely on autogamy (Uphof 1938; Tucker 1986; Razifard et al. 2017a).

Some data about the biogeography of Elatinaceae point to the origin and distribution of the family in the direction of temperate regions (Cai et al. 2016). This information seems to go against some of the advantages commonly related to autogamy: colonization from a single propagule, less investment in the androecium, and successfully reproducing in locations where pollinators are limited, for example, in artic environments (Baker 1955; Cheptou 2012; Pannell 2015).

The two families are currently circumscribed in the clade [Elatinaceae + Malpighiaceae] + Centroplacaceae, characterized by axile placentation, crassinucellate ovules, no endothelium, and persistent sepals on the fruits (Xi et al. 2012). Support for the clade is low due to the absence of morphological information for Centroplacaceae, and floral anatomy and embryological data of this family could help better explain the evolution of the clade.

The Floral Anatomy Of Elatinaceae

The floral anatomy analysis of Elatinaceae revealed that the presence of a siphonostele is a characteristic exclusive to this group among the closest Malpighiales (Table 1). The remaining characteristics, in contrast to the other Malpighiales families, reinforce how selection acted on the floral architecture of Elatinaceae in favor of autogamy and partially explain the numerous morphological differences between this family and Malpighiaceae.

The anatomical characters that are evidence of autogamy are the reduction in the number of microsporangia (Endress and Stumpf 1990; Battjes et al. 1994), precocious germination of pollen grains inside the anthers (Mazer and Delesalle 1998; Mann et al. 2021), and the small investment in attracting and rewarding pollinators, including the absence of floral nectaries (Mazer and Delesalle 1998; Goodwillie et al. 2010).

The absence of physical barriers that prevent self-fertilization is a strong indicator of floral adaptations to autogamy (Garnock-Jones 1976). In this work, the most extreme modifications to self-fertilization observed were the gynostegium and germination in situ. Pollen grain germination in situ can occur in both chasmogamous and cleistogamous species but must occur in cleistogamous species (Lord 1981). Interestingly, there are records of this type of process in Malpighiaceae; pollen grains have been observed germinating inside indehiscent anthers of cleistogamous flowers of *Gaudichaudia* spp. and *Janusia guaranitica* A. Juss. (Anderson 1980).

From an ecological perspective, autonomous self-fertilization is a strategy that guarantees reproduction (Zhang and Li 2008) and promotes establishment after long-distance dispersal when crossbreeding is not possible (Baker 1955; Llyoid 1992; Morgan and Wilson 2005; Passos et al. 2021). This can be related to the emergence of Elatinaceae, which underwent long-distance dispersal during the Eocene long after the emergence of Malpighiaceae at the beginning of the Cretaceous (Cai et al. 2016).

Since there are herbaceous species with an aquatic habit in Elatinaceae (Davis and Chase 2004), many anatomical floral characteristics of this group result from environmental adaptations and often evolutionary reversals. However, it is still possible to infer the evolution of the floral structure in the group based on its floral anatomy.

Of the characters observed, the presence of vascular complexes feeding adjacent carpels, placenta projecting to the interior of the locule, and hypostase on the ovules occur in all the Elatinaceae species we studied. The presence of differing adaxial and abaxial sepal surfaces was exclusively observed in *Bergia*, while gamosepalous calyx are only present in *Elatine*; these are possible synapomorphies for the genera.

Although uncommon in angiosperms, a reduction to one sepal trace is understandable considering physiological aspects (Puri 1951) since the plants are reduced in size, with tiny flowers, and adapted to aquatic environments. The reduced flowers can also be explained by a decrease in allocating resources to floral display (Goodwillie et al. 2010); autogamy is known to occur in some family species and, consequently, these species do not require pollinators (Uphof 1938; Tucker 1986).

Autogamy in Elatinaceae is frequently associated with cleistogamy in submersed flowers (Uphof 1938; Tucker 1986). However, we do not know if all the species we analyzed are cleistogamous because there is only information about the reproductive system of *Elatine triandra* (Uphof 1938). Despite this, aquatic, cleistogamous plants commonly lack endothecium thickening, as recognized here for *E. triandra*, since the wall of the microsporangia does not differentiate for active dehiscence (Maheshwari 1950). Additionally, in cleistogamous plants, a reduced corolla and reduced androecium are expected (Lord 1981). Other evidence of cleistogamy observed in this work was the androecium fused to the gynoecium in *Elatine gratioloides*, which points to an extreme specialization that favors autogamy. This fusion between the anthers of antisepalous stamens and the stigmas forms the gynostegium (sensu Endress 2016).

Conclusions

The floral anatomy characters we recorded for Elatinaceae that are shared with Malpighiaceae are the presence of glandular connective and hypostase. The scarcity of floral anatomy synapomorphies between these two families is expected because Malpighiaceae originated and diversified long before Elatinaceae. Therefore, the characters recorded in the extant representatives of Elatinaceae could result from rapid diversification where selection acted on floral morphology in favor of autogamous processes. Thus, we demonstrate how comparative studies based on phylogenetic relationships are relevant to understanding reversions (autapomorphies) and evolutionary novelties toward a specific type of reproductive system.

Declarations

Authors contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Stéphani Karoline Vasconcelos Bonifácio, André Márcio Amorim and Denise Maria Trombert Oliveira. The first draft of the manuscript was written by

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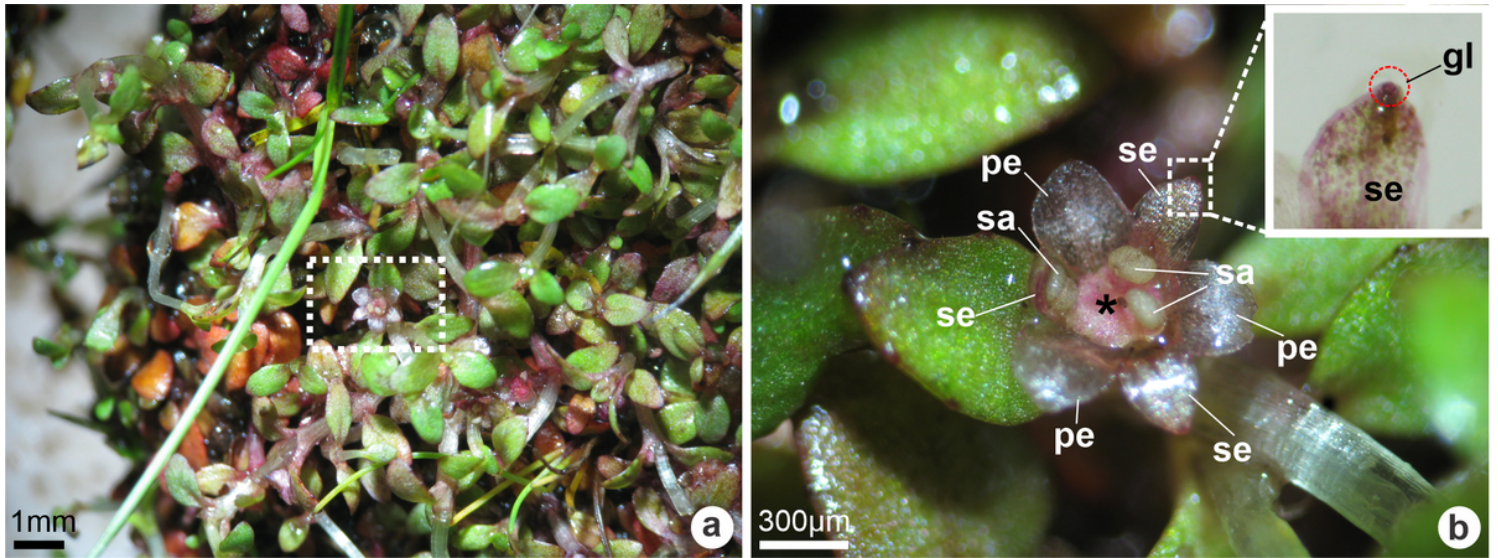
Figures

Figure 1

Elatine lindbergii. **a** General view of the plant; the rectangle highlights a flower. **b** Detail of a flower at anthesis; the insertion (on the right) is a magnification of the sepal showing the apical gland. *asterisk* gynoecium, *gl* calyx gland, *pe* petal, *sa* stamen, *se* sepal (Photo by S.K.V. Bonifácio)
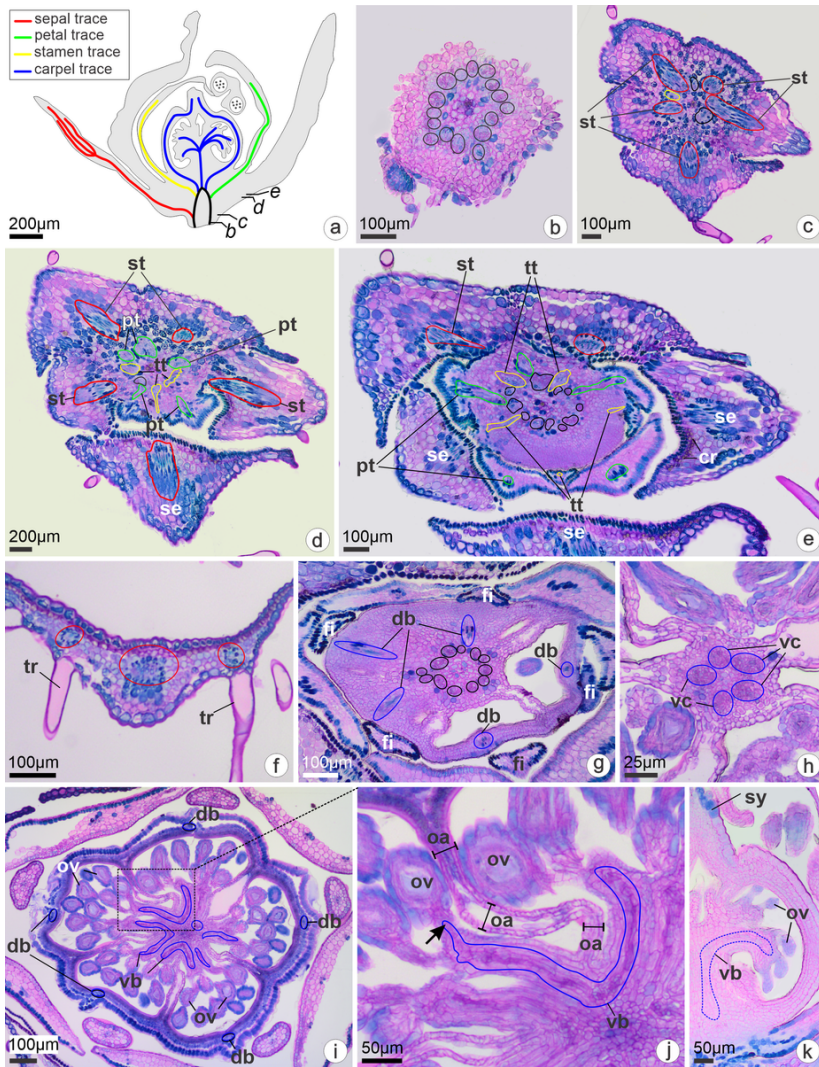

Figure 2

Floral vasculature of *Bergia perennis*. Longitudinal (a, k) and transverse (b–j) sections of floral buds. **a** Reconstruction of a floral bud showing the general pattern of the vasculature of the perianth; the letters indicate the corresponding following images. **b** General view of the pedicel showing the eustele vascular cylinder. **c–f** Vasculature of the perianth and androecium. **g–j** Vasculature pattern of the carpels; in **i**, note the ventral complexes that each supply two adjacent carpels; the arrow indicates the margin between adjacent carpels. **k** Apical vasculature of the placenta. *cr* crystal, *db* dorsal bundle, *fi* filament, *oa* ovary wall, *ov* ovule, *pt* petal trace, *se* sepal, *st* sepal trace, *sy* stigma, *tr* trichome, *tt* stamen trace, *vb* ventral bundle, *vc* ventral complex
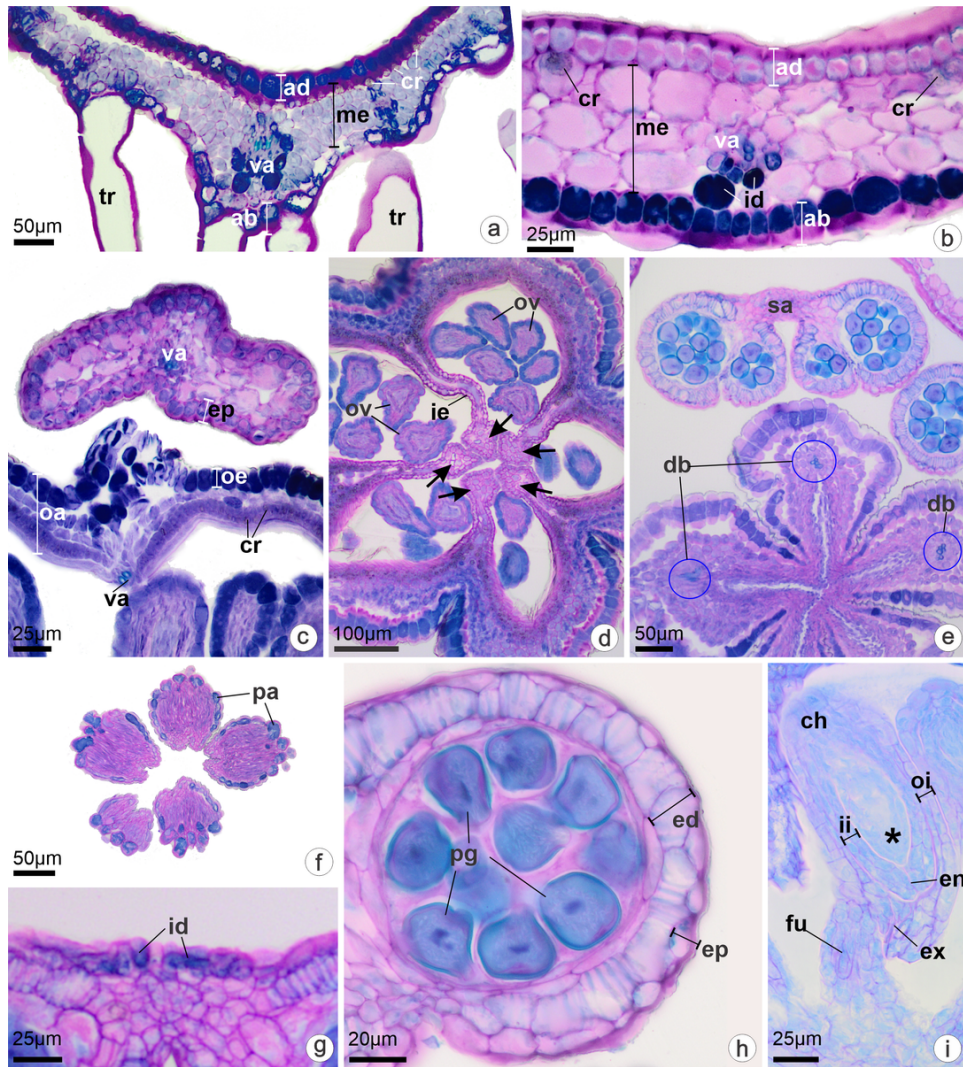

Figure 3

Floral anatomy of *Bergia perennis*. Transverse (a–h) and longitudinal (i) sections of a floral bud. **a** Detail of a sepal showing crystals in the subepidermal layer and trichomes on the abaxial surface of the epidermis. **b** Epidermis and mesophyll in the middle region of a petal. **c** General view of a filament and detail of the ovary wall. **d** Apical view of the ovary showing the compitum (the arrows indicate the independent ventral margins of the carpels). **e** General view of the style showing the single stylar canal and an apical view of a stamen; observe the tetrasporangiate anther with introrse dehiscence. **f** General view of the five stigmas; note the papillose epidermis. **g** Detail of the glandular connective. **h** Detail of a microsporangium showing the parietal layers and pollen grains. **i** General view of an ovule on the placenta. *asterisk* megasporangium, *ab* abaxial surface of the epidermis, *ad* adaxial surface of the epidermis, *ch* chalaza, *cr* crystal, *db* dorsal bundle, *ed* endothecium, *en* endostome, *ex* exostome, *ep* epidermis, *fu* funiculus, *id* phenolic idioblast, *ie* inner epidermis of the ovary, *ii* inner integument, *me* ovary mesophyll, *oa* ovary wall, *oe* outer epidermis of the ovary, *oi* outer integument, *ov* ovule, *pa* papillae, *pg* pollen grain, *sa* stamen, *tr* trichome, *va* vascular bundle
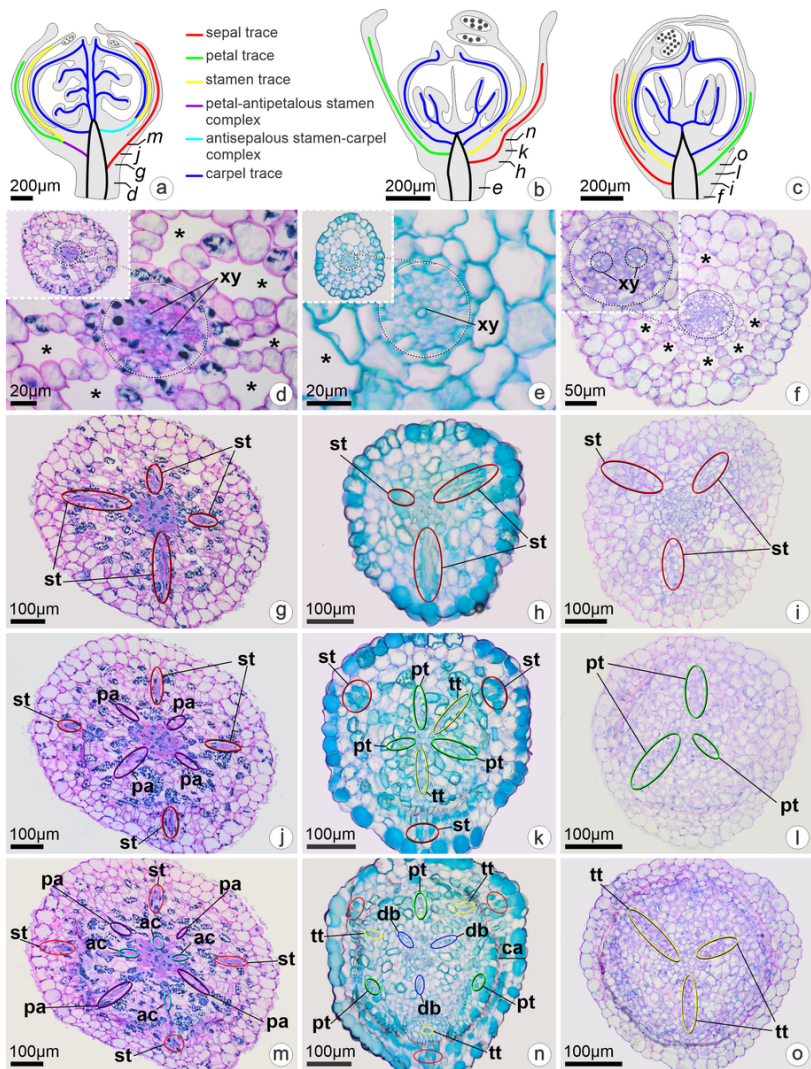
Figure 4

Floral vasculature of *Elatine*. Longitudinal (a–c) and transverse (d–o) sections of floral buds (a–k, m–o) and young fruit (l): *E. gratioloides* (a, d, g, j, m), *E.lindbergii* (b, e, h, k, n), and *E. triandra* (c, f, i, l, o). **a–c** Reconstructions of floral buds in longitudinal section showing the vasculature pattern; the letters indicate the corresponding following images. **d–f** General view of the pedicel showing the arrangement of the vascular cylinder; asterisks indicate intercellular spaces. **g–o** Emission of the perianth and androecium vasculature. *ac* antisepalous stamen-carpel complex, *db* dorsal bundle, *pa* petal-antipetalous stamen complex, *pt* petal trace, *st* sepal trace, *tt* stamen trace, *xy* xylem
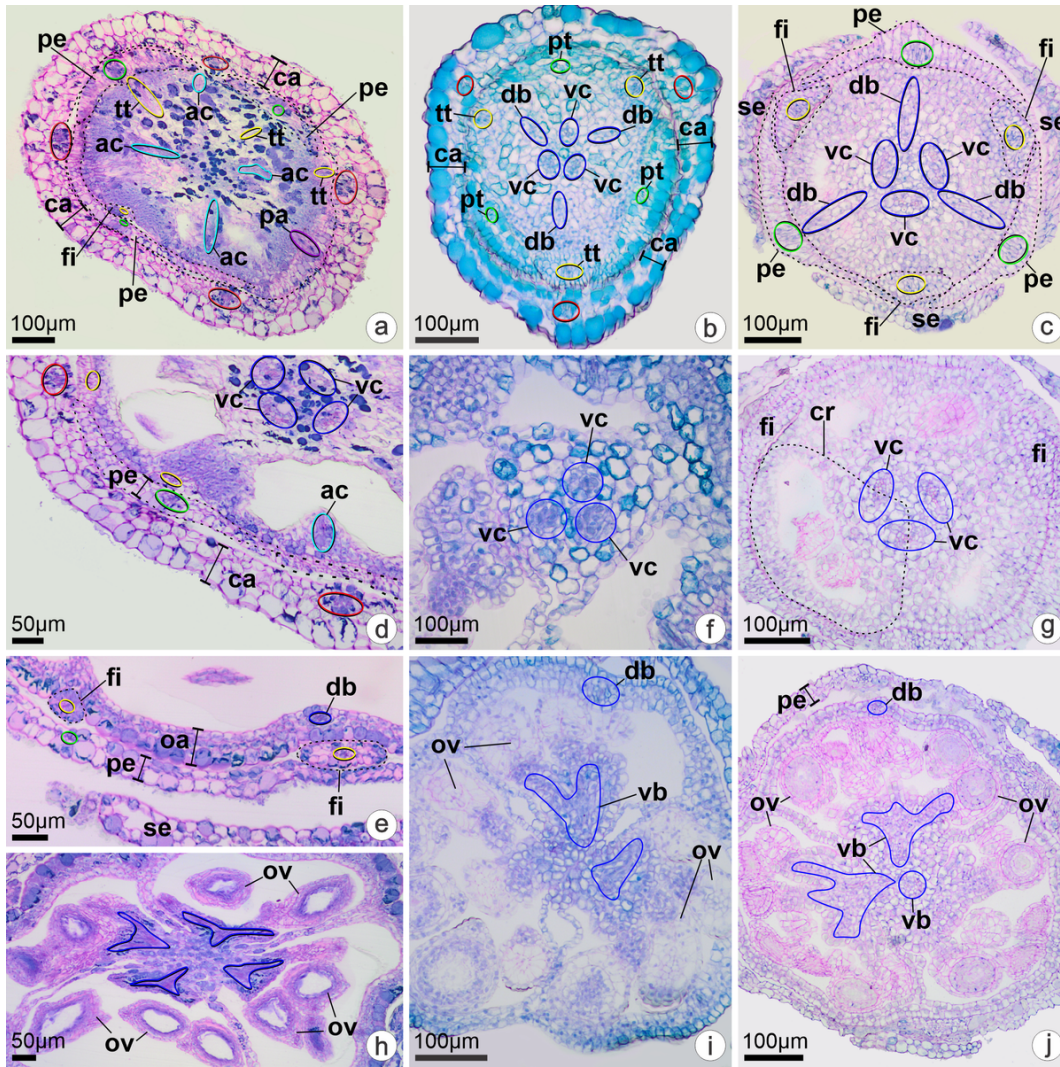
Figure 5

Carpel vasculature of *Elatine* in transverse sections of floral buds (a–f, h–j) and young fruit (g): *E.gratioloides* (a, d–e, h), *E. lindbergii* (b, f, i), and *E.triandra* (c, g, j). The colors on the images follow those indicated in fig. 4. **a–c** Initial emission of peripheral carpel vasculature (dorsal bundles). **d–g** Emission of intercarpellary complexes; observe the three ventral complexes. **h–j** Emission of ovule vasculature. *ac* antisepalous stamen-carpel complex, *ca* calyx, *db* dorsal bundle, *fi* filament, *oa* ovary, *ov* ovule, *pa* petal-antipetalous stamen complex, *pe* petal, *pt* petal trace, *se* sepal, *tt* stamen trace, *vb* ventral bundle, *vc* ventral complex
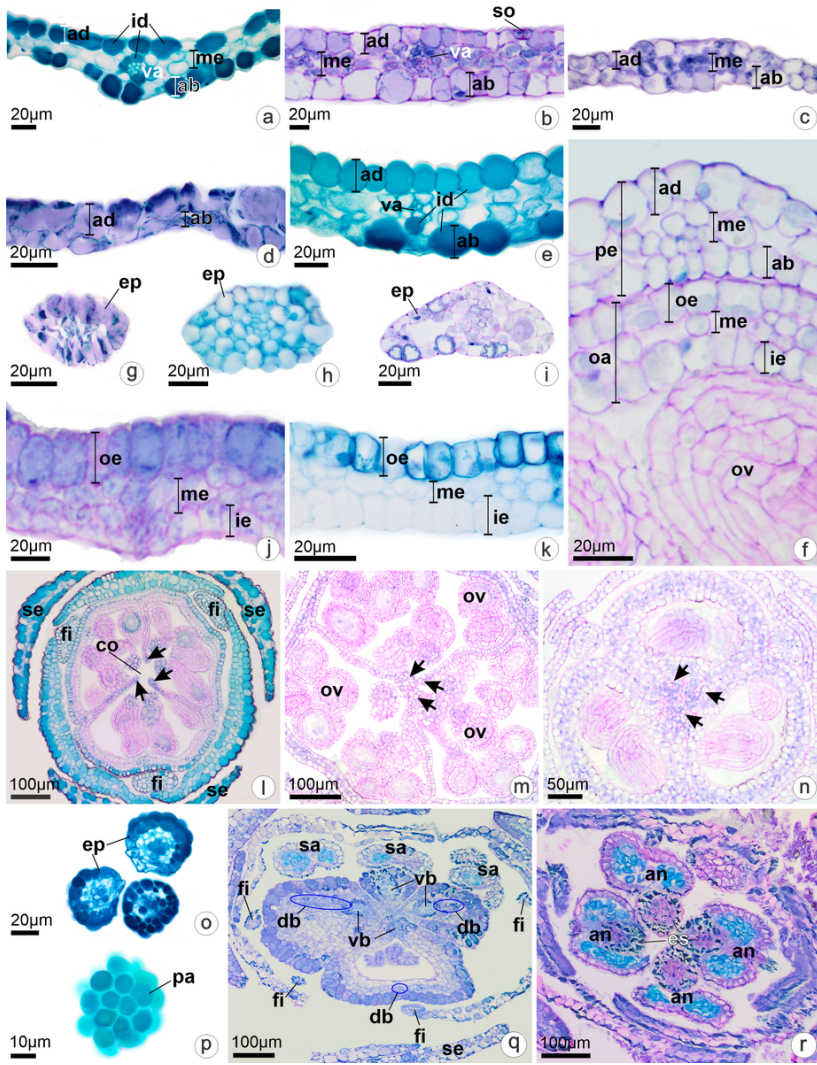
Figure 6

Floral anatomy of *Elatine* in transverse section: *E.gratioloides* (b, d, g, j, q−r), *E. lindbergii* (a, e, h, k−l, o−p), and *E.triandra* (c, f, i, m−n). **a−c** General view of the middle region of the sepal. **d−e** General view of the middle region of the petal. **f** Detail of the middle region of the petal and ovary. **g−i** General view of the filaments. **j−k** Detail of the ovary wall. **l−n** Apical region of the ovary; see the compitum in l-m. **o-r** Apical region of the carpel; note the free styles in o-q, the papillose stigma in p and the fusion of the apical region of the ovary to the anthers in r (gynostegium). *arrow* free ventral portion at the compitum, *ab* abaxial surface of the epidermis, *ad* adaxial surface of the epidermis, *an* anther, *co* compitum, *db* dorsal bundle, *ep* epidermis, *es* stigma, *fi* filament, *id* phenolic idioblast, *ie* internal ovary epidermis, *me* mesophyll, *oa* ovary, *oe* outer ovary epidermis, *ov* ovule, *pa* papilla, *pe* petal, *sa* stamen, *se* sepal, *so* stoma, *va* vascular bundle, *vb* ventral bundle
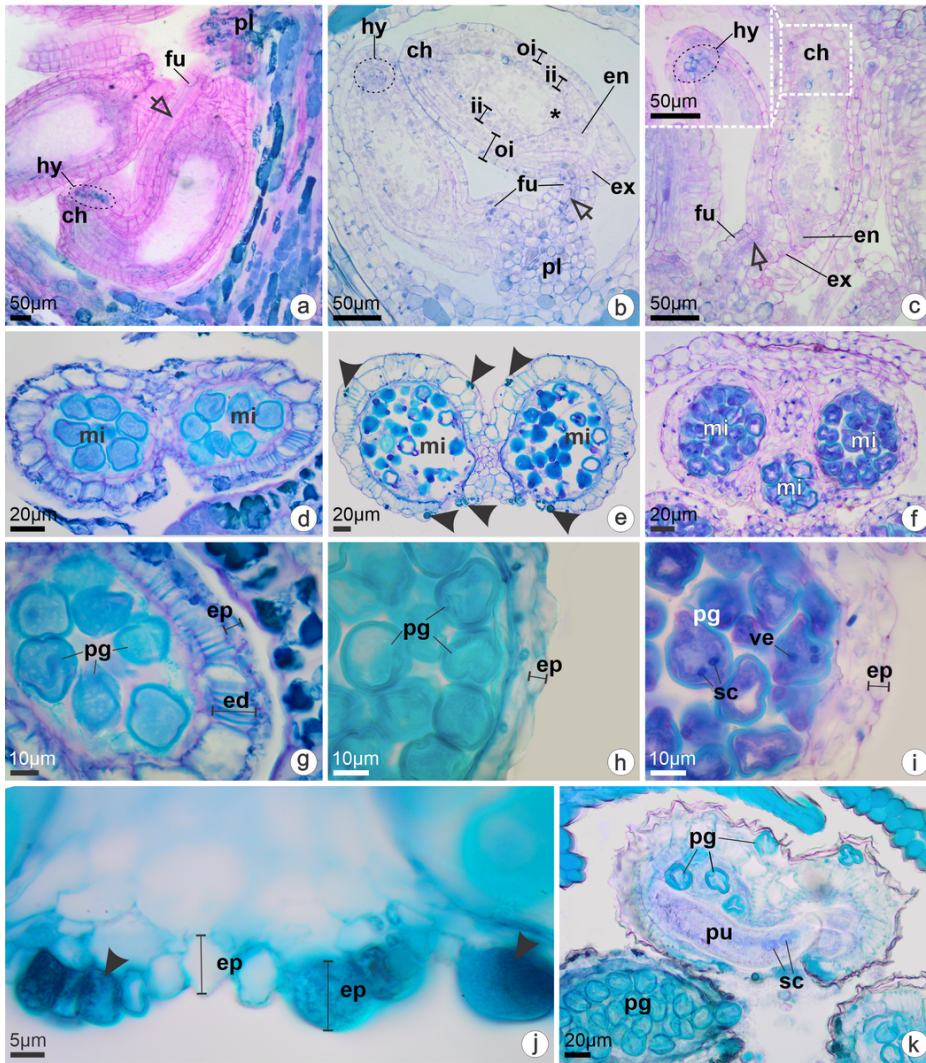
Figure 7

Anatomy of the ovules (a–c) and anthers (d–k) of *Elatine* in longitudinal (a–c) and transverse (d–f) sections of floral buds: *E. gratioloides* (a, d, g), *E. lindbergii* (b, e, h, j–k), and *E. triandra* (c, f, i). **a–c** Insertion of the ovule on the placenta; c highlights the hypostase. **d–f** Mature anthers. **g–i** Detail of the microsporangium walls. **j** Epidermis showing idioblasts. **k** Anther showing pollen grains. *asterisk* nucellus, *arrowhead* secretory epidermis cell, *arrow* vasculature of the ovule, *ch* chalaza, *ed* endothecium, *en* endostome, *ep* epidermis, *ex* exostome, *fu* funiculus, *hy* hypostase, *ii* inner integument, *mi* microsporangium, *oi* outer integument, *pl* placenta, *pg* pollen grain, *pu* pollen tube, *sc* sperm cells, *ve* vegetative cell
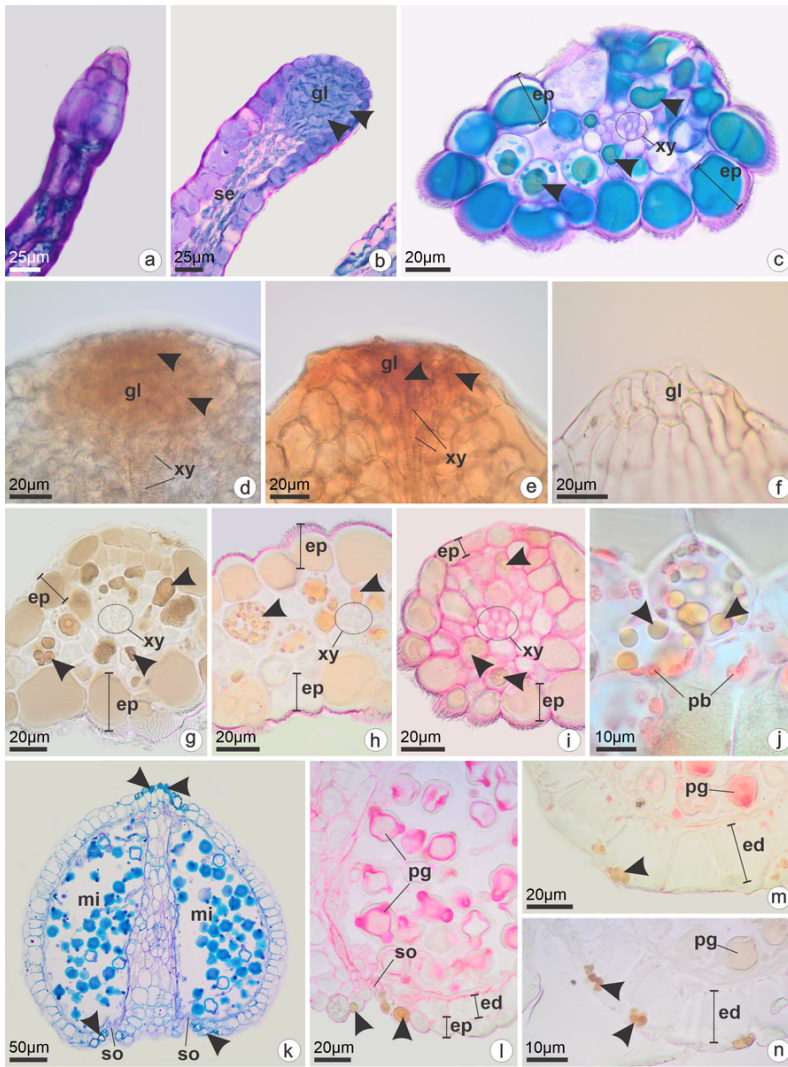
Figure 8

Secretory structures and histochemistry of Elatinaceae flowers. Longitudinal (a–b, k) and transverse (c, g–j, l–n) sections of sepals (a-j) and anthers (k-n) and entire dissected sepals (d-f): *Bergia perennis* (a), *Elatine gratioloides* (b, d), *E. lindbergii* (c, e, g–n), and *E. triandra*(f). **a** Vestigial gland on the marginal region of a sepal. **b–c** Vestigial gland at the apex of a sepal. **d–f** Apical region of the sepals in unstained sections; note the presence of vascular tissue in *d* and *e*, in which the apex is highlighted by the natural color of the cells. **g–j** Calyx glands submitted to histochemical tests: brown, presence of phenolic compounds (*g*); pink, lipids (*h*) and pectic compounds (*i*); and red, proteins (*j*). **k** General view of an anther. **l–n** Anthers submitted to histochemical tests for pectic compounds (*l*), proteins (*m*), and phenolic compounds (*n*). *arrowhead* idioblast, *ed* endothecium, *ep* epidermis, *gl* gland, *mi* microsporangium, *pb* protein bodies, *pg* pollen grains, *se* sepal, *so* stomium, *xy* xylem