Brazilian Journal of Botany
https://doi.org/10.1007/s40415-021-00779-9

STRUCTURAL BOTANY - ORIGINAL ARTICLE

Revisiting pericarp structure, dehiscence and seed dispersal in Galipeeae (Zanthoxyloideae, Rutaceae)

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Received: 16 October 2021 / Revised: 6 December 2021 / Accepted: 13 December 2021

Abstract

Rutaceae is a large family, particularly variable in fruits which have traditionally been used in the delimitation of infrafamilial taxa. Within the family, tribe Galipeeae stands out due to the high diversity of fruits with an exceptional complexity of structure and development, as well as various strategies of seed and fruit dispersal. To better understand the morphological evolution of the tribe, we selected distinct types of fruits occurring in *Erythrochiton* (subtribe Galipeinae)*, Esenbeckia*, *Helietta* and *Metrodorea* (subtribe Pilocarpinae) and comparatively analyzed their structure and development under light microscopy. *Erythrochiton* has a follicarium, *Esenbeckia* and *Metrodorea* capsules, and *Helietta* a samarium. All species investigated have multiplicative pericarp, resulting in large fruits containing many layers of fibers in the endocarp. The follicarium and capsules grow equally by diffuse cell divisions, but the samarium grows more markedly in the median dorsal part of mericarps, forming wings associated with an aerenchyma, which reduces their specific weight, facilitating dispersal by wind. The loculicidal capsules open along a dorsal dehiscence line, while the ventricidal follicles of the follicarium open along the ventral slit directed by the ventral vascular bundle. Both loculicidal capsules and follicaria dehisce explosively, and the seeds are ejected by the endocarp, which detaches from the pericarp. In addition to the novel observations in Galipeeae, a correlation between the degree of carpel union and the timing of schizocarpy is suggested for the first time.

Keywords Anemochory · Autochory · Capsule · Development · Follicarium · Sapindales

1 Introduction

Rutaceae (order Sapindales) consist of 154 genera and 2100 species worldwide (Kubitzki et al. 2011), with a high degree of endemism in Brazil, where 34 genera and around 206 species are found (Pirani and Groppo 2020). Most Rutaceae occur in tropical and subtropical environments in mountainous or lowland habitats in the Neotropical region (Porter and Elias 1979; Morton and Telmer 2014). In Brazil, the phytogeographic domains of the Atlantic Rain Forest and the Amazon Forest stand out as the main centers of the family's diversity (Pirani 1999; Colli-Silva and Pirani 2019).

The family exhibits a wide range of fruit types (APG IV 2016), and this diversity was used by Engler (1931), who subdivided Rutaceae into six subfamilies based on fruit types and other characters. Recent phylogenetic studies have shown that the englerian subfamilies are not monophyletic and that many homoplasies likely occurred in carpels and fruits throughout the evolutionary history of the family (Groppo et al. 2008, 2012; El Ottra et al. 2019). Consequently, alternative infrafamilial classifications have been proposed based on molecular features, varying from two (Groppo et al. 2012) to three (Kubitzki et al. 2011; Morton and Telmer 2014) or six subfamilies (Appelhans et al. 2021). Since this latest classification is based on the largest number of molecular regions studied thus far (six nuclear and plastid markers), as well as morphological and karyological characters, we have adopted it in our study.

Although Rutaceae is well known for the modified berry of *Citrus* spp., usually designated as hesperidium, there is a wide variation in fruits in Galipeeae, which reflects the diversity of floral morphologies (Pirani 1999). Tribe Galipeeae (Zanthoxyloideae, Appelhans et al. 2021), formerly Cusparieae in Engler's monograph (1931), is exclusively Neotropical with 33 genera and

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about 226 species (Kallunki and Pirani 1998), concentrated in two main centers of diversity: the Atlantic Rain Forest and the Amazon Forest (Colli-Silva and Pirani 2019). The tribe is composed of two subtribes, Galipeinae and its sister group, Pilocarpinae (Bruniera et al. 2015; Cole and Groppo 2020). The subtribe Galipeinae differs from Pilocarpinae mainly for having zygomorphic flowers due to the common presence of abaxial staminodes in most taxa, whereas Pilocarpinae has actinomorphic flowers with fully fertile haplostemonous androecium (Engler 1931; Groppo et al. 2012). Also, in several genera of Galipeinae, a slight to strong zygomorphy is stablished by means of petals of different sizes and positions at anthesis, often with two erect adaxial lobes flanking the two fertile stamens, and three abaxial lobes. In addition, Galipeinae possess tubular flowers, whereas Pilocarpinae develops widely opened dish-shaped flowers at anthesis. The floral tube usually develops in Galipeinae by the postgenital coherence of petals and adherence of filaments to petals through intertwining trichomes (or by the congenital fusion of the same organs) while in the Pilocarpinae the corolla usually shows the postgenital connection of petals only in bud stage, through interlocked papillae (Pirani et al. 2010; Kubitzki et al. 2011; El Ottra et al. 2013, 2019).

Most taxa of Galipeeae have dry autochorous fruits (Kubitzki et al. 2011). Regardless of such predominance, *Hortia* has zoochoric coriaceous berries, *Balfourodendron* has samaras, and *Helietta* has a samarium. These genera were recently transferred from Toddalioideae to Galipeeae (Groppo and Pirani 2012; Groppo et al. 2012; Cole and Groppo 2020). Thus, Galipeeae exhibits an impressive variety of fruit types in its current circumscription, which is strongly supported by molecular data.

Classifications of fruit types are based on fruit morphology, structure of the gynoecium and mode of dispersal (see Radford et al. 1974; Spjut 1994). Despite the progress of macromorphological studies, the nomenclature, types and characteristics used in taxonomical classifications based solely on macromorphological features often neither reflect homologies nor the evolutionary history of the groups. An example of such a feature is the case of the capsule, which was found to exhibit homoplasy in Galipeeae (e.g., Groppo et al. 2008; Appelhans et al. 2021). Efforts to understand fruit anatomy and ontogeny should be employed to better circumscribe taxa and elucidate the evolutionary pathways of structural evolution within angiosperm clades (Bobrov and Romanov 2019; Lima et al. 2021). Hence, this study aimed to analyze the structure, development and dehiscence of fruits in selected genera of Galipeeae, revisiting their morphological classifications, aspects of seed dispersal and schizocarpy in the tribe.

2 Materials and methods

Morphological analysis – We analyzed anthetic flowers, immature, mature and dehiscent fruits from vouchers deposited in the Herbarium SPF (Instituto de Biociências, Universidade de São Paulo, IB-USP) and fresh fruits collected from live plants cultivated in the Department of Botany of IB-USP, using a Leica M125 stereomicroscope. Digitalized material from the virtual herbarium Reflora (http://reflora.jbrj.gov.br/reflora/herbarioVirtual) was also analyzed. We investigated macromorphological characters of most genera of Galipeeae, with emphasis in *Erythrochiton*, *Esenbeckia*, *Helietta* and *Metrodorea* in which we also undertook anatomical analyses. Macromorphological features observed were mainly related to the type of the fruit, timing and degree of schizocarpy and dehiscence zones.

Anatomical analysis – We studied fruits of *Erythrochiton brasiliensis* Nees & Mart. (J.H.L. El Ottra 236), *Esenbeckia grandiflora* Mart. (J.H.L. El Ottra 241), *Helietta glaziovii* (Engl.) Pirani (J.R. Pirani 5259, 5325) and *Metrodorea nigra* A.St.-Hil. (G.O. Paschoalini 2). *Erythrochiton brasiliensis* and *M. nigra* were collected from cultivated plants at the Department of Botany of IB-USP, while *E. grandiflora* and *H. glaziovii* were collected in Grão-Mogol, Minas Gerais state, Brazil. Vouchers were deposited in Herbarium SPF. All studied taxa belong to subfamily Zanthoxyloideae (Appelhans et al. 2021), tribe Galipeeae, with *Erythrochiton* included in subtribe Galipeinae, and the remaining three genera in subtribe Pilocarpinae.

For the anatomical study, fruits were divided in three main developmental stages based on size and general aspects of the pericarp: (1) young fruit; (2) immature fruit; (3) mature fruit. All fruits were fixed in FAA (Formalin, Acetic acid and 50% ethyl Alcohol; Johansen 1940), dehydrated in a tertiary butanol series, embedded in Paraplast (Leica Microsystems, Heidelberg, Germany) and sectioned using a Leica RM 2145 rotary microtome (Leica Microsystems, Wetzlan, Germany). Sections 8-12 µm thick were stained with astra blue and safranin (Gerlach 1984) and mounted in Entellan (Merck KGaA, Darmstadt, Germany). The slides were examined using a Leica DM 4000B microscope equipped with a Leica DFC 425 digital camera (Leica Microsystems, Wetzlan, Germany).

Terminology and tree mapping – For morphological analysis, we used Radford et al. (1974) for general terms and Spjut (1994) for the specific fruit terminology. For anatomical descriptions, we followed Roth (1977) and Bobrov and Romanov (2019).

Additionally, we mapped on a cladogram of Galipeeae (based on Cole and Groppo 2020 and references therein) the fruit types accordingly to the terminology herein proposed for each genus of the tribe. The identification of fruit types in species not ontogenetically studied is based on the morphological survey of herbarium materials (see Supplementary Table 1) and descriptions from the literature (Emmerich 1978; Albuquerque 1976; Kaastra 1982; Kallunki 1992, 1994, 1998a, b, 2009; Kallunki and Pirani 1998; Skorupa 1998, 1999, 2000; Skorupa and Pirani 2004; Groppo and Pirani 2005, 2012; Groppo et al. 2005; Pirani 1998, 2004, 2005; Pirani and Kallunki 2007; Bruniera et al. 2011; Kubitzki et al 2011; Dias et al. 2013; Fernandez-Hilario and Arteaga 2017; Brito et al. 2019; Pirani and Groppo 2020). Furthermore, besides the terminology used as proposed by Spjut (1994), we additionally defined as a follicle a multicarpellate gynoecium that in fruiting stage consistently developed a single fruitlet, though we are aware that the lack of detailed structural and developmental data of these taxa may change this typification latter. Conversely, a same species that developed one or all carpels in fruitlets was considered to have a follicarium, even when it had only one fruitlet developed in some fruits. Likewise, when more than one type of fruit is described for the same genus, all of them were considered in our mapping. In these cases, new studies are needed to elucidate whether the divergences correspond to differences in the interpretation of fruit type or to a higher structural diversity.

3 Results

We distinguished three types of fruits in the species analyzed: follicarium in *Erythrochiton*, capsules in *Esenbeckia* and *Metrodorea*, and samarium in *Helietta* (Fig. 1).

Erythrochiton brasiliensis – *Morphology*. *Erythrochiton brasiliensis* has mostly apocarpous anthetic gynoecium, only connate at the base and at the apex. The fruit is a cordate to elliptical follicarium (Fig. 1a–d), with rounded base and obtuse apex with a carena. It is frequently formed by five one-seeded fruitlets (sometimes two seeds develop per fruitlet), and usually all fruitlets develop to dehiscence. Each fruitlet is trullate to rhombic (Fig. 2a, b), greenish colored in young fruits, greenish to reddish in immature fruits, reddish to brownish and subglabrous when mature. Fruits have a coriaceous or fleshy surface when young, punctate with oil cavities (Fig. 2c), becoming scarious and woody at maturity. Dispersion of the seeds occurs by autochory, and the dehiscence is ventricidal.

Anatomy. Exocarp is uni-stratified (Fig. 2c, d), formed by juxtaposed polygonal cells, columnar when young, rectangular to cubic in immature fruits, with primary walls (Fig. 2d). Mesocarp is multi-stratified (Fig. 2c–e) with diffuse multiplicative growth. The mesocarp outermost layer (hypodermis) is composed of juxtaposed columnar cells which become thick walled and sclerified (immature stage, Fig. 2d). The mesocarp has many phenolic idioblasts, vascular bundles and secretory cavities immersed in a parenchyma matrix with very frequent crystalliferous idioblasts containing raphides (Fig. 2c). Vascular bundles are seen running in axial and transverse directions throughout the mesocarp, with the ventral bundles being larger and thus more conspicuous (Fig. 2e; Suppl. Fig. 1a, b). The endocarp is multilayered since floral stage, increasing the number of layers throughout development (Fig. 2c, f; multiplicative growth). Endocarp cells are rectangular to cubic in young fruits. In later stages, most endocarp cells have wavy-shaped periclinal walls, with irregular wall thickening (Fig. 2f), except in the inner flanks and ventral region of carpels, where the cells maintain the same shape as in younger stages (polygonal) and without thickening. External layers of the endocarp are arranged perpendicularly to the cells with wavy, sinuous walls in the axial position (Fig. 2f), generating opposite tensions in the tissue. Endocarp detaches from the mesocarp in mature stage, forming a beak in its median dorsal region (Fig. 2e). The elastic ejection of the seeds performed by the endocarp occurs due to mechanical tension generated between the dorsal region (with wavy thickened cell walls) and inner flanks and ventral region (thin-walled polygonal cells). The limited extension of the region of the union among carpels at the ovary apex is ruptured early during fruit development (early schizocarpy), giving rise to the mostly separated fruitlets (follicarium, Fig. 1a, b). Additionally, no dehiscence line is observed in the dorsal or ventral regions. The loss of water generates a tension in the lignified tissues, and the fruit breaks in an explosive way (autochory) along the ventral bundle in the ventral slit (Fig. 1c), ejecting the seeds (Fig. 2e).

Esenbeckia grandiflora – *Morphology*. *Esenbeckia grandiflora* has a syncarpous gynoecium according to morphological observations of anthetic flowers. The fruit is a globose to prolate capsule (Fig. 1e–i), truncate at the base, emarginate at the apex and usually pentalocular (rarely four locules), with one seed or sometimes two collateral seeds in each locule (Fig. 3a–e). Young and immature fruits are greenish colored, turning brownish and glabrous in mature stage. The surface is indurate when young, becoming woody when mature, muricate with spiny protuberances (Figs. 1f-i, 3c–f). Dispersion of the seeds occurs by autochory, and dehiscence is simultaneously septicidal and loculicidal.

Anatomy. Exocarp is uni-stratified (Fig. 3g), formed by juxtaposed polygonal cells, frequently cubic, sometimes slightly columnar to rectangular with primary walls in young fruits and lignified secondary walls in mature fruits (Fig. 3g). Mesocarp is multi-stratified (Fig. 3f–h)

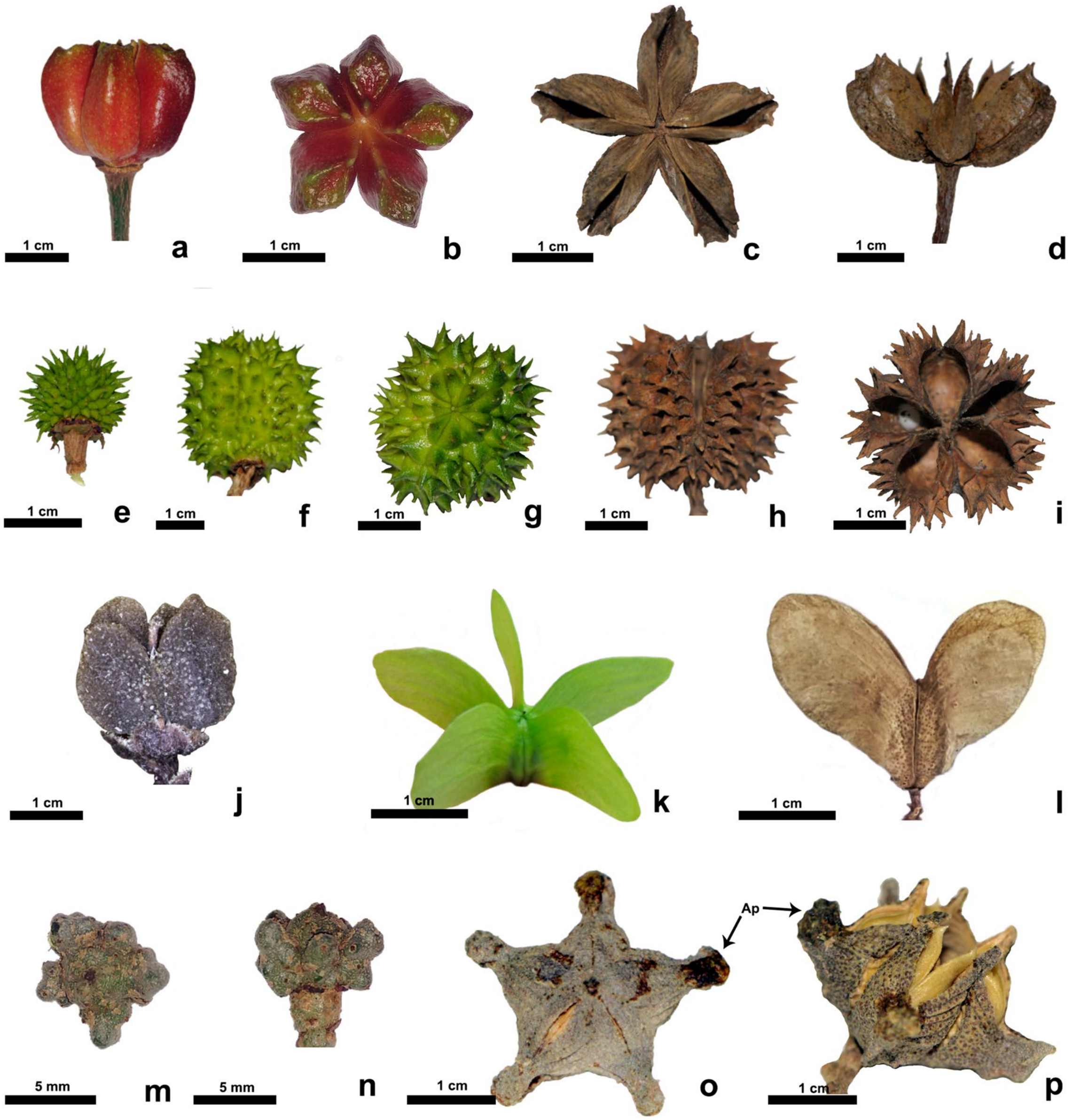


Fig. 1 Morphology of fruits in Galipeeae (Rutaceae) at different developmental stages. **a**–**b**, **f**–**g**, **k**, **m**–**n** Immature fruits. **c**–**d**, **h**–**i**, **l**, **o**–**p** Mature fruits. **e**, **j** Young fruits. **a**, **d**, **e**, **f**, **h**, **j**, **l**, **n**, **p** Lateral views. **b**, **c**, **g**, **i**, **k**, **m**, **o** Frontal views. **a**–**d** Follicarium of *Erythrochiton brasiliensis* Nees & Mart. Note early schizocarpy (**a**–**b**) and ventricidal dehiscence in '**c**'. **e**–**i** Capsule of *Esenbeckia grandiflora* Mart. Note late schizocarpy (**e**–**i**), septicidal (**h**–**i**) and loculicidal (**i**) dehiscence. **j**–**l** Samarium of *Helietta glaziovii* (Engl.) Pirani. Note septicidal dehiscence line discernable from immature (**k**) to mature fruit stages (**i**). **m**–**p** Capsule of *Metrodorea nigra* A.St.-Hil. Note late schizocarpy (**m**–**p**), septicidal and loculicidal dehiscence (**o**, **p**), and apophysis (Ap)

with diffuse multiplicative growth, composed of chlorophyll (outer region) and ground (inner region) parenchyma, secretory cavities (Fig. 3d, f), abundant phenolic idioblasts (Fig. 3f, g) and some crystalliferous idioblasts containing raphides. Vascular bundles extend in axial and transverse directions throughout the mesocarp (Fig. 3e, f; Suppl. Fig. 1c–e). Larger bundles are found in ventral and apical regions (Suppl. Fig. 1e). The endocarp is multilayered since the floral stage, increasing the number of layers throughout development of the fruit (Fig. 3b, d, f) by multiplicative growth. Endocarp cells are rectangular in young fruits and elongated in later stages, stretching in the ventral region (Fig. 3h). In a later stage, endocarp cells become fusiform and sclerified. Dehiscence occurs likely due to the loss of water by the lignified tissues with divergent orientations (Fig. 3i), releasing the seeds in an explosive autochoric way. The breakdown occurs in the separation tissue, consisting of a line of structurally weak parenchyma that develops from

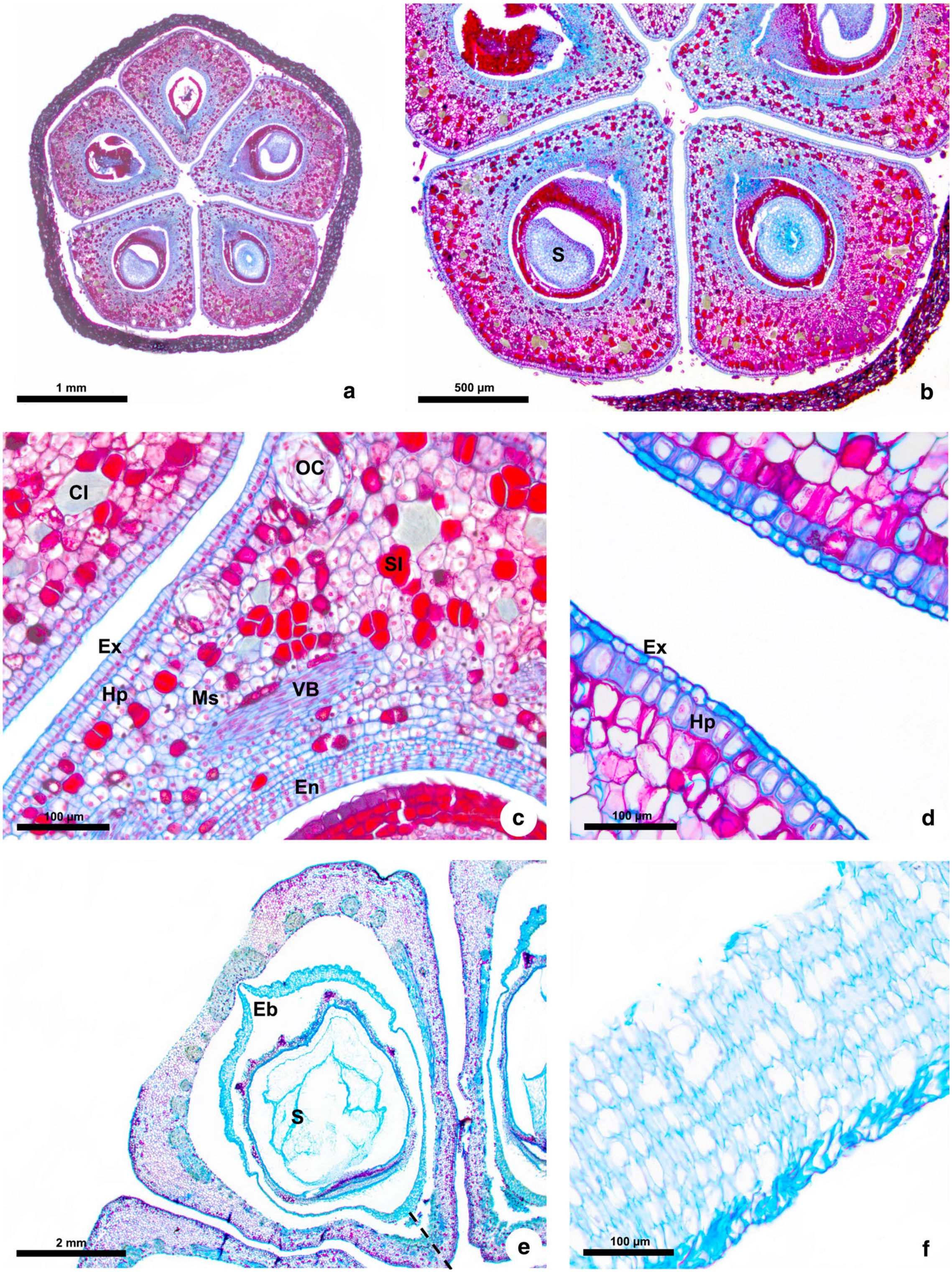
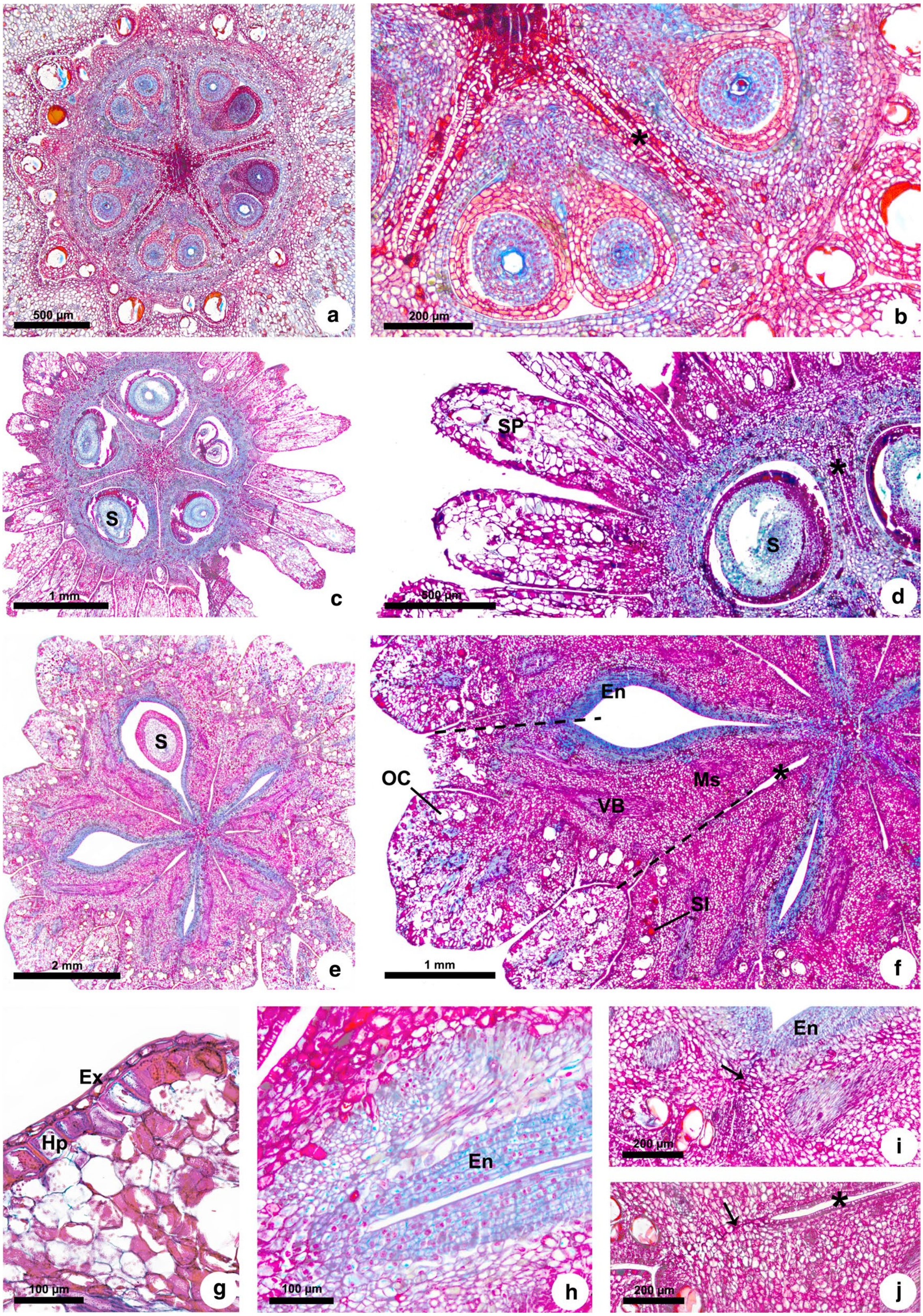


Fig. 2 Structure and development of the follicarium of *Erythrochiton brasiliensis* Nees & Mart. **a**–**d** Young fruit. **e**–**f** Mature fruit. **a**, **e** General view. **b** Detail of the one-seeded follicles. **c** Pericarp composed of a uni-stratified exocarp, hypodermis, parenchymatous mesocarp containing vascular bundles, oil cavities and phenolic and crystalliferous idioblasts, in addition to the developing endocarp with several layers of cells. **d** Exocarp and mesocarp. Note the sclerenchymatic hypodermis. **e** Endocarp detachment from the mesocarp. Note the dehiscence region of the follicle along the ventral bundle (dashed line). **f** Detail of the endocarp. CI, crystalliferous idioblast; Eb, endocarp beak; En, endocarp; Ex, exocarp; Hp, hypodermis; Ms, mesocarp; OC, oil cavity; S, seed; SI, secretory idioblast; VB, vascular bundle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◂**Fig. 3** Structure and development of the capsule of *Esenbeckia grandiflora* Mart. **a**–**b** Young fruit. **c**–**d** Immature fruit. **e**–**j** Mature fruit. **a**, **c**, **e** General view. **b** Early developing pericarp containing two fertilized ovules. **d** Developing pericarp with spiny protuberances. **f** Mature pericarp composed of a uni-stratified exocarp, hypodermis, parenchymatous mesocarp containing vascular bundles, oil cavities and phenolic idioblasts, in addition to the multilayered endocarp. Note the septicidal and loculicidal dehiscence regions (dashed lines). **g** Exocarp and mesocarp. Note the sclerenchymatic hypodermis. **h** Detail of the endocarp in the ventral region. **i** Separation tissue (arrow) of the loculicidal dehiscence line. **j** Separation tissue (arrow) of the septicidal dehiscence line. Asterisk, septal slit; En, endocarp; Ex, exocarp; Hp, hypodermis; Ms, mesocarp; OC, oil cavity; S, seed; SI, secretory idioblast; SP, spiny protuberance; VB, vascular bundle

the immature stage in the median dorsal region of exo- and mesocarp (Fig. 3i) and outer (lateral) flanks (Fig. 3j). Additionally, unfused regions of the septa at the inner (lateral) flanks of the locules (Fig. 3a–f, j) may contribute to this breakdown since these create regions of weakness in the septa radii; these slits are present since early floral stages.

Helietta glaziovii – *Morphology*. *Helietta glaziovii* has a syncarpous gynoecium according to morphological observations of anthetic flowers. The fruit is a star-shaped samarium (Fig. 1j–l), with a reniform base and emarginate apex, separating into four to five fruitlets (rarely three) at maturity, each with a single seed (Fig. 4a–d). The median dorsal region of each fruitlet (samarid) forms a scarious, leathery, puberulent wing (Fig. 1l). The fruitlets are greenish in young and immature fruits, turning brownish to grayish in the mature stage (Fig. 1j–l). Dispersion of the samarids occurs by anemochory, and dehiscence is septicidal, the fruitlets separating only in mature fruits (late schizocarpy).

Anatomy. Exocarp is uni-stratified (Fig. 4e–i), composed of juxtaposed cubic to slightly columnar cells with primary walls (Fig. 4h, i). Peltate glandular trichomes were observed in young and immature fruits (Fig. 4e). Mesocarp is multi-stratified (Fig. 4g, h) and formed by multiplicative growth with diffuse cellular divisions occurring in young and immature fruits. These divisions are more pronounced in the median dorsal region of each carpel, forming the wings (Fig. 4a, b, d, e, g, h). Most of the mesocarp is composed of chlorophyll or ground parenchyma formed by cubic to elliptical cells with primary walls (Fig. 4e–h). Secretory cavities are found close to the exocarp (Fig. 4h), and phenolic idioblasts and crystalliferous idioblasts are sparsely present. Vascular bundles are observed running in axial and transverse directions throughout the mesocarp (Fig. 4e, h; Suppl. Fig. 1f, g). Ventral bundles are larger (Suppl. Fig. 1f), and an aerenchyma (Fig. 4f) is formed during wing development in the young and immature stages (Fig. 4a, b, d). The endocarp is multilayered since early stages (Fig. 4c), increasing the number of layers throughout fruit development (with fewer layers than all other species observed herein) by multiplicative growth, giving rise to rectangular to polygonal cells (Fig. 4i). These cells are widely elongated and lignified in mature fruits (Fig. 4g). The dehiscence occurs due to rupture in the separation tissue, consisting of a line of structurally weak parenchyma that develops in outer (lateral) flanks of each fruitlet (Fig. 4h, i). The unfused region of the septa at the inner (lateral) flanks of adjacent locules (Fig. 4c, d, h), which is present from the floral stage, may also contribute to the separation of samarids in later stages since these create regions of weakness in the septa radii.

Metrodorea nigra – *Morphology*. *Metrodorea nigra* has a syncarpous gynoecium according to morphological observations of anthetic flowers. The fruit is a pentalocular capsule (Fig. 1m–p), globose to star-shaped with a truncate base and rounded apex, each locule with one or two collateral seeds (Fig. 5a–d). The median dorsal outer region of each locule forms a prominently clavate structure distally in the fruit (apophysis, Fig. 1o, p). The color pattern is a maculation of green and brown. The texture is indurate, and the surface is rugose, punctate and glabrous. Dispersion of the seeds occurs by autochory, and the dehiscence is simultaneously septicidal and loculicidal (Fig. 1p).

Anatomy. Exocarp is a multi-stratified periderm (Fig. 5e) with desquamating outer cell layers differentiated in immature to mature fruits. Mesocarp is multi-stratified (Fig. 5b, d–g) with multiplicative growth, diffuse in lateral and ventral regions, and more pronounced in direction to the upper median dorsal region (Fig. 5c). The mesocarp is composed of ground parenchyma (Fig. 5d, g) with elliptical cells with primary walls, secretory cavities close to the exocarp and phenolic idioblasts (Fig. 5e, h, i). Vascularization increases markedly in number and size in late stages, including the vascular bundles inside the median clavate dorsal protuberance of each locule (Fig. 5b–d; Suppl. Fig. 1 h–j). The endocarp is multilayered since floral stage, increasing the number of layers throughout development by multiplicative growth (Fig. 5f). It is composed of polygonal, thin-walled cells in the early stage. In later stages, it is composed mostly of sclerenchymatous tissue, consisting of oblique fibers, except at the periphery (Fig. 5g). The endocarp is thinner and beak shaped in its median dorsal region (Fig. 5d, g, h). The endocarp layer stretches in the ventral region. In later stages, endocarp cells become fusiform and sclerified (Fig. 5g, h). Dehiscence likely occurs due to the loss of water by the lignified tissues with divergent orientations, releasing the seeds in an explosive autochoric way. The separation tissue, containing large intercellular spaces (Fig. 5h), differentiates in the immature stage. This tissue occurs in the median dorsal region of each locule (Fig. 5d, g; loculicidal dehiscence) and in the outer flanks (Fig. 5d; septicidal dehiscence), associated with the unfused regions of the septa at the inner flanks of locules (present from the floral stage; Fig. 5b, d, i).

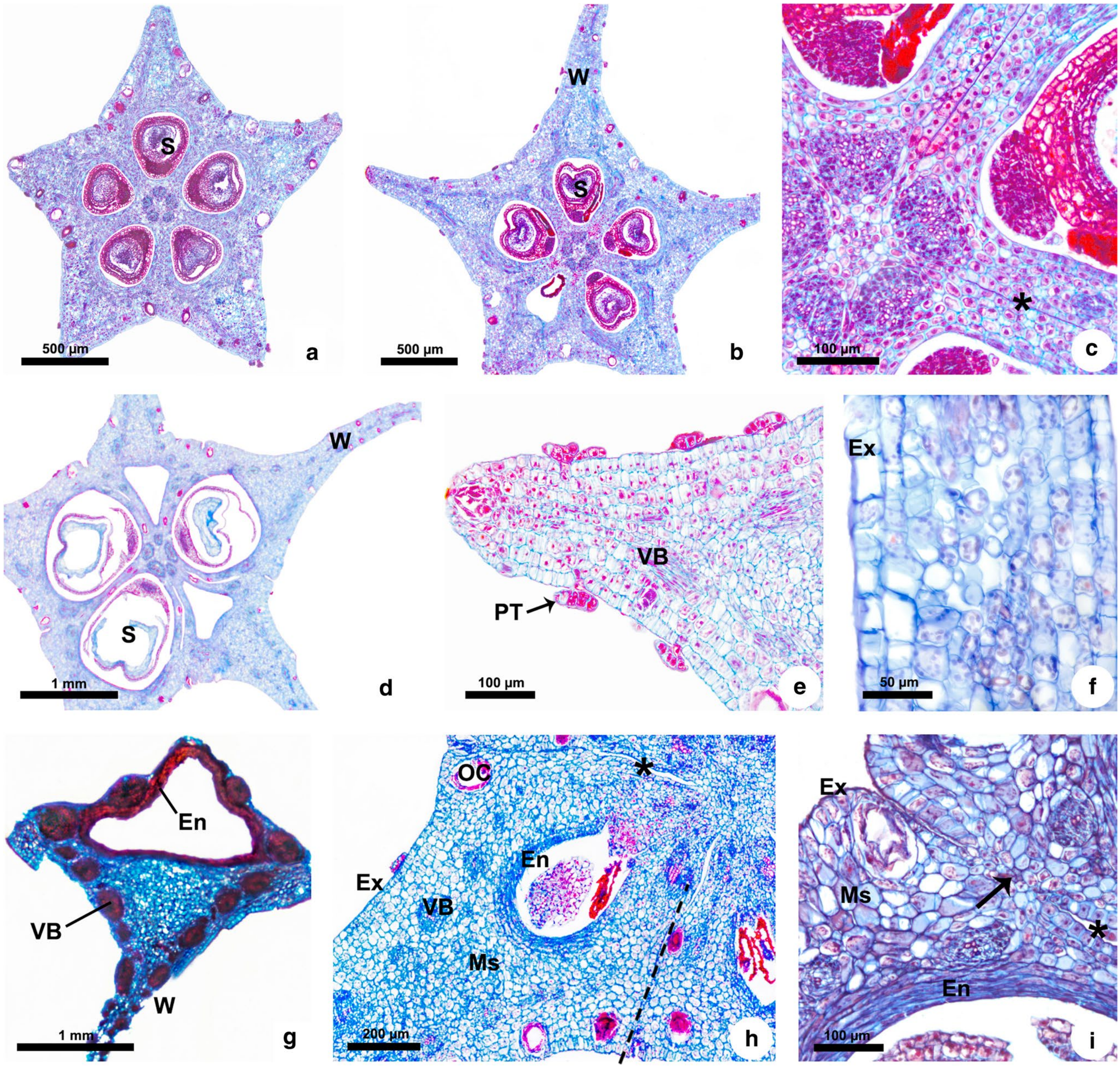


Fig. 4 Structure and development of the samarium of *Helietta glaziovii* (Engl.) Pirani. **a**–**c**, **e** Young fruit. **d**, **f**, **h**, **i** Immature fruit. **g** Mature fruit. **a**–**b**, **d** General view. Distinct stages of wing development. **c** Detail of the septal slits. **e** Early stage of the wing elongation covered by peltate glandular trichomes. **f** Aerenchyma in the wing. **g** Mature samarid with lignified endocarp and pronounced lignification of the fibers in the vascular bundles. **h** Pericarp composed of a uni-stratified exocarp, parenchymatous mesocarp containing vascular bundles and oil cavities, in addition to the multilayered endocarp not sclerified at this stage. Note the septicidal dehiscence region (dashed line). **i** Detail of the separation tissue (arrow) of the septicidal dehiscence line (dashed line in '**h**'). Asterisk, septal slit; En, endocarp; Ex, exocarp; Ms, mesocarp; OC, oil cavity; PT, peltate trichome; S, seed; VB, vascular bundle; W, wing

4 Discussion

The study of the structure and development of fruits in Sapindales provides important information about the evolutionary history of plant lineages, in addition to indicating seed dispersal strategies (Bobrov and Romanov 2019; Tölke et al. 2017; Lima et al. 2021; this study). The analysis of selected genera of Galipeeae, a highly diverse clade, allows us to make valuable and reliable inferences, but it is first necessary to adopt a terminology that reflects the set of fruit characters, as discussed below.

Morphology and typology of the fruits – Morphological treatments of Rutaceae have been carried out since Engler

(1931) in monographs, floras and taxonomic descriptions at the genus and species levels. As several researchers have employed different concepts and treatments for fruits of Galipeeae and a range of characters have been prioritized over others, it becomes essential to update terminology and provide guidelines for future descriptions. Spjut's (1994) terminology, due to its describing both the multiple fruit—when it occurs—and its carpological units, seems to us the most suitable to use in Galipeeae from a morphological perspective. In this tribe, the anthetic carpels show various degrees of congenital or postgenital connation, and the gynoecium varies from apocarpy to syncarpy (Gut 1966; Ramp 1988; Barroso et al. 1999; Kubitzki et al. 2011). In addition, the fruitlets may remain connate or exhibit various degrees of schizocarpy during the fruit development. Thus, an accurate analysis is needed for each case.

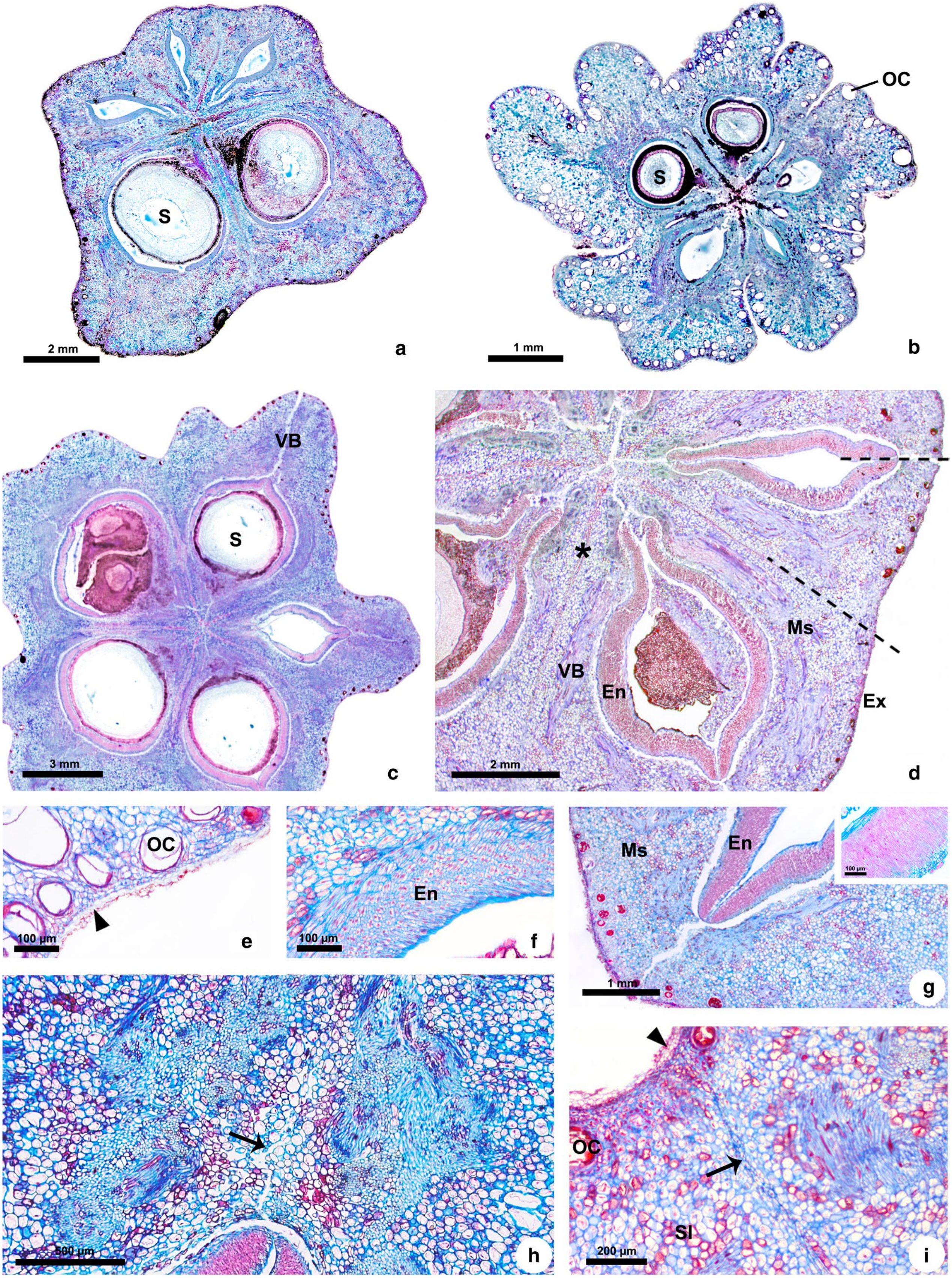
Erythrochiton. Kallunki (1992) claims the occurrence of schizocarpy in some species of *Erythrochiton* with carpels that are mostly free at the ovary level, except at their bases and apices. In *E. brasiliensis*, fruits may remain axially united at the base, but the fruitlets are easily detached. For this genus, Albuquerque (1976) used the term "capsule" with slightly coherent carpids on the sides, perhaps based on the classification of Hertel (1959), which includes follicular, capsular and schizocarpous fruits in the same "capsuloid" category. According to our ontogenetic analysis, this classification does not represent the structural and developmental pattern found for the fruit of *Erythrochiton*. Based on Spjut's (1994) classification and our observations presented herein, the fruit of *E. brasiliensis* should be classified as a multiple fruit of the follicarium type, with each unit designated as follicle, with each follicle opening along its ventral slit (Kubitzki et al. 2011).

Esenbeckia. This genus has a septicidal and loculicidal capsule with a smooth, muricate, tuberculate or echinate surface, varying between species and within a species (Kaastra 1982; Barroso et al. 1999; Kubitzki et al. 2011). Spjut (1994) highlights *Esenbeckia flava* Brandegee as an example of coccarium since the fruit opens into valves. However, in *Esenbeckia* there are various degrees of gynoecium connation, and schizocarpy is restricted to the moment of the dehiscence, contradicting the main feature of a coccarium. Septicidal capsules are similar to schizocarpous fruits since the dehiscence occurs along the commissures of carpels in both but vary in the timing of fruit units' separation (Spjut 1994). In this sense, the analysis of the stage of development in which the separation among fruitlets takes place is essential (Cronquist 1981; Spjut 1994).

Metrodorea. This genus is closely related to *Esenbeckia* and also has syncarpous gynoecium according to morphological analyses. Souza et al. (2008) classify the fruit of *M. nigra* as a schizocarp, coccarium, as well as a capsule based on the type of dehiscence. Based on Hertel (1959), the fruit of *M. nigra* would be capsuloid due to seed dispersal through openings in certain zones and schizocarpaceous due to the separation of the ovary into mericarps. In contrast, Barroso et al. (1999) used loculicidal capsule to designate the fruit of *Metrodorea,* while Kaastra (1982) described it as a septicidal and loculicidal capsule. The term coccarium (schizocarpaceous coccoid for Hertel 1959) does not correspond to what we observed in *Metrodorea* since the gynoecium has neither a schizocarpous developmental pattern nor dehiscence by both ventral and dorsal sides (Spjut 1994; this study). Therefore, capsule (Kaastra 1982; Barroso et al. 1999; Kubitzki et al. 2011; Dias et al. 2013; Pirani and Groppo 2020) is here considered a better definition for this fruit, with locules that remain joined from the base to the apex, displaying septicidal and loculicidal dehiscence after maturation. An apophysis may or may not be present at the fruit tip (Kaastra 1982). If present, it acts as a limiting factor for loculicidal dehiscence as it prevents tissue breakage in its region (Kaastra 1982). The apophysis is characteristic of *Metrodorea*, likely having been lost only in *M. mollis* Taub. (Dias et al. 2013).

Dehiscent fruits with seeds detached are the most common type in Rutaceae, and they were retrieved in the evolutionary reconstruction by Appelhans et al. (2021) as the probable ancestral character state in the family, as well as in Zanthoxyloideae, and it was conserved in most extant Galipeeae, in which only two transitions to winged fruits occurred (*Balfourodendron* and *Helietta,* see below).

Helietta. This genus has a syncarpous gynoecium, on morphological grounds, which develops into winged fruitlets. It is closely related to *Balfourodendron* (Pirani 1998) and also to *Esenbeckia* (Cole and Groppo 2020; Appelhans et al. 2021), but while the latter have capsules, the former keeps the carpels united in fruit, forming a single dispersal unit, i.e., the samara. *Helietta* develops a samarium, formed by fruitlets that separate at maturity, called samarids, which are in fact the dispersal units of the plant (Pirani 1998; this study). Four stages of formation of a samarium from a fertilized gynoecium of *H. glaziovii* are depicted in Pirani (1998). *Helietta* and *Balfourodendron* stand out as being the only anemochorous genera of Galipeeae (Fig. 6), but samaras evolved independently at least four additional times in Rutaceae, according to Appelhans et al. (2021): in the North American genus *Ptelea* and in the Australian *Pentaceras,* both belonging to Zanthoxyloideae, and also in three genera of the distantly related Cneoroideae: *Bottegoa* from Africa and in the clade formed by the Neotropical *Spathelia* and *Sohnreyia*. Considering the current phylogenetic positioning of anemochorous taxa of Galipeeae (Fig. 6), it is likely that *Helietta* has retained schizocarpy due to the septicidal dehiscence, as present in its sister genus *Esenbeckia* and in the closely related *Metrodorea*, while *Balfourodendron* has lost the ability to segregate carpels as dispersal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◀**Fig. 5** Structure and development of the capsule of *Metrodorea nigra* A.St.-Hil. **a** Young fruit. **b**, **f** Immature fruit. **c**–**e**, **g**–**i** Mature fruit. **a**–**c** General view. **d** Mature pericarp composed of an exocarp, parenchymatous mesocarp containing vascular bundles, oil cavities and phenolic idioblasts, in addition to the multilayered sclerenchymatous endocarp. Note the septicidal and loculicidal dehiscence regions (dashed lines). **e** Exocarp and mesocarp. Note the presence of periderm (arrowhead). **f** Detail of the non-lignified endocarp. **g** Detail of the mature pericarp at the loculicidal dehiscence region. Note the detachment of the lignified endocarp (inset) **h** Separation layer (arrow) of the loculicidal dehiscence line with large intercellular spaces. **i** Separation tissue (arrow) of the septicidal dehiscence line. Asterisk, septal slit; En, endocarp; Ex, exocarp; Ms, mesocarp; OC, oil cavity; S, seed; SI, secretory idioblast; VB, vascular bundle

units. The schizocarpous development of the fruit of *Helietta* is also noted by Barroso et al. (1999).

Pericarp structure – *Exocarp*. In the Galipeeae taxa studied here, *Esenbeckia* and *Helietta* have typical uniseriate exocarp, whereas *Erythrochiton and Metrodorea* show exceptions to this pattern. The presence of periderm in the capsules of *Metrodorea* is notable and was also observed by Souza et al. (2008). These authors attribute a mixed origin to the periderm, formed by cell divisions of epidermal and subepidermal layers in young fruits. The phellogen produces cells only in the beginning of the fruit development, and its walls promptly undergo deposition of suberin (Roth 1977). Although a rare phenomenon, the appearance of periderm in fruits has been previously reported for Sapotaceae (Roth 1977), in this case, originated only by exocarp initials. Lignified uniseriate exocarp occurs in members of both Pilocarpinae and Galipeinae, such as *Ertela trifolia* (L.) Kuntze, *Esenbeckia febrifuga* (A.St.-Hil.) A. Juss. ex Mart., *Esenbeckia leiocarpa* Engl. and *G. jasminiflora* (Afonso 2018). Some appendages may also be found in the exocarp of Rutaceae regardless the fruit type: multicellular glandular trichomes are found in *Helietta*, as well in *Esenbeckia*, *Dictamnus* and *Ruta* (Brückner 1991; Afonso 2018; Bobrov and Romanov 2019; this study), the last two genera distantly related to Galipeeae. Likewise, multicellular glandular trichomes have been found in *Balfourodendron*, which is closely related to *Helietta* (Pirani 1998, 1999) and to *Esenbeckia* (Groppo et al. 2008, 2012; Appelhans et al. 2021). Trichomes are quite common in fruits and might have taxonomic implications at the genus or species level. In fact, the juice sacs, characteristic of the hesperidium endocarp of *Citrus*, are a well-documented specialization of a coating structure that initiates its ontogeny in a similar way to regular glandular trichomes (Roth and Lindorf 1972; Roth 1977).

Mesocarp. All species analyzed have a homogeneous parenchymatous mesocarp. It is noteworthy that fruits so different in morphology and mode of dispersion have almost the same mesocarp composition, a fact that may be related to their close phylogenetic relationship. The only exception is the follicarium of *Erythrochiton*, with the outermost layer of mesocarp (hypodermis) sclerified. In this sense, *E. brasiliensis* has fruits similar in structure to those of *Pilocarpus* (Pilocarpinae, Souza et al. 2005), *Conchocarpus minutiflorus* Groppo & Pirani and *C. pentandrus* (A.St.-Hil.) Kallunki & Pirani (Galipeinae, Afonso 2018), either due to the presence of an outermost sclerenchymatous layer (Souza et al. 2005; Afonso 2018) or by their mostly apocarpous fruit from early stages (Gut 1966; Ramp 1988; El Ottra et al. 2013, 2019).

The fruits of *Helietta* and *Balfourodendron* develop wings in the median dorsal region of each unit by cell proliferation associated with aerenchyma differentiation, which reduces the specific weight of the fruit (Afonso 2018; this study). Detailed descriptions of the structure of the remaining genera with winged fruits in Rutaceae (*Bottegoa, Pentaceras, Ptelea, Spathelia, Sohnreyia*) are still lacking. Other Sapindales, such as some Sapindaceae, also develop similar wings (many Paullinieae) with extensive intercellular spaces in fruits, as found in *Dodonea* (Weckerle and Rutishauser 2005; Paoli and Sarti 2008), which aid the "flight" of the fruit over greater distances, forming part of the anemochorous apparatus (van der Pijl 1982). As several known populations of *H. glaziovii* are found along riversides (Pirani 1998), it is likely that wings with aerenchyma may also help samarids to float on water.

Endocarp. All the fruits studied have multiplicative endocarps, regardless the dehiscence type. Among the Rutaceae, *Citrus* spp. are the best-known examples in which extensive meristematic activity of the endocarp forms juice sacs (Roth 1977). In Pilocarpinae and Galipeinae, mainly periclinal divisions occur in the inner epidermis of mature ovaries, which will later form the sclerified endocarp. In *M. nigra* (Souza et al. 2004; El Ottra et al. 2019), *Esenbeckia* spp. and *B. riedelianum* (Afonso 2018), the inner epidermis forms one to two layers, while in *Helietta* spp. (Afonso 2018; this study) it forms three to more layers. Similarly, *Adiscanthus fusciflorus* Ducke, *Dryades cyrtantha* (Kallunki) Groppo & Kallunki, *Ertela bahiensis* (Engl.) Kuntze and *Neoraputia trifoliata* (Engl.) Emmerich ex Kallunki have one to two layers, *Conchocarpus macrocarpus* (Engl.) Kallunki & Pirani, *C. obovatus* (Nees & Mart.) Kallunki & Pirani, *Sigmatanthus trifoliatus* Huber ex Emmerich and *Hortia oreadica* Groppo, Kallunki & Pirani have more than three layers, and *C. odoratissimus* (Lindl.) Kallunki & Pirani and *Rauia resinosa* Nees & Mart. have more than four layers. In some Galipeinae, even more pronounced multiplication is also common, and four to nine layers can occur in *C. ruber* (A.St.-Hil) Bruniera & Groppo, *Raputiarana subsigmoidea* (Ducke) Emmerich, *Rauia nodosa* (Engl.) Kallunki, *Ravenia infelix* Vell. and *Spiranthera odoratissima* A.St.-Hil. (El Ottra 2014; El Ottra et al. 2019).

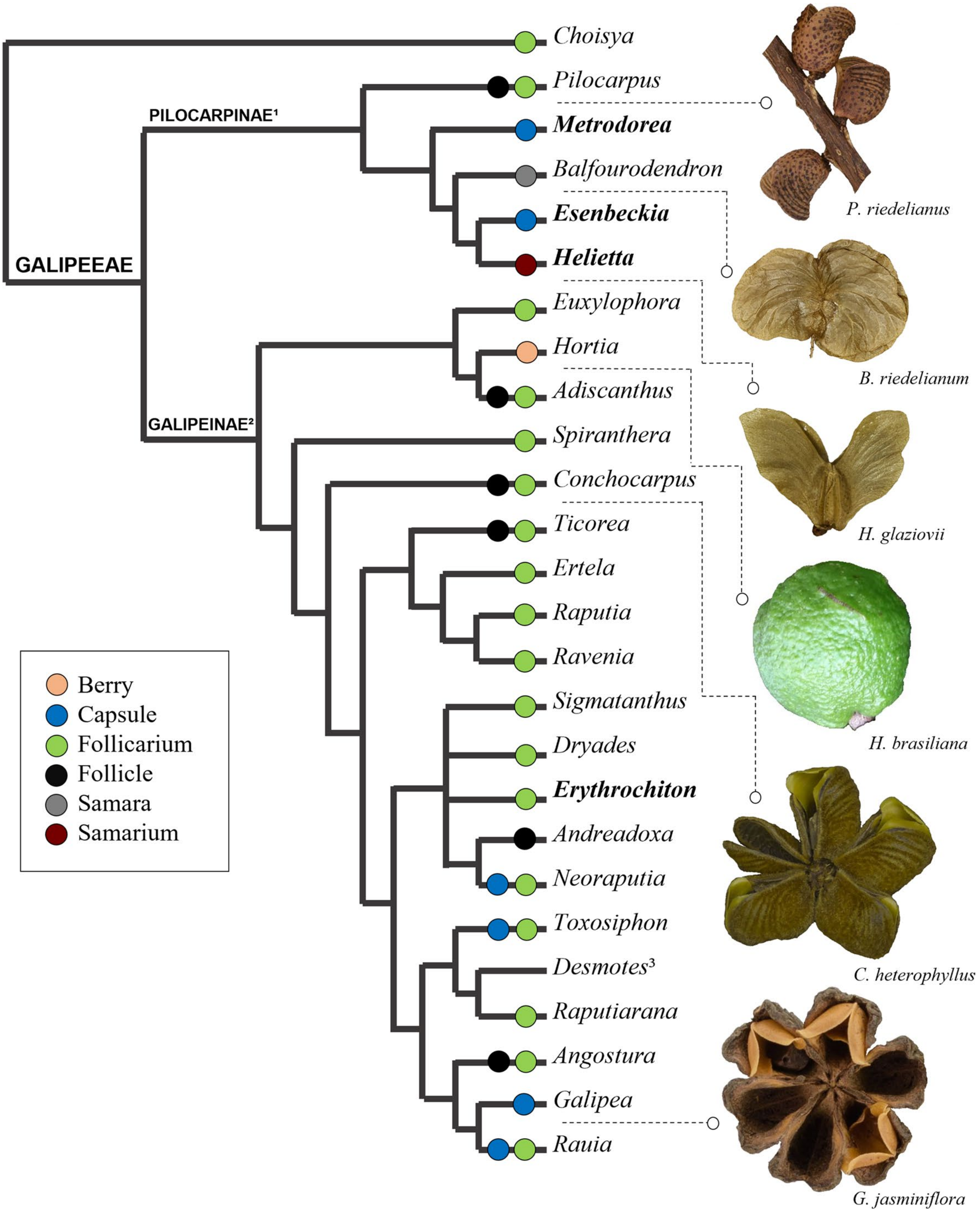

Fig. 6 Cladogram of Galipeeae (based on Cole and Groppo 2020) with the types of fruits mapped upon the genera. Data obtained from literature and personal observations in the herbarium (Suppl. Table 1). Picture of bacca of *Hortia brasiliana* Vand. ex. DC. taken from a fresh fruit by Henrique Moreira; pictures of the remaining dry fruits taken from herbarium specimens: *Balfourodendron riedelianum* (Engl.) Engl. - P. Dias 217; *Conchocarpus heterophyllus* (A.St.-Hil.) Kallunki & Pirani - N.P. Taylor 1577; *Galipea jasminiflora* (A.St.-Hil.) Engl. - A.P. Duarte 5978; *Helietta glaziovii* (Engl.) Pirani - J.R. Pirani CFCR 8878, E. Tameirão Neto 2674; *Pilocarpus riedelianus* Engl. - L.A.M. Silva 1489

Almost all mature fruits of Pilocarpinae and Galipeinae described to date have a multiplicative lignified endocarp composed of fibers elongated in perpendicular planes (Souza et al. 2005, 2008; Afonso 2018; this study), a required feature for the explosive dehiscence observed in many members of Galipeeae.

Dehiscence and schizocarpy – The mechanical stress in the lignified endocarp combined with further strongly regulated physiological processes in the entire fruit wall is responsible for the dehiscence in follicles and capsules (Roth 1977; Bobrov and Romanov 2019). In Rutaceae with loculicidal dehiscence (e.g., *Esenbeckia* and *Metrodorea*), the dehiscence line comprises a constriction of the sclerified endocarp in the median dorsal region of the locule (endocarp beak) combined with a separation tissue formed by a weakened linear arrangement of the parenchyma, which extends through the entire mesocarp to the exocarp. Although a ventricidal dehiscence line (or separation tissue) was not found in *Erythrochiton*, the ventral bundle in the region of the ventral slit associated with an abrupt reduction in the mesocarp in this region likely plays the role of the dehiscence line, guiding the rupture of the pericarp along the ventral slit. According to Roth (1977), this type of dehiscence is commonly observed in follicles and corresponds to the most primitive dehiscence type in fruits. Alternatively, recent studies in *Orixa* (Zanthoxyloideae, Rutaceae) have showed complex mechanical forces acting in the dehiscence of different layers of the pericarp, leading to a similar rupture of the mesocarp driven by hygroscopic movements of the endocarp (Huang and Fu 2021). Further detailed studies on this matter are still needed for Galipeeae.

The splitting of the separation tissue or the rupture of the dehiscence zone in mature fruits occurs due to a tension generated by the drying processes (xerochastic mechanism) in the sclerified endocarp (Hartl 1957, 1958; Roth 1977; Leins and Erbar 2010; Bobrov and Romanov 2019). The follicles of the follicaria in *Erythrochiton* as well as the capsules of *Esenbeckia* and *Metrodorea* open with great kinetic energy such that the elastic endocarp ejects the seeds outwards, resulting in the splitting of the endocarp in two parts (not splitting in *Erythrochiton*). It is known that elastic forces may operate in different directions due to the distinct orientation of the fruit fibers in the endocarp. In addition, the tissue of contraction (endocarp) may exert more pressure than the tissue of resistance (mesocarp along with endocarp beak), leading to seed ejection in autochorous fruits (Roth 1977; Huang and Fu 2021), as observed here. The endocarp that detaches from the fruit, assisting seed ejection, is a common feature in Rutaceae (Hartl 1957, 1958) and thought to have evolved multiple times in the family (Groppo et al. 2008; Huang and Fu 2021). Nevertheless, dehiscent fruits "from which the seeds become detached and are often forcibly expelled" were recently inferred as the ancestral character state in Rutaceae (Appelhans et al. 2021).

The schizocarpy seems to be profoundly affected by the extension of the ovary union between carpels, especially by the degree of fusion between carpel flanks (septa). The septicidal dehiscence of *Esenbeckia, Metrodorea* and *Helietta* results not only from a dehiscence line but is likely aided by unfused areas (slits) in the septa radii. Septicidal dehiscence, referred to here as late schizocarpy (i.e., near the moment of seed expulsion), is apparently facilitated by the occurrence of these slits in these three genera herein analyzed. Conversely, in gynoecia where the ovary is mostly apocarpous, united only at the base and/or apex in limited areas, schizocarpy occurs very early in fruit development, as observed in *Conchocarpus* and *Erythrochiton* (El Ottra et al. 2013; Afonso 2018; 2019; this study). Thus, the larger the degree of union between carpel at the ovary level, the later the fruit will split in fruitlets. In fact, flowers of many Galipeinae have carpel flanks free at the ovary level along most of its extent (El Ottra et al. 2013, 2019; this study); as a consequence, the schizocarpy takes place quite precociously. A similar occurrence is seen in *Pilocarpus pennatifolius* Lem. and *P. riedelianus* (Souza et al. 2003), as well as in *Adiscanthus* (El Ottra et al. 2019). According to Gut (1966), members of the subfamily Rutoideae (at that time including the Galipeeae genera) have weak carpel connections when compared to the formerly designated subfamilies Aurantioideae and Toddalioideae (the former is monophyletic and a fully syncarpous subfamily, while the latter are currently placed in other subfamilies in the classification of Appelhans et al. 2021), but are also mostly syncarpous groups (Ramp 1988; Groppo et al. 2012; Appelhans et al. 2021).

This developmental diversity found in Rutaceae makes it complex to identify the fruit type for some genera (e.g., capsule vs. schizocarp). Although each type of fruit appears to have evolved consistently within each genus in Galipeeae (Fig. 6), contradictory descriptions in the literature may reflect distinct interpretations made by the authors or a higher structural diversity not completely understood to date. In *Galipea*, the fruits are described by Pirani (2004) as capsules or schizocarps, while fruits of *Neoraputia* were described by Kallunki (2009) as mericarps. Also, variability occurs at the infrageneric level in *Rauia*, with species differing in the degree of gynoecium union in the anthetic stage (El Ottra et al. 2019) and likely, as a consequence, also differing in the fruit type. *Neoraputia* is characterized by carpels with the ovary base congenitally fused at the flanks and center (El Ottra et al. 2019), which may result in schizocarpous fruits (Kallunki 1990, 2009). A similar pattern is observed in anthetic gynoecia of *Rauia* (El Ottra et al. 2019), with fruits that undergo late schizocarpy (Pirani 1999). Some authors have hypothesized that "hemisyncarpous" and "eusyncarpous" follicles (what we refer to as aggregate fruits) evolved as transitional forms between follicles sensu stricto and capsules (Bobrov and Romanov 2019; and references therein). Thus, we could assume that an analogous situation might have occurred in Galipeeae, considering the follicarium as a transitional form between follicles and capsules (Fig. 6). Future evolutionary studies should clarify this hypothesis. In the evolutionary reconstruction of ancestral character states presented by Appelhans et al. (2021), two types of dehiscent fruits were distinguished: those with seeds detached (as seen in Galipeeae) and those with seeds attached. The former was recovered as the ancestral character state for the family and conserved in several genera of all subfamilies, with independent transitions to other types, such as berry, hesperidium, drupaceous, samara, and the dehiscent type with seeds attached, which evolved multiple times within the large clade Zanthoxyloideae.

Our results shed new light on the understanding of the complex fruits of Galipeeae. We found different types of fruit in the four genera analyzed, in addition to a likely relationship between the schizocarpous development and the type of dehiscence with the structure of the ovary. Early schizocarpy is related to reduced carpel union in the anthetic ovary and was observed in *Erythrochiton*, while late schizocarpy can be considered a consequence of the higher degree of union between carpel flanks, occurring in *Esenbeckia*, *Metrodorea* and *Helietta*. Loculicidal dehiscence occurs through the disruption of the pericarp along a median dorsal separation tissue found in *Esenbeckia* and *Metrodorea*, whereas the ventricidal dehiscence observed in *Erythrochiton* is likely directed by the ventral vascular bundle in the ventral slit. The seeds are ejected from the fruit through an explosive dehiscence likely due to xerochastic tension generated on the endocarp fibers, which are oriented in different planes. Other types of fruit are present in Galipeeae, and further studies are needed to verify their classification and developmental patterns, which can greatly increase the structural diversity observed in this study.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40415-021-00779-9.

Acknowledgements This work was supported by Grants from 'Fundação de Amparo à Pesquisa do Estado de São Paulo' (FAPESP; proc. #2014/18002-2, #2015/09776-7 and #2018/14853-9), awarded to JRP, JHLEO and GOP, respectively. The first author was also granted with a short-term scholarship from National Council for Scientific and Technological Development (CNPq) (Grant ID: #144153/2018-1). We are grateful to Henrique Moreira for making a picture of a fruit of *Hortia* available for us. We also thank the technician of the Laboratory of Plant Anatomy (IB-USP) Tássia C. Santos, for her assistance to the first author in the laboratory.

Authors' contributions GOP, JRP and JHLEO involved in conceptualization; GOP, JRP, DD and JHLEO took part in methodology; GOP, JRP, DD and JHLEO took part in formal analysis and investigation; GOP, JRP, DD and JHLEO involved in writing—original draft preparation; GOP, JRP, DD and JHLEO involved in writing—review and editing; GOP, JRP and JHLEO involved in funding acquisition; JRP and JHLEO took part in supervision. All authors have read and approved the final version of the manuscript.

Declarations

Conflict of interest Diego Demarco is the editor-in-chief of the Brazilian Journal of Botany, and this article was entirely handled by an associate editor.

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