



How pollen and ovule development underlay dioecy in *Chloroluma gonocarpa* (Sapotaceae)

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Abstract

Previous studies have determined that *Chloroluma gonocarpa* (Sapotaceae), is a species that has cryptic dioecy. This type of sexual system is characterized by flowers that are morphologically perfect (both sexual whorls are present) but functionally pistillate or staminate (in each type of flower one of the sexual whorls is non-functional). In *C. gonocarpa* the pistillate flowers present well-developed stigma, functional ovules, and staminodes, while the staminate flowers present a poorly developed stigma, collapsed ovules, and pollen-producing anthers. In angiosperms, the abortion of sexual organs can occur at different stages of development (from pre-meiosis to post-meiosis), that is why we conducted an anatomical analysis of both flower types at various developmental stages. Using light microscopy, we described the processes of sporogenesis and gametogenesis to establish when the staminate flowers lose their pistillate function. To achieve this, we collected, fixed, and processed the flowers following conventional anatomical techniques for observation under a light microscope. Our findings reveal that pollen development occurs only in staminate flowers, while ovule development begins in both types of flowers but ceases in staminate flowers due to post-meiosis abortion. In contrast, normal development continues in pistillate flowers. These results suggest that dioecy in *C. gonocarpa* may have arisen from a gynodioecious pathway.

Keywords Abortion · Embryo sac · Gynodioecious · Pistillate flowers · Staminate flowers

Introduction

In angiosperms there is a large record of literature on the possible variants between monoecy and dioecy and the evolution of unisexual flowers. Two types of unisexual flower morphologies are recognized (Mitchell and Diggle 2005). The first is type I, whose flower is unisexual by abortion (the development of both sexual whorls is initiated but development stops in one). The second is type II, whose flower is unisexual by inception (the floral meristem initiates only one sexual whorl). Mitchell and Diggle (2005) and Diggle et al. (2011) outlined four stages of loss of sexual organ function

in the development of unisexual flowers: before the initiation of the stamen or carpel primordia (stage 0); early in the development of the stamen or carpel (stage 1); pre-meiosis (stage 2); and post-meiosis (stage 3). Diggle et al. (2011) reviewed flower unisexuality in 292 angiosperm taxa and found that developmental arrest of a sexual organ occurs with equal frequency at each of the four stages in both staminate and pistillate flowers. This suggests that there are no particular stages of androecium or gynoecium development that are repeatedly affected in the evolutionary origin of unisexual flowers. Furthermore, these authors suggested that the stage of sexual organ arrest that characterizes these taxa is not necessarily the stage of arrest associated with the evolutionary origin of floral unisexuality for that lineage. Diggle et al. (2011) also propose the existence of six processes associated with the cessation of organ development: (i) cell death by collapse and/or degeneration of cells and tissues; (ii) programmed cell death (PCD); (iii) parenchymatization: cessation of cell division without cell death or degradation; (iv) developmental arrest; (v) alteration in the timing of flower development or maturation; and (vi) infertile or non-viable pollen (female flowers only).

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Sapotaceae is a family of flowering plants with a pantropical distribution. It comprises three subfamilies: Chrysophylloideae, Sapotoideae, and Sarcospermatoideae, and includes 73 genera (Swenson and Anderberg 2005; Swenson et al. 2023). Species in the Sapotaceae family have actinomorphic flowers, stamens opposite the corolla lobes, a superior ovary, and a simple style (Ayensu 1972; Pennington 2004; Swenson and Anderberg 2005).

Within the Sapotaceae family, there are different sexual systems. These include hermaphroditism, where each individual has bisexual flowers; monoecy, where staminate and pistillate flowers are present on the same individual; and dioecy, where staminate flowers are found on one individual and pistillate flowers on another (Pennington 2004). Recently, gynomonocious flowers have been described in species of the genus *Planchonella* (Méndez and Munzinger 2010) and in *Chrysophyllum marginatum*, Sapotaceae. (Sigrist et al. 2021). In both cases, the studies included analyses of the morphology of the flowers and the floral biology of the species.

It has been suggested that the frequency of species with unisexual flowers in the Sapotaceae is probably higher than thought but misidentified because of the hermaphroditic appearance of some flowers (Pennington 2004). In particular, cryptic dioecy, i.e. species with unisexual flowers in which one or both morphs appear to be hermaphrodite, by retaining non-functional sexual organs (Mayer and Charlesworth 1991), could be present in Sapotaceae. However, purported non-functional organs of unisexual angiosperm flowers are often referred to as “aborted” without providing data on how they lost their function (Diggle et al. 2011). Different disciplines should be integrated to better understand the mechanism leading to flower unisexuality (Diggle et al. 2011). So far, the only anatomical study on a dioecious species of Sapotaceae is that of Judkevich et al. (2023) on *Chrysophyllum gonocarpum* (currently recognized as *Chloroluma gonocarpa* (Mart. & Eichler) Baill. ex Aubrév. according to Swenson et al. 2023). Anatomical studies describing the processes of sporogenesis and gametogenesis to confirm or explain in detail a sexual system are scarce for hermaphrodite species and non-existent for unisexual flowering species of Sapotaceae (Bhatnagar and Gupta 1970; Zavaleta Mancera and Engleman 1995). The latest published work on the anatomical study of the ovule in a species of Sapotaceae is that of Zavaleta Mancera and Engleman (1995) in *Manilkara zapota*, a hermaphroditic species.

C. gonocarpa (subfamily Chrysophylloideae) is a South American species found in south-eastern Brazil, northern Argentina, Paraguay, and Bolivia (Cronquist 1964; Swenson et al. 2023). *C. gonocarpa* is a species that is acquiring agricultural interest since its fleshy fruits are consumed by

humans after cooking in syrup (Pirondo et al. 2018). This species has been recently confirmed to be cryptically dioecious (Judkevich et al. 2023). In many cases the anatomical study of pollen and ovule development has been important to help determine or fully understand the sexual system of different species of angiosperms (Anderson et al. 2000; Benevides et al. 2013; Judkevich et al. 2022a, b; Li et al. 2010; Strittmatter et al. 2002). However, the processes of sporogenesis and gametogenesis in this species, and the moment at which the development of the ovule stops in staminate flowers, are so far unknown.

Considering the absence of studies that anatomically describe the development of ovules and pollen grains in non-hermaphroditic species of Sapotaceae, this study aims to (1) describe the processes of sporogenesis and gametogenesis in *C. gonocarpa*, and (2) determine the stage at which ovule development stops in the staminate flowers of this species.

Materials and methods

Field observations of *C. gonocarpa* were made at the experimental field of the Universidad Nacional del Nordeste (FCA-UNNE), located in Corrientes (Argentina). Observations were made during three consecutive years on 46 trees of the same age, all of which have an identification number. Half of these trees always produce fruit (trees with pistillate flowers) and the other half have never produced fruit (trees with staminate flowers). Photographic recording of the flowers and their analysis under stereoscopic microscopy were carried out on all the trees. For anatomical studies, flowers and flower buds were collected from seven randomly selected trees, four pistillate and three staminate. The fresh material collected was fixed with FAA (formaldehyde, acetic acid and alcohol). The vouchers were deposited in the CTES herbarium: ARGENTINA. PROV: Corrientes, Capital, 20 Dec 2017, tree number 2, pistillate flowers, Ana Gonzalez 521; idem, tree number 8, pistillate flowers, Ana Gonzalez 522; idem, tree number 9, staminate flowers, Ana Gonzalez 523; idem, tree number 12, staminate flowers, Ana Gonzalez 524; idem tree number 15, staminate flowers, Ana Gonzalez 525; idem, tree number 27, pistillate flowers, Ana Gonzalez 526; idem, tree number 39, pistillate flowers, Ana Gonzalez 527.

From each collected sample, 10 buds or flowers at different stages of development were anatomically analyzed, focusing on the anthers and ovary. For anatomical analysis, the plant material was processed following conventional techniques of alcoholic dehydration and paraffin embedding (Gonzalez and Cristóbal 1997; Johansen 1940). Subsequently, serial longitudinal and transverse sections of

12–15 μm thickness were made with Microm HM350 rotary microtome (Microm International, Walldorf, Germany). Serial sections were stained with safranin and astra blue (Luque et al. 1996) and mounted with Canada balsam. The sections were analyzed and photographed using a Leica DM LB2 light microscope (Leica Microsystems) equipped with a Leica ICC50 HD digital camera. In addition, fresh pollen samples were stained with Sudan III to detect the presence of pollenkitt (Kraus and Arduin 1997).

Results

Development of stamen and staminodes

The staminate flowers (Fig. 1a–c) have five stamens with basifixed anthers and long filaments (Fig. 1a, b). In pistillate flowers (Fig. 1d–f), the staminodes (Fig. 1e) are much smaller than the stamens of staminate flowers. The anatomy of the anthers, including the anther wall and the development of pollen grains in staminate flowers, as well as the structure of the staminode in pistillate flowers, is described below.

Staminate flowers

Stamens are typical of angiosperms, composed of a filament and tetrasporangiate bithecal anthers. Initially the anther primordium is formed by a protodermis and meristematic cells (Fig. 1g). The protodermis develops into the epidermis, while the meristematic cells divide mitotically in different planes until the young anther acquires a tetralobed shape (Fig. 1h). In each lobe the subepidermal cells continue to divide until they form the multi-layered wall of the anthers (Fig. 1i, j), while the innermost cells divide to form the sporogenous tissue. After differentiation of the sporogenous tissue, the secretory tapetum, 3–4 middle layers and the unistratified endothecium are distinguished in the anther wall (Fig. 1k, n). The cells of the sporogenous tissue differentiate into microspore mother cells that are surrounded by callose. As the microspore mother cells increase in volume, the cells of the middle layers adjacent to this tissue begin to collapse. In the connective tissue the vascular bundle is already differentiated, and numerous cells with phenolic compounds and some laticifers (coming from the filament) are identified (Fig. 1m). The interocular septum is formed by parenchymatous cells with intensely coloured cytoplasm consisting of phenolic compounds (Fig. 1l). The stomium region begins to delimit (Fig. 1l).

Microspore mother cells divide by meiosis generating tetrads surrounded by callose, the cells of the secretory tapetum begin to show a retracted cytoplasm (Fig. 2a). The

callose dissolves and the microspores are released into the loculus (Fig. 2b). The tapetal cells have now completely collapsed as have most of the cells in the middle layer. Endothecium cells increase in volume and differential growth is observed, the cells are larger towards the connective tissue and smaller towards the stomium region. Fibrous thickening develops in all cells of the endothecium. Few stomata are observed in the epidermis surrounding the locules (Fig. 2c).

Microspores differentiate into pollen grains, through a mitotic division in the microspores forms vegetative and generative cells (Fig. 2d, e). The cells of the interocular septum collapse so that there is fusion of the locules. Subsequently, as a consequence of the collapse of the stomium cells, the anthers undergo longitudinal dehiscence releasing pollen just before floral anthesis (Fig. 2f). In fresh material, pollenkitt is observed on the surface of the pollen grains (Fig. 2g) which reacts positively to Sudan III indicating its lipid nature.

Pistillate flowers

The staminodes are formed only by filaments consisting of epidermis and parenchyma, there is no anther or pollen development (Fig. 2h–j). The mature staminodes have a central vascular bundle and laticifers (Fig. 2j).

Development of ovule and embryo sac

In both pistillate and staminate flowers the ovary is (3–4)–5 locular, with axillary placentation and one anatropous, unitegmic, and tenuinucellate ovule per locule. The following describes the development of the ovule and embryo sac in both types of flowers, and the differences between them.

Pistillate flowers

The ovule primordium appears on the placenta as a small dome formed by cells with dense cytoplasmic content (Fig. 3a). A series of mitotic divisions in different planes increases the size of the ovule primordium and at the same time causes it to curve (Fig. 3b, c).

It is possible to distinguish the nucellus and the initiation of the single tegument when the primordium reaches a 90° bend (Fig. 3d). When the nucellus exceeds 90° a single archesporial cell appears beneath the nucellar cells (Fig. 3e). When the unitegmic tegument closes to form the micropyle, the archesporial cell directly becomes the megaspore mother cell (Fig. 3f). As a result of the meiotic division of the latter, a dyad is formed first (Fig. 3g) and then a tetrad of megaspores oriented parallel to the longitudinal direction of the ovule. As these divisions occur, the innermost layer of the integument differentiates into an integumentary tapetum.

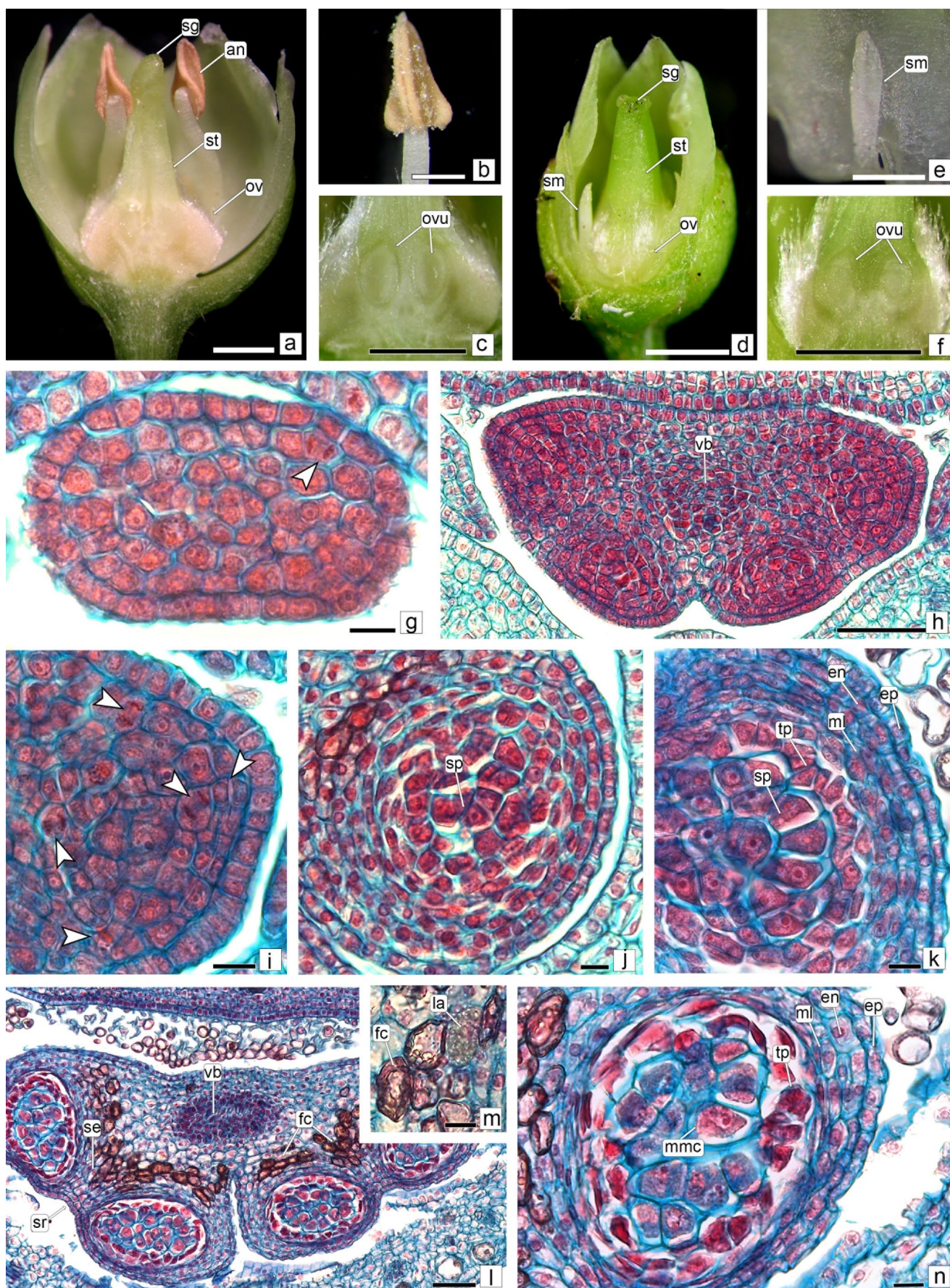


Fig. 1 Staminate and pistillate flowers. **a-c** Staminate flower. **b** Anther. **c** Longitudinal section of ovary with ovules. **d-f** Pistillate flower. **e** Staminode. **f** Longitudinal section of ovary with ovules. **g-n** Development of anthers of staminate flowers. **g** Anther primordium (dividing cells marked with white arrow). **h** Young tetralobed anther. **i** Detail of a lobe with dividing cells (white arrows). **j** Young sporangium. **k** Sporangium with sporogenous tissue. **l** Anther in the stage of the microspores mother cell. **m** Detail of cells with phenolic compounds and cell with latex. **n** Detail of sporangium with mmc in which meiosis has already started. Abbreviations: an = anther, en = endothecium, ep = epidermis, fc = phenolic compounds, la = laticiferous, ml = middle layers, mmc = microspore mother cell, ov = ovary, ovu = ovule, se = septum, sg = stigma, sm = staminode, sp = sporogenous tissue, sr = stomium region, st = style, tp = tapetum, vb = vascular bundle. Scales: **a, c-d, f** = 1 mm, **b, e** = 0.5 mm, **g, i-k, m-n** = 10 μ m, **h, l** = 50 μ m

The three apical megaspores of the tetrads collapse and the chalazal megaspore develop directly into a functional megaspore (Fig. 3h). The megaspore undergoes mitotic divisions and consequently increases considerably in volume and the nucellar epidermis ruptures (Fig. 3i). The result of the divisions is an embryo sac of the *Polygonum*-type (Fig. 4a-d), with seven cells and eight nuclei, including an egg cell and two synergids (with the filar apparatus) in the micropylar region, a binucleated central cell, and three antipodal cells in the chalazal region. The mature ovule is anatropous and consists of a single massive-type integument surrounding the embryo sac and a short funiculus (Fig. 4e).

Staminate flowers

In these flowers the development of the ovule is similar to that of the pistillate flowers from the primordium of the ovule stage until the meiosis of the megaspore mother cell (Fig. 5a-f). From this stage, development may stop after the formation of the tetrad (Fig. 5g, h), or it may continue until the embryo sac is formed (Fig. 5i-l) and then collapse afterwards (Fig. 5m). In both cases, after the collapse of the tetrad or sac, the rest of the ovule also degenerates completely (Fig. 5n). Abortion begins on the megagametophyte and extends to the nucellus. At anthesis, the ovule is completely disintegrated. The funicle does not show any signs of disintegration (Fig. 5n).

The ovules of both types of flowers are vascularized. Longitudinal sections of the ovule from lateral, posterior and frontal views show how a single vascular bundle enters from the placenta (Fig. 6a), passes through the funiculus where it branches (Fig. 6b) and innervates the single integument (Fig. 6c). The small vascular bundles are predominantly procambial. They do not reach to innervate the micropyle.

Discussion

Stamen and staminodes

The mature anther wall of Sapotaceae consists of an epidermis, endothecium, 2–3 middle layers and secretory tapetum (Johri et al. 1992). In *C. gonocarpa*, the anther wall of the staminate flowers is similar to that described for the family. The only difference is that there are 3–4 intermediate layers. The epidermis of the anthers of *C. gonocarpa* has some stomata. The presence of stomata on the anther wall has previously been reported in *Mimusops elengi*, a hermaphrodite species of the Sapotaceae (Bhatnagar and Gupta 1969, in Johri et al. 1992). The stomata in the anthers would participate in the dehiscence process by facilitating anther dehydration (Keijzer et al. 1987). In pistillate flowers, the staminodes are reduced to the staminal filament and anthers are completely absent. In *C. gonocarpa*, pistillate flowers are completely unisexual because the stamens, which are reduced to staminodes without anthers, make pollen production infeasible.

The only known study of pollen development in Sapotaceae was conducted on *M. elengi* by Bhatnagar and Gupta (1970). In the staminate flowers of *C. gonocarpa*, pollen development occurs similarly to that of *M. elengi*, and is completely absent in pistillate flowers in which only staminodes of parenchymatous tissue are present.

Droplets of pollenkitt, a substance of lipidic origin, have been observed on the surface of mature pollen of *C. gonocarpa*. This same substance has also been found in another species of the family, *Chrysophyllum marginatum* (Sigrist et al. 2021). The pollenkitt has been described in numerous angiosperms in which it would facilitate pollen dispersal, prevent pollen dehydration, allow pollen to remain attached during transport, and facilitate adherence to different parts of the flower and to the body of the pollinator, among other functions (Pacini and Hesse 2005). The flowers of *C. gonocarpa* also show entomophilous pollination (pers. obs.), as has been recorded in other species of the genus, so the presence of pollenkitt would favor this type of pollination.

Ovule and embryo sac

Ovule development is normal in pistillate flowers but shows abnormalities in staminate flowers. Mature ovules in *C. gonocarpa* are anatropous, unitegmic and tenuinucellate, with a *Polygonum*-type embryo sac. These characteristics are found in both pistillate flowers and those that mature before abortion in staminate flowers. This is consistent with the typical ovules described for Sapotaceae (Johri et al. 1992; Pennington 2004). Unitegmy is also a predominant

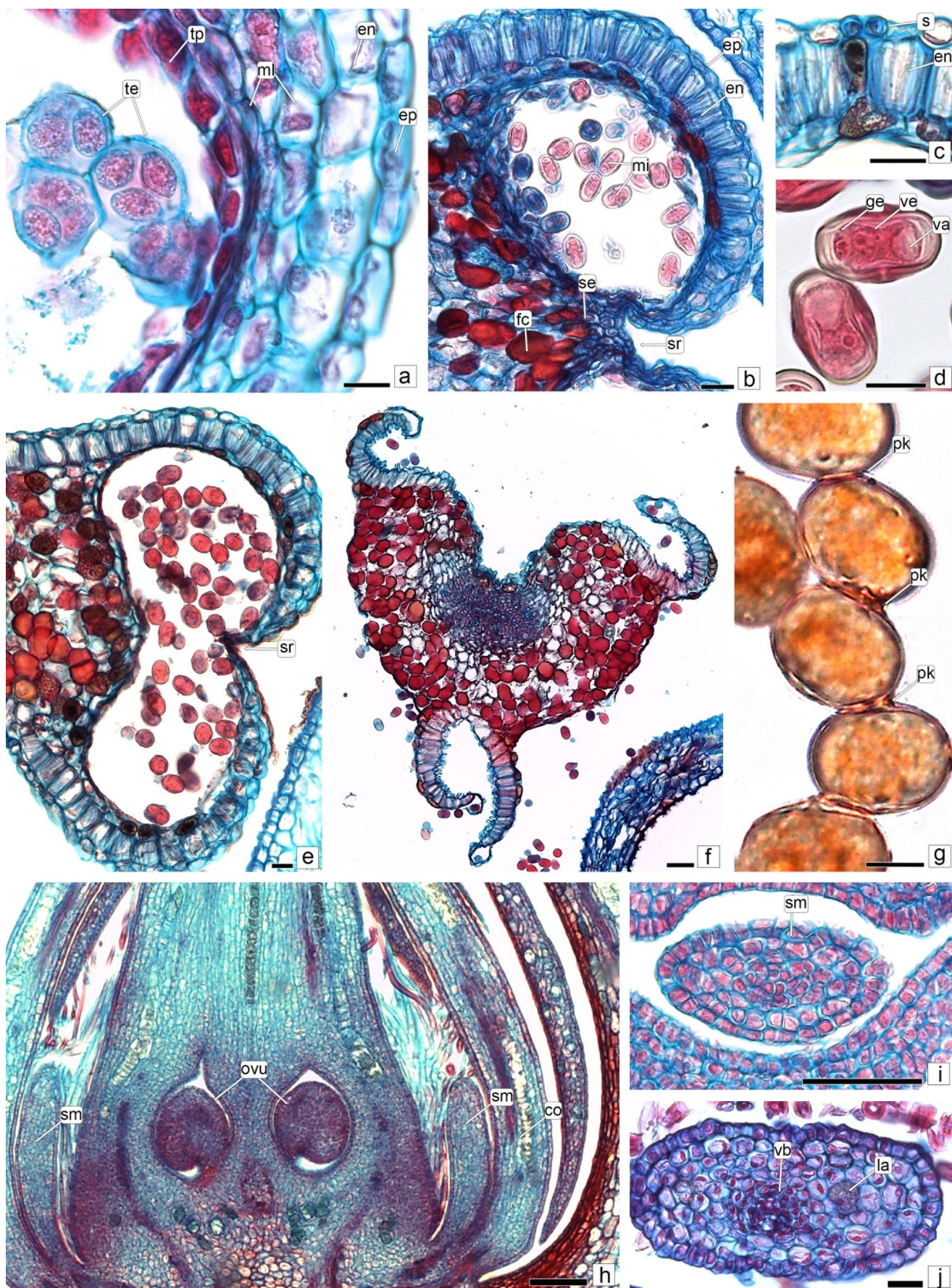


Fig. 2 Staminate and pistillate flowers. **a-g** Staminate flower. **h-j** Pistillate flower. **a** Detail of callose tetrads and wall of anther. **b** Sporangium in microspore stage, endothecium shows thickening. **c** Detail of stomata in the area of the endothecium. **d** Microspores, the one above with vegetative and generative cells. **e** Theca with fusion of locules. **f** Anther dehiscence. **g** Mature pollen grains colored with Sudan III, attached by pollenkitt. **h** Longitudinal section of pistillate flower bud at the ovary and staminodes. **i** Cross section of the staminode of a flower bud. **j** Cross section of the staminode of a flower at anthesis. Abbreviations: co=corolla, en=endothecium, ep=epidermis, fc=phenolic compounds, ge=generative cell, la=latexiferous, mi=microspores, ml=middle layers, mmc=microspore mother cell, ov=ovary, ovu=ovule, pk=pollenkitt, s=stomata, se=septum, sm=staminode, sp=sporogenous tissue, sr=stomium region, te=tetrads, tp=tape-tum, va=vacuole, vb=vascular bundle, ve=vegetative cell. Scales: **a, d, g**=10 μm , **b-c, e, j**=20 μm , **f, i**=50 μm , **h**=100 μm

characteristic in the ovules of the Asterides, the clade to which the Sapotaceae belongs (Endress 2011; Rudall 2021).

The early degeneration of the nucellus during the development of the ovule, which leaves the embryo sac in direct contact with the inside of the integument, has already been described as common in certain tenuinucellated ovules of angiosperms (Endress 2011) and is found in both pistillate and staminate flowers of *C. gonocarpa*. In Sapotaceae it has previously been described in *Manilkara sapota*, a hermaphroditic species (Zavaleta Mancera and Engleman 1995).

In tenuinucellate ovules an endothelium, also known as integumentary tapetum, usually forms within the inner integument (Endress 2011; Kapil and Tiwari 1978), as seen in the interior of the single tegument of *C. gonocarpa*. The integumentary tapetum is found in certain Rosides and Asterides and would participate in the nutrition of the embryo sac during its development (Endress 2011; Kapil and Tiwari 1978). However, there are cases in which the endothelium presents certain abnormalities that cause the collapse of the embryo sac (Kapil and Tiwari 1978). This has been documented in Asteraceae taxa, such as *Helianthus* (Savchenko 1973) and *Smallanthus sonchifolius* (Ibañez et al. 2017). In the case of *Helianthus* the cuticle of the endothelium becomes thick, cutting off the nutrient supply to the embryo sac, so it collapses while the endothelium proliferates due to excessive nutrient accumulation (Savchenko 1973). Meanwhile, in *S. sonchifolius* a proliferation of endothelium cells is observed prior to the collapse of the embryo sac (Ibañez et al. 2017). On the other hand, it has been observed that in *Arabidopsis* plants subjected to salt stress, the first symptom of ovule abortion is the synthesis of callose in the endothelium (Sun et al. 2004). Although there is no proliferation of the endothelium in the ovules of staminate flowers of *C. gonocarpa*, the cell walls of the endothelium appear to be thicker in the ovules of staminate flowers than in those of pistillate flowers. Future comparison of the endothelium of both flower types, either ultrastructural or studies to detect DNA damage will help detect possible abnormalities in the

endothelium of staminate flowers and determine if this tissue causes ovule abortion.

The ovules of both *C. gonocarpa* flower types are vascularized from the funiculus and have ramified branches running along the tegument. Evidently, the supply of water and nutrients is not a determining factor in the abortion of the ovules of the staminate flowers. In general, massive ovules and ovules that develop into large seeds are associated with vascularization in the integuments (Endress 2011). Seeds of *C. gonocarpa* reach 2.1 cm in length and have been described as having a profuse vascular supply running along the raphe, branching at the chalaza and with numerous post-chalaza branches running along the sides of the seed up to the micropyle (Beltrati et al. 1983; Felippi et al. 2010).

Sexuality

The present study demonstrates that in staminate flowers of *C. gonocarpa*, the loss of pistillate function also occurs by abortion of ovules early during megasporogenesis (at the megaspore tetrad stage) or near maturity during megagametogenesis. In both cases, the cell death continues in the surrounding tissues of the ovule, leading to its complete degeneration. In other Angiosperm species, sterility of ovules in staminate flowers occurs by abnormal development of the integuments and nucella with subsequent degeneration of their cells (*Opuntia robusta*, Hernández-Cruz et al. 2019), by abortion of the megagametophyte during megagametogenesis (*Cylindropuntia wolfii*, Ramadoss et al. 2022) or by heterochrony, i.e., the ovules develop normally but begin their senescence before floral anthesis (*Consolea* spp., Strittmatter et al. 2008). An extreme case of reduction has been seen in dioecious species of Rubiaceae in which the ovules are represented only by small undifferentiated parenchyma bodies protruding from the placenta (Judkevich et al. 2022a).

Based on the results obtained in this study, and in accordance with the classification proposed by Mitchell and Diggle (2005) for unisexual flowers in angiosperms, the flowers of *C. gonocarpa* are classified as Type I, which exhibit rudiments of the nonfunctional stamen (pistillate flowers) or pistil (staminate flowers). In staminate flowers, the ovules are aborted by developmental arrest which occurs post-meiosis (stage 3). Pistillate flowers, on the other hand, have staminodes composed by parenchyma (without signs of tissue abortion) already as smaller flower buds. Further ontogenetic studies showing the early stages of pistillate flowers are needed to determine the process involved in the arrest of anther development leading to staminode formation. Among the species listed by Diggle et al. (2011), there is only one genus belonging to the Sapotaceae family: *Planchonella*,

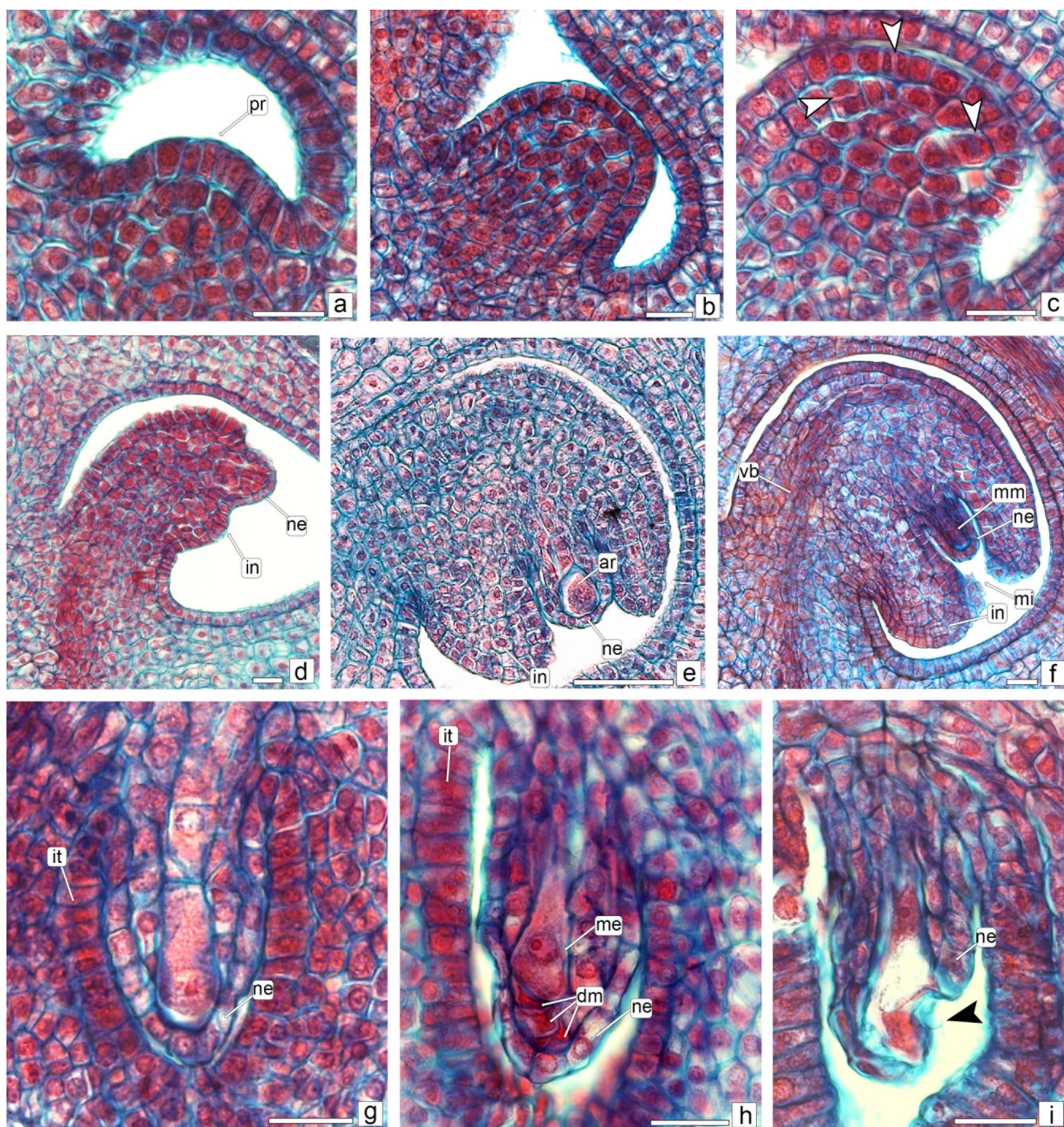


Fig. 3 Development of the ovule and embryo sac in pistillate flowers. **a-d** Primordium of the ovule; arrowheads in **c** Indicate dividing cells. **e** Ovule with archesporial cell covered by nucellar epidermis. **f** Ovule at megaspore mother cell stage. **g** Detail of nucella with dyad of megaspore. **h** Linear tetrad with three aborted apical megaspores. **i** Young embryo sac, the arrowhead points to a region of the nucellar

epidermis that has been ruptured by the enlarged embryo sac. Abbreviations: ar = arquesporial cell, dm = degenerate megaspores, in = integument, it = integumentary tapetum, me = megaspore, mi = micropyle, mm = megaspore mother cell, ne = nucellar epidermis, pr = ovule primordium, vb = vascular bundle. Scales: **a-d, f-i** = 20 μm , **e** = 50 μm

which is gynodioecious and shows stage 2 of androecium sterility (pre-meiosis).

Dioecy can evolve directly from hermaphroditism or through intermediate stages, such as gynodioecy,

androdioecy, and monoecy (Bawa 1980). Dufay et al. (2014) analyzed the gynodioecy-dioecy pathway in Angiosperms and found that several angiosperm families have dioecious species that may have evolved from gynodioecious

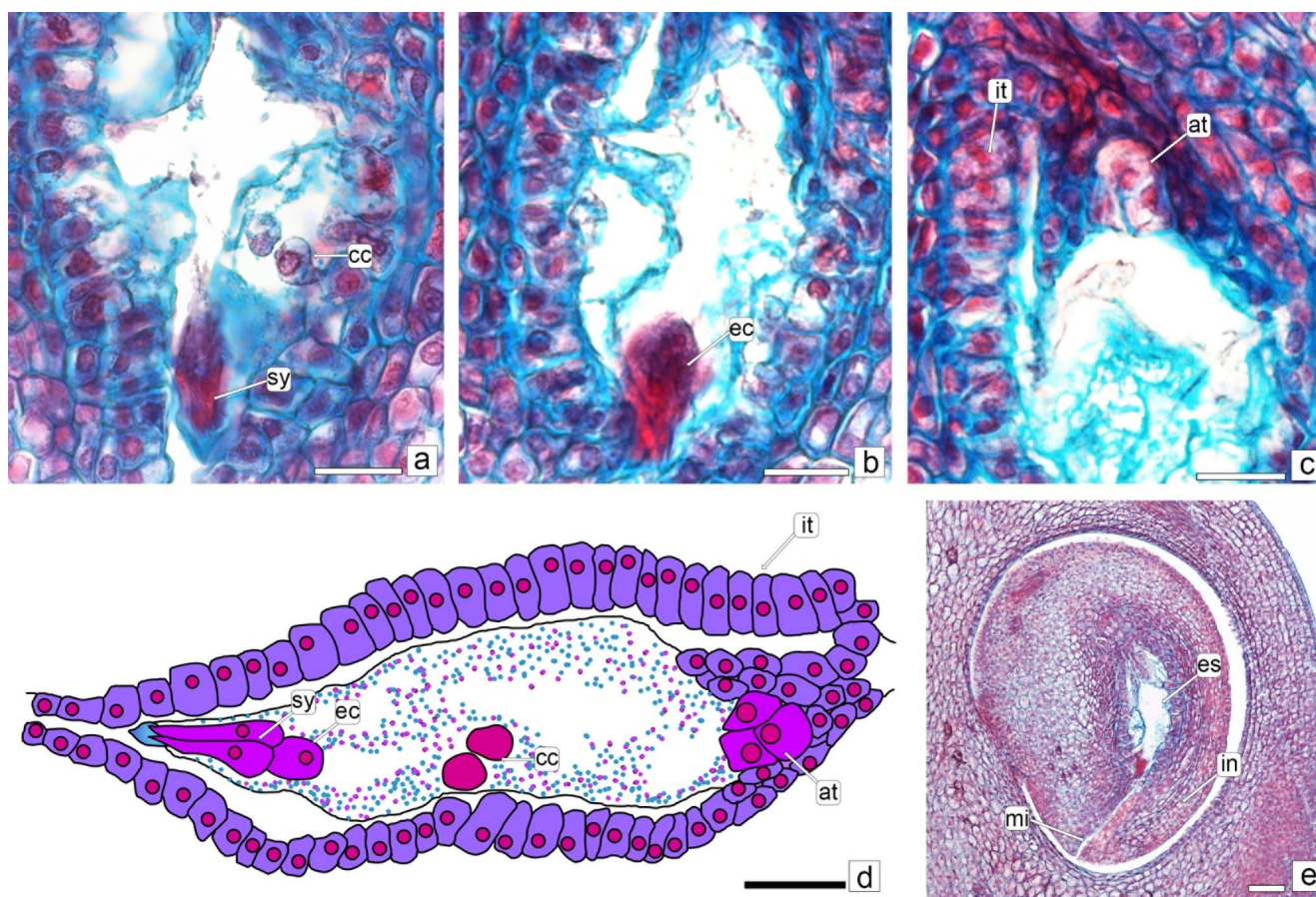


Fig. 4 Embryo sac in pistillate flowers. **a-d** Embryo sac. **a** One synergid and binucleated central cell. **b** Egg cell. **c** Antipodes. **d** Scheme of the complete embryo sac. **e** Ovule. Abbreviations: at = antipodal

cells, cc = central cell, ec = egg cell, es = embryo sac, in = integument, it = integumentary tapetum, mi = micropyle, sy = synergid. Scales: **a-d** = 20 μ m, **e** = 50 μ m

precursors. These precursors are taxa in which pistillate and hermaphroditic flowers are borne on different plants. One such family is Sapotaceae, within this family gynodioecy has been mentioned in the genera *Planchonella* (Havran et al. 2021) and *Pleioluma* (Swenson et al. 2018), and in *Bequaertiodendron magalismontanum* (Steyn and Robbertse 1988). In the latter species, hermaphrodite plants produce fewer fruits and fewer seeds than pistillate plants but contribute pollen to the population. It is therefore considered that this species is in transition from a gynodioecy to a complete dioecy (Steyn and Robbertse 1989). Considering that in *C. gonocarpa*, the staminate flower initiates as a hermaphrodite flower, but at maturity the stigma is poorly developed (Judkevich et al. 2023) and as found in the present work, the ovule eventually aborts during embryo sac development, we hypothesize that the dioecy in *C. gonocarpa* originated from a gynodioecious ancestor.

Conclusion

In Angiosperms, unisexuality has evolved independently numerous times but is poorly studied from an anatomical point of view. This is the first study in the family Sapotaceae to provide anatomical evidence for the arrested development of ovules in staminate flowers. The pistillate flowers of *C. gonocarpa* are unisexual because their stamens are replaced with staminodes without anthers. On the other hand, the staminate flowers are unisexual due to underdeveloped stigma/style and the abortion of ovules after meiosis. In addition, the results obtained here suggest that in *C. gonocarpa* this sexual system may have been derived from gynodioecy.

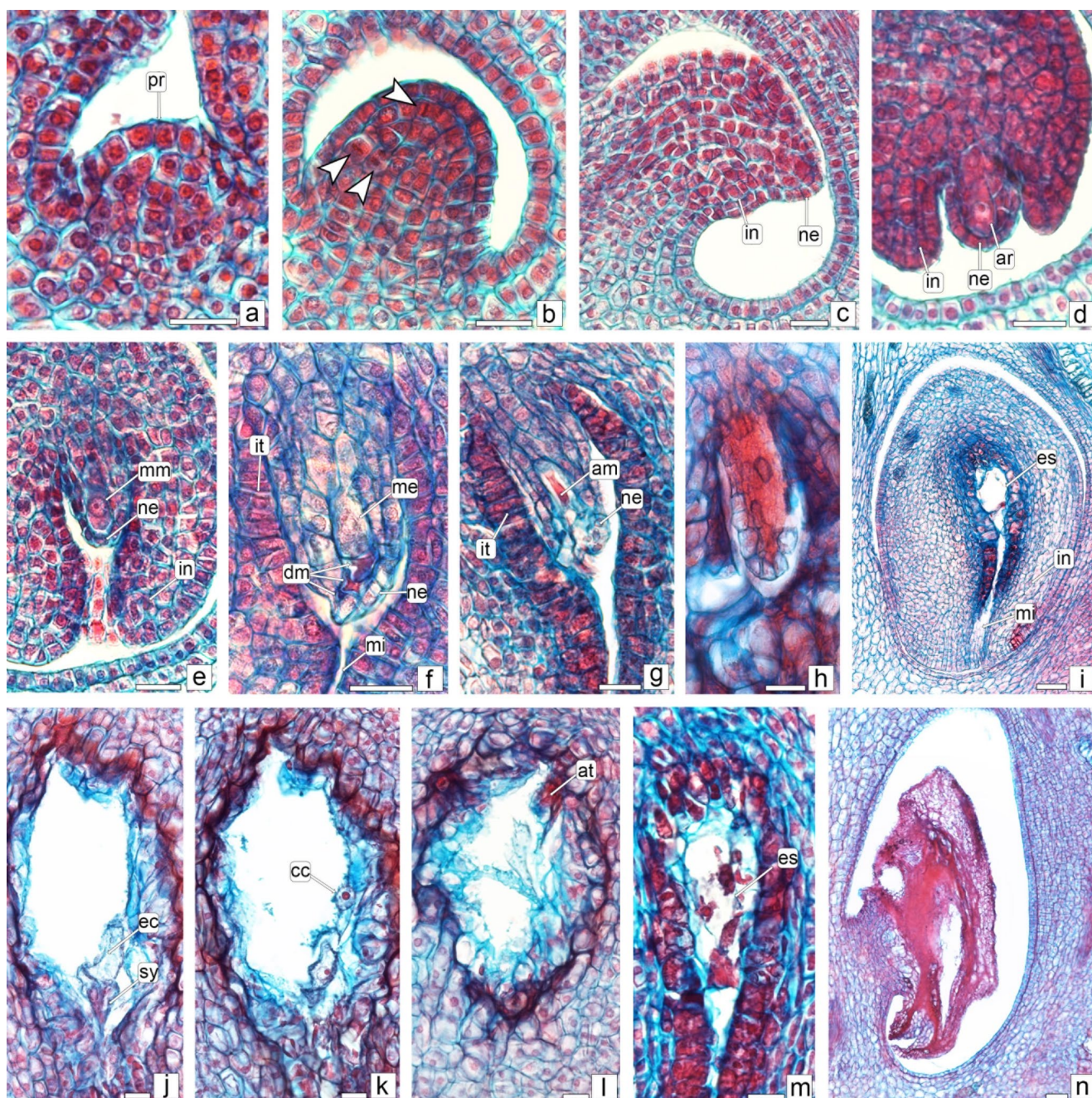


Fig. 5 Development of the ovule and embryo sac in staminate flowers. **a-c** Primordium of the ovule; arrowheads in **b** indicate dividing cells. **d** Detail of archesporial cell and nucellar epidermis. **e** Megaspore mother cell. **f** Linear tetrad with the three apical megaspores aborted. **g** Aborted basal megaspore. **h** Abortion of tissues surrounding the megaspore. **j-l** Embryo sac. **m** Aborted embryo sac. **n** completely

aborted ovule. Abbreviations: am = aborted megaspore, at = antipodal cells, ar = arquesporial cell, dm = degenerate megaspores, es = embryo sac, in = integument, it = integumentary tapetum, me = megaspore, mi = micropyle, mm = megaspore mother cell, ne = nucellar epidermis, pr = ovule primordium. Scales: **a-h, j-m** = 20 μ m, **i, n** = 50 μ m

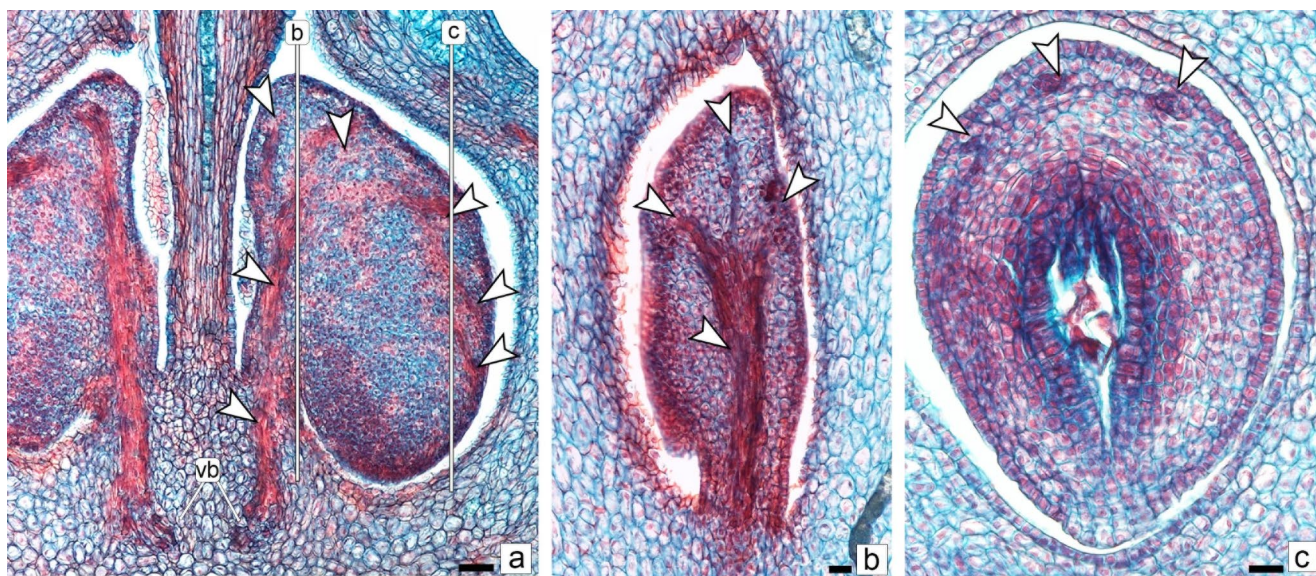


Fig. 6 Vascularization of the ovules. **a–b** Ovules of staminate flowers. **a** Lateral longitudinal view, the line **b** Marks the height at which the section in figure **b** is made. **b** Dorsal longitudinal view. **c** Ovule of pistillate flower, frontal longitudinal view. The arrowheads mark the vascular bundles. Abbreviations: vb=vascular bundle. Scales: **a** = 50 μ m, **b–c** = 20 μ m

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Data availability All data generated or analysed during this study are included in this published article.

Declarations

Competing interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

Author's contributions AMG and PAL provided the general idea of the paper. PAL provided the plant material. AMG provided laboratory supplies and equipment and also collected the vouchers deposited at the herbarium. MDJ conducted the field observations, collected and processed the plant material to make the histological preparations, took the microscopy photos, and prepared the figures. MDJ wrote the manuscript in collaboration with AMG. All authors read and approved the manuscript.

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