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ORIGINAL ARTICLE

Androecium anatomy of *Isertia laevis*, a polysporangiate species of Rubiaceae

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Abstract

In this study, we performed an anatomical analysis of the polysporangiate anthers and the development of pollen in *Isertia laevis* (Rubiaceae) with the aim to elucidate the internal structure of these atypical anthers. For this purpose, flowers in successive stages of development were dissected and the anthers were processed for conventional anatomical analysis. The material was examined using light microscopy and scanning electron microscopy. The present study shows that the anthers of *Isertia laevis* have two thecae divided into multiple microsporangia. This division is due to the presence of transverse and longitudinal septa formed of parenchyma and idioblasts with crystals. The septa appear together with the microsporangia and remain in the mature anther, even developing fibrous thickening. As the anther matures, the idioblasts in the septa accumulate crystals until they break, facilitating the separation of the septa from the outer wall of the theca, and thus apparently assisting the process of dehiscence. The mature anther opens through the longitudinal dehiscence of each theca. In addition to the anatomy of the anther, the development and morphology of the pollen, and the presence of orbicules are described. The structure of the anthers of *I. laevis* is discussed with other polysporangiate species in the Rubiaceae and angiosperms.

Keywords Isertieae · Multiple sporangia · Pollen development · Structure

Introduction

The stamens are the male reproductive parts of angiosperm flowers and are formed of the anther where the pollen is produced, and the filament, which is responsible for nourishing and supporting the anther (Scott et al. 2004). In this group of plants, the anthers generally have a uniform organization represented by two thecae joined by vascularized connective tissue, each theca containing two sporangia in which the pollen develops (D'Arcy and Keating 1996). However, some species deviate from this pattern of organization and their flowers have anthers with a larger number of sporangia. These anthers are mentioned in the literature as follows: polysporangiate, septate, or multilocellate anthers (Robbrecht 1984; Kirkbride 1985; Tobe and Raven 1986; Sudhakaran et al. 1995; Baumgratz et al. 1996; Tsou and Johnson 2003; Oliveira et al. 2011; Pandey and Pandey 2013; Suaza-Gaviria et al. 2016; Amaral et al. 2017; Lima et al. 2019; Robayo et al. 2020). In these anthers, the sporogenous tissue is divided by sterile tissue, a characteristic that has developed independently in about 30 families of angiosperms (Endress and Stumpf 1990).

In recent years, there has been growing interest in studying the anatomical structure of this type of anther in species from different families, such as Annonaceae (Tsou and Johnson 2003), Clusiaceae (Amaral et al. 2017), Loranthaceae (Suaza-Gaviria et al. 2016; Robayo et al. 2020), Melastomataceae (Baumgratz et al. 1996; Lima et al. 2019) and Primulaceae (Pandey and Pandey 2013). The Rubiaceae is one of the angiosperm families with the largest number of species, ca. 13000, 620 genera, and with a cosmopolitan

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distribution (Govaerts et al. 2011). Despite being one of the most species-rich families, Rubiaceae only presents a few polysporangiate taxa. It has been only mentioned in species of few genera, such as *Isertia* Schreb. (tribe Isertieae; Boom 1984; Dávila and Vicentini 2016), *Kerianthera* J.H. Kirkbr. (tribe Isertieae; Kirkbride 1985; Oliveira et al. 2011), *Dictyandra* Welw. ex Hook. f. (tribe Pavetteae; Robbrecht 1984), and *Calycosiphonia* Pierre ex Robbrecht ((tribe Coffeeae; Robbrecht 1981; Sonké et al. 2007). Endress and Stumpf (1990) made a general description of the polysporangiate anthers of *Isertia hypoleuca* Benth., but only in the young stage of the anther. On the other hand, the anthers in species of of *Kerianthera*, *Dictyandra*, and *Calycosiphonia*. have only been referred to in the context of taxonomic studies, so their anatomical structure and development is still unknown.

We recently had the opportunity to obtain fixed samples of *Isertia laevis* (Triana) B.M. Boom with flowers in different stages of development; Boom (1984) had cited the presence of polysporangiate anthers in this species. *I. laevis* is a shrub or small tree up to 12 m in height, with large membranous leaf blades (29–68 × 14–36 cm), and pauciflorous inflorescences. The flowers are bisexual; calyx green, cup-shaped, 6–7-lobed; corolla white, salverform, 6–7-lobed, with a tube 32–60 mm long; 6–7 stamens alternating with the corolla lobes; anthers are fused to the corolla tube by a short filament. The gynoecium consists of an inferior ovary, single style, and a 4-lobed stigma. The ovary has five locules with more than one ovule in each locule (Boom 1984). As part of a phylogenetic study, Bremer and Thulin (1998) described the anthers of *I. laevis*, with each of the two thecae being divided into about 160 small chambers, organized in eight longitudinal rows. A photo of a transverse section of the anther was shown, but an anatomical description was not included.

Isertia laevis has a wide distribution, from the South East of Central America (Nicaragua to Panama) and the North, Mid-Western, and Mid-Central of South America (Colombia, Venezuela, Ecuador: Amazonas; Peru, Brazil: Acre, Amazonas; Bolivia), especially in Andean humid forest. *Isertia laevis* mainly grows in secondary and successional forests, being common on roadsides, but it also inhabits primary rain forests (Boom 1984; Grandtner and Chevrette 2013).

An anatomical study of the polysporangiate anthers and the development of pollen in *Isertia laevis* has been carried out in order to provide detailed information on the structure of this particular type of anther in the Rubiaceae.

Materials and methods

Flowers and buds of *Isertia laevis* fixed in formalin, acetic acid, and alcohol (FAA) were dissected, and the anthers were processed for anatomical analysis. The voucher number is as follows: Ecuador, Napo province, 27 March 1980. *J. Branbyge & E. Asanza 30638* (AAU). Additional herbarium specimens examined: Ecuador, Napo province, Lago Agrio to Quito road 52 km from Lago Agrio just before Lumbaqui, 0° 0′ N, 77° 25′ W, 1650 m, 28 Ago 1983, *Balslev H. & B. Boom 4400* (AAU); idem, Pastaza province, Shell-Mera rain forest, 1° 29′ S, 78° 3′ W, 1050 m, 05 Ago 1968, *Holm-Nielsen L. B. & S. Jeppesen 500* (AAU); idem, Curaray, ridge NE of Destacamento, 1° 21′ S, 76° 56′ W, 250 m, 19 March 1980, *Holm-Nielsen, L. B. et al. 22104* (AAU); idem, Lorocachi, 2 km del Río Curaray, 1° 38′ S, 75° 58′ W, 200 m, 23 May 1980, *Jaramillo J. et al. 30712* (AAU); idem, Río Pastaza, River banks between the outlets of Río Bobonaza and Río Ishpingo, 2° 34′ S, 76° 43′ W, 275 m, 22 July 1980, *Ollgaard B. 25010* (AUU).

For light microscopy (LM) observations, the anthers were dehydrated and embedded in paraffin (Johansen 1940; modified by Gonzalez and Cristóbal 1997). Serial transverse and longitudinal sections (12 μm thick) were made using a Microm HM350 rotary microtome (Microm International, Walldorf, Germany). The sections were stained with safranin and astra blue (Luque et al. 1996), and mounted in synthetic Canada balsam. The presence of crystals was verified by observation with light microscopy with polarized filters (PLM). Observations and digital images were acquired using a Leica DM LB2 (Leica Microsystems) light microscope equipped with a Leica DATA digital camera.

To observe orbicules with scanning electron microscopy (SEM), mature anthers were opened, dehydrated in a graded ascending acetone series, and then critical point dried using liquid CO_2 (Denton Vacuum, DCP-1, Pleasanton, NJ) and sputter-coated with gold-palladium (Denton Vacuum, Desk II, Pleasanton, NJ). Analysis was performed using a Jeol LV 5800 (JEOL, Tokyo, Japan) at 10 kV in the Service of Electron Microscopy facility at the Universidad Nacional del Nordeste (Corrientes, Argentina).

Samples of FAA-fixed pollen were acetolized according to Erdtman (1966) and analyzed by LM and SEM. An average of 30 pollen grains was measured. The terminology used follows Punt et al. (2007).

Results

Anther morphology

The anthers of *Isertia laevis* are narrowly oblong or narrowly elliptic, between 6 and 8 mm long (Fig. 1a–b, f). Each anther consists of two parallel thecae, with a distinctly bullate surface (Fig. 1a–b) each bulla reflecting the position of an underlying sporangium; each theca contains multiple sporangia (more than 70) in the form of spherical or ellipsoidal chambers, which can be observed in both transverse section (Fig.

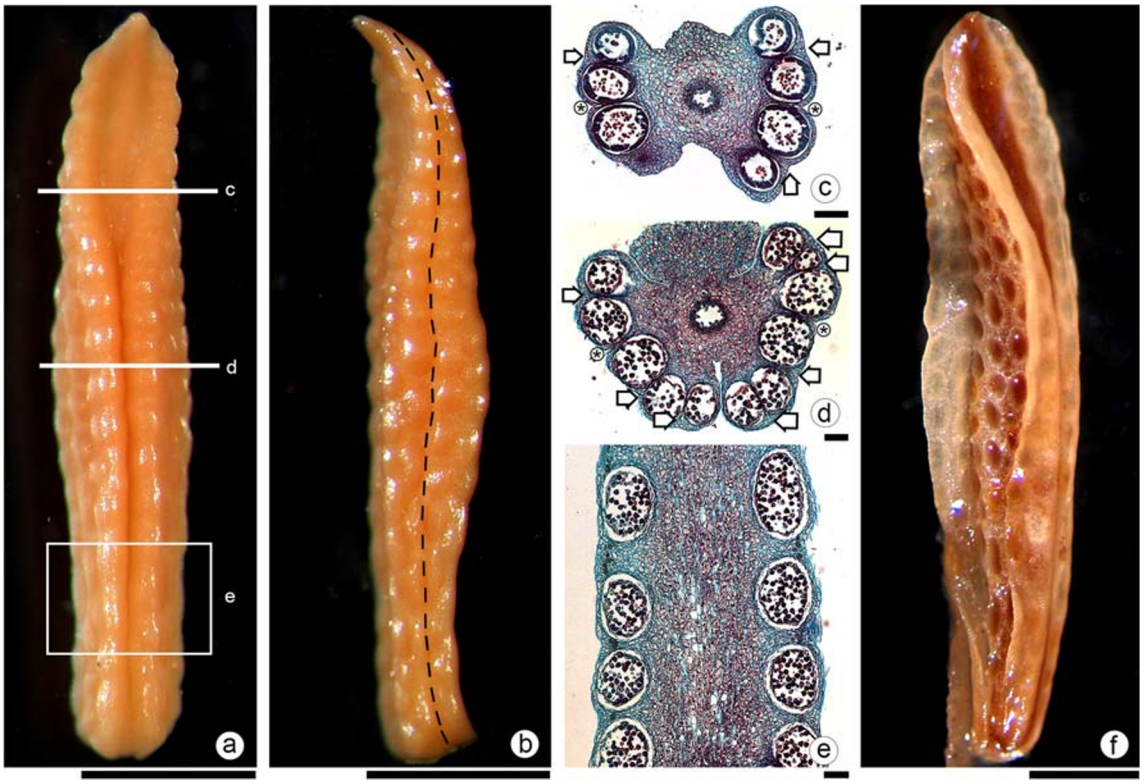

Fig. 1 Polysporangiate anthers of *Isertia laevis*. (**a**) Frontal view of an indehiscent anther. (**b**) Lateral view of an indehiscent anther, dotted line indicates the dehiscence line. (**c**, **d**) Transverse sections (LM) of indehiscent anthers with different number of sporangia showing the region of the stomium (*) and the region of rupture (arrows). (**e**) Longitudinal section (LM) of the anther showing the multiple sporangia. (**f**) Dehiscent anther. Scale bar: a–d, f = 1 mm, c–e = 100 μm

1c–d) and longitudinal section (Fig. 1e). Although the anther has multiple sporangia, each theca only has one longitudinal dehiscence line (Fig. 1b–f).

Anther anatomy and pollen development

Already in the young anther, the numerous microsporangia are separated by septa that divide each theca, both longitudinally and horizontally (Fig. 1c–d). Each microsporangium is formed by numerous groups of sporogenous tissue, comprised of cells larger than those surrounding it, with dense cytoplasm and a conspicuous nucleus; these cells divide into several planes (Fig. 2a–b). The septa separating the sporogenous tissues are formed of 1–3 layers of parenchyma cells containing druse crystals of calcium oxalate (Fig. 2b). The connective tissue is of polygonal parenchyma cells, and it has a concentric vascular bundle with inner xylem (Fig. 2c).

As the anther matures, the microsporangia increase in volume and the sporogenous tissue cells differentiate into microspore mother cells (mmc), surrounded by a callose layer (Fig. 2d). The mmc undergoes meiotic division (Fig. 2e–f) producing tetrahedral tetrads (Fig. 2g). At this stage, each microsporangium is surrounded by the secretory tapetum, formed by elongated cells with dense cytoplasm and a single middle layer (Fig. 2d–g). The endothecium is uni-stratified towards the epidermis and multi-stratified towards the connective tissue and septa between the microsporangia. The unicellular epidermis is continuous throughout the anther, lacking stomata. It is observed that the xylem of the vascular bundle is broken in the connective tissue, so that the vascular bundle is hollow (Fig. 2h).

The callose surrounding the tetrads disintegrates and consequently the microspores are released into the locus of the pollen sacs (Fig. 3a). Three apertures in each microspore can already be recognized; the nucleus is conspicuous and has a central position (Fig. 3a). The microspores increase their volume and the central nucleus is displaced by a large vacuole (Fig. 3b). In some microspores, an oncus (formed of intine and protoplast) can be seen protruding from the apertures (Fig. 3b). It has been observed that there may be a time delay in the anther, as some pollen sacs have tetrads while others already have microspores.

In the zones of union between the septa and the external wall of the anther, the deposition of calcium oxalate crystals continues in the form of crystalline sand that accumulates in the idioblasts that already have druses (Fig. 3d–h). One stomium region develops longitudinally in the middle of each theca between the median septum and anther wall (Figs. 1b–d and 3d–e). These areas show less development of parenchyma cells under the epidermis, which determines the future line of

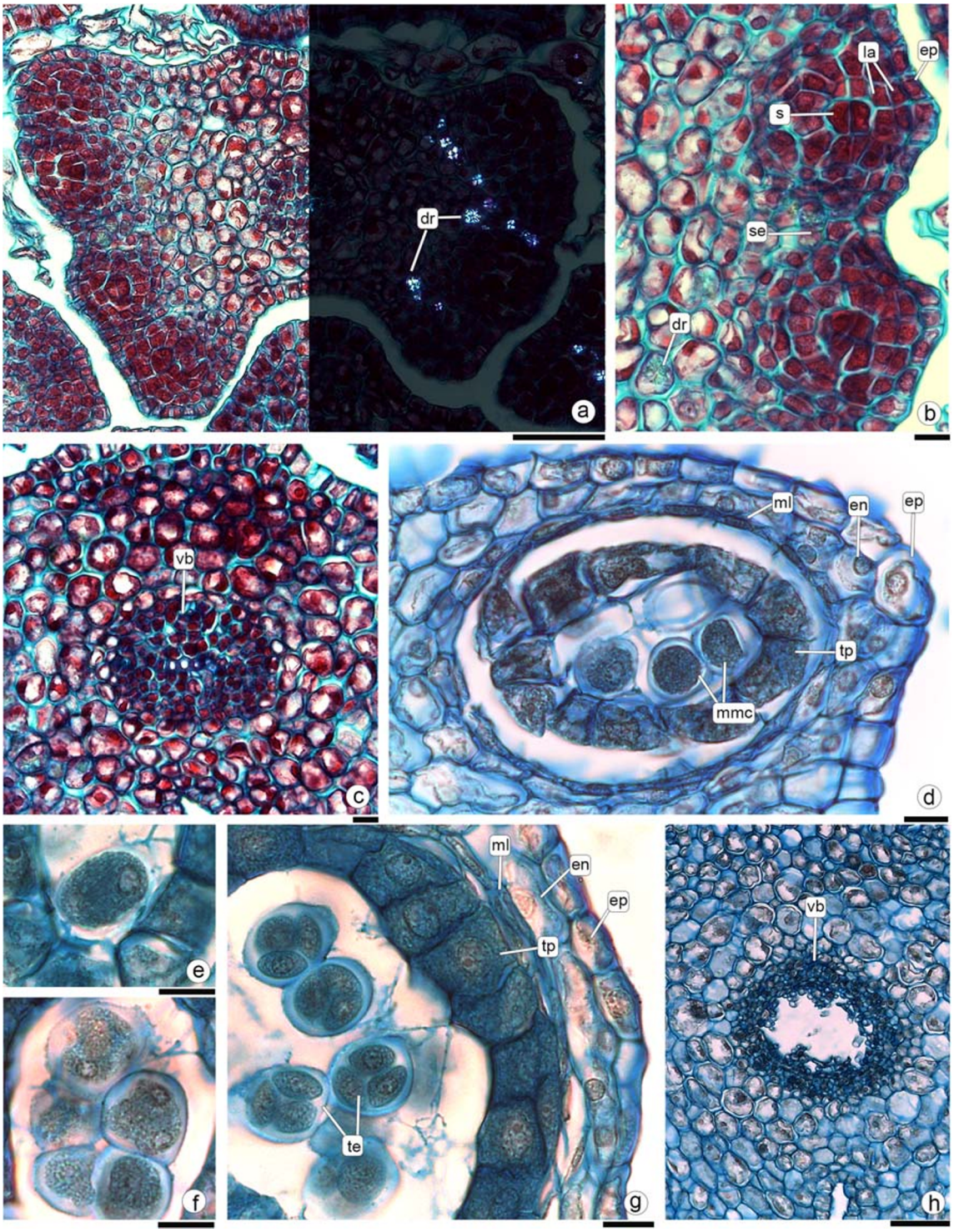

Fig. 2 Light microscopy of transverse section in young anther. (**a**) Anther with multiple sporangia, right half with polarized light to observe crystals. (**b**) Detail of two sporangia separated by a septum. (**c**) Connective and vascular bundle. (**d**) Sporangium in stage of microspore mother cell. (**e**, **f**) microspore mother cell in meiotic division. (**g**) Sporangium with tetrads. (**h**) Detail of the hollow vascular bundle. dr, druses; en, endothecium; ep, epidermis; mmc, microspore mother cell; ml, middle layer; s, sporogenous tissue; se, septum; te, tetrad; tp, tapetum; vb, vascular bundle. Scale bar: a, h = 50 μm, b–g = 10 μm

dehiscence of each theca (Fig. 3d–e). In the rest of the septa, the area that connects with the external wall of the anther is formed of several cell layers (Fig. 3d–f). In addition, it is observed that the idioblasts with druses, present in the septa that separate the microsporangia, accumulate crystalline sand, thus increasing their volume (Fig. 3g–h).

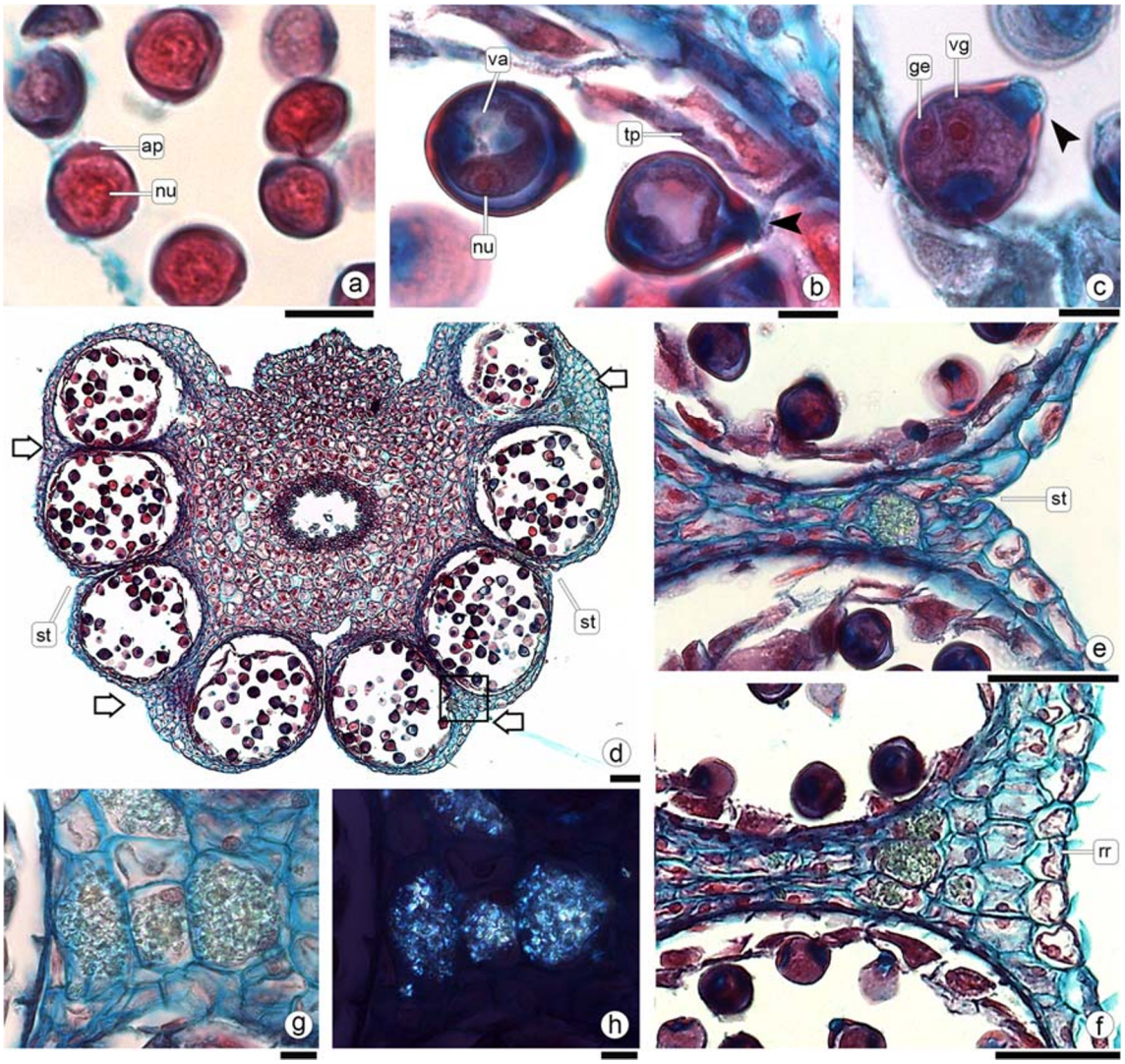

Fig. 3 Light microscopy of transverse section of mature, pre-dehiscent anther. (**a**) Free microspore with central nucleus. (**b**) Microspore with a large vacuole and protruding oncus (arrowpoint), note the degenerated tapetal cell. (**c**) Pollen grain with vegetative and generative cells, note the protruding oncus (arrowpoint). (**d**) Transverse section of the anther showing the multiple sporangia and the stomium region. (**e**) Detail of the stomium region. (**f**) Detail of the rupture region near to septa from box in (**d**). (**g**) Crystal idioblasts between the sporangia observed with LM. (**h**) Crystal idioblasts observed with PLM. ap, aperture; ge, generative cell; nu, nucleus; rr, rupture region; st, stomium region; tp, tapetum; va, vacuole; vg, vegetative cell. Scale bar: a–c, g–h = 10 μm, d–f = 50 μm

Later, the tapetal cells collapse and the nucleus of the microspore divides mitotically, giving rise to pollen grains with vegetative and generative cells (Fig. 3c). The cells of the endothecium in all the septa, and those under the epidermis in the wall of the anthers, develop fibrous thickening (Fig. 4a–f). The accumulation of crystals (druse + sand) in the septa causes the rupture of the idioblasts that contain them (Fig. 4a–e). These multiple regions of rupture, added to that of the stomium region, cause the multiple pollen sacs in each theca to coalesce before dehiscence (Fig. 4f).

In the stomium region, a minor development and the small size of the cells cause the opening of the anther by longitudinal dehiscence and the pollen grains are released by longitudinal dehiscence (Figs. 1f and 4g–h). Some smaller, collapsed, empty pollen grains (presumably aborted) are also observed (Fig. 4b).

Pollen grains and orbicules

Each pollen grain is oblate-spheroidal (Fig. 5a–b). In non-acetolyzed grains, three apertures and the protruding onci are observed (Fig. 5a). In the acetolyzed grains (Fig. 5b–c) the protruding onci have been destroyed and it can be observed in detail that the openings are colpoidorate and the exine sculpturing is smooth. The dimensions of the pollen grain are as follows: polar view 30.17–35.55 μm, equatorial view 33.36–37.76 μm, and exine thickness 0.87–1.10 μm.

The orbicules are very abundant and are randomly scattered on the inner tangential side of the cells of the tapetum and on the tapetal membrane (Fig. 5d). They are spherical to slightly elongate with a smooth surface (Fig. 5e). They have a diameter of 100–400 nm.

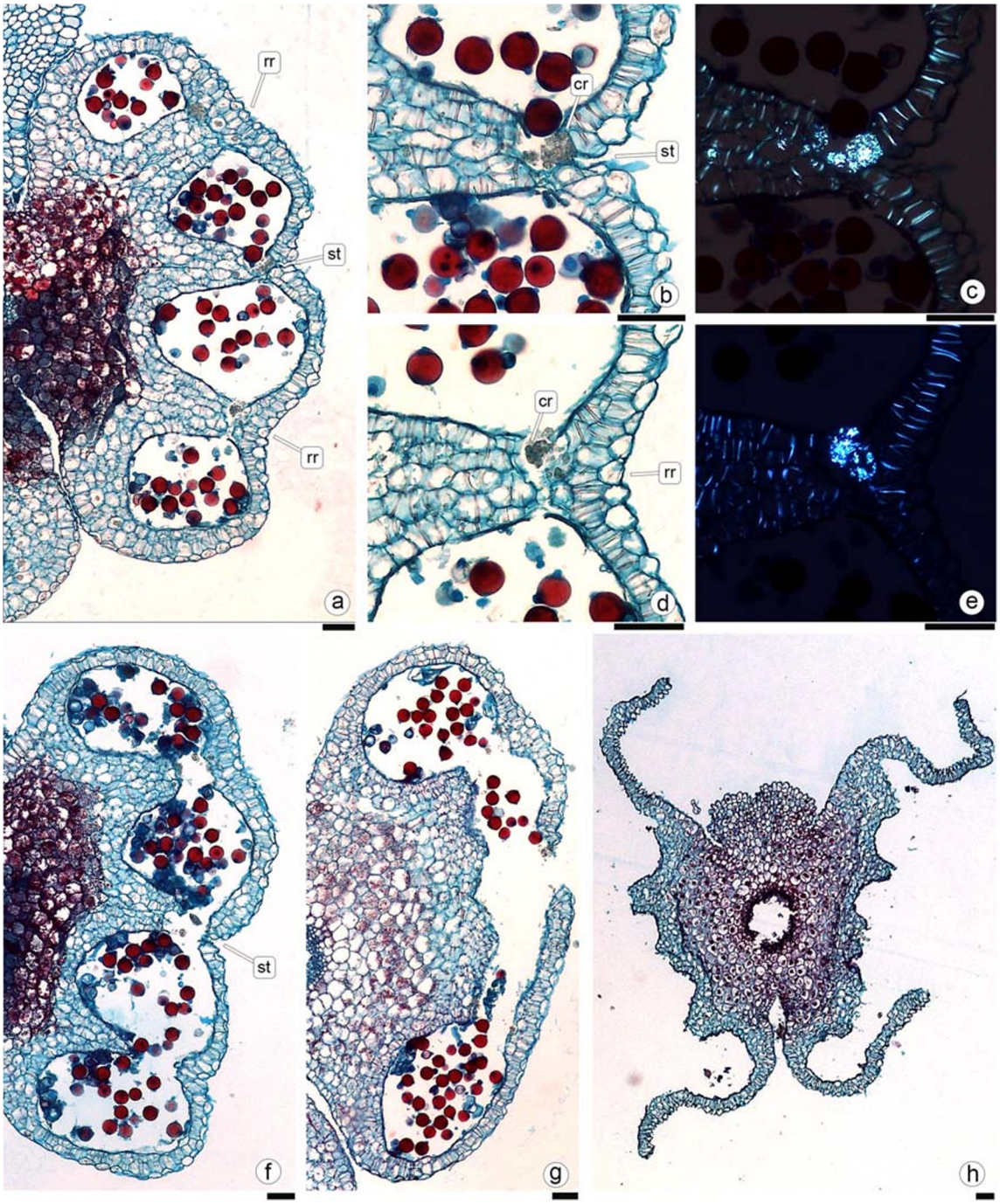

Fig. 4 Light microscopy of pre-dehiscent (**a**–**f**) and dehiscent anthers (**g**–**h**). Transverse section of one theca showing four sporangia separating by septa. (**b**, **c**) Detail of the stomium region showing fibrous thickenings in endothecium, and crystals with LM (**b**) and PLM (**c**). (**d**, **e**) Detail of the rupture region and crystals with LM (**d**) and PLM (**e**). (**f**) Transversal section of one thecae showing fusion of four sporangia located on the same plane in a single pollen sac. (**g**) Rupture of stomium. (**h**) Dehiscent anther. cr, crystals; rr, rupture region; st, stomium region. Scale bar: 50 μm

Discussion

The anatomical study carried out in the androecium of *Isertia laevis* revealed that the septa that create the polysporangial structure are formed in the young anther, simultaneously with the sporogenous tissue. The septa remain until the time of dehiscence. Each microsporangium presents the typical structure found in most angiosperms, with the typical layers, epidermis, endothecium, middle layer, and tapetum, that surround and accompany the formation of microspores and later the pollen grains (Scott et al. 2004). In *I. laevis*, the septa separating the multiple pollen sacs have these same layers,

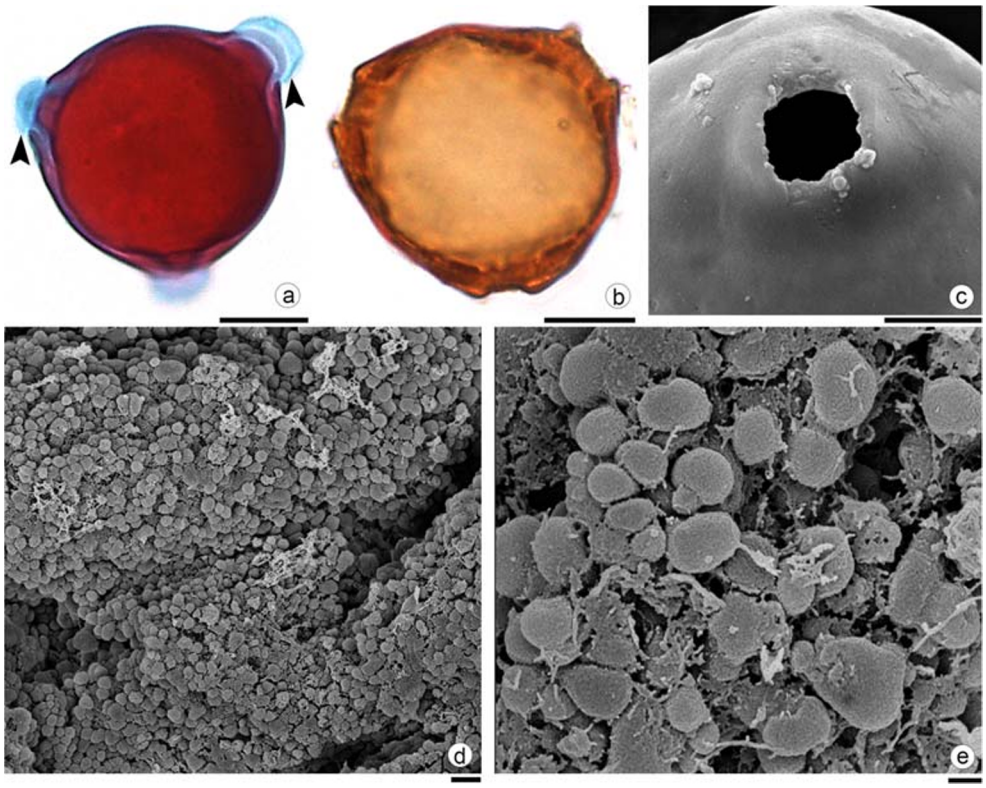

Fig. 5 Mature pollen grain and orbicules with light microscopy (**a**–**b**) and SEM (**c**–**e**). (**a**) Non-acetolized pollen grain with protruding oncus (arrows). (**b**) Acetolized pollen grain. (**c**) Detail of the aperture and the exine. (**d**) General view of the orbicules. (**e**) Detail of the orbicules. Scale bar: a–b = 10 μm, C = 5 μm, d = 1 μm, e = 200 nm

except for the epidermis, so they were described as the parenchymatous-type or P-type according to the types proposed by Endress and Stumpf (1990) and Tsou and Johnson (2003).

In angiosperms, the occurrence and variability of the polysporangiate anther has been summarized in several studies, the main ones being those by Lersten (1971) and Endress and Stumpf (1990), who recorded such anthers in 16 and 18 families, respectively. The former authors recognize two types of septa: parenchymatous, of primary origin from the beginning of sporogenous tissue differentiation, and tapetal septa with a secondary origin from an initially homogeneous sporogenous tissue, the first type being present in *Isertia*.

An androecium with polysporangiate anthers produced by divisions of the four microsporangia with the bithecal structure (anther with two thecae and four microsporangia) is quite frequent. Parenchymatous septa were described in *Aegiceras corniculatum* (L.) Blanco (Primulaceae, Endress and Stumpf 1990; Pandey and Pandey 2013), in some Loranthaceae, like *Aetanthus mutisii* (Kunth) Engl. (Suaza-Gaviria et al. 2016) and *Psittacanthus schiedeanus* (Loranthaceae; Robayo et al. 2020), and in species of *Microlicia* D. Don (Melastomataceae; Lima et al. 2019). In Onagraceae, the septa can be tapetal or parenchymatous; this character has been used as a synapomorphy at the generic level (Tobe and Raven 1986; Endress and Stumpf 1990).

Endress and Stumpf (1990) proposed that the parenchymatous septa have an early origin, (along with sporogenic tissue differentiation), whereas the tapetal septa have a secondary origin (from an initially homogeneous sporogenic tissue). Similar variation in septate anthers was described in Annonaceae, where Tsou and Johnson (2003) recognized two types of septa called T-septa (tapetal) and P-septa (parenchymatous); however, they described both types as of primary origin, i.e., present from the beginning of sporogenous tissue differentiation and homologous in ontogeny.

Another variation in polysporangiate anthers occurs when there is no organization in the thecae. This occurs in taxa of different families, among which we can mention: some species of Clusiaceae, where the multiple locelli (microsporangia) are at the apex of the filament (Amaral et al. 2017); in *Rhizophora mucronata* Lam. (Rhizophoraceae) in which the anthers present discontinuous linear rows of sporangia below the epidermis (Sudhakaran et al. 1995); and in *Rafflesia* sp. (Rafflesiaceae), the anthers have many sporangia arranged irregularly in their middle zone, whereas they are arranged in two concentric rings with less sporangia towards the apex (Endress and Stumpf 1990). On the other hand, in species of *Viscum* L. (Viscaceae), the multiple sporangia are arranged irregularly on the surface of the anther (Endress and Stumpf 1990).

The species analyzed here, *Isertia laevis*, has a bithecal organization and longitudinal dehiscence. This organization

had already been described by Endress and Stumpf (1990) in another species of the same genus, *I. hypoleuca*; however, as the study was carried out in a single, young stage of anther development, data obtained for this species are incomplete. On the other hand, in the other genera of Rubiaceae in which species with polysporangiate anthers are known, bithecal organization and longitudinal dehiscence is only mentioned for *Kerianthera preclara* (Kirkbride 1985) and *K. longiflora* (Oliveira et al. 2011). Unfortunately, the polysporangiate anthers are only mentioned as part of the taxonomic description in these other genera and there are no details of the anatomical structure of the anthers and how the multiple pollen sacs are formed.

Kerianthera was treated as part of the tribe Condamineeae (Kirkbride 1985), especially for the presence of semaphyllous calycophylls, valvate aestivation of corolla and winged seeds (Oliveira et al. 2011). However, the genus position was reinvestigated using both the molecular and morphological approaches, and it was relocated in the tribe Isertieae (Delprete 1996; Robbrecht and Manen 2006; Andersson and Antonelli 2005), the easily observable multi-locellate anthers being the main synapomorphy of the tribe.

A structure known as the placentoid was recently described in the anthers in three species of Rubiaceae (tribe Gardenieae, Judkevich et al. 2020b): *Tocoyena formosa* (Cham. & Schltdl.) K. Schum., *Randia calycina* Cham., and *R. heteromera* Judkevich & R.M. Salas (Judkevich et al. 2020a). The placentoid is a tissue that grows from the septum and invades the locules (Chatin 1866); however—unlike the septa—it does not divide them completely and therefore it does not generate a polysporangiate anther state. The placentoid in these species can also be tapetal (species of *Randia*) or parenchymatous with endothecial thickening (*T. formosa*). The placentoid could be considered an intermediate state in the development of septate anthers, with identical structural organization as septa.

The process of dehiscence in a tetrasporangiate anther normally occurs due to a sequence of events that include the development of endothecium thickening, lysis of the septum between the pollen sacs of each theca, and rupture of the stomium (Wilson et al. 2011). However, in the polysporangiate anthers of *Isertia laevis*, it has been observed that the septa between the multiple pollen sacs of each theca develop fibrous thickening similar to that of the endothecium. The presence of fibrous thickening in the septa of polysporangiate anthers with bithecal organization and longitudinal dehiscence has not been previously described in other species that have this type of anther. In conventional, tetrasporangiate anthers of Rubiaceae, the presence of septal thickening has also been documented in two other species of the family, i.e., *Cordiera concolor* (Cham.) Kuntze and *Genipa americana* L., both from the tribe Gardenieae (Judkevich et al. 2020b). It seems that the presence of fibrous thickening of the septa in the anthers would be a characteristic only found so far in some Rubiaceae species. Although this characteristic characteristic is not mentioned in the standard mechanism of dehiscence summarized by Wilson et al. (2011), the anthers are opened longitudinally in a conventional way. In *C. concolor* and *G. americana*, dehiscence occurs after the stomium region breaks, even if the septum does not undergo lysis (Judkevich et al. 2020b). In *I. laevis*, these septa are detached from the endothecium because the idioblasts with druses that are found in the septa, accumulate crystalline sand until the cell breaks. Finally, longitudinal dehiscence occurs due to the rupture of the stomium, in which the crystals also participate.

Moreover, accumulation of crystals in the septum and stomium has been described in the family Solanaceae (Horner and Wagner 1980; Bonner and Dickinson 1989) and also in other Rubiaceae species, like *Cephalanthus glabratus* (Spreng.) K. Schum. (Romero et al. 2017) and elsewhere *Terenna gracilipes* (Hayata) Ohwi (Vinckier and Smets 2005). Furthermore, the presence of druses has been mentioned in the dehiscence region of *I. hypoleuca* (Endress and Stumpf 1990), although their possible role was not discussed. In Solanaceae, the degeneration of the stomium and septum has been associated with the development of crystals in their cells, suggesting that these crystals would be necessary for anther dehiscence (Horner and Wagner 1980; Bonner and Dickinson 1989). On the other hand, in *Terenna gracilipes*, Rubiaceae, when anther dehiscence occurs, the stomium breaks and thus releases the crystals into the locules, so that the surface of the pollen grains is covered with them, and it was suggested that the crystals might provide a visual signal to pollinators and thus improve pollination (Vinckier and Smets 2005). However, in *I. laevis*, the pollen grains are released with their surface free of crystals which has been suggested as being more directly related to the process of anther dehiscence, as proposed by Romero et al. (2017) for *Cephalanthus glabratus*.

Although the precise function of the division of the anther into multiple sporangia is not yet determined, there are several hypotheses that would explain its possible role. One of them was proposed by Lersten (1971) who suggests that this type of anther is an intermediate state in an evolutionary trend towards a reduction in the size of anthers and their sporogenous tissue. A second hypothesis, which is one of the most accepted so far, was suggested by different authors (Pacini et al. 1985; Lima et al. 2019), and it proposes that the presence of septa would increase the contact surface between the tapetum and the developing pollen which would benefit pollen nutrition. In accordance with this second hypothesis, Tsou and Johnson (2003) suggested that species in the family Annonaceae with multiple septa in the anthers have larger pollen grains and a thicker exine than those without multiple septa. However, in Rubiaceae more polysporangiate species should be analyzed to

see if there is any relationship between this state of the anthers and the size of the pollen grains and thickness of the exine.

Another hypothesis was given in Melastomataceae (Patel et al. 1984; Baumgratz et al. 1996), in which it is suggested that the septa can modify the pattern of pollen release, ensuring that not all pollen is released at the same time when the first pollinator visits the flower, so that more pollinators would encounter some pollen. This hypothesis is also supported by Robayo et al. (2020) for *Psittacanthus schiedeanus* (Schltdl. & Cham.) Blume ex Schult. (Loranthaceae). Field observations involving plant-pollinator interaction are recommended for both *Isertia laevis* (primarily sphingophilous, but also vistited by hummingbirds, according to Wolff et al. 2003) and the other species of Rubiaceae that have polysporangiate anthers.

On the other hand, the development of pollen grains in *I. laevis* follows the conventional pattern of most angiosperms; the sporogenous tissue differentiates into the microspore mother cells that produce microspores through meiosis, which mature to form pollen grains that are released from the anther during dehiscence (Scott et al. 2004). In the species analyzed, the mature pollen grains are oblate-spheroidal, 3-colpoidorate. These characteristics are common to other species in the genus *Isertia*, such as *I. hypoleuca* and *I. spiciformis* DC. (Huysmans et al. 1998b). However, *I. laevis* differs from these in that it has a smooth (psilate) exine in contrast to both species that have a perforated exine. In the non-acetolyzed grains of *I. laevis*, the protrusion of oncus has been observed in each aperture, which is a structure composed of intine and protoplast (Hyde 1955). The presence of oncus in pollen grains has been reported in other angiosperms (Hyde 1955; Tilney and Van Wyk 1997) and even in other species of Rubiaceae from different tribes (Tilney et al. 2014; Yue et al. 2017; Romero et al. 2017; Judkevich et al. 2020b), and their function is still not clarified.

In addition, orbicules have been found in the anthers of Isertia laevis. The orbicules are sporopolenin particles that are found in the walls of the tapetal cells and often on the pollen surfaces, and which are present in more than 72 families of angiosperms (Huysmans et al. 1998a; Vinckier and Smets 2002; Ruggiero and Bedini 2020). They have been widely mentioned and described in numerous species of the Rubiaceae family, varying in abundance, size, shape, and ornamentation (Huysmans et al. 1997; Verellen et al. 2007; Verstraete et al. 2011). In *I. laevis* they are abundant, spherical to slightly elongate in shape and with a smooth surface. Spherical and smooth-surfaced orbicules represent the plesiomorphic condition in Rubiaceae (Verstraete et al. 2011). According to the classification by Vinckier and Smets (2002) of orbicule typology in Gentianales, the orbicules of *I. laevis* correspond to type III (smooth, more or less spherical) coinciding with that also found by these authors for *I. spiciformis*.

Conclusions

Until now, studies carried out on polysporangiate species of Rubiaceae had a taxonomic focus, so the anatomy and developmental pathway of this unusual type of anther were unknown. The present study described the anatomical structure of the polysporangiate anther of *Isertia laevis* during different stages of development in detail for the first time.

This study reveals that the septa in *I. laevis* are of the parenchymatous-type, present in the anthers from the sporogenous tissue stage and are preserved even after dehiscence with the development of fibrous thickening similar to that of the endothecium. The presence of septa with fibrous thickening is, currently, a unique feature of this species. In addition, it was found that the accumulation of calcium oxalate crystals in the idioblasts of the septa plays an important role in anther dehiscence.

Field studies to observe the pollination mechanism in this species, and other polysporangiate species of the Rubiaceae, are necessary to interpret the possible functional role of this type of anthers in the family.

The results obtained can be used as a basis for comparison with the other Rubiaceae genera that present polysporangiate anthers and to evaluate the taxonomic importance of this character in the family.

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Author contribution statement RMS provided the plant material and gave data on the environment of the species. MDJ processed the plant material to make the histological preparations, took the microscopy photos, and prepared the figures. MDJ and AMG performed the anatomical interpretations. MDJ and AMG contributed in the discussions and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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