

Structural analysis and developmental stages of domatia of *Schinus terebinthifolius* Raddi (Anacardiaceae)

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Abstract The domatium presents a diversity of forms, and the understanding of the structural aspect of the domatia is very important because it can provide diagnostic characters for families and genera, and favors the establishment of mutualistic relations. The objectives of the present study were to characterize the developmental stages of the domatia of *Schinus terebinthifolius* Raddi. (Anacardiaceae) located at the base of the leaflet, and based on these data to determine if the structural aspects provide good shelter for mites and ants. Domatia were observed under a stereomicroscope and then processed following standard techniques for light and scanning electron microscopy. The domatia of *S. terebinthifolius* develop early during the development of the leaves and go through four stages of development. The domatia have a spiraling architecture with internal chambers that contain trichomes and stomata, suggesting good conditions of shelters for arthropods. Deposits of phenolic compounds and calcium crystals were identified in the domatia, suggesting an investment in alternative forms of protection from herbivory. Structural analysis showed the developmental stages of domatia and how the architecture of these structures can provide efficient shelter for arthropods, thereby contributing to the protection of these plants from herbivory and facilitating ecological studies.

Keywords Anatomy · Defense strategies · Ecological relationships · Morphology

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1 Introduction

Domatia are small structures that often harbor predaceous arthropods which are potentially beneficial to the plant (Agrawal et al. 2000). These structures are very important for taxonomy because they provide diagnostic characters for botanical families and genera (Nickol 1998; Michelangeli 2000; Kim and Ngondya 2010; Leroy et al. 2010). In this regard, these structures have been reported in approximately 277 families and more than 2000 species of Angiospermae (Agrawal and Karban 1997).

In addition to their contribution to taxonomy, domatia also play an important ecological role. Numerous studies have reported the establishment of a mutualistic relationship between domatia-bearing plants and mites (Agrawal 1997; Norton et al. 2001; Romero and Benson 2005). Domatia favor mites by providing them favorable environments for oviposition and the effectiveness survival of mite offspring, in addition to providing protection against desiccation and attack by predators (Pemberton and Turner 1989; Grostal and O'Dowd 1994; Norton et al. 2001; Romero and Benson 2005). Mites in turn remove spores and hyphae of fungal pathogens, in addition to protecting the plant from attack by phytophagous insects (Roda et al. 2001; Kreiter et al. 2003; Romero and Benson 2005). This mutualistic relationship has been increasingly drawing the attention of ecologists, who seek to understand more completely the interactions between domatia and mites (Nishida et al. 2006).

The diversity of forms of domatia may favor these interactions. The principle types are in the form of pits or pouches and dense tufts of hair between the main and secondary veins (Romero and Benson 2005; Moraes et al. 2011), or a combination of tufts of hairs with pits and pouches (O'Dowd and Willson 1991; Romero and Benson

2005). Studies on *Cinnamomum camphora* (L.) J. Presl (Lauraceae) revealed that the different forms of domatia could be related to specific taxa of mites (Nishida et al. 2006). Domatia that are in the form of pouches are preferentially inhabited by herbivorous or fungivorous mites, and the domatia that are in the form of pits are commonly used by carnivorous mites (Nishida et al. 2006). The coevolution of domatia and mites was proposed by Pemberton and Turner (1989); however, there is little concrete evidence to support this hypothesis.

Studies aim at characterizing the morphology and anatomy of the different forms of domatia are rare, and most of them have been mainly dedicated to investigating the ecological relationships involving mites (O'Dowd 1989; Agrawal 1997; Norton et al. 2001; Romero and Benson 2004). However, some authors have dedicated themselves to investigating morphoanatomical aspects and the development of the different forms of domatia (Tillberg 2004; Nishida et al. 2006; Leroy et al. 2008; Leroy et al. 2010; Richards and Coley 2012). Solís and Ferrucci (2006) described morphoanatomical characteristics of the domatia of two species of *Cardiospermum* L. (Sapindaceae) in order to collect data that contribute to the systematic of the genus. In addition, Solís-Montero et al. (2009) characterized the domatia of nine species in *Mortoniendron* Standl. & Steyerl. (Malvaceae), with the aim of evaluating if there is any relation between the domatia type and the species. Val and Dirzo (2003) investigated how the ontogeny of domatia of *Cecropia peltata* L. (Cecropiaceae) can influence the interaction with mites, causing changes in plant defense strategies. Leroy et al. (2008) found changes in leaf blade tissue during the formation of domatia of *Hirtella physophora* Mart. & Zucc. (Chrysobalanaceae).

The species in question, *Schinus terebinthifolius* Raddi (Anacardiaceae), is dioecious, and can be found in the form of medium-sized trees (3–4 m) or small bushes. The leaves are compound, imparipinnate, and with the petiole and rachis narrowly winged. The leaflets are opposite or sub-opposite, usually sessile and may be oval or elliptical in shape (Cronquist 1981). Two types of domatia have been described for this species, one located in the region of the junction of the secondary veins with the midrib, referred to as “tufts of hair,” and the other located at the base of the leaflet, referred to as the “cavity form” (Barros 1961). Wiggers et al. (2005) reported a high diversity of mites inhabiting the domatia of *S. terebinthifolius* located at the base of the leaflet, and the great majority of fungivorous and predatory mites.

Following this reasoning, some anatomical characteristics of leaf may be modified by pressures exerted by the mutualistic relationship. Thus, this study aims to characterize the stages of development of the of *S. terebinthifolius*

located at the base of the leaflet and determine if there are developmental strategies of structure.

2 Materials and methods

Individual leaflets of *S. terebinthifolius* Idem were collected during the month of January 2014, in the Grussaí/IQUIPARI lagoon complex (Complexo Lagunar de Grussaí/IQUIPARI; 21°26'54"S and 41°03'45"W), in the municipality of São João da Barra in the state of Rio de Janeiro, Brazil.

For structural and development of domatia analyses we select 10 individuals from which five buds and five fully expanded leaves were collected. Later, domatia at different stages of development were removed from the base of buds and leaves. For morphological characterization of the domatia, samples were observed and images captured with a camera (Power Shot A640, CANON, New York, USA) attached to a stereomicroscope (Zeiss Stemi SV 11).

Histochemical tests were performed using freehand sections in all development stages of domatia. The sections were then deposited on slides and submitted to different reagents to determine the chemical groups present in the cells of the domatia, such as lugol to determine the presence of starch (Berlyn and Miksche 1976); ruthenium red for detection of mucilage/pectic (Chamberlain 1932); ferric chloride for identification of phenolic compounds (Johansen 1940); sudan IV to verify the presence of lipids (Gerlach 1984), and for identification of calcium oxalate crystals was utilized polarized light microscopy. Standard control procedures were performed simultaneously. Observation of cuts and obtaining the images were made using a light microscope Axioplan ZEISS (Oberkochen, Germany), using camera Cannon Power Shot A640 and software Analysis[®]-LINK/ISIS/ZEISS (Oxford, UK). The chemical composition of the crystals, which was calcium oxalate, was confirmed because they were insoluble in acetic acid and soluble in chloridric acid (Mclean and Cook 1958).

For anatomical analysis, the material was fixed in a solution of 2.5% glutaraldehyde, 4% formaldehyde and 0.05 M sodium cacodylate buffer at pH 7.2, then washed in the same buffer and postfixed in 1% osmium tetroxide and 0.05 M sodium cacodylate buffer for 2 h at ambient temperature. Next, the samples were dehydrated in an ascending series of acetone. After dehydration, the material was infiltrated and embedded in epoxy resin (Epon 812 Polybed[®], Warrington, USA). Semi-thin sections of approximately 2–5 µm were obtained and stained with 1% toluidine blue and 1% borax buffer (Johansen 1940). The slides were then observed and documented with a camera (PowerShot A640, CANON, New York, USA) attached to

a light microscope (Axioplan, ZEISS, Oberkochen, Germany).

The thickness of the domatia, mesophyll, and number of mesophyll cells were calculated from cross sections of the domains. We examined 25 fields for each domatium developmental stage. The images obtained were processed and analyzed using image processing digital system Image Pro-Plus.

Domatia were also subjected to scanning electron microscopy. For this, the domatia were fixed and dehydrated following the same methods used for optical microscopy. Then, the samples were subjected to critical-point drying with CO₂ (CPD 030, Baltec, Heerbrugg, Switzerland) and adhered to stubs using carbon adhesive tape and covered with a layer of approximately 20 nm of gold (SCD 050, Baltec, Heerbrugg, Switzerland). Images were obtained using a ZEISS-DSEM 962 scanning electron microscope (Oberkochen, Germany) at a voltage of 25 kV.

3 Results

The rachis of *S. terebinthifolius* is characterized by being cylindrical in shape in the distal region and pseudo-winged in the basal region of the leaflets. This wing-like rachis is most developed in the region of the third leaflet, as can be seen in Fig. 1. This structure is very close to the base of the leaflets, with folds forming an enclosure called the domatium (Fig. 1–13). Domatia were observed only at the base of the second and third leaflets, while none of the first leaflets analyzed had domatia (Fig. 1). Only this form of domatium was found in individuals investigated.

Four stages of development of domatia were characterized. The initial stage (IS) is characterized by small projections that emerge from the winged structures located at the base of the leaflets (Fig. 2, 6, 10). At this stage, a great quantity of trichomes are distributed over the entire surface of this projection (Fig. 6). Transverse sections at this stage revealed that the winged projections show undifferentiated mesophyll, and a subepidermal layer consisting of 2–5 layers of cells underlying the two surfaces of epidermis (Fig. 14). The adaxial epidermal cells are higher than those of the abaxial epidermis, and a thickening of the cuticular layer on both sides was found (Fig. 15, 16).

The second stage, called the initial intermediate stage (IIS), exhibits further development of these projections and a slight curvature of the structure, in which is formed a concave region facing the abaxial surface of the leaves (Fig. 3, 7, 11). This event resulted in an increase in the mesophyll between the adaxial and abaxial surfaces (Fig. 17). In IIS, the subepidermal layer is comprised of

three layers of cells below the adaxial surface and is absent from the abaxial surface (Fig. 18, 19).

The third stage, called the late intermediate stage (LIS), has differentiated mesophyll, consisting of palisade and spongy parenchyma, differently of earlier stages (Fig. 20, 22). Exhibits more accentuated curvature of the projection compared to the second stage (IIS). The curvature is the result of the continuous process of developing and folding of the projection upon itself resulting in a domatium, and culminating in the initial formation of a structure similar to a capsule/enclosure (Fig. 4, 8, 12). In this stage, the domatia are at different points of folding, causing cell compaction in different regions of the structure (Fig. 20, 22). In the basal region of the insertion of the rachis where there is less compaction of the tissues, intercellular spaces are present between the cells of the mesophyll (Fig. 20). On the other hand, in the median region, mesophyll and the epidermis presented more cellular compaction (Fig. 21). In the region distal to the rachis, cellular compaction was more intense in the region of mesophyll. A larger number of cells were also observed on the abaxial surface of the epidermis that were smaller than the cells of the epidermis of the adaxial surface (Fig. 22).

The last stage of development, called the complete development stage (CDS) of the domatia, a capsule/enclosure is already fully formed due to process of continuous folding of the projections (Fig. 5, 9, 13). The anatomical characteristics encountered at this stage were similar to those encountered in the LIS, differing only with respect to the lesser degree of cellular compression in the middle region of this structure (Fig. 23–25).

Through the morphometric analysis of the domatium, it was possible to verify a reduction of the domatia thickness, the mesophyll thickness, and the number of mesophyll cells in the LIS and CDS stages when compared to the IS and IIS stages (Table 1). These results help to understand the processes of folding of the domatium.

In the transverse section of the rachis, more details of formation of the domatium can be observed, leaving their enclosure inclined toward facing the abaxial surface of the leaf (Fig. 26, 29). The internal structure of the domatium is organized by spiral chamber that were produced by the folding (Fig. 27, 30). It is in this chamber that mites can be found (Fig. 28) among the tectory trichomes (Fig. 27) and the glandular trichomes (Fig. 30). Stomata are restricted to just inside of the domatium (Fig. 32). The microchemical test with ferric chloride detected the presence of phenolic compounds (Fig. 33), and polarized light microscopy revealed the presence of calcium oxalate crystals of druse type in mesophyll cells (Fig. 34). There were no starches, lignin or lipid droplets detected.

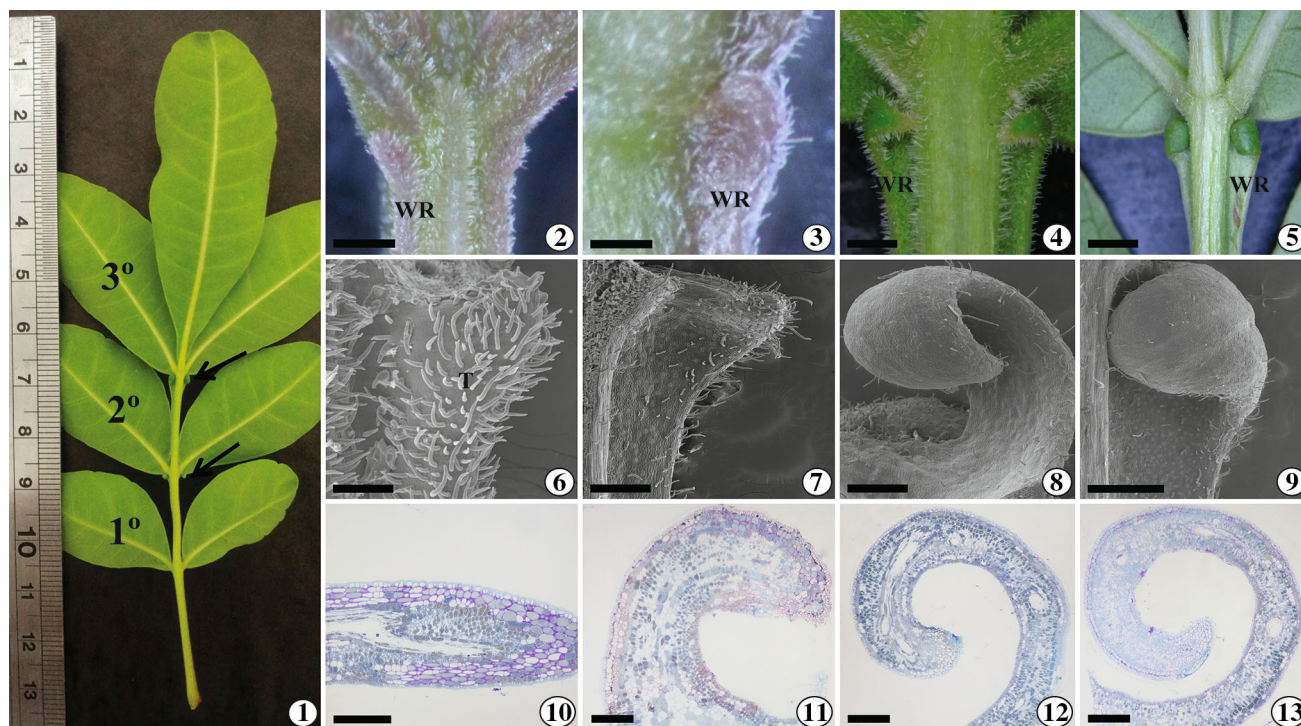


Fig. 1–13 Characterization of the stages of development of domatia of *Schinus terebinthifolius*. **1** Overview of a leaf showing domatia at the base of the leaflets. **2, 6, 10** Initial stage of development. **3, 7, 11** Initial intermediate stage of development. **4, 8, 12** Late intermediate stage of development. **9, 13** Complete development stage. **1–5** Images taken using a stereomicroscope. **6–9** Images taken using a scanning electron microscope. **10–13** Images taken using an optical microscope. Identification of leaflets (1° , 2° , 3°); Domatia (arrows); Wing-like rachis (WR); Trichomes (T). Bars: **2, 3** 1 mm; **4, 5** 2 mm; **6** 1 mm; **7** 100 μm ; **8** 200 μm ; **9** 500 μm ; **10–13** 200 μm

4 Discussion

The best-known types of domatia are structures found on the abaxial surfaces of leaves, usually in the region of the contact between the midrib and secondary veins on the edges of the leaves (Agrawal 1997; Moraes et al. 2009, 2011). According to Barros (1961), *S. terebinthifolius* present two types of domatia: (1) tuft of hair; (2) cavity shaped. However, only the second type was found and analyzed in this work. In this study, we utilized the terminology capsule/enclosure type of domatia to differentiate them from the cavity-shaped domatia that have already be unclassified between veins of leaves of other species of plants.

The ontogeny of the leaves domatia of *S. terebinthifolius* occurs along with the development of the leaves, suggesting that they contribute to the early establishment of the mutualistic relationships with ants or mites, thus providing protection throughout the life cycle of the leaf (Brouat and McKey 2000).

The development of domatia on only the second and third leaflets is a constant characteristic of all leaves of *S. terebinthifolius*, suggesting that the distribution of domatia is an intrinsic genetic characteristic of the species and not a response to environmental variables. Similar patterns of

distribution of domatia were observed in *Miconia tristis* Spring. and *M. doriana* Cogn. (Melastomataceae) (Souza and Marquete 2000), and in *Miconia sellowiana* Naudin (Melastomataceae) (Larcher de Carvalho et al. 2012). However, the hypothesis that the distribution of domatia is part of a strategy for the mutualistic relationship cannot be ruled out.

The formation of domatia in *S. terebinthifolius* can be explained by the processes of development and folding of the rachis winged structures located at the base of the leaflets. This pattern of formation of domatia was described by Leroy et al. (2008), for pouch-shaped domatia of *Hirtella physophora* (Chrysobalanaceae). Structural alterations of tissue also contribute to the formation of domatia. In general, the compaction of parenchyma cells is one of the main causes of the folding of leaves during the domatia development (Santos and Almeida 1995; Nishida et al. 2006). Similar processes were encountered in the present study for the formation of capsule-/enclosure-type domatia of *S. terebinthifolius*. Another factor that can contribute to the folding of the winged structure is the existence of smaller and more numerous cells in the abaxial epidermis in comparison with the adaxial epidermis. Studies of development of leaves reported with the primary characteristic for the winding of the leaves (Paiva et al. 1993).

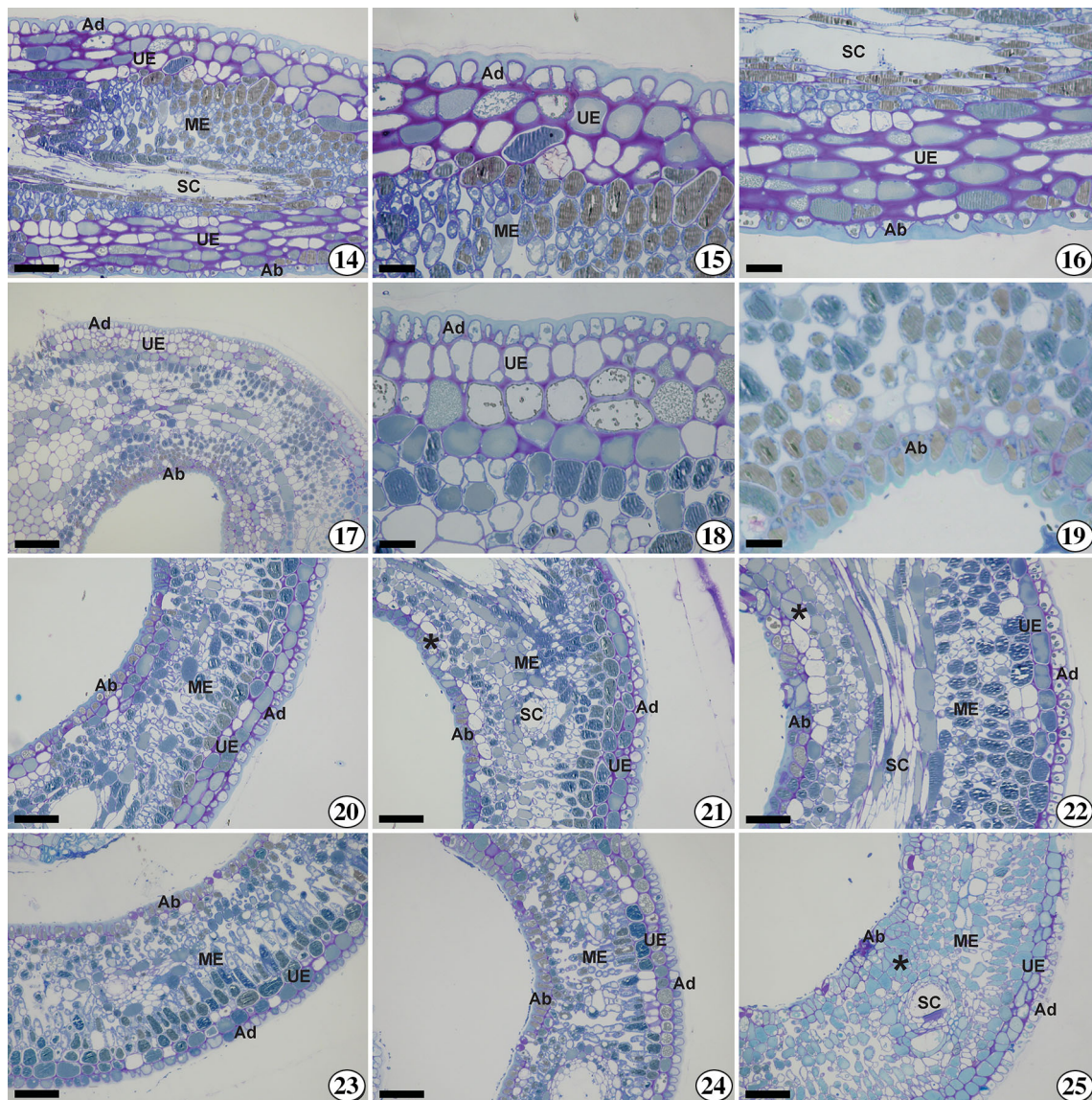


Fig. 14–25 Anatomical characterization of the domatia of *Schinus terebinthifolius*. **14–16** Transverse section of a domatium in initial stage. **17–19** Transverse section of a domatium in initial intermediate stage. **20–22** Transverse section of a domatium in late intermediate stage. **23–25** Transverse section of a domatium in complete development stage. Asterisks indicate cell compaction. Adaxial epidermis (Ad); Subepidermal layer (UE); Mesophyll (ME); Secretory canal (SC); Abaxial epidermis (Ab). Bars: **14, 17, 20, 21** 100 μm ; **15, 16, 18, 19, 22, 24, 25** 50 μm

Table 1 Mean and standard deviation of the anatomical parameters of the domatium of *S. terebinthifolius*

Anatomical traits	Stage of development			
	Initial stage (IS)	Initial intermediate stage (IIS)	Late intermediate stage (LIS)	Complete development stage (CDS)
Domatia thickness	291.3838 \pm 8.1750	333.2125 \pm 5.6361	208.2194 \pm 15.5115	179.4092 \pm 5.2395
Mesophyll thickness	261.3644 \pm 6.8318	300.8347 \pm 6.3196	175.1440 \pm 11.4536	147.4134 \pm 7.0792
Number of mesophyll cells	19.2667 \pm 2.0517	18.6000 \pm 1.4541	11.4000 \pm 1.6818	10.5333 \pm 2.0999

The internal organization of chambers in domatia can provide efficient shelter for species of mites, as confirmed by the presence of these arthropods within these chambers.

Wiggers et al. (2005) showed that *Lorryia formosa* Cooreman (Tydeidae) is the most abundant species of mites in domatia of the *S. terebinthifolius*, representing

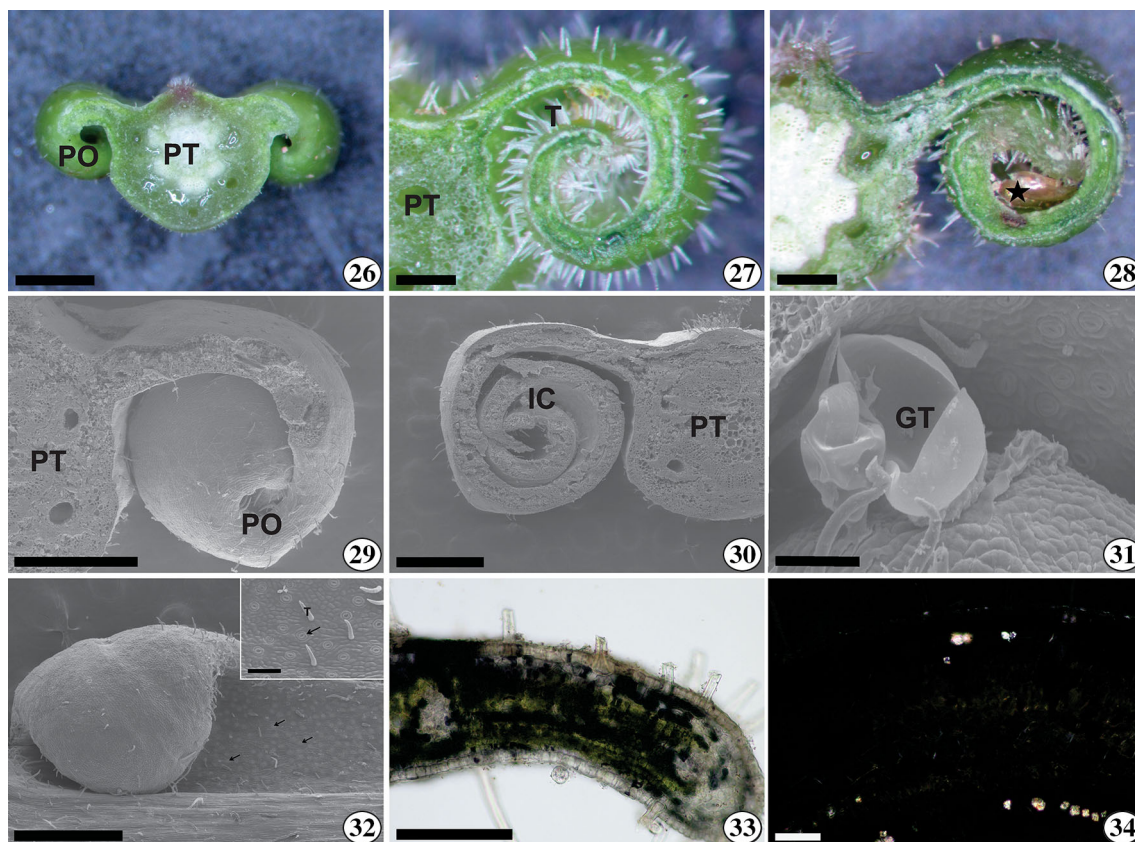


Fig. 26–34 Morphological aspects of domatia of *Schinus terebinthifolius* in complete development stage. **26** Transverse section of rachis detailing the spiral organization of a portion of a domatium. **27** Transverse section showing the presence of trichomes inside of a domatium. **28** Presence of mites in the interior of a domatium. **29** Front view of a domatium. **30** Transverse section of a domatium. **31** Glandular trichomes inside of a domatium. **32** Stomata inside of a domatium. Detail highlighting the presence of stomata inside of a domatium. **33** Detection of phenolic compounds in a domatium. **34** Calcium crystals in a domatium. **26–28** Stereomicroscopy. **29–32** Scanning electron microscopy. **33** Light microscopy and i polarized light microscopy. Petiole (PT); Pore (PO); Trichomes (T); Internal chambers (IC); Glandular trichomes (GT); Mite (star); Stomata (arrow). Bars: **26** 2 mm; **27, 28** 1 mm; **29** 200 μ m; **30, 31, 32** 500 μ m; **33** 100 μ m; **34** 50 μ m

95.9% of the total mites observed. In addition, the same authors reported that mites were found in different stages of development, in the form of eggs, juveniles and adults, proving that domatia of the *S. terebinthifolius* are good environment for the shelter and for spawn mites. This characteristic may be part of a strategy for the mutualistic relationship in that provides a more comfortable and camouflaged shelter for the arthropods. This reinforces the hypothesis defended by several authors that the establishment of this relationship between plant and arthropods can be beneficial to both (Walter and O’Dowd 1992; Agrawal 1997; Agrawal et al. 2000; Norton et al. 2001; Romero and Benson 2004).

The occurrence of trichomes inside of domatia has already been described for other species of plants and is considered to serve as a physical defense for mites, as well as contribute to the diet of these organisms, since these trichomes can trap pollen and mold spores that the mites will eat (Romero and Benson 2005; Moraes et al. 2011). The presence of stomata inside of domatia indicates that

they can act jointly with trichomes to establish a more humid environment, thus providing mites with protection from desiccation (O’Dowd and Willson 1991; Romero and Benson 2005).

Another strategy against herbivory of this type of domatia of *S. terebinthifolius* include the presence of phenolic compounds and calcium crystals. Herbivores mites were rarely found in domatia of the *S. terebinthifolius* (Wiggers et al. 2005) that can be related to large amounts of phenolic compounds and crystals present in the leaves. Phenolic compounds may inhibit herbivores since they are astringent substances that promote unpalatability to herbivores including phytophages (Roshchina and Roshchina 1993; Carvalho et al. 2000). But this species also has low levels of herbivory. In *Cecropia peltata* L. (Cecropiaceae), Val and Dirzo (2003) reported the presence of phenolic compounds in domatia, suggesting an inefficient interaction between the arthropods and plants in the protection against herbivory, since the plant is developing additional resources for protection from herbivory.

The presence of druse crystals can also be related to protection against herbivores. Some studies have linked increased amounts of calcium crystals on plants that suffer attacks from herbivores (Molano-Flores 2001; Nakata 2003; Franceschi and Nakata 2005). The presence of calcium crystals in the domatia of *S. terebinthifolius* may be another indication of the investment these plants make in alternative forms of protection against herbivory.

Structural analysis showed that the stages of development and the architecture of domatia can provide a more efficient shelter for mites that in turn can contribute to plant protection. Various plant strategies are related to the distribution of domatia, the development and folding of the lateral projections for the formation of domatia to shelter mites, and phenolic compounds and druse crystals for efficient protection against herbivory, thereby corroborating ecological studies. Furthermore, this study provides information that can also contribute to the study of the systematic of this plant family and genus.

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