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Original Paper

Anatomy of the floral nectaries of selected species of Gardenieae (Rubiaceae)

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Abstract
Rubiaceae is one of the five most species-rich families of Angiosperm, its flowers are characterized by a nectariferous disc surrounding the base of the style; however, there are almost no studies on anatomy of these structures in the family. The aim of this study was to describe for the first time the anatomy of the floral nectaries in the tribe Gardenieae (Rubiaceae). Flowers from *Cordiera concolor*, *Genipa americana*, *Randia calycina*, *Randia ferox*, *Randia heteromera*, *Randia micracantha*, and *Tocoyena formosa* were collected, photographed, and processed using conventional techniques for observation with optical and scanning electron microscopies. In all species the nectary is differentiated histologically into epidermis, nectary and sub-nectary parenchyma, and vascular bundles. Nectar is exuded via nectarostomata. The nectaries have reproductive function, and are structural and mesenchymal during the floral stage. Whereas after corolla has fallen they are persistent nectaries, with post-floral secretion and extranuptial function. In addition, floral visitors that appeared on open flowers and after corolla fall were recorded.

Key words: floral structure, nectaries, post-floral secretion, Rubiaceae.

Resumo
Rubiaceae é uma das cinco famílias mais ricas em espécies de Angiospermas, suas flores são caracterizadas por um disco nectarífero ao redor do estilete. No entanto, quase não há estudos sobre a anatomia destas estruturas na família. O objetivo deste trabalho foi descrever pela primeira vez a anatomia dos nectários na tribo Gardenieae (Rubiaceae). Flores de *Cordiera concolor*, *Genipa americana*, *Randia calycina*, *Randia ferox*, *Randia heteromera*, *Randia micracantha* e *Tocoyena formosa* foram coletadas, fotografadas e fixadas. Os nectários foram processados utilizando técnicas convencionais de observação em microscopia óptica e microscopia eletrônica de varredura. Em todas as flores o nectário é histologicamente diferenciado em epiderme, parênquima nectarífero, parênquima subnectarífero e traços vasculares. O néctar é exsudado através de estômatos. Os nectários tem função reprodutiva e são estruturados e mesenquimais na fase floral. Após a queda da corola são nectários persistentes com secreção pós-floral e função extranupcial. Também foram registados os visitantes florais que apareceram em flores abertas e após a queda da corola.

Palavras-chave: estrutura floral, nectarios, secreção pós-floral, Rubiaceae.

Introduction

Over time, numerous classifications have been proposed to characterize and typify nectaries, some were based on location as floral nectary (FN) and extrafloral nectary, according to whether the nectary is in the flowers or in the vegetative organs respectively (Caspary 1848). Schmid (1988) used the terms reproductive nectary for those located in flowers, fruits, inflorescences, bracts and pedicels, and extra-reproductive nectary when located

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in strictly vegetative organs. Nectaries are also classified according to their function as nuptial nectary, for those that participate in the pollination process, and extranuptial nectary, for those that are not involved with pollination (Delpino 1868–1874) but also maintain an anti-herbivore community in the plant that is responsible, for example, for reducing predation on flower buds (Rivera 1997).

In the case of the FN, the persistence of the floral parts on which they are placed is also considered in the classification, so those FN associated with floral parts that fall after fertilization (*e.g.*, stamens, petals) are called deciduous nectaries, while persistent nectaries are those associated with floral parts that do not fall after pollination (*e.g.*, receptacle, ovary) and during fruit differentiation (Smets 1968, 1988; Smets & Cresens 1988). Persistent nectaries may remain intact in the flower without producing any nectar or they may have post-floral secretion for a period of time after anthesis or during fruit differentiation, in which case there are authors who consider that they would have a role in protecting the development fruit against predators (Santos & Del-Claro 2001; Amorim & Oliveira 2006; Falcão *et al.* 2014). Among the persistent nectaries are the so-called pericarpial nectaries, which are formed after fertilization (*e.g.*, in *Erythrina speciosa* Tod., Paiva 2009).

Regardless of the position on the plant, other classifications consider the anatomical structure and mode of nectar secretion: non-structural nectaries do not present a structure different from that of the neighboring tissues, contrasting with structural nectaries (Zimmermann 1932). Based on anatomical features, Vogel (1977) proposed the terms mesenchymal nectary, which consists of glandular and storage tissues that normally secrete nectar in interstitial spaces or in the apoplast of the cells which is then exuded through stomata; epithelial nectary, consisting of a permeable glandular epidermis, often with an underlying glandular tissue; and trichomatic nectary, when the secretion is released by glandular trichomes. Moreover, Fahn (1979) described that nectaries were generally formed by epidermis and parenchymatous tissue (with vascular bundles), while Durkee (1983) considered three tissues: epidermis, secretory tissue (parenchymal tissue that separates epidermis from vascularized tissue), and subglandular parenchyma with vascular bundles (as synonymous to Fahn's parenchymatous tissue). Finally, Nepi (2007) proposes the terms epidermis, nectary parenchyma, and sub-nectary parenchyma with vascular bundles. The modified, usually open and anomocytic, stomata were called first nectarthodes by Schmid (1988) and later, nectarostomata by Ronse De Craene & Smets (1991).

It is common the presence of floral nectaries in plants that are pollinated by insects or birds (Fahn 1982), in which these secretory structures are involved in the pollination process. The floral nectaries can be found in almost all parts of flower (sepal, petal, stamen, receptacle, ovary, and style) and they can produce a wide chemically variety of sweetened exudates (Fahn 1982; Bernardello 2007; Tölke *et al.* 2019). In addition, the morphology and location of nectaries can be useful for establishing relationships between taxa in systematic treatments (Fahn 1982; Bernardello 2007; Nepi 2007; Almeida *et al.* 2013; Tölke *et al.* 2018; Phukela *et al.* 2020).

Rubiaceae is one of the five most species-rich families of Angiosperms (up to 13,000 species), divided into three subfamilies (Cinchonoideae, Ixoroideae, and Rubioideae), 40 tribes, and ca. 620 genera (Govaerts *et al.* 2020). This family have flowers with tetracyclic arrangement, predominantly actinomorphic symmetry, the stamens are epipetalous and in the same number as the number of corolla lobes (the filaments are fused with the corolla-tube), and the ovary is almost always inferior; also, a nectary disc (ND) surrounding the basis of the style with nectarostomata is almost universally present (Robbrecht 1988; Simpson 2010). Despite the uniformity of traits of floral nectaries in the family, the studies in Rubiaceae have mostly focused on nectar composition (Freeman *et al.* 1991), or on ecological aspects of the nectar (Falcão *et al.* 2014; Amorim & Oliveira 2006; Salinas-Esquivel *et al.* 2013; Queiroga & Moura 2017; Avila Jr. & Freitas 2011). There are few studies that describe their anatomy and these are mainly focused on species of the tribe Spermacoceae (subfamily Rubioideae, Galati 1991; Galetto 1998; Florentín *et al.* 2016).

In the tribe Gardenieae (Subfamily Ixoroideae), anatomical studies have been carried out on different organs such as leaves, stems, and colleters (Koek-Norman 1972; Dave *et al.* 1987; Coelho *et al.* 2006; Erbano & Duarte 2010; Judkevich *et al.* 2015; Miguel *et al.* 2016); however, studies describing the anatomy of the floral nectaries of this tribe are still unknown. In the Neotropics, Gardenieae comprises 18 woody genera, among which there are dioecious and monoecious species. Here we studied the ND of seven Neotropical species of this tribe: *Tocoyena formosa* (Cham. & Schltdl.) K. Schum, *Cordiera concolor* (Cham.) Kuntze, *Genipa americana* L., *Randia calycina* Cham, *Randia ferox* (Cham. & Schltdl.) DC, *Randia micracantha* (Lillo) Bacigalupo, and *Randia heteromera* Judkevich & R.M. Salas. The only monoecious species with monoclinous flowers is *T. formosa*, while the other species are dioecious with diclinous flowers (Judkevich *et al.* in press).

Considering all this, the objective of the present study was: 1) to perform an anatomical analysis of the floral nectaries of seven selected species of Gardenieae; 2) to compare the morphology of nectaries between the nectaries of pistilate and staminate flowers in the dioecious taxa; and 3) compare anatomically with the nectaries of other species of the family.

Material and Methods

Material from *Tocoyena formosa*, *Cordiera concolor*, *Genipa americana*, *Randia calycina*, *Randia ferox*, *Randia micracantha*, and *Randia heteromera* were collected and photographed in the field. Voucher specimens were deposited in the herbarium Carmen Cristóbal (CTES) of the Instituto de Botánica del Nordeste, Corrientes, Argentina (voucher numbers in the Appendix S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>). Flowers were collected in the field and preserved in formalin-acetic acid-alcohol (5 mL formalin, 5 mL acetic acid, and 90 mL 70% ethanol; Johansen 1940).

Using a digital caliber (Wembley 5940) 20 flowers of each species (of the material collected in different localities, see Appendix S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>) were measured and calculated mean values from: the total length of the flower, the diameter of the ovary (including the hypanthium), and the diameter of the nectary. In addition, to know the average number of nectarostomata per nectary in each species, the nectarine epidermis was dissociated from the nectary with sodium hypochlorite and then stained with safranin (D'Ambrogio de Argüeso 1986). The total number of nectarostomata was counted with a Leica DM LB2 (Leica Microsystems) light microscope.

To light microscopy (LM) analysis, five anthetic flowers were taken from two random specimens of each species fixed. The nectaries removed from these flowers were dehydrated in an ascending series of alcohols and embedded in paraffin (Johansen 1940; modified by Gonzalez & Cristóbal 1997). The samples were cut into 12 μm sections using a Microm HM350 rotary microtome (Microm International, Walldorf, Germany), stained with safranin and Astra blue (Luque *et al.* 1996) and mounted in synthetic Canada balsam. Observations and digital images were made using a Leica DM LB2 (Leica Microsystems) light microscope equipped with a Leica DATA digital camera in the plant anatomy laboratory of the Instituto de Botánica del Nordeste. Nepi's terminology was followed to describe the anatomy of the nectaries of Gardenieae (Nepi 2007).

For scanning electron microscopy (SEM) nectaries from fixed anthetical flowers were dehydrated in an increasing 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). The samples were analyzed with a Jeol LV 5800 (JEOL, Tokyo, Japan) at 10 kV in the Service of Electron Microscopy facility at the Universidad Nacional del Nordeste.

Results

Floral morphology

The flowers respond to the typical characteristics of the family, they are actinomorphic, the calyx is synsepalous, the corolla is tubular sympetalous, haplostemonous, and the ovary is inferior (Fig. 1). Only the anthers of the monoclinous flower of *Tocoyena formosa* and of the staminate flowers of the dioecious species produce pollen. The anthers of the pistilate flowers are sterile and indehiscent, and thus are staminodes. The gynoecium consists of a style, a stigma, and an ovary that has ovules in the monoclinous and pistilate flowers, while in the staminate flowers a poorly developed carpelode was formed with rudimentary ovules (composed only by undifferentiated masses of parenchyma). There are variations in the length of the flowers, in the number of floral organs (perianth and stamens), and in the color of the corolla among species (Fig. 1; Tab. 1). Staminate flowers have smaller hypanthium (Fig. 1b,f,j,l) then pistilate flowers (Fig. 1c,e,g,k,m).

Floral nectaries

All species have a ring-shaped floral nectary on top of the inferior ovary, surrounding the base of the style (Figs. 2-4). The nectary disc is green or yellowish, glabrous (Figs. 2; 4).

In the dioecious species, the pistilate flowers have an ovary of larger diameter than the staminate flowers; the ND is proportionally larger in most of the pistilate flowers (Figs. 1a,c,e,g,i,m; 3b-e,g). *Randia micracantha* is the only species with the opposite relation of size (Fig. 3f). In *Tocoyena formosa* (Fig. 3a) and in both types of *Genipa americana* flowers (Fig. 3c), the surface of the ND is markedly depressed in the direction of the style base. In the other species the surface is generally convex and may have a slight inclination towards the style (Fig. 3b,d-g). On the other hand, the ND of both types of flowers in *G. americana* and the staminate flowers of *R. calycina* and *R. ferox* are smoothly undulated in its outer surface (Fig. 4f-g,i,l,p) as result of the anthers pressure on the disc during flower development (personal observation).

A smooth cuticle covers the nectaries (except in *R. ferox* which is striated, Fig. 4n) with no sign of rupture (Fig. 4b,e,h,k,n,q,t). The

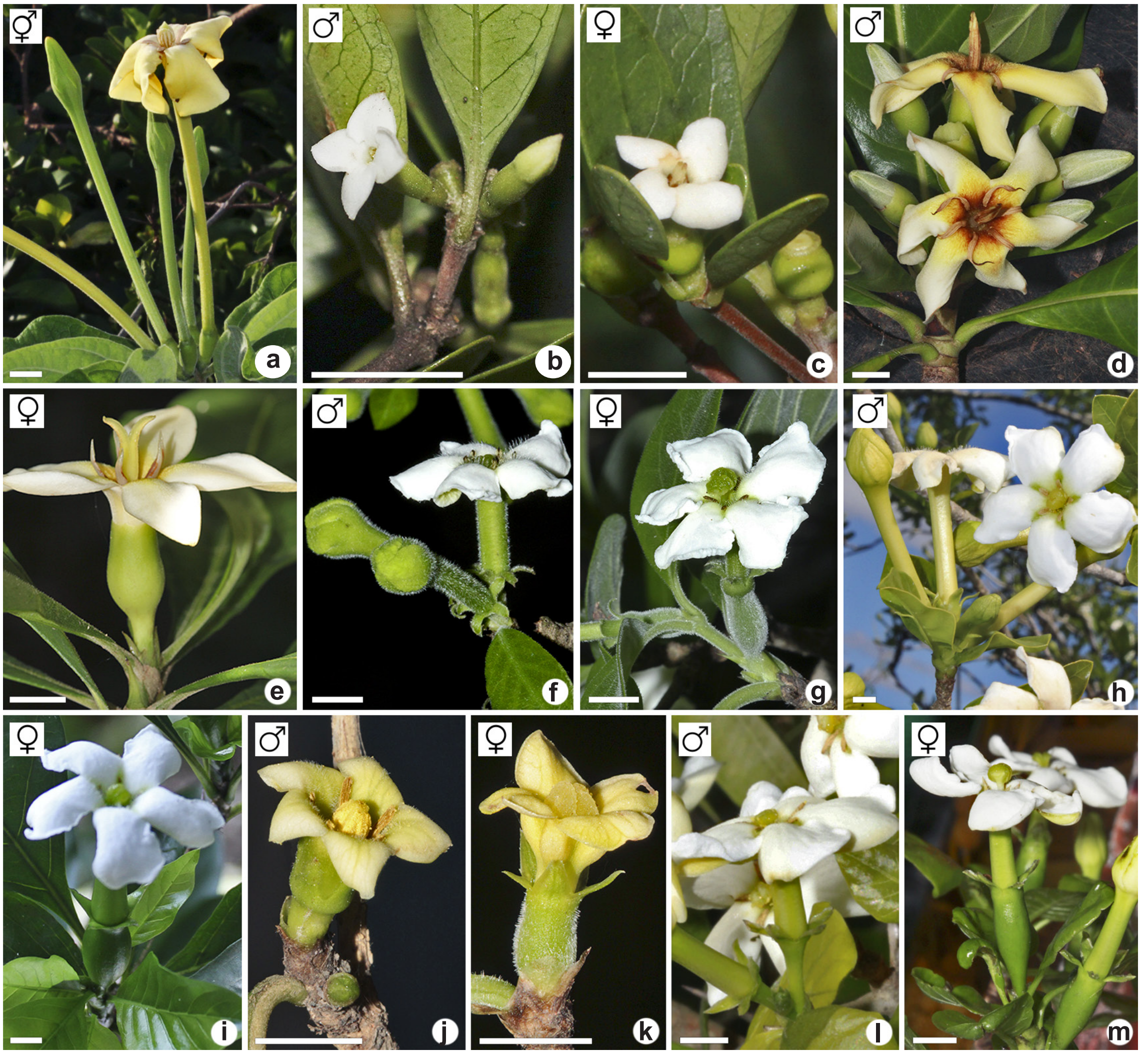

Figure 1 – a-m. Flowers of the analyzed species of Gardenieae – a. monoclinous *Tocoyena formosa* (⚥); b-m. diclinous species, staminate (♂) and pistilate (♀) respectively – b-c. *Cordiera concolor*; d-e. *Genipa americana*; f-g. *Randia calycina*; h-i. *Randia ferox*; j-k. *Randia micracantha*; l-m. *Randia heteromera*. Scale bar: a, d-e = 1 cm; b-c, f-m = 0.5 cm.

Table 1 – General features of the flowers of the analyzed Gardenieae species. Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower.

Species	Flower length (mm)	Ovary width (mm)*	Number of pieces of perianth and androecium	Corolla color
Tocoyena formosa	⚥: 100-120	⚥: 5	5-merous	yellowish
Cordiera concolor	♀: 5-6	♀: 2.5	4-merous	white
	♂: 5-6	♂: 1.4		
Genipa americana	♀: 38-50	♀: 13.0	5–6-merous	white
	♂: 35-40	♂: 7.0		
Randia calycina	♀: 27-32	♀: 3.7	5-merous	white
	♂: 16-20	♂: 2.0		
Randia ferox	♀: 40-50	♀: 8.0	5-merous	white
	♂: 40-50	♂: 3.5		
Randia heteromera	♀: 20-27	♀: 3.0	5–6(–7)-merous	white
	♂: 20-23	♂: 1.5		
Randia micracantha	♀: 10-13	♀: 2.5	5-merous	yellowish green
	♂: 10-14	♂: 2.0		

* As the ovary is inferior, these values include the hypanthium.

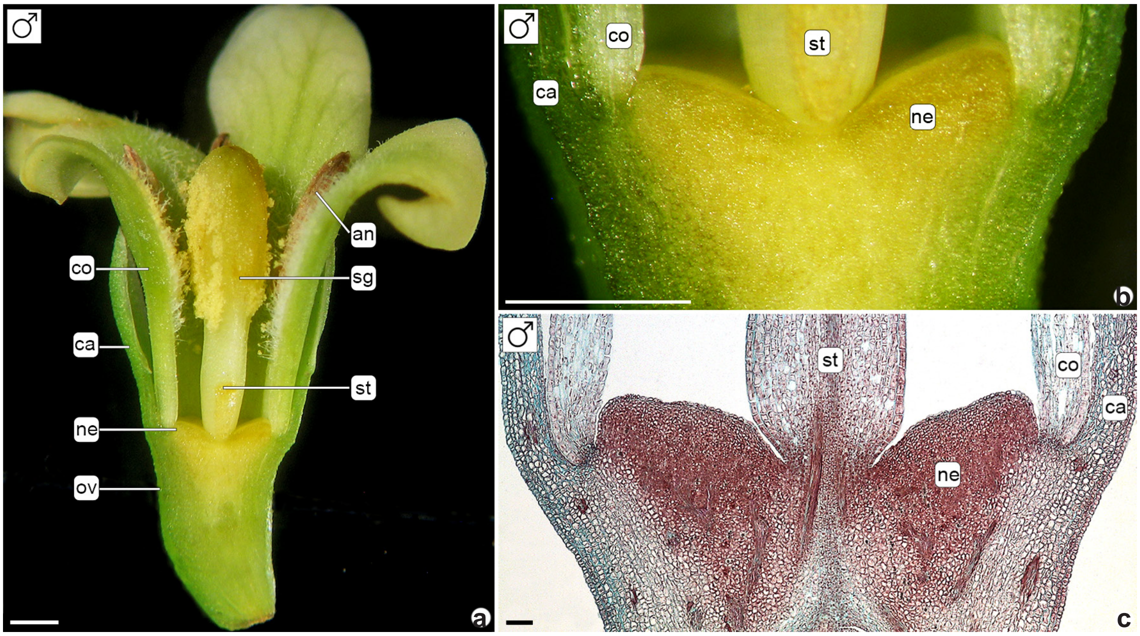

Figure 2 – a-c. Position of the nectary in the flower (longitudinal sections of the staminate flower of *Randia micracantha* in which the nectary can be seen surrounding the base of the style) – a. anthetic flower; b. detail of the nectary area from a fresh flower; c. detail of the nectary area, with light microscopy. Abbreviations: an = anther; ca = calyx; co = corolla; ne = nectary; ov = ovary; sg = stigma; st = style. Scale bar: a, b = 1 mm; c = 100 µm.

nectar is secreted through nectarostomata (Fig. 4b,e,h,k,n,q,t), mostly distributed at the apex of the nectary and on the surface close to the style base. The biggest nectaries present a large number of nectarostomata (Tab. 2), therefore the pistilate flowers of dioecious species present more nectarostomata than the staminate flowers (except in *R. micracantha*).

Anatomically, the ND is formed by epidermis and nectary and sub-nectary parenchyma (Figs. 5-6). The epidermis is glabrous and covered by a cuticle that is remarkably thick (Figs. 5b,f-g,l; 6c,g,k,o). The nectarostomata are anomocytic and are at the same level as the surrounding epidermal cells or slightly elevated, but they may be sunken in *Randia ferox* (Fig. 6g). In all cases they are open and have small sub-stomatic chambers (Figs. 5b,f-g,l; 6c,g,k,o).

The nectary parenchyma is composed of several layers of small cells, polygonal or circular in section, with dense cytoplasm, thin walls, and a conspicuous nucleus (Figs. 5a-b,d-e,i-k; 6a-b,e-f,i-j,m-n). There are druses in all species, except in *Randia heteromera*, and cells with phenolic compounds only in *C. concolor*.

Sub-nectary parenchyma presents larger and more laxly arranged cells than those of nectary parenchyma. This tissue is vascularized by small vascular bundles formed by xylem and phloem, which branch from the nearby hypanthium bundles (Figs. 5c,h,m; 6d,h,l,p). Except for *R. heteromera*, in this tissue the rest of the species show druses randomly distributed (Figs. 5c,h,m; 6d,h,i,p). Cells with phenolic compounds are abundant and randomly distributed in *C. concolor* (Fig. 5e,h) but are rare in *T. formosa* (Fig. 5c), and absent in the nectaries of the remaining species (in *G. americana* they are found in other organs of the flower -calyx, corolla, anthers, and ovary-).

In the species analyzed, differences were found in the shape of the epidermal cells (polygonal, rectangular, and quadrangular), in

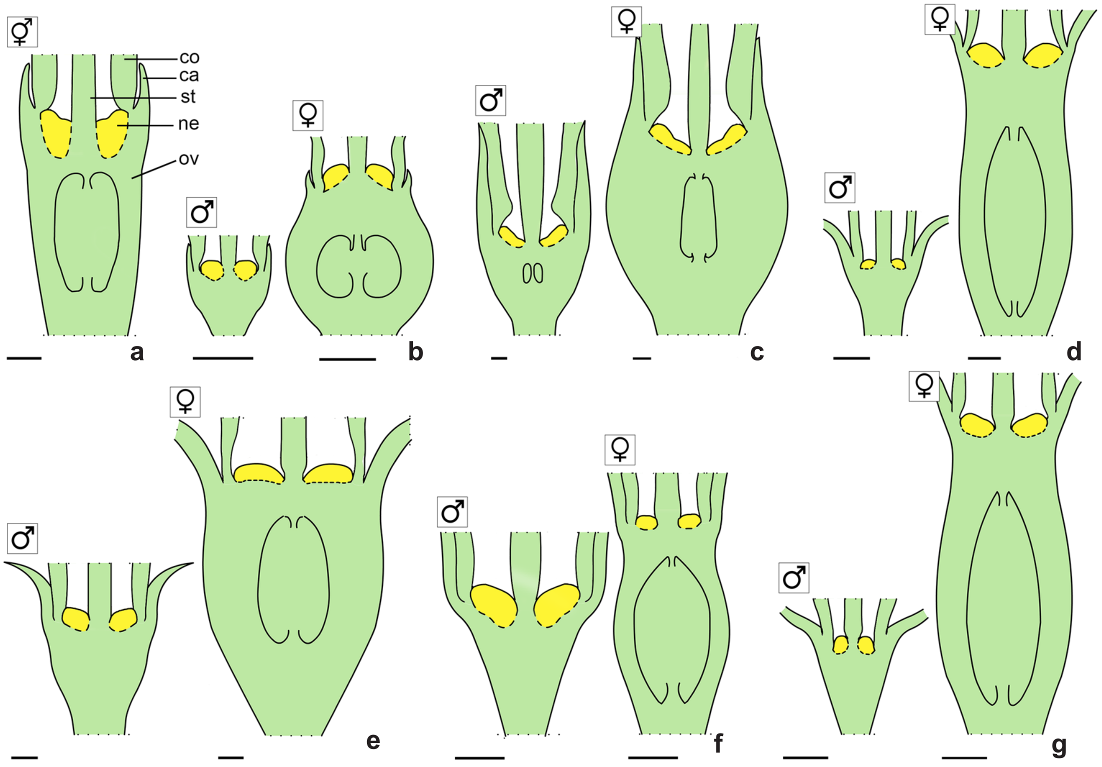

Figure 3 – a-g. Schematic median longitudinal sections of the base of the flowers showing the position of nectaries (in yellow) in monoecious and dioecious species of Gardenieae – a. *Tocoyena formosa*; b. *Cordiera concolor*; c. *Genipa americana*; d. *Randia calycina*; e. *Randia ferox*; f. *Randia micracantha*; g. *Randia heteromera.* Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower. Abbreviations: ca = calyx; co = corolla; ne = nectary; ov = ovary; st = style. Scale bar: 1 mm.

the number of nectarostomata, and between the proportion of the nectary parenchyma and the sub-nectary parenchyma, in *T. formosa* and *C. concolor* the proportion of nectary parenchyma is higher than that of sub-nectary parenchyma, in *R. micracantha* the opposite occurs, while in the remaining species both tissues are found in similar proportion (Tab. 2).

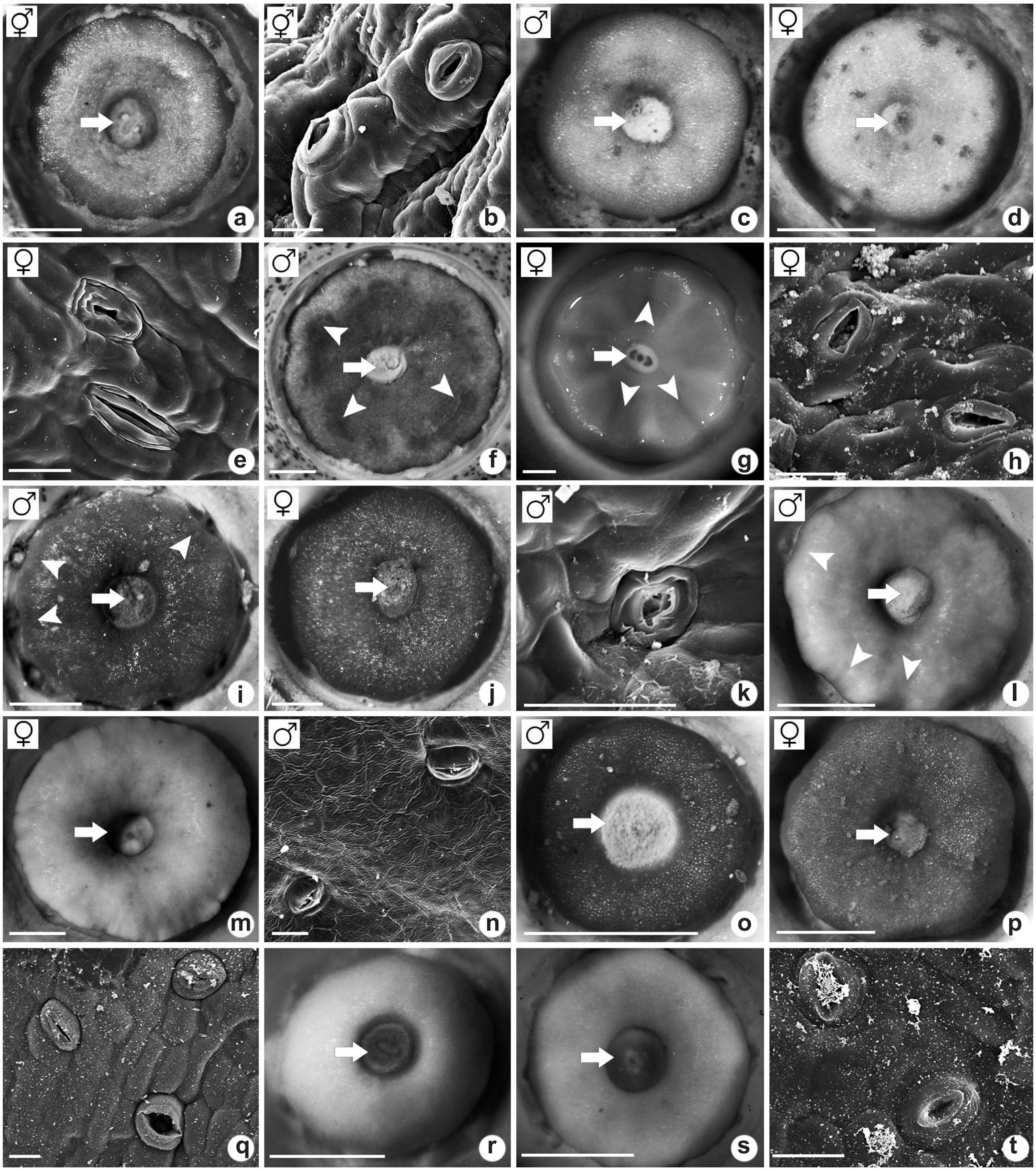

Figure 4 – Surface views of nectariferous disc (a, c-d, f-g, i-j, l-m, o-p, r-s, with LM) and details of nectarostomata (b, e, h, k, n, q, t, with SEM) in monoecious and dioecious Gardenieae species – a-b. *Tocoyena formosa*; c-e. *Cordiera concolor*; f-h. *Genipa americana*; i-k. *Randia calycina*; l-n. *Randia ferox*; o-q. *Randia micracantha*; r-t. *Randia heteromera*. White arrows indicate the base of the style (removed). Arrowhead points to the undulations on the surface of the nectary produced by the pressure of the anthers during flower development. Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower. Scale bar: a, f-g, i-j, l-m, o-p, r-s = 1 mm; b, e, h, k, n, q, t = 20 μm; c-d = 0.5 mm.

Table 2 – Diameter, epidermal cell shape, number of nectarostomata, and proportion of parenchymatic tissues of the nectaries in the analyzed Gardenieae species. Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower. Abbreviations: nt = nectarostomata; np = nectary parenchyma; sp = sub-nectary parenchyma.

Species	**Nectary diameter (mm)**	**Epidermal cell shape in in longitudinal section of the nectary**	**N° of Nt on the nectary**	**Relative proportion of parenchymatic tissues**
Tocoyena formosa	⚥: 2.5	Polygonal	⚥: 143	np > sp
Cordiera concolor	♀: 0.9 ♂: 0.7	Polygonal or rectangular (elongated in the longitudinal axis)	♀: 31 ♂: 30	np > sp
Genipa americana	♀: 6.6 ♂: 4.5	Rectangular	♀: 2133 ♂: 1752	np = sp
Randia calycina	♀: 2.7 ♂: 2.1	Polygonal or rectangular (elongated in the longitudinal axis)	♀: 221 ♂: 123	np = sp
Randia ferox	♀: 4.6 ♂: 3.4	Polygonal or rectangular (elongated in the longitudinal axis)	♀: 628 ♂: 38	np = sp
Randia heteromera	♀: 2.3 ♂: 1.7	Polygonal or quadrangular	♀: 362 ♂: 279	np = sp
Randia micracantha	♀: 1.4 ♂: 2.5	Polygonal or rectangular (elongated in the transverse axis)	♀: 89 ♂: 152	np < sp

Field observations

In all analyzed species the floral nectaries are active with evident secretion and visited by bees, butterflies, and moths when the flower is open (Fig. 7a-c). These nectaries are also visited by bees and ants (the latter more frequently) after the fall of the corolla in flowers of all taxa (Fig. 7d-l), and even during the first stages of fruit differentiation (Fig. 7j).

Discussion

Brown (1938) and Robbrecht (1988) mentions as an almost universal characteristic of Rubiaceae flowers the presence of a floral nectary surrounding the base of the style. Although in this research it was not possible to study the composition of the substance secreted by these glandular structures, taking into account their anatomical characteristics and the presence of floral visitors whose feeding is exclusively nectar [*Pompeius verna* -Lotts & Naberhaus (2020)-, and *Aellopos titan* -Moraga (2018)-, both Lepidoptera], we can confirm that they are floral nectaries. These nectaries correspond to the so-called epigynous nectaries, a term that has been used by Manns & Bremer (2010) to designate the floral nectaries of the tribe Naucleeae (Rubiaceae), and by Vaes *et al.* (2006) for *Spermacoce* spp., tribe Spermacoceae.

In all species of Gardenieae studied here the nectary morphology is quite uniform. Only a difference in the size of the nectary was observed between the pistilate and staminate flowers in dioecious species. The nectar in the species analyzed is secreted through nectarostomata, which is the most common mode of nectar release in Eudicots (Nepi 2007; Roy *et al.* 2017) and has also been recorded in the few species studied in the family (Galati 1991; Galetto 1998; Florentín *et al.* 2016). The nectarostomata found are anomocytic, which is in agreement with Bahadur *et al.* (1971) who describes this type of nectarostomata in 26 species of Rubiaceae.

Only few studies detail the anatomy of floral nectaries in Rubiaceae species (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>), most of which are from the tribe Spermacoceae (Galati 1991; Galetto 1998; Florentín *et al.* 2016). These species share with the Gardenieae here analyzed general characteristics such as the location of the nectary in the flower, its annular or disc shape, and the presence of nectarostomata through which they secrete nectar. However, differences were found in some aspects. While in Gardenieae the annular nectary is entire, in some species of the tribe Spermacoceae as in *Borreria* and *Diodia* it is bipartite (Galati 1991) and in *Oldenlandia* *salzmanni* it is bi or tetrapartite (Florentín *et al.* 2016). In addition, within the species analyzed here, a variation in the inclination of the nectariferous disc surface can be observed, particularly in *Genipa americana* with a markedly depressed surface in the direction of the style base. All this variation in the morphology of the nectariferous disc (number of lobes, inclination of the disc surface, etc.) was described by Robbrecht (1988) in different genera of Rubiaceae who even attributes to the disc of a group of Gardenieae the characteristic of being fused with the base of the calyx-tube. The latter differs from the species analyzed in the present study. However, it is important to emphasize that although most of the species in the family have a floral nectariferous disc, this can have a great variety of morphologies in different taxa of Rubiaceae. On the other hand, while Gardenieae nectaries have both the cuticle and the epidermis smooth (except in *R. ferox* whose cuticle is striated), in Spermacoceae the cuticle is always striated and the epidermis is papillate (Galati 1991; Florentín

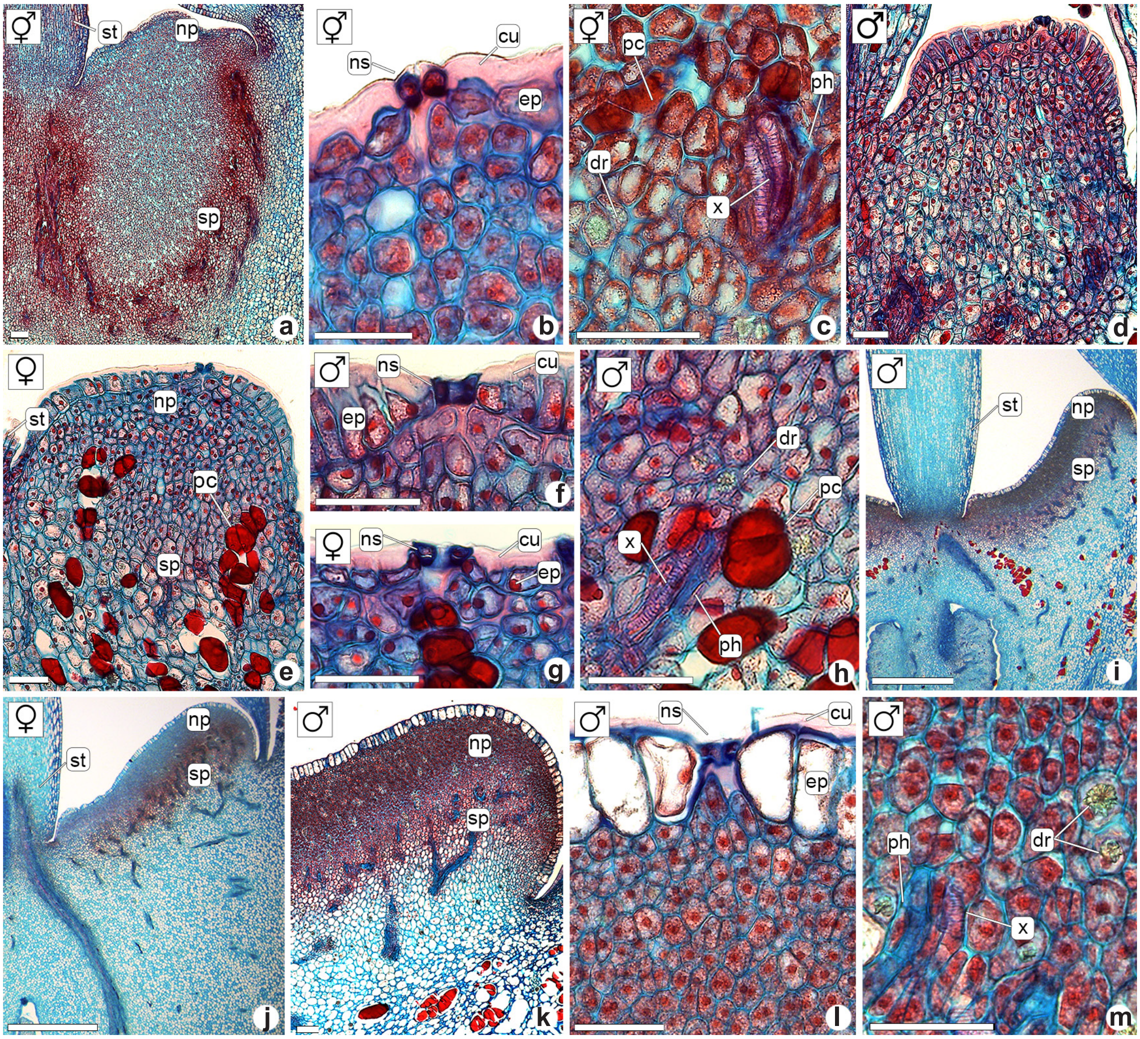

Figure 5 – a-m. Anatomy of nectariferous disc (ND) of *Tocoyena formosa* (a-c), *Cordiera concolor* (d-h), and *Genipa americana* (i-m) in longitudinal section – a, d-e, i-k. details of a portion of ND showing the nectary and sub-nectary parenchyma; b, f-g, l. epidermis, nectarostomata, cuticle, and a portion of nectary parenchyma; c, h, m. details of the vascular traces in sub-nectary parenchyma. Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower. Abbreviations: cu = cuticle; dr = druse; ep = epidermis; np = nectary parenchyma; pc = phenolic compounds; ph = phloem; sp = sub-nectary parenchyma; st = style; x = xylem. Scale bar: a, k = 100 µm; b-h, l-m = 50 µm; i-j = 1 mm.

et al. 2016). *Oldenlandia salzmanni* it also differs from the species of Gardenieae in having a nectarostoma with 5–7 subsidiary cells arranged in a rosette (Florentín *et al.* 2016). In the other Rubiaceae species the type of nectarostoma is not mentioned.

Following Nepi (2007), in the Gardenieae species, the sub-nectary parenchyma has vascular

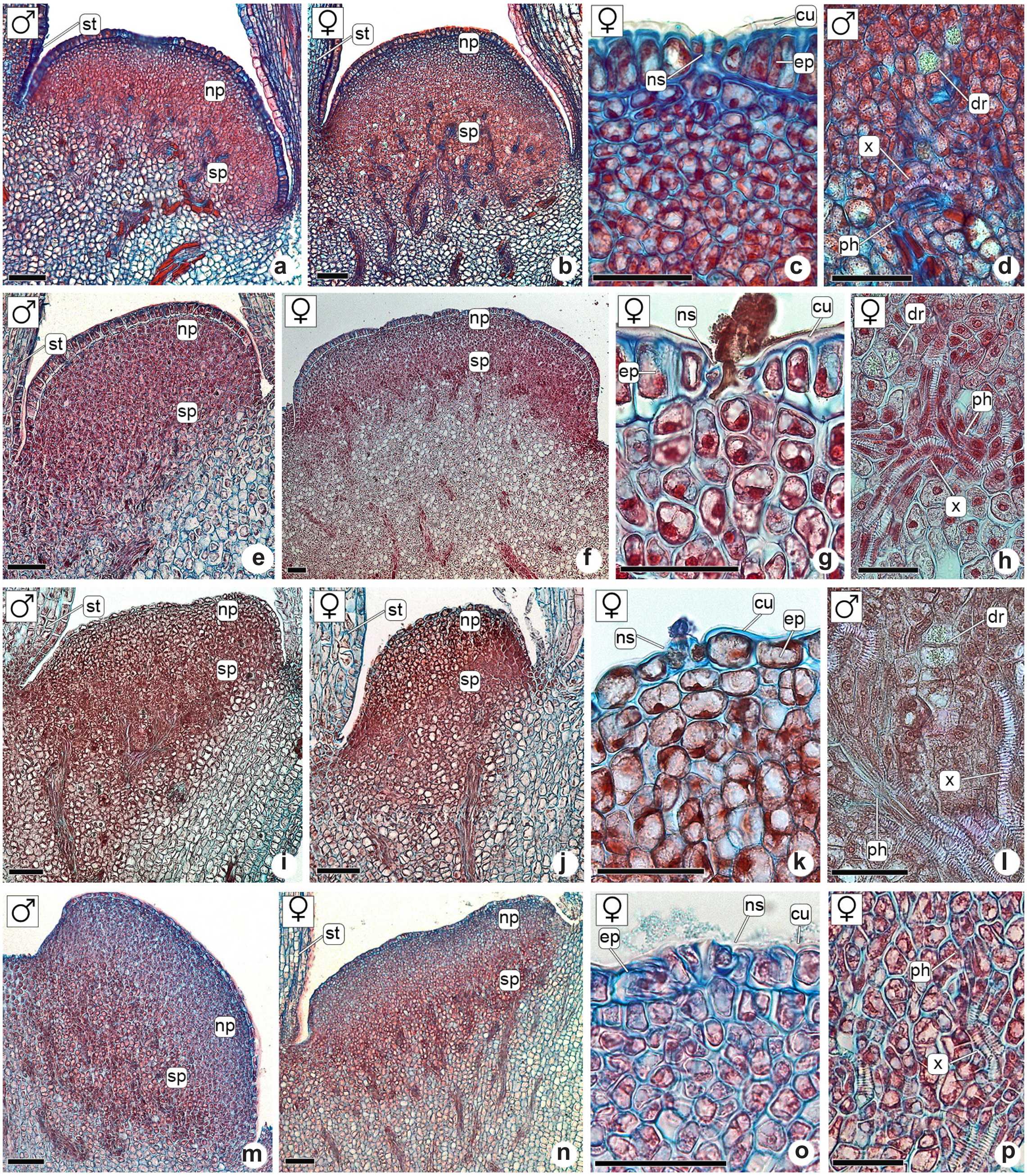

Figure 6 – a-p. Anatomy of nectariferous disc of *Randia calycina* (a-d), *Randia ferox* (e-h), and *Randia micracantha* (i-m) in longitudinal sections – a-b, e-f, i-j, m-n. details of a portion of ND showing the nectary and sub-nectary parenchyma; c, g, k, o. epidermis, nectarostomata, cuticle, and a portion of nectary parenchyma; d, h, l, p. detail of the vascular traces in sub-nectary parenchyma. Simbols: ⚥ = monoclinous flower; ♀ = pistilate flower; ♂ = staminate flower. Abbreviations: cu = cuticle; dr = druse; ep = epidermis; np = nectary parenchyma; ph = phloem; sp = sub-nectay parenchyma; st = style; x = xylem. Scale bar: a-b, e-f, i-j, m-n = 100 µm; c-d, g-h, k-l, o-p = 50 µm.

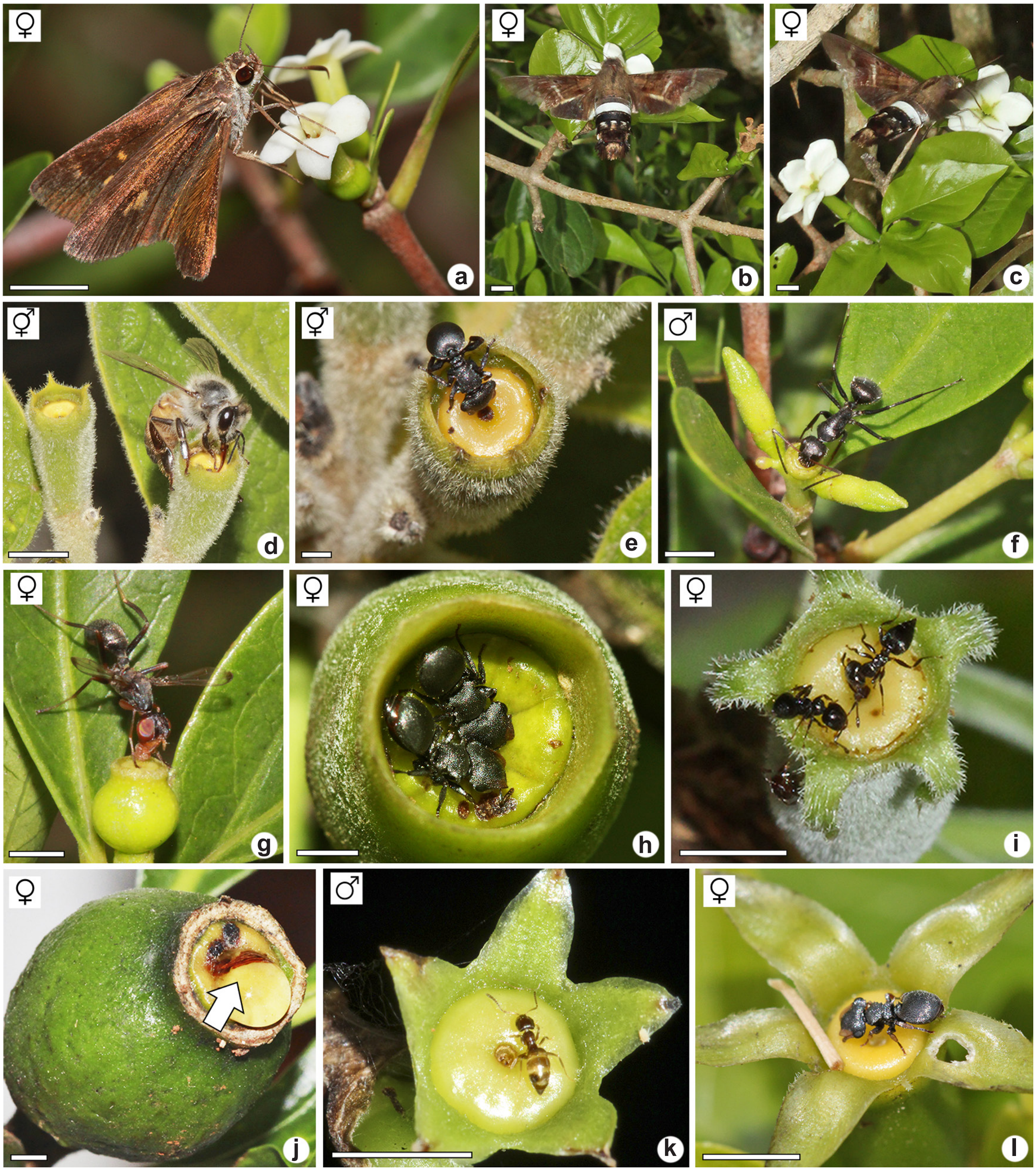

Figure 7 – a-l. Floral visitors in flowers (a-c) and in nectaries with post-flower secretion (d-l) – a. *Cordiera concolor*, floral visitor: Lepidoptera, Hesperiidae, *Pompeius verna*; b-c. *Randia heteromera*, floral visitor: Lepidoptera, Sphingidae, *Aellopos titan*; d-e. *Tocoyena formosa* – d. floral visitor: Hymenoptera, Apidae, *Eucerini sp*; e. floral visitor: Hymenoptera, Formicidae, *Cephalotes sp.*; f-g. *Cordiera concolor* – f. floral visitor: Hymenoptera, Formicidae, *Camponotus sp*; g. floral visitor: Diptera, Syrphidae, *Ocyptamus sp.*; h. *Genipa americana*, with Hymenoptera, Formicidae, *Cephalotes sp.*; i. *Randia calycina*, with Hymenoptera, Formicidae, *Crematogaster sp.*; j. *Randia ferox*, unripe fruit with nectar drop (white arrow); k. *Randia micracantha*, with Hymenoptera, Formicidae, *Camponotus sp.*; l. *Randia heteromera*, Hymenoptera, Formicidae, *Cephalotes sp*. Scale bar: a = 0.5 cm; b-c = 1 cm; d-l = 2 mm.

bundles of xylem and phloem. However, in the Rubiaceae species analyzed by Galati (1991; Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>) it was reported an epidermis and a secretory tissue with traces of phloem only, while in the species of Rubiaceae analyzed by Galetto (1998; Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>) it was mentioned a secretory parenchyma with vascular traces of xylem and phloem. These authors have probably used Fahn's (1979) terminology since they do not mention a subdivision of the parenchyma as in the other classifications. However, given the authors' descriptions of the tissue that composes the nectaries and the presence of vascular bundles, they could be considered similar to those of Gardienieae in terms of anatomical organization. We consider that the terminology suggest by Nepi (2007) more suitable since it is the most commonly used by other authors. For this reason, in the data in Table S2 (available on supplementary material <https://doi.org/10.6084/m9.figshare.20233767.v1>), the terms were standardized with Nepi's terminology in order to make the results obtained in this and other studies of Rubiaceae more understandable. Only in *O. salzmanni* there is no mention of the presence of vascular traces (Florentín *et al.* 2016), and the published photographs do not show a differentiation of the parenchymal tissue as in the other species. Generally, nectaries present vascular bundles of only phloem, or phloem and xylem (Fahn 1979; Nepi 2007) as is the case in the Gardenieae species analyzed here. Nectar water may be derived from both xylem and phloem or only phloem, although the rest of the nectar components including carbohydrates and amino acids can be derived from different sources such as phloem sap, cytological activity or degeneration of certain parts of the nectary, among others (Nepi 2007). However, in some cases the nectary may lack vascular bundles and receive irrigation from the vasculature of the organs nearby (Fahn 1979; Nepi 2007) as could be the case of *O. salzmanni*. Finally, in *Hamelia patens*, *Heterophyllaea pustulata*, *Manettia cordifolia*, and *Oldenlandia salzmanni*, the nectaries present raphides, which differs from the studied Gardenieae that present druses. Considering the scarcity of anatomical studies of nectaries in Rubiaceae, it is necessary to perform similar studies in other taxa of the family in order to establish structural patterns of nectaries in Rubiaceae.

Based on field observations it was found that, in the species analyzed, the nectaries are still visited by animals even after the corolla had fallen, in both staminate and pistilate flowers. It is possible that the thick cuticle observed in these nectaries protects them against desiccation, helping them to persist longer in the flower. In Rubiaceae, this type of nectary that persists after the fall of the corolla was mentioned in a few species and some hypotheses were expressed for its function. In *Hamelia patens* (tribe Hamelieae) the floral nectary would act as an extrafloral nectary during fruit formation, despite being found also in flower stage (Koptur 1992; Galetto 1998). In *Amaioua guianensis* (tribe Gardenieae) the possible function would be to protect the fruit against predators (Amorim & Oliveira 2006). This possible function was also proposed for *Alibertia verrucosa* (tribe Gardenieae) in which an extrafloral nectary continues to secrete nectar during fruiting (Santos & Del-Claro 2001; Falcão *et al.* 2014). In this species, Falcão *et al.* (2014) carried out a detailed study in which they showed that the plant invests more energy in the intermediate size fruits producing more nectar if compared with the youngest and mature fruits. They assumed that this occurs because the youngest fruits that are damaged can be aborted avoiding future energy expenditure, whereas in mature fruits a chemical modification could occur making the seeds less attractive or the hardening of the pericarp would act as a physical barrier preventing the predator's accessibility to the seed.

In *Palicourea rigida* (tribe Psychotrieae) the epigynous nectary of the flower which persists in the fruit was described as pericarpial, with the post-floral secretion being consumed by ants, which protect the vegetative parts of the plant instead of the fruit (Del-Claro *et al.* 2013). Finally, in *Tocoyena formosa*, Sanz-Veiga *et al.* (2017) nectaries are called post-floral pericarpial nectaries, which continue to secrete copious amounts of nectar during fruit differentiation and are visited by ants, but which do not provide protection to the fruit against seed predators. We have observed in *T. formosa* that not only ants visit these nectaries. Bearing in mind that the flowers of each inflorescence in this species have an asynchronous development, it can be assumed that those nectaries could help attract more pollinators for the flowers that mature later. The latter could also be valid for the dioecious species studied since staminate plants have inflorescences with asynchronous development of their flowers. In these species the persistence of nectaries could favor pollination by ensuring that pollinators visit the flowers of the staminate inflorescences. However, in the pistilate plants it is necessary to carry out more observations to be able to hypothesize the role of the persistence of nectaries in the fruits of the species studied. Considering all the studies on the possible role of nectaries with post-flower secretion, it is evident that their function is not simple. Probably multiple mutualists benefit from their presence, either participating in the plant-pollinator interaction or favoring the defense against herbivory to both the vegetative parts of the plant and the developing fruit.

In this study we could conclude that the nectary disc of the species analyzed of Gardenieae can be classified as: reproductive, structural, and mesenchymal, which act as floral nectaries during the floral stage, with a nuptial function, whereas after the corolla has fallen and during fruit differentiation they are persistent nectaries, with post-floral secretion, and maybe with an extranuptial function.

Despite the apparent uniformity of the nectariferous discs in Rubiaceae, this work shows that there are some variations in the morphology and anatomy of this structure, thus more studies are needed to enable broader discussions including character evolution in the group. Among the characteristics that should be considered in future studies to establish differences between the nectaries of different Rubiaceae groups are the following: variation of morphology of the nectary disc (entire, bi or tetrapartite), variations in the epidermis (papillate, smooth), type of nectarostomata (with or without subsidiary cells), variation of cuticle (smooth or striated), anatomical organization, type of vascular traces (phloem and xylem, only phloem), presence and type of crystals (druses, raphides), among others. Future studies should take account of characteristics such as detailed observations of the nectar secretion period and of floral visitors during floral anthesis, after the fall of the corolla, and during the differentiation of the fruits. In the case of dioecious species these observations should be made comparatively between pistilate and staminate flowers. In this way the plant/insect interactions maintained by these nectaries could be established.

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References

Almeida OJG, Cota-Sánchez & Paoli AAS (2013) The systematic significance of floral morphology, nectaries, and nectar concentration in epiphytic cacti of tribes *Hylocereeae* and *Rhipsalideae* (Cactaceae). Perspectives in Plant Ecology, Evolution and Systematics 15: 255-268.

Amorim FW & Oliveira PE (2006) Estrutura sexual e ecologia reprodutiva de *Amaioua guianensis* Aubl. (Rubiaceae), uma espécie dióica de formações florestais de cerrado. Revista Brasileira de Botânica 29: 353-362.

Avila Jr RS & Freitas L (2011) Frequency of visits and efficiency of pollination by diurnal and nocturnal lepidopterans for the dioecious tree *Randia itatiaiae* (Rubiaceae). Australian Journal of Botany 59: 176-184.

Bahadur B, Rajagopal T & Ramayya N (1971) Studies on the structural and developmental variation and distribution of stomata in the Rubiaceae. Botanical Journal of the Linnean Society 64: 295-310.

Bernardello G (2007) A systematic survey of floral nectaries. *In*: Nicolson SW, Nepi M & Pacini E (eds.) Nectaries and nectar. Springer, Dordrecht. Pp. 19-128.

Brown WH (1938) The bearing of nectaries on the phylogeny of flowering plants. Proceedings of the American Philosophical Society 79: 549-594.

Caspary R (1848) De nectariis. Elverfeld, Bonn. 56p.

Coelho VPM, Agra MF & Vasconcellos Barbosa MR (2006) Estudo farmacobotânico das folhas de *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. (Rubiaceae). Brazilian Journal of Pharmacognosy 16: 170-177.

Dave Y, Kuriachen PM & Thomas V (1987) Development, structure and senescence of colleters in *Gardenia lucida* Roxb. (Rubiaceae). Acta societatis Botanicorum Poloniae 57: 3-7.

D'Ambrogio de Argüeso A (1986) Manual de técnicas de histología vegetal. Ed. Hemisferio Sur, Buenos Aires. 83p.

Del-Claro K, Guillermo-Ferreira R, Almeida EM, Zardini H & Torezan-Silingardi HM (2013) Ants visiting the post-floral secretions of pericarpial nectaries in *Palicourea rigida* (Rubiaceae) provide protection against leaf herbivores but not against seed parasites. Sociobiology 60: 217-221.

Delpino F (1868-1874) Ulteriori osservazioni e considerazioni sulla dicogamia nel regno vegetale. Atti della Società Italiana di Scienze Naturali 11: 265-332 (1868); 12: 21-141, 179-233 (1869); 13: 167-205 (1870); 16: 151-349 (1873); 17: 266-407 (1874).

Durkee LT (1983) The ultrastructure of floral and extrafloral nectaries. *In*: Bentley B & Elias T (eds.) The biology of nectaries. Columbia University Press, New York. Pp. 1-29.

Erbano M & Duarte MR (2010) Morfoanatomia de folha e caule de *Genipa americana* L., Rubiaceae. Brazilian Journal of Pharmacognosy 20: 825-832.

Fahn A (1979) Secretory tissues in plants. Academic Press, London. 302p.

Fahn A (1982) Plant anatomy. 3rd ed. Pergamon Press, Oxford. 544p.

Falcão JCF, Dáttilo W & Izzo TJ (2014) Temporal variation in extrafloral nectar secretion in different ontogenic stages of the fruits of *Alibertia verrucosa* S. Moore (Rubiaceae) in a Neotropical savanna. Journal of Plant Interactions 9: 137-142.

Florentín MN, Cabaña Fader A & Gonzalez AM (2016) Morpho-anatomical and morphometric studies of the floral structures of the distylous *Oldenlandia salzmannii* (Rubiaceae). Acta Botanica Brasilica 30: 585-601.

Freeman E, Worthington RD & Jackson MS (1991) Floral nectar sugar compositions of some South and Southeast Asian species. Biotropica 23: 568-574.

Galati BG (1991) Estudios embriológicos em la tribu Spermacoceae (Rubiaceae). Parte I: anatomia floral. Megasporogenesis. Megagametogenesis. Boletín de la Sociedad Argentina de Botánica 27: 7-20.

Galetto L (1998) Estructura floral y composición química del néctar en tres especies de Rubiaceae. Kurtziana 26: 83-98.

González AM & Cristóbal CL (1997) Anatomía y ontogenia de semillas de *Helicteres Lhotzkyana* (Sterculiaceae). Bonplandia 9: 287-294.

Govaerts R, Ruhsam M, Andersson L, Robbrecht E, Bridson DM, Davis AP, Schanzer I & Sonké B (2020) World checklist of Rubiaceae. Facilitated by the Royal Botanic Gardens, Kew. Available at <http://wcsp.science.kew.org/>. Access on 29 April 2020.

Johansen DA (1940) Plant microtechnique. McGraw-Hill, New York. 523p.

Judkevich MD, Salas RM & Gonzalez AM (2015) Revisión de *Randia* (Rubiaceae) en Argentina, taxonomía y morfoanatomía. Boletín de la Sociedad Argentina de Botánica 50: 607-625.

Judkevich MD, Salas RM & Gonzalez AM (2020, in press) Anther structure and pollen development in species of Rubiaceae and anatomical evidence of pathway to morphological dioecy. Annals of the Brazilian Academy of Sciences, Río de Janeiro.

Koek-Norman J (1972) The wood anatomy of Gardenieae, Ixoreae and Mussaendeae (Rubiaceae). Acta Botanica Neerlandica 21: 301-320.

Koptur S (1992) Plants with extrafloral nectaries and ants in everglades hábitats. Florida Entomologist 75: 38-50.

Lotts K & Naberhaus T (2020) Butterflies and moths of North America. Version 3/19/2020. Available at <http://www.butterfliesandmoths.org/>. Access on 19 March 2020.

Luque R, Sousa HC & Kraus JE (1996) Métodos de coloração de Roeser (1972): modificado - e Kropp (1972) visando a substituição do azul de astra por azul de alcião 8GS ou 8GX. Acta Botanica Brasilica 10: 199-212.

Manns U & Bremer B (2010) Towards a better understanding of intertribal relationships and stable tribal delimitations within Cinchonoideae *s.s.* (Rubiaceae). Molecular Phylogenetics and Evolution 56: 21-39.

Miguel EC, Cunha M, Miguel TBAR & Barros CF (2016) Ontogenesis secretion and senescence of *Tocoyena bullata* (Vell.) Mart. (Rubiacaeae) colleters. Plant Biology 18: 851-858. DOI: 10.1111/plb.12473

Moraga C (2018) Área de Conservación Guanacaste. *Aellopos titan* (Sphingidae). Version 3/19/2020. Available at <https://www.acguanacaste.ac.cr/paginas-de-especies/insectos/101-sphingidae/4299-i-aellopos-titan-i-sphingidae>. Access on 19 March 2020.

Nepi M (2007) Nectary structure and ultrastructure. *In*: Nicolson SW, Nepi M & Pacini E (eds.) Nectaries and nectar. Springer, Dordrecht. Pp. 129-166.

Paiva EAS (2009) Ultrastructure and post-floral secretion of the pericarpial nectaries of *Erythrina speciosa* (Fabaceae). Annals of Botany 104: 937-944.

Phukela B, Adit A & Tandon R (2020) A snapshot of evolutionary history of floral nectaries across angiosperm lineages. *In*: Tandon R, Shivanna KR & Koul M (eds.) Reproductive ecology of flowering plants: patterns and processes. Springer Singapore, Singapore. Pp. 105-129.

Queiroga D & Moura RF (2017) Positive relation between abundance of pericarpial nectaries and ant richness in *Tocoyena formosa* (Rubiaceae). Sociobiology 64: 423-429.

Rivera GL (1997) Biología reproductiva en Bignoniaceae de Argentina. Tesis Doctoral. Universidad Nacional de Córdoba, Córdoba. 234p.

Robbrecht E (1988) Tropical woody Rubiaceae. Opera Botanica Belgica 1: 1-271.

Ronse De Craene LPR & Smets E (1991) The floral nectaries of *Polygonum s.l.* and related genera (Persicarieae and Polygoneae): position, morphological nature and semophylesis. Flora 185: 165-185.

Roy R, Schmitt AJ, Thomas JB & Carter CJ (2017) Nectar biology: from molecules to ecosystems. Plant Science 262: 148-164.

Salinas-Esquivel L, Arizmendi MC, Domínguez CA, Castillo-Guevara C & Lara C (2013) Producción de nectar y flores como rasgos compensatorios al robo de nectar en *Bouvardia ternifolia* (Rubiaceae): experimentos de campo. Botanical Sciences 91: 85-92.

Santos JC & Del-Claro K (2001) Interação entre formigas, herbívoros e nectários extraflorais em *Tocoyena formosa* (Cham. & Schlechtd.) K. Schum. (Rubiaceae) na vegetação do cerrado. Revista Brasileira de Zoociências 3: 77-92.

Sanz-Veiga PA, Jorge LR, Benitez-Vieyra S & Amorim FW (2017) Pericarpial nectary-visiting ants do not provide fruit protection against pre-dispersal seed predators regardless of ant species composition and resource availability. PLoS ONE 12: e0188445.

Schmid R (1988) Reproductive versus extra-reproductive nectaries - historical perspective and terminological recommendations. The Botanical Review 54: 179-232.

Simpson MG (2010) Plant Systematics. 2nd ed. Elsevier/ Academic Press, Boston. 752p.

Smets E (1968) Localization and systematics importance of the floral nectaries in the Magnoliate (Dicotyedons). Bulletin du Jardin Botanique National de Belgique 56: 51-76.

Smets E (1988) La présence des "nectaria persitentia" chez les Magnoliophytina (Angiosperms). Candollea 43: 709-716.

Smets EF & Cresens EM (1988) Types of floral nectaries and the concept of "character" and "character state"- a reconsideration. Acta Botanica Neerlandica 37: 121-128.

Tölke ED, Bachelier JB, Lima EA, Galetto L, Demarco D & Carmello-Guerreiro SM (2018) Diversity of floral nectary secretions and structure, and implications for their evolution in Anacardiaceae. Botanical Journal of Linnean Society 187: 209-231.

Tölke ED, Capelli NDV, Pastori T, Alencar AC, Cole TC & Demarco D (2019) Diversity of floral glands and their secretions in pollinator attraction. *In*: Mérillon J-M & Ramawat KG (eds) Co-evolution of secondary metabolites. Springer, Cham. Pp. 1-46. DOI: 10.1007/978-3-319-76887-8_48-3

Vaes E, Vrijdaghs A, Smets EF & Dessein S (2006) Elaborate petals in Australian Spermacoce (Rubiaceae) species: morphology, ontogeny and function. Annals of Botany 98: 1167-1178.

Vogel S (1977) Nektarien und ihre ökologische Bedeutung. Apidologie 8: 321-336.

Zimmermann J (1932) Über die extrafloralen nektarien der Angiospermen. Beihefte zum Botanischen Centralblatt 49: 99-196.

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