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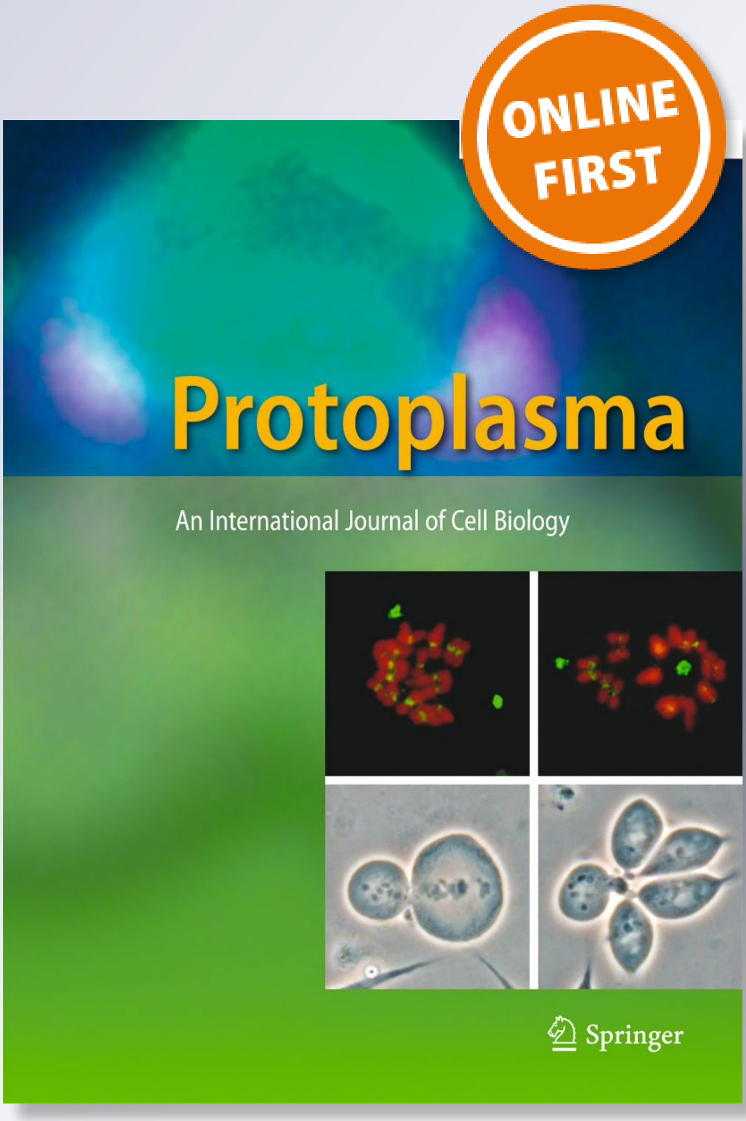

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ORIGINAL ARTICLE

Floral nectaries in Sapindaceae *s.s.*: morphological and structural diversity, and their systematic implications

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Abstract We investigated the morphology and structure of the floral nectary in 11 Neotropical genera belonging to the subfamilies Dodonaeoideae and Paullinioideae (Sapindaceae) from southern South America representing three tribes (Dodonaeaeae, Paullinieae, and Melicocceae), in relation to other floral traits in species with contrasting morphological flower characteristics. Nectary organization was analyzed under light, stereoscopic, and scanning electron microscopes; *Diplokeleba floribunda* N.E. Br. was also observed using transmission electron microscopy. Our comparative data may contribute to the understanding of floral nectary evolution and systematic value in this family. The nectaries were studied in both staminate and pistillate flowers. All the floral nectaries are typical of Sapindaceae: extrastaminal, receptacular, structured, and persistent. The anatomical analysis revealed a differentiated secretory parenchyma and an inner non-secretory parenchyma; the nectary is supplied by phloem traces and, less frequently, by phloem and xylem traces. Nectar is secreted through nectarostomata of anomocytic type. The anatomical analysis showed the absence of nectary in the three morphs of *Dodonaea viscosa* flowers. Nectary ultrastructure is described in *D. floribunda*. In this species, the change in nectary color is related to progressive accumulation of anthocyanins during the functional phase. We found relatively small variation in the nectary structural characteristics compared with large variation in nectary morphology. The latter aspect agreed with the main infrafamilial groupings revealed by recent phylogenetic studies, so it is of current valuable systematic importance for Sapindaceae. In representatives of Paullinieae, the reduction of the floral nectary to 4–2 posterior lobes should be interpreted as a derived character state.

Keywords Sapindaceae floral nectary · Morphology · Structure · Nectarostomata · Color change

Introduction

Sapindaceae *s.l.* comprises approximately 144 genera and 1900 species distributed in tropical and subtropical regions (Acevedo-Rodríguez et al. 2017). The species may exhibit tree, shrub, or climber habit and are mostly monoecious, rarely dioecious or polygamous. Flowers are often functionally unisexual by reduction. Phylogenetic analyses in Sapindaceae inferred from nuclear and plastid sequence data have notably changed the subfamilial, tribal, and generic concepts initially proposed by Radlkofer (1931-1934), and together with this, the morphological criteria considered in different groupings have also changed (Savolainen et al. 2000; Soltis et al. 2000; Harrington et al. 2005, 2009; Thorne and Reval 2007; Buerki et al. 2009, 2010, 2012). Harrington et al. (2005, 2009) circumscribed Sapindaceae in a broad sense, including Aceraceae and Hippocastanaceae, and recognized four subfamilies. However, in a more recent phylogenetic analysis, Buerki et al. (2010) recovered most of the traditional classification of Sapindaceae *s.s.* by delimiting the subfamilies Dodonaeoideae Burnett (including *Diplokeleba* N.E. Br.) and

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Sapindoideae Burnett, identifying Aceraceae, Hippocastanaceae, and the new family Xanthoceraceae. But the most recent phylogenetic analysis by Buerki et al. (2011) keeps Xanthoceroideae as one of the four subfamilies within Sapindaceae *s.l.*

In relation to the tribal and generic concepts, the 14 tribes originally recognized by Radlkofer (1931–1934) have been demonstrated to be highly para- and polyphyletic, with the exception of the monophyletic Paullinieae nested in a paraphyletic Thouinieae (Buerki et al. 2009). Recently, the supertribe Paulliniodae including the genera of Thouinieae, except for *Guindilia* Gillies ex Hook. & Arn. (Acevedo-Rodríguez et al. 2017), has been proposed.

Nectaries are glandular tissues that synthesize and secrete nectar regularly; they are located in vegetative and reproductive organs (Schmid 1988). Floral nectaries are widespread in angiosperms; they have appeared and evolved independently in different lineages in response to selective pressures imposed by interactions with a wide spectrum of pollinators. Since floral nectaries play a crucial role in pollination biology of numerous plant species, the nectary and nectar features are linked with the diversification of flowering plants (Cruden et al. 1983; Simpson and Neff 1983; Vogel 1997; Perret et al. 2001; Bronstein et al. 2006). Floral nectaries are enormously diverse; they can vary in number, topographic position, shape, duration, color, structure, and mode of secretion and release (Brown 1938; Elias and Gelband 1976; Fahn 1979; Smets 1986; Smets and Cresens 1988; Vogel 1997; Pacini et al. 2003; Bernardello 2007). Nectaries can be morphologically labile or rather constant among plant groups; thus, they can be a valuable source of information for comparative and phylogenetic studies (Bernardello 2007). However, basic traits of the nectary structure can be conserved in different groups of plants (Nores et al. 2013).

The floral nectary is a taxonomically useful character in Sapindaceae *s.s.* because it is well represented and morphologically heterogeneous. However, the diversity, structure, and development of floral nectaries within Sapindaceae have been scarcely explored and the information is still not representative. The receptacular nectary is considered an important morphological synapomorphy for the order Sapindales (Gadek et al. 1996; Gallant et al. 1998; Ronse De Craene et al. 2000; Solís and Ferrucci 2009; Bachelier and Endress 2008; Zini et al. 2014). The extrastaminal nectary position has been used to discriminate Sapindaceae *s.l.* from other families of this order, such as Rutaceae, Meliaceae, and Nitrariaceae, which usually have intrastaminal nectaries (Judd et al. 1999; Ronse De Craene et al. 2000; Soltis et al. 2005; Ronse De Craene and Haston 2006; Bernardello 2007; Bachelier and Endress 2008; Acevedo-Rodríguez et al. 2011; Bachelier et al. 2011). Intrastaminal nectaries are quite unusual within Sapindaceae; they have only been cited for the genus *Dodonaea* Mill. (e.g., Reiche 1896; West 1984; Ferrucci 2005; da Silva et al. 2013). The morphology of nectaries may be annular or unilateral; the former state includes a continuous nectary tissue surrounding the receptacle, such as lobed-, thimble-, or dish-shaped discs and cupular or bicupular discs, whereas the latter state is unilateral and can be semiannular or divided into four or two lobes (e.g., Radlkofer 1931–1934; Ferrucci 1991, Ferrucci and Acevedo-Rodríguez 1998; Buerki et al. 2009, 2010; Ferrucci and Urdampilleta 2011a, b; Acevedo-Rodríguez 2003, 2011, 2012; Acevedo-Rodríguez et al. 2011). Ronse De Craene et al. (2000) report floral ontogeny and anatomy of *Koelreuteria paniculata* Laxm., providing an anatomical description of the annular nectary. Ning-Xi and Wu (2005) contribute with information of the structural and developmental characteristics of the annular nectary of *Litchi chinensis* Sonn. The anatomy and ontogeny of unilateral nectaries in *Cardiospermum* L. and *Urvillea* Kunth were also reported (Solís and Ferrucci 2009; Zini et al. 2014); anatomical and ultrastructural aspects of the nectary in *Koelreuteria elegans* subsp. *formosana* (Hayata) F. G. Mey. have been recently studied (Avalos et al. 2016).

The color is a distinct feature of the floral nectaries that varies widely among taxa and that can be used to recognize taxonomic groups (Bernardello 2007). In angiosperms, nectaries are usually greenish when chlorophyll presumably prevails in their plastids, and they can remain greenish or change in color with age, through the formation of carotenes and/or flavonoids (Bernardello 1986; Vezza et al. 2006; Horner et al. 2007; Gouvêa et al. 2008; Giuliani et al. 2012; Paiva 2012). However, most nectaries of Sapindaceae do not seem to undergo a notable color change during the flowering phase.

The primary goals of the present paper were (a) to analyze 11 species of 11 genera, representing both subfamilies recognized for Sapindaceae *s.s.*, to test whether floral nectaries retain a permanent pattern of the position and structure, and to test whether their structure is a significant taxonomic trait at different levels within the family. To this end, a special focus was made on the morphoanatomical similarities and differences among species and (b) to analyze the distinctive changes of the nectary color in *Diplokeleba floribunda*, at the macroscopic, anatomical, and ultrastructural levels, because it is an unusual phenomenon within Sapindaceae. The results from this study will contribute to the understanding of the pollination biology of the species; moreover, in conjunction with the current scheme of classification, the results will help to determine how nectary diversity is distributed and how nectaries evolved within the family.

Material and methods

Plant material

Open flowers and floral buds at different developmental stages (pre-anthesis, anthesis, and post-anthesis stages) were fixed in formalin, acetic acid, and ethanol (FAA) for anatomical and scanning electron microscopy examinations. Pistillate flowers of *Dodonaea viscosa* and staminate flowers of *Llagunoa glandulosa* (Hook. & Arn.) G. Don derived exclusively from herbarium specimens. In the present study, we follow the classification proposed by Acevedo-Rodríguez et al. (2011) for infrafamiliar grouping. In the preparation of the figures, species were ordered according to the morphology of the nectary, from annular to unilateral, semidisc to four-lobed.

Subfamily Dodonaeoideae

Tribe Dodonaeaeae

Diplokeleba floribunda N.E. Br. Argentina. Prov. Corrientes. Dpto. Capital, en quebrachal a orillas del río Paraná, 23.12.2008, *Ferrucci* et al. *2834a*; Molina Punta, 11.12.1976, *Martínez Crovetto & Schinini 10639*; Santa Ana, 17.12.2007, *Ferrucci* et al. *2828*.

D. viscosa Jacq. Argentina. Prov. Entre Ríos. Dpto. La Paz, 18.11.2015, *Lattar* et al. *24*. Bolivia. Dpto. Chuquisaca. Prov. Chuquisaca, 19.01.1994, *Hunziker* et al. *12657*.

L. glandulosa (Hook. & Arn.) G. Don. CHILE. Región de Coquimbo. La Serena. 01.08.2000, *Landrum 9826*.

Magonia pubescens A. St.-Hil. BOLIVIA. Dpto. Santa Cruz. Prov. Cordillera, 18.07.2013, *Ferrucci* et al. *3124*; Germán Busch, 2.5 km S de ruta 4. 20.07.2013, *Ferrucci* et al. *3134*.

Subfamily Sapindoideae

Tribe Paullinieae

Allophylus edulis (A. St.-Hil., A. Juss. et Cambess.) Hieron. ex Niederl. Argentina. Prov. Corrientes. Dpto. Capital. Corrientes, 1.08.2008, *Ferrucci & Lezcano 2836*; Dpto. Gral. Paz. Ea. La Flecha, Lomas de Vallejos, 27.08.1979, *Ferrucci* et al. *152*.

Houssayanthus incanus (Radlk.) Ferrucci. Argentina. Prov. Chaco. Dpto. 1° de Mayo, 19.04.2006, *Ferrucci* et al. *2710*; ib., 1.04.2007, *Meza Torres* et al. *598*.

Lophostigma plumosum Radlk. Bolivia. Dpto. Cochabamba. Prov. Campero, 12.04.2006, *Ferrucci* et al. *2681*; ib., 24.07.1994, *Ferrucci* et al. *864*.

Paullinia elegans Cambess. Argentina. Prov. Corrientes. Dpto. Capital, 17.12.2007, *Ferrucci* et al. *2827*; Dpto. Monte Caseros, 12.01.2007, *Ferrucci* et al. *2716*.

Serjania meridionalis Cambess. Argentina. Prov. Corrientes. Dpto. San Cosme, 24.02.2007, *Ferrucci & Meza Torres 2751*; Dpto. Ituzaingó, 23.04.2007, *Meza Torres* et al. *616*.

Thinouia mucronata Radlk. Argentina. Prov. Misiones. Dpto. San Ignacio, 12.02.2008, *Ferrucci* et al. *2832*; ib., 22.01.2010, *Keller & Ferrucci 8175*.

Tribe Melicocceae

Melicoccus lepidopetalus Radlk. Argentina. Prov. Corrientes. Dpto. Capital. Corrientes, 10.8.2008, *Zini & Ferrucci 1*; Corrientes, *Zini & Ferrucci 2*; Corrientes, 10.9.2008.

The voucher specimens were deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES), Argentina.

Light microscopy For preparing permanent slides, the material was processed by dehydration through an ethanol series with a pre-impregnant rinsing of tertiary butyl alcohol (Gonzalez and Cristóbal 1997) and infiltration in Histoplast® paraffin (Biopack, Buenos Aires, Argentina), according to Johansen (1940). Flowers were sectioned transversely and longitudinally (10–12 μm thick) with a rotary microtome; the sections were stained with astra blue–safranin (Luque et al. 1996) and mounted with synthetic Canada balsam (Biopur, Buenos Aires, Argentina).

Five fresh nectaries of floral buds and flowers at anthesis and post-anthesis in *D. floribunda* were transversally and longitudinally hand-sectioned with a razor blade and mounted in water–glycerin. All morphological and anatomical features were observed and photographed using a Leica MZ6 stereomicroscope and a Leica DM LB2 compound microscope (Leica, Wetzlar, Germany), both equipped with a digital camera.

To macroscopically analyze the color change of nectary in *D. floribunda*, observations were made for 5 days on 20 staminate flowers and 10 pistillate flowers labeled on ramifications isolated in a laboratory. Floral visitors and free pollinated flowers were observed in the field.

Scanning electron microscopy Fixed flowers were dissected and dehydrated through a series of increasing ethanol solutions. The material was then critical point-dried with solvent-substituted liquid carbon dioxide and coated with a thin layer of gold palladium. Photomicrographs were obtained using a JEOL 5800 LV at 20 kV (JEOL USA, Peabody, MA, USA).

Transmission electron microscopy Five nectaries of *D. floribunda* at two developmental stages (yellow and red) were sectioned and pre-fixed in 1% glutaraldehyde and 4% formaldehyde in phosphate buffer (pH 7.2) for 2 h. The samples were then post-fixed in 1.5% O_sO_4 at 2 °C in the same buffer for 3 h. Then, they were dehydrated in ascending graded series of acetone and embedded in Spurr's resin. Ultrathin sections of 70 nm were made using a Reichert ultramicrotome and stained with uranyl acetate and lead citrate (Zarlavsky

2014). The sections were examined under a JEOL-JEM 1200 Ex II transmission electron microscopy (TEM) at 85 kV.

Results

Floral morphology

The main morphological floral features of the 11 studied species are compared in Table 1. The species have two flower morphs, morphologically perfect flowers but functionally pistillate with indehiscent anthers and non-functional pollen grains, and staminate flowers with a gynoecium reduced to a small pistillode (in *D. viscosa* functional hermaphrodite flowers are also present); flowers are gathered in a single thyrsi or in inflorescences of multiple order, axillary or terminal. The flowers are small to comparative large, actinomorphic or oblique zygomorphic. The calyx can be tetramerous or pentamerous, with two outer sepals and two or three inner ones; sepals distinct or connate at the base, with or without short glandular hairs on their margin. The corolla, generally present, is choripetalous and has four or five petals, often with glandular trichomes on both faces or only on the adaxial one. Petals usually possess a ventral appendage, with or without a crest, and a deflexed appendage. The nectary is extrastaminal. A well-developed androgynophore or gynophore may be present. Staminate flowers have eight (9–12, rarely more) stamens, filaments distinct or connivent at the base. The filaments are cylindrical; the anthers are basifixed or dorsifixed, with longitudinal dehiscence. Pistillate flowers exhibit short and indehiscent stamens. The syncarpous gynoecium usually has three, rarely two, carpels. The ovary is superior, with one or two (exceptionally eight) anatropous, hemianatropous, or campilotropous ovules per carpel; the style is long and filiform or short, with papillate stigmatic lobes or branches.

Nectary morphology

Nectaries of pistillate and staminate flowers do not differ in position, shape, or structure in most of the studied species, except for *D. viscosa*. The nectary is located on the receptacle in *A. edulis*, *D. floribunda*, *M. pubescens*, *M. lepidopetalus*, and *T. mucronata* and at the base of the androgynophore or gynophore in *H. incanus*, *L. plumosum*, *P. elegans*, and *S. meridionalis*. The hypothetic intrastaminal nectary of *D. viscosa* is observed as an outgrowth on the receptacle (gynophore) only in pistillate and hermaphroditic flowers, whereas this bulge is not observed in staminate ones; the receptacle becomes more bulky at the fruiting stage. An unusual feature in the nectary of *L. glandulosa* was observed; it is a posterior unilateral broad disc, unequally lobed that is welded on the calyx tube, and reaches the margin of the calyx lobes (one, two, and four sepals). In all species, the nectary persists after the end of the flowering phase and later in the fruit.

The nectaries are annular and glabrous in *A. edulis*, *M. lepidopetalus*, and *T. mucronata* (Figs. 1a, c, e and 2a–c) and are orange, light brown, and white, respectively. In these species, they are somewhat hidden underneath the petal appendages at anthesis. The disc is four- (five-) lobed in *A. edulis* and *M. lepidopetalus*; the lobes alternate with sepals in both species. In functionally pistillate flower of the latter species, the receptacle is thick between the stamens and gynoecium, appearing to be a continuation of the nectary; however, such region is devoid of secretory parenchyma, indicating a strictly extrastaminal position of the nectary.

In *D. floribunda*, the nectary is annular and bicupular, with the outer margin waved; the number of waves is equal to that of petals (5); the crenate is internal, with number of waves equal to that of stamens (8) (Figs. 1i and 3a). The nectary color changes from yellow to red during the flowering phases. In *M. pubescens*, the nectary is annular but unequal, pale yellow, with two well-differentiated regions: the anterior deeply folded (it appears to be fragmented in small portions), glabrous, and the posterior bicupular, the external laminae slightly three-lobed and higher than the internal one, pubescent (Figs. 1l–o and 3g). Both species are devoid of petal appendages, and the nectaries are exposed at anthesis.

The nectary of *L. plumosum* is unilateral, notably developed toward the posterior side of the flower, with a four-lobed, whitish, and glabrous margin (Figs. 1q and 2d). It is covered by the four petals, each one with a short appendage that has a highly folded crest. In *H. incanus*, *P. elegans*, and *S. meridionalis*, the nectary is unilateral, fragmented into four lobes, two posterior and two anterior ones; they are whitish in the three species (Figs. 1s, u, w and 4a–c). The morphological differences between anterior and posterior lobes of the nectary lie in their relative dimension, shape, and presence or absence of simple trichomes. In *H. incanus* and *P. elegans*, the posterior nectary lobes are ovoid, obtuse at apex (Fig. 1u, w); few simple trichomes are present only in *P. elegans*, whereas in *S. meridionalis*, nectary lobes have a nearly circular outline (Fig. 1s). The anterior nectary lobes of *H. incanus*, *P. elegans*, and *S. meridionalis* are slightly smaller than the posterior ones and exhibit an elliptic outline (Fig. 1s, u, w). In the three species, each nectary lobe is entirely enclosed by its own petal appendages. The two posterior petals have more elaborate appendages, consisting of a crest and a deflexed appendage. The nectary of *L. glandulosa* is unilateral, expanded toward the posterior side of the flower, flat, slightly undulated, glabrous, and completely exposed at anthesis (Fig. 5a, d, e). In *H. incanus*, *L. glandulosa*, *L. plumosum*, *M. pubescens*,

Table 1 Flower features of species examined

Characters	Taxa								
	Diplokeleba floribunda. Dodonaeoideae, *Dodonaeaeae*	*Dodonaea viscosa.* Dodonaeoideae, *Dodonaeaeae*	*Llagunoa glandulosa.* Dodonaeoideae, *Dodonaeaeae*	*Magonia pubescens.* Dodonaeoideae, *Dodonaeaeae*	*Allophylus edulis.* Sapindoideae, *Paullinieae*	*H. incanus* *P. elegans* *S. meridionalis*. Sapindoideae, *Paullinieae*	*Lophostigma plumosum.* Sapindoideae, *Paullinieae*	*Thinouia mucronata.* Sapindoideae, *Paullinieae*	*Melicoccus lepidopetalus.* Sapindoideae, *Melicocceae*
Flower symmetry	Actinomorphic	Actinomorphic	Zygomorphic	Zygomorphic	Zygomorphic	Zygomorphic	Zygomorphic	Actinomorphic	Actinomorphic
Flower size	Medium (5–6.5 mm long)	Medium (2,5–6 mm)	Medium (8–12-mm diam)	Large (24–33 mm long)	Small (2–4 mm long)	Medium (4.5–6 mm long)	Small (2.5–3.5 mm long)	Small (2.5–4 mm long)	Medium (4–6 mm long)
Calyx	Pentamerous	Tetramerous or pentamerous	Pentamerous	Pentamerous	Tetramerous or (pentamerous)	Pentamerous	Pentamerous	Pentamerous	Tetramerous or (pentamerous)
Corolla	Pentamerous	–	–	Pentamerous	Tetramerous or (pentamerous)	Tetramerous	Tetramerous	Pentamerous	Tetramerous or (pentamerous)
Petal shape	Ovate or oblong, with pubescent margin	–	–	Oblanceolate, pubescent	Spatulate, ciliate, equal	Obovate, oblong, two posterior symmetrical and two anterior asymmetric	Obovate, subequal	Spatulate, equal	Ovate or oblong, with woolly margin
Basal appendage	–	–	–	–	Bifid to bipartite	With bipartite crest in pp[a] With emarginated crest in pp[b] With a 2-corniculiform crest in pp[c]	Short, bifid, very folded	Bipartite free portion, hairy divergent branches	Bifid, minute, linear, woolly--pubescent
Stamens number	8	(5) 8–10	8	8	8	8	8	(5–) 8	8 (9–12)
Androgynophore	–	–	–	–	–	Glabrous[a,c] Puberulent[b]	Glabrous	–	–
Pistillate flower: gynoecium	3-carpellate ovary ovoid, style curved, trifid stigma with short branches welded	3 (4)-carpellate, ovary trigonous subspherical, style filiform, trifid stigmatic branches	3-carpellate, ovary trigonus, ovoid, style filiform, stigma subcapitate	3-carpellate ovary ovoid-lobate, style filiform, curved, stigma capitate	(2)-3-carpellate, ovary (2)-3-lobed, style gynobasic, filiform style, stigma (bifid) trifid	3-carpellate, ovary trigonous, obovoideus, style shorter or not than the 3 stigmatic branches	3-carpellate, ovary trigonous, style filiform, stigma trifid	3-carpellate, stipitate, ovary trigonous, obovoideus, filiform style, stigma trifid	2-carpellate, ovary ovoid, slightly flattened, style brief, stigma bilobed
Floral nectary morphology	Annular, bicupular	–	Unilateral, broad disc welded on the calyx tube	Annular, unequal	Annular, 4 (5)-lobed	Unilateral, 4 lobed, 2 anterior lobes and 2 posterior ones	Unilateral, margin 4-lobed	Annular, slightly 5-lobed	Annular, 4 (–5)-lobed

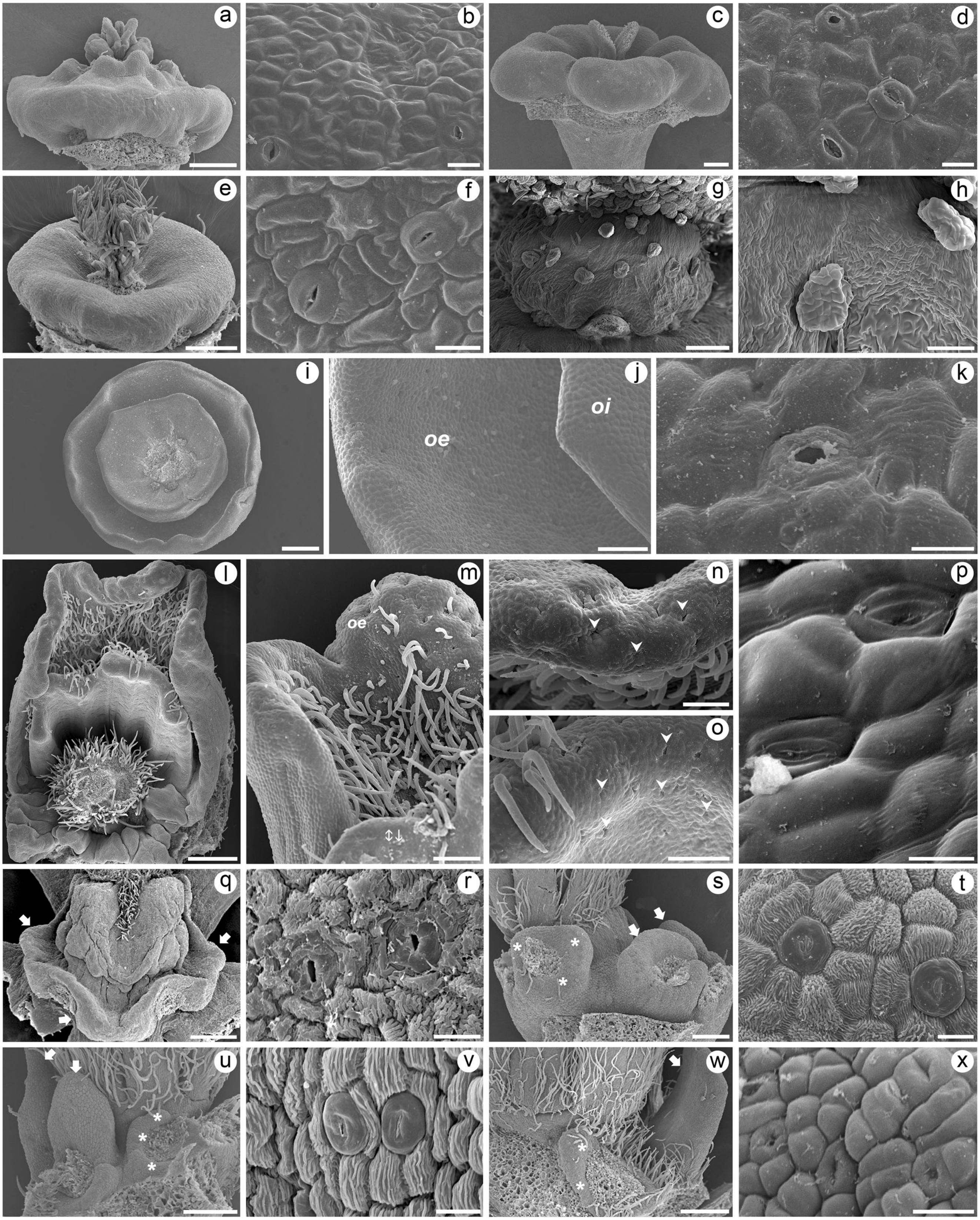
Infrafamiliar grouping follows Acevedo et al. (2011). Flower whorls in parentheses indicate that it is rare

– Absent, *pp* posterior petal

[a] *H. incanus*

[b] *P. elegans*

[c] *S. meridionalis*



P. elegans, and *S. meridionalis*, the nectary is part of the floral oblique monosymmetry, whereas in *A. edulis*, the radially symmetric nectary does not fit into the overall floral zygomorphy.

◀ **Fig. 1** SEM micrographs of floral nectaries. **a**, **b** *Allophylus edulis.* **c**, **d** *Melicoccus lepidopetalus.* **e**, **f** *Thinouia mucronata.* **g**, **h** *Dodonaea viscosa.* **i**–**k** *Diplokeleba floribunda*. **l**–**p** *Magonia pubescens.* **q**, **r** *Lophostigma plumosum.* **s**, **t** *Serjania meridionalis.* **u**, **v** *Houssayanthus incanus.* **w**, **x** *Paullinia elegans.* **a**, **c**, **e** Annular nectaries. **g** Receptacular ring. **b**, **d**, **f**, **k**, **p**, **r** Nectary epidermis with nectarostomata. **h** Epidermis of with glandular hairs. **i** Bicupular annular nectary. **j** Inner (*oi*) and outer (*oe*) edges of bicupular annular nectary. **l** Unequal annular nectary. **m** Inner and outer (*oe*) edges of bicupular portion of the unequal annular nectary. **n** Detail of outer edge with sunken nectarostomata (*arrowheads*). **o** Detail of inner edge with sunken nectarostomata (*arrowheads*). **q** Incompletely annular nectary (*arrows*). **s**, **u**, **w** Unilateral nectary, fragmented in four lobes, two posterior (*arrows*) and two anterior (*asterisk*). **t**, **v**, **x** Details of epidermis of posterior nectary lobe with nectarostoma *Scale bars*: **a**, **c**, **e**, **i**, **q**, **s**, **u**, **w** = 1 mm; **b**, **d**, **f**, **h**, **k**, **r**, **t**, **v**, **x** = 50 μm; **g**, **m** = 200 μm; **j**, **l** = 500 μm; **n**, **o** = 100 μm; and **p** = 10 μm

Nectary structure

The anatomical traits of the nectaries are identical in both morphs of all species; the nectary tissue consists of an epidermis, several layers of nectary (secretory) parenchyma, subnectary (non-secretory) parenchyma, and vascular bundles. *D. viscosa* is treated independently because it presents notable differences.

Epidermis Epidermal cells are polygonal to subrectangular in superficial view. The cuticle is smooth in *L. glandulosa*, *A. edulis*, *D. floribunda*, *M. lepidopetalus*, and *P. elegans*, (Figs. 1b, d, k, x and 5b) striated in *L. plumosum*, *H. incanus*, and *S. meridionalis* and (Fig. 5r, v, t) and slightly striated in *M. pubescens* and *T. mucronata* (Fig. 1f, p). Anomocytic nectarostomata are present in all the studied species. In *A. edulis*, *M. lepidopetalus*, and *T. mucronata*, stomata are most frequent on the external margin of the disc (Fig. 2u–w). In *D. floribunda*, nectarostomata are restricted to the adaxial surface of the external ridge and the abaxial surface of the internal ridge, and in *M. pubescens*, they are confined to the base and edges of the posterior, bicupular region, the site where nectar accumulates (Fig. 3k). In *L. glandulosa*, however, nectarostomata are homogeneously distributed all over the nectary surface (Fig. 5c, g, h). In *H. incanus*, *P. elegans*, and *S. meridionalis*, nectarostomata are scattered over the two posterior nectary lobes facing the adaxial surface of the petal; they are absent in the anterior nectary lobes, except in *S. meridionalis* which exhibits few of them. In *L. plumosum*, stomata are found on the margin of the incomplete disc (Fig. 2x). Nectarostomata are situated at the same level of the epidermis in *M. lepidopetalus*; sunken relative to the epidermal cells in *D. floribunda* and *L. glandulosa* (Figs. 1k, 3e, and 5h); at the same level of the epidermis in the base and sunken at the edges of the posterior and bicupular region in *M. pubescens* (Figs. 1n, o and 3k, l); and at the same level or slightly above the epidermis in *T. mucronata*, *H. incanus*, *P. elegans*, and *S. meridionalis* (Figs. 2w and 4m–o).

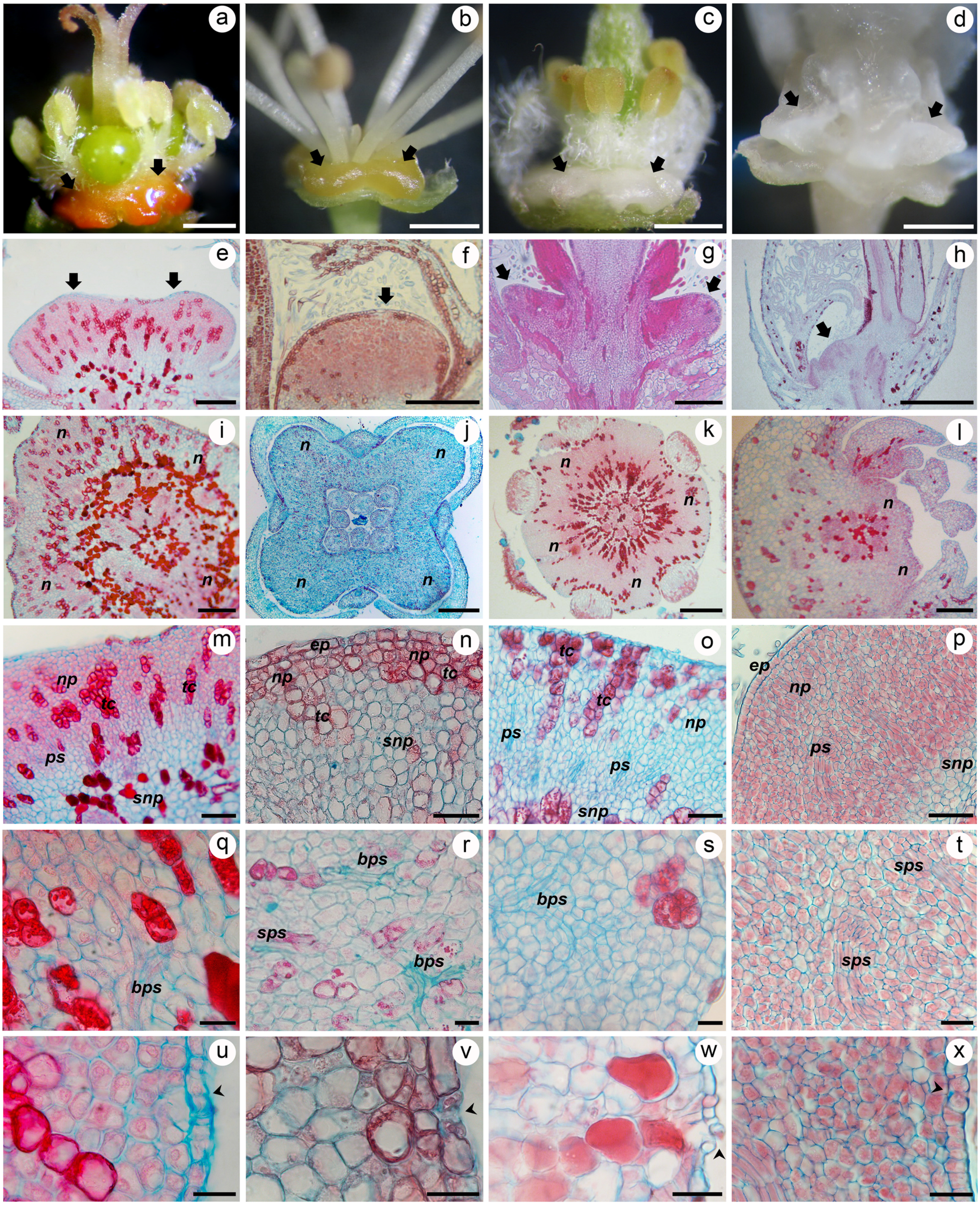
Transversal sections show uniseriate epidermis with quadrangular or subrectangular cells. The epidermal cells are thin-walled and have granular cytoplasm and conspicuous nucleus. Nectarostomata can be open or closed, and the substomatal chamber is small or almost absent. Idioblasts containing tannin are often present in the epidermis of *A. edulis*, *H. incanus*, *M. lepidopetalus*, *T. mucronata*, *P. elegans*, and *S. meridionalis.*

In *M. pubescens*, simple trichomes are observed on the surface of the concavity of the posterior bicupular structure, whereas they are absent in the anterior region of the nectary (Fig. 3h–j). Another remarkable characteristic of this species is that the reduced anterior region and the outer surface of the external laminae have slightly papillate epidermal cells with dense cytoplasm and conspicuous nucleus, and both surfaces are devoid of nectarostomata (Fig. 3h, n).

Nectary parenchyma This tissue consists of several layers of isodiametric cells with minute intercellular spaces between them; the cells are thin-walled, the nucleus is large, vacuoles are generally small, and the cytoplasm is densely stained, often with granular content. In *A. edulis*, *M. lepidopetalus*, *T. mucronata*, *S. meridionalis*, and *M. pubescens*, cells with tannin are relatively common either in groups or isolated throughout the tissue (Figs. 2m–o and 3k). In *H. incanus* and *P. elegans*, the first two or three subepidermal cell layers have idioblasts containing tannins, with this characteristic being more evident on the upper surface of the anterior and posterior nectary lobes (Fig. 4d, e, g, h, j, k). In addition, cells containing druses are abundant throughout the nectariferous tissue in all species, except in *S. meridionalis* where they were not observed (Fig. 4f, i, l). In *L. plumosum*, the parenchyma is more homogeneous due to the absence of tannins and the low occurrence of druses (Fig. 2l, p). The nectary parenchyma in *L. glandulosa* is composed of about five cell layers and is characterized by the presence of small-sized cells (Fig. 5f).

The secretory parenchyma occupies specific areas of the nectary in *D. floribunda* and *M. pubescens*. In the former species, this tissue comprises the whole inner ridge and nearly half of the outer ridge of the bicupular nectary (Fig. 3b–d); in *M. pubescens*, this parenchyma is observed in approximately two thirds of the outer laminae and in the entire inner laminae (Fig. 3h–j).

Subnectary parenchyma This parenchyma is characterized by polygonal thin-walled cells with large vacuoles. These cells are bigger and have much less dense cytoplasm than those of the secretory tissue, although cells with granular content are also observed. In *A. edulis*, *M. lepidopetalus*, *T. mucronata*, *L. plumosum*, and *L. glandulosa*, this parenchyma is located immediately below the secretory tissue (Figs. 2e–k and 5f). Numerous tannic idioblasts are observed in *A. edulis*, *M. lepidopetalus*, and *T. mucronata* (Fig. 2m–o),



as well as druses in *M. lepidopetalus*. In *D. floribunda*, the subnectary parenchyma is located in the outer half of the outer ridge of the nectary (Fig. 3b, c), and in *M. pubescens*, this parenchyma occupies the entire anterior region of the nectary and the peripheral layers of the outer posterior laminae (Fig. 3h–j). In *H. incanus*,

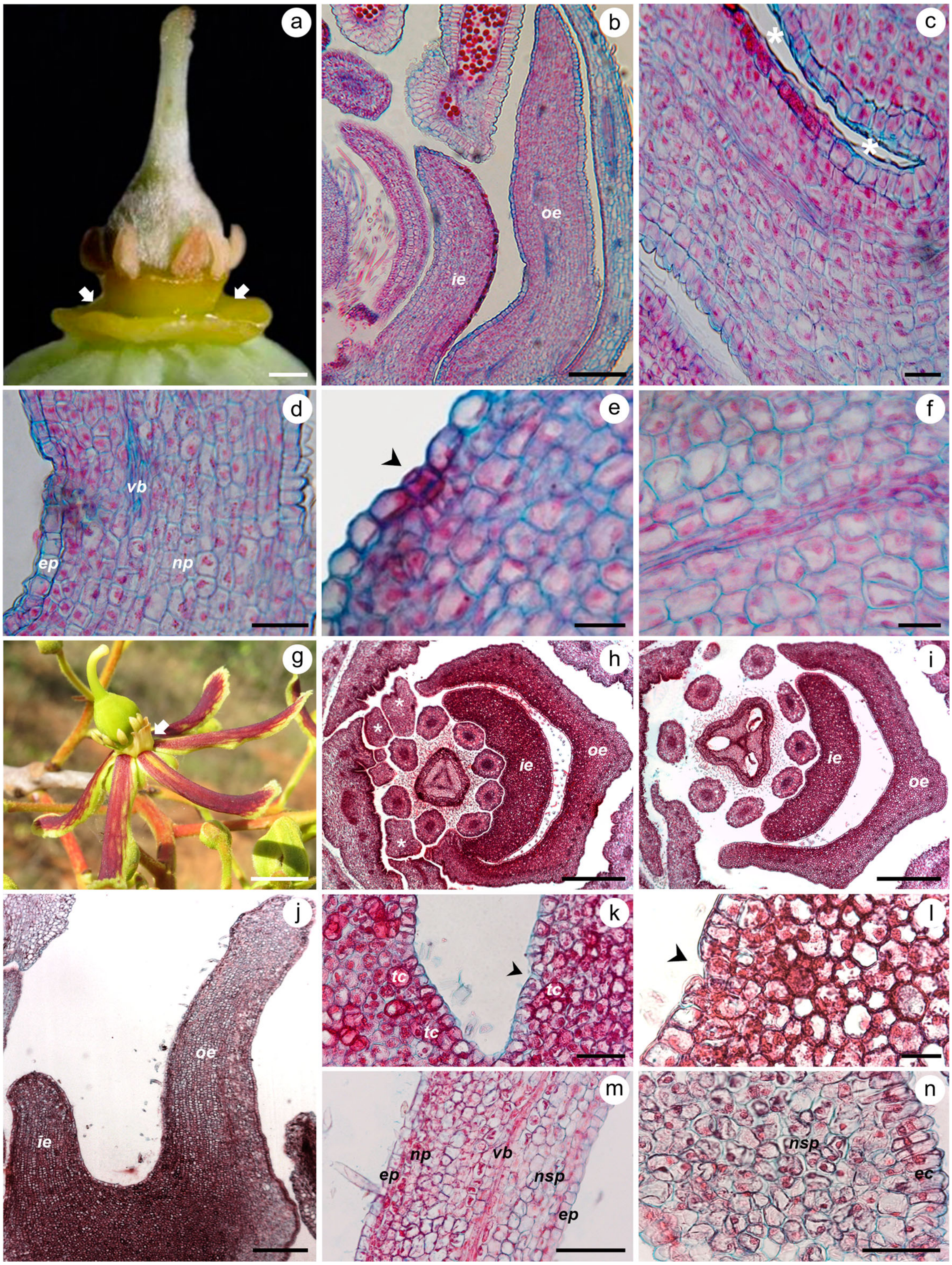
◀ **Fig. 2** Morphoanatomy of annular and incompletely annular floral nectaries. **a**, **e**, **i**, **m**, **q**, **u** *Allophylus edulis.* **b**, **f**, **j**, **n**, **r**, **v** *Melicoccus lepidopetalus.* **c**, **g**, **k**, **o**, **s**, **w** *Thinouia mucronata.* **d**, **h**, **l**, **p**, **t**, **x** *Lophostigma plumosum*. **a**–**c** Flowers devoid of sepals and petals, showing the location of annular nectary (*arrows*). **d** Flower devoid of perianth, with incompletely annular nectary. **e**–**h** Longitudinal sections of nectary (*arrows*). **i**–**l** Cross sections of nectary (*n*). **m**–**p** Details of nectary tissue, epidermis (*ep*), nectary parenchyma (*np*), subnectary parenchyma (*snp*), tannin cells (*tc*), and phloem strands (*ps*). **q**–**t** Details of simple (*sps*) and branched (*bps*) phloem strands. **u**–**x** Nectarostomata in cross sections (*arrowhead*). *Scale bars*: **a**–**d** = 1 mm; **e**–**g**, **i**, **l** = 100 μm; **h**, **j** = 500 μm; **k** = 200 μm; **m**–**p** = 50 μm; and **q**–**x** = 20 μm

P. elegans, and *S. meridionalis*, the ground parenchyma surrounds the petal base (Fig. 4d–f, j–l).

Vasculature The vascular bundles supplying the nectaries come from the central ectophloic stele. In *A. edulis*, *M. lepidopetalus*, *T. mucronata*, *L. plumosum*, and *D. floribunda*, the vasculature consists of 15–20 phloem strands usually branched in the subnectary parenchyma (Figs. 2q–t and 3f). These strands branch again and reach the first layers of the secretory tissue. Each one of the posterior nectary lobes of *H. incanus*, *P. elegans*, and *S. meridionalis* is innerved by four or five phloem strands, which branch until they reach the third or the fourth subepidermal layers of the secretory tissue (Fig. 4g–i). The anterior nectary lobes have few phloem strands in *H. incanus* and *S. meridionalis* (Fig. 4j, l), whereas they are not vascularized in *P. elegans* (Fig. 4k). In all the species mentioned earlier, the phloem strands are composed of sieve elements, companion cells, and parenchymatous cells. In *M. pubescens*, the vascular strand consists of both phloem and xylem tissues; thus, bundles consist of sieve tubes, tracheal elements, and companion and parenchymatous cells. In this species, the vascular strands reach the base of the secretory tissue, as well as the top of the inner and outer lamellae of the posterior region; vasculature is absent in the anterior part of the nectary (Fig. 3j, m). In *L. glandulosa*, vascular bundles of the sepal vasculature are directed toward the nectary. These bundles consist of phloem and xylem that reach the subnectary parenchyma up to the two most basal layers of the secretory parenchyma (Fig. 5f, i).

Structure of the hypothetic nectary in *D. viscosa* The intrastaminal ring observed in perfect and pistillate flowers anatomically does not correspond to a nectary; instead, it represents an outgrowth of the gynophore (Figs. 1g and 6a–h). The epidermis lacks nectarostomata but exhibits scattered viscous glandular hairs, which are also present on the pistillode of staminate flowers and on the gynoecium of perfect and pistillate flowers (Figs. 1h and 6c–e, h), as well as simple non-glandular trichomes, as occurs in other floral parts (Fig. 6e). The epidermis has a thick cuticle. In longitudinal sections, the epidermal cells are rectangular or quadrangular in staminate flowers and hermaphrodite flowers and radially elongated in pistillate flowers (Fig. 6c–e, h). Vascular bundles are absent in the outgrowth of the gynophore. The subepidermal tissue consists of two to five layers of rounded collenchymatous cells and parenchymatous cells below (Fig. 6d, e, h). Idioblasts containing tannins or other dense components are also observed (Fig. 6f–h). Intercellular spaces are conspicuous in deeper parts of the tissue (Fig. 6d, h).

Nectary color change in *D. floribunda* Anthetic pistillate and staminate flowers were found simultaneously in different inflorescences of the same individual, with dominance of the latter floral morphs. From floral bud stage to floral senescence, the color of the nectary ranges from green to dark red and finally becomes almost black, even within the same inflorescence (Fig. 7a). Young floral buds exhibit greenish nectaries partially covering the developing stamens (Fig. 7b). Anatomically, the nectariferous tissue is green, with the exception of the epidermal layer which is colorless. However, in floral buds, the nectary changes from green to yellow 3–4 days before anthesis (Fig. 7c). The epidermis remains colorless, and the secretory parenchyma is yellow (Fig. 8a). When flowers start to open, at about 7:00 a.m., the corolla expands completely and reflexes backwards; in staminate flowers, the filaments stretch gradually and the anthers begin to dehisce. In contrast, pistillate flowers have small and indehiscent stamens throughout the entire flowering phase. Instead, the gynoecium is well developed and has greenish and a shiny stigmatic surface (Fig. 7d). In both types of flowers, droplets of nectar are visible on the concave surface of the nectary bicupular structure. In the afternoon, the exposed nectary either remains yellow or turns light orange (Fig. 7e). One day after anthesis, the nectary turns orange red. Nectar droplets are observed both in the morning and in the afternoon (Fig. 7f). The pollen in anthers of staminate flowers has been released. The accumulation of reddish pigment is anatomically detected due to the appearance of spots starting in the epidermal cells and continuing in deeper layers of the secretory tissue (Fig. 8b). Between 2 and 4 days after anthesis, nectary color changes from orange red to dark red (Fig. 7g). Scarce nectar may be still present or absent. In the field, bees, wasps, and ants (*Camponotus* spp.) visit staminate flowers. The former two land yellow and red nectaries; they stay on the yellow nectaries longer than on the orange red or dark red ones. The ants move inside the inflorescences in search of nectar. Some nectaries are dry and exhibit blackened patches. In free-pollinated pistillate flowers, the stigma is brownish, the style is incurved, and the ovary is bulky. Five days after anthesis, the sepals, petals, and stamens start to wilt. The nectary is purple or black, and the stamens fall down (Fig. 7h). The accumulation of red pigments extends from the epidermis to the nectariferous tissue (Fig. 8c, d).



Ultrastructure The yellow nectary shows active secretory cells with thin wall, large nucleus, and electron-dense cytoplasm with dictyosomes, numerous vesicles, mitochondria with well-developed cristae, portions of rough endoplasmic reticulum, chromoplastids, and some vacuoles (Fig. 9a, b). Secretory cells in dark red nectaries have cytoplasm with rough endoplasmic reticulum, mitochondria, vacuoles, amyloplasts, and vesicles (Fig. 9c). However, at this stage, some cells show signs of degeneration; the cytoplasm is highly vacuolated, and organelles and membranes are undergoing degradation (Fig. 9d).

◀ **Fig. 3** Morphoanatomy of annular floral nectary. **a**–**f** *Diplokeleba floribunda*. **g**–**n** *Magonia pubescens*. **a** Pistillate flower with annular bicupular nectary (*arrows*). **b** Longitudinal section of bicupular nectary, with inner (*ie*) and outer (*oe*) edges delimiting the concavity. **c** Detail of the concavity where nectar accumulates (*asterisk*). **d** Outer edge of nectary: adaxial epidermis (*ep*), nectary parenchyma (*np*) with vascular bundles (*vb*). **e** Detail of epidermis with nectarostomata (*arrowhead*). **f** Detail of vascular bundles. **g** Pistillate flower with annular unequal nectary. **h** Cross section of flower, at the base of nectary, showing the anterior, simple region of nectary (*asterisk*) and the posterior and bicupular region with inner (*ie*) and outer (*oe*) edges. **i** Median cross section of flower, showing the inner (*ie*) and outer (*oe*) edges of the nectary. **j** Longitudinal section of the posterior part, showing inner (*ie*) and outer (*oe*) edges. **k** Detail of the cavity where nectar accumulates; note tannin cells (*tc*) in nectary parenchyma and epidermis with nectarostomata (*arrowhead*). **l** Nectarostomata (*arrowhead*). **m** Longitudinal section of outer edge with epidermis (*ep*), simple trichomes, nectary parenchyma (*np*), and subnectary parenchyma (*snp*) with vascular bundles (*vb*). **n** Simple anterior region of nectary with papillose epidermal cells (*ec*) and non-secretory parenchyma (*nsp*). *Scale bars*: **a**, **g** = 1 mm; **b**, **c**, **h**, **i** = 500 μm; **d** = 200 μm; **e**, **f**, **k**, **n** = 50 μm; **j** = 250 μm; and **l** = 20 μm

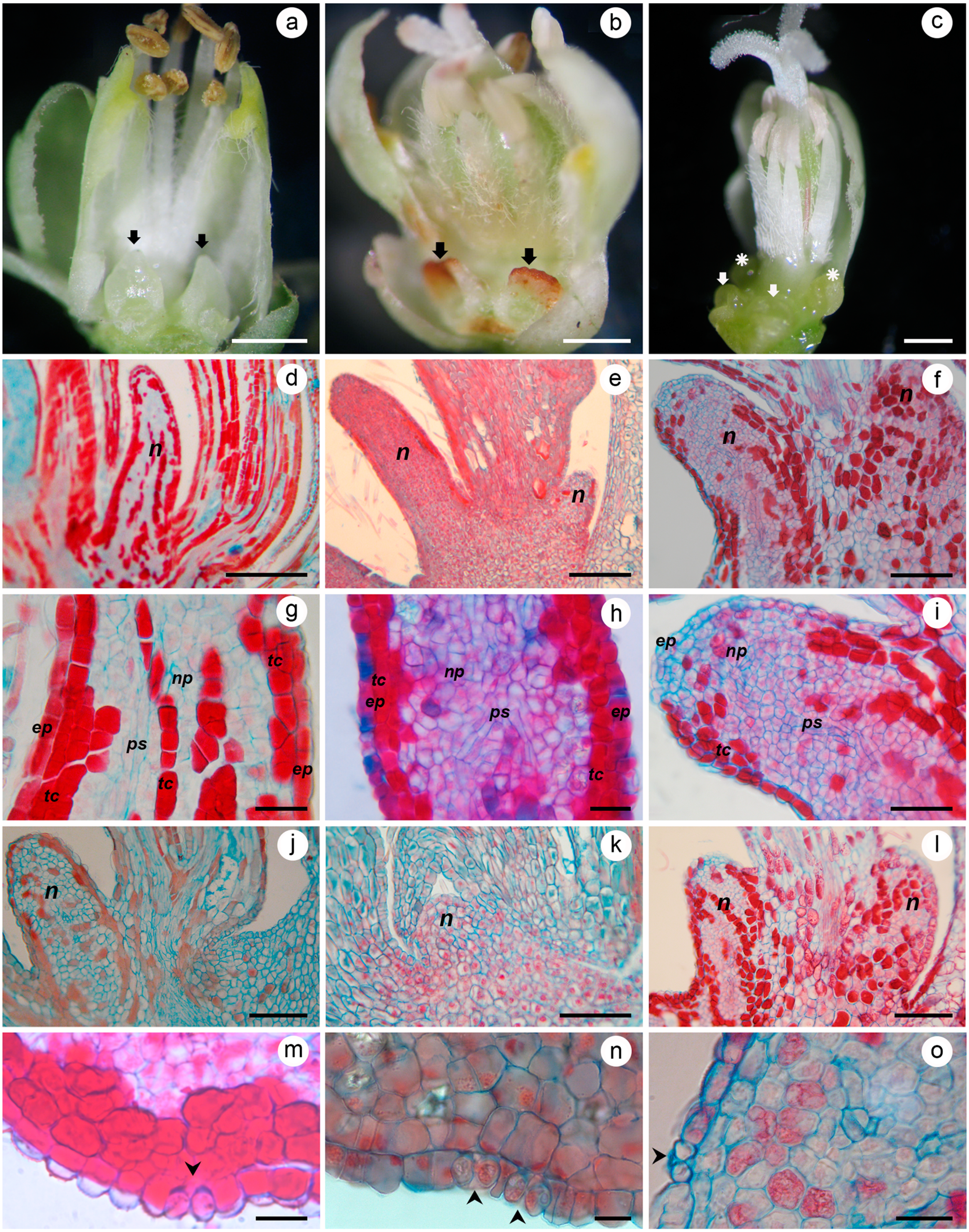
Discussion

Morphology and structure of the floral nectaries

The nectaries of most species examined here are located on the receptacle, as reported for other species of the family and related families of Sapindales (Cronquist 1981; Gadek et al. 1996; Ronse De Craene et al. 2000; Ning-Xi and Wu 2005; Solís and Ferrucci 2009; Zini et al. 2014). However, the location of the nectary was less evident in *L. glandulosa* due to its extensive development on the calyx tube. Therefore, more detailed investigations involving ontogeny are necessary in this nectary. Association of nectaries with other floral parts (essentially petals) is a very common feature in the family. We have previously determined that petals and nectaries can originate simultaneously from a common meristematic group of cells or both can originate at more distant ontogenetic stages. The latter condition has evolved within the *Paullinieae* tribe (Solís and Ferrucci 2009; Zini et al. 2014). The extrastaminal position of floral nectaries is also consistent with that previously reported for other Sapindaceae *s.l.* (Judd et al. 1999; Ronse De Craene et al. 2000; Soltis et al. 2005; Ronse De Craene and Haston 2006; Bernardello 2007; Acevedo-Rodríguez et al. 2011). The evolutionary study conducted by Ronse De Craene and Haston (2006) revealed that intrastaminal nectary is plesiomorphic for Sapindales and that extrastaminal nectary is derived for Sapindaceae *s.l.*

Considering nectary nature and duration, nectaries of the studied species correspond to the persistent type (Smets 1986; Smets and Cresens 1988). According to the histological differentiation, nectaries are of structural (Zimmermann 1932) and mesenchymatic types (Vogel 1977). The nectariferous tissue is well differentiated, consisting of epidermis with modified stomata (i.e., nectarostomata) and nectary and subnectary parenchyma. However, these traits were absent in all of the three floral morphs of *D. viscosa*, indicating the absence of a nectar-secreting role. Nectarostomata are present in 14 genera studied to date (Ning-Xi and Wu 2005; Solís and Ferrucci 2009; Zini et al. 2014; Avalos et al. 2016; present study), representing the most frequent via of nectar release within Eudicots (Fahn 1952, 1979; Durkee et al. 1981; Davis and Gunning 1992; Fahn and Shimony 2001; Matthews and Endress 2005; Nepi 2007; Konarska 2014). In Sapindaceae, the distribution of nectarostomata on the nectary surface can be linked with the nectary morphology. In species with annular nectary, the nectarostomata were mostly located on the outer margin of the disc (Ning-Xi and Wu 2005; present study) or exceptionally on the middle of the thimble-shaped nectary in *K. elegans* (Avalos et al. 2016). In the unilateral nectaries of *Cardiospermum grandiflorum* Sw., *C. heringeri* Ferrucci, and *C. integerrimum* Radlk., nectarostomata are numerous on the top of two horn-like lobes (Solís and Ferrucci 2009; Zini et al. 2014). In the four-lobed nectaries of *H. incanus*, *P. elegans* (present study), and *Urvillea chacoensis* (Solís and Ferrucci 2009), nectarostomata were present only on the posterior lobes. Thus, the lack of nectarostomata on the anterior lobes can suggest that they are not involved in nectar exudation. A comparable situation was reported in some species of Lamiaceae, in which only one part of the gynoecial nectary bears nectarostomata (Dafni et al. 1988; Zer and Fahn 1992; Naghiloo et al. 2014). The nectary of *Rosmarinus officinalis* L. is asymmetrically four-lobed, and the smaller lobes have no nectarostomata, but the nectar produced by the whole nectary flows through the intercellular spaces to the longer lobe, where it is released. One-sided nectary is interpreted as an adaptation to the horizontal position of the flower (Zer and Fahn 1992), although this correlation does not apply to Sapindaceae since flowers do not have a horizontal position.

Vascularization in nectaries of the Sapindaceae may be relatively poorly or rich. The vascular bundles consisting of only phloem elements are present in most studied species of the family (Ning-Xi and Wu 2005; Solís and Ferrucci 2009; Zini et al. 2014; Avalos et al. 2016; present study) and are largely present in angiosperms (e.g., Frey-Wyssling 1955; Fahn 1979). In this study, we have found that both phloem and xylem elements can be present in nectaries of Sapindaceae. In addition, vasculature is absent in some reduced parts of the glandular structure, as observed in the anterior part of the nectary of *M. pubescens* (present study) and the two smaller (anterior) nectary lobes of *U. chacoensis* (Solís and Ferrucci 2009). These observations support a directly proportional relationship between the size of the structure and the rich vascularization suggested by Carlquist (1969).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◀ **Fig. 4** Morphoanatomy of unilateral floral nectary, fragmented in four nectary lobes. **a**, **d**, **g**, **j**, **m** *Houssayanthus incanus.* **b**, **e**, **h**, **k**, **n** *Paullinia elegans*. **c**, **f**, **i**, **l**, **o** *Serjania meridionalis.* **a** Staminate flower devoid of part of perianth, showing two posterior nectary lobes (*arrows*). **b** Pistillate flower devoid of petals and sepals, showing two posterior nectary lobes (*arrows*). **c** Pistillate flower devoid of perianth, showing four nectary lobes, two posterior (*arrows*) and two anterior (*asterisk*). **d**–**f** Longitudinal section of a posterior nectary lobe (*n*). **g**–**i** Detail of posterior nectary lobe, showing epidermis (*ep*), nectary parenchyma (*np*), tannin cells (*tc*), and phloem strands (*ps*). **j**–**l** Longitudinal section of an anterior nectary lobe (*n*). **m**–**o** Detail of nectarostomata in cross section (*arrowheads*). *Scale bars*: **a**–**c** = 1 mm; **d**, **e** = 200 μm; **f**, **j**–**l** = 100 μm; **h**, **i** = 50 μm; **g** = 25 μm; and **m**–**o** = 20 μm

Nectary color change in *D. floribunda*

In angiosperms, when nectary color change occurs, it may be through the formation of carotenes in chromoplasts and/or the accumulation of flavonoids in the vacuoles (Bernardello 1986; Vezza et al. 2006; Horner et al. 2007; Gouvêa et al. 2008; Giuliani et al. 2012; Paiva 2012). Regarding color change from yellow to red in *D. floribunda*, TEM images showed that the chromoplasts have disappeared at the red phase; instead, some amyloplasts are undergoing degradation. Therefore, carotenoid substances could be responsible for the yellow color of nectaries but not for the red one. On the contrary, in *Nicotiana langsdorffii* Weinm. × *N. sanderae* Hort. (Solanaceae), the macroscopical changes of the nectary from green to orange during nectar secretion are explained ultrastructurally by conversion of amyloplasts to chromoplasts (Horner et al. 2007). This phenomenon was later observed during color change from pale yellow to red in *Boswellia sacra* Flueck. (Burseraceae; Giuliani et al. 2012) and in *Swietenia macrophylla* King (Meliaceae; Paiva 2012). The ultrastructure of the secretory cells of *D. floribunda* indicating abundant endoplasmic reticulum, dictyosomes, vesicles, plastids, and mitochondria is a frequent characteristic in nectar-producing parenchyma (e.g., Fahn 1979, 1988; Durkee 1982; Belmoente et al. 1994; Radice and Galati 2003; Paiva and Machado 2008; Mosti et al. 2013; Antón and Kaminska 2015). The most common organelles in the nectary of *D. floribunda* at the post-anthesis stage were the Golgi complex with vesicles, endoplasmic reticulum, vacuole with content, and plastids; in *K. elegans*, a large vacuole, mitochondria, and rough endoplasmic reticulum have been reported at a similar stage (Avalos et al. 2016). As in *K. elegans*, nectary

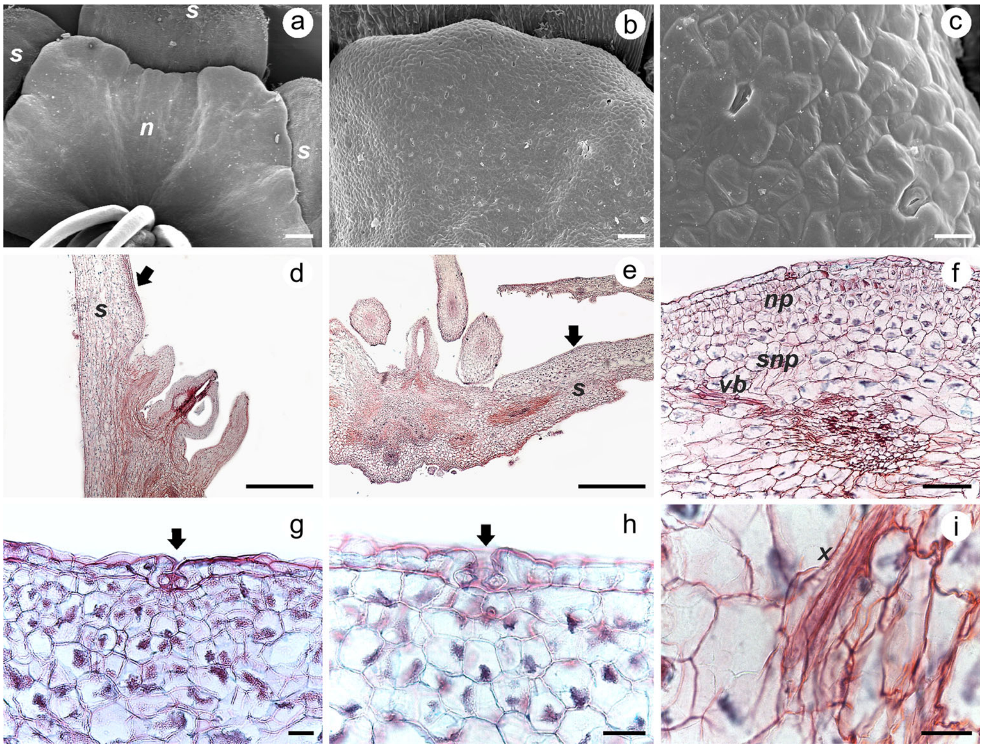

Fig. 5 SEM and LM micrographs showing the nectary morphology and structure in *Llagunoa glandulosa*. **a** Unilateral nectary (*n*) on sepals (*s*). **b** Numerous sunken nectarostomata on the nectary surface. **c** Detail of epidermis with nectarostomata. **d**–**e** Flower longitudinal (**d**) and cross (**e**) sections showing a portion of sepal (*s*) with the nectary on it (*arrow*). **f** Detail of nectary tissue with nectary parenchyma (*np*) and subnectary parenchyma (*snp*) with vascular bundle (*vb*). **g**, **h** Closed (**g**) and open (**h**) nectarostomata (*arrows*). **i** Detail of xylem element (*x*) in the subnectary tissue. *Scale bars*: **a**, **d**, **e** = 500 μm; **f** = 100 μm; and **c**, **g**–**i** = 20 μm

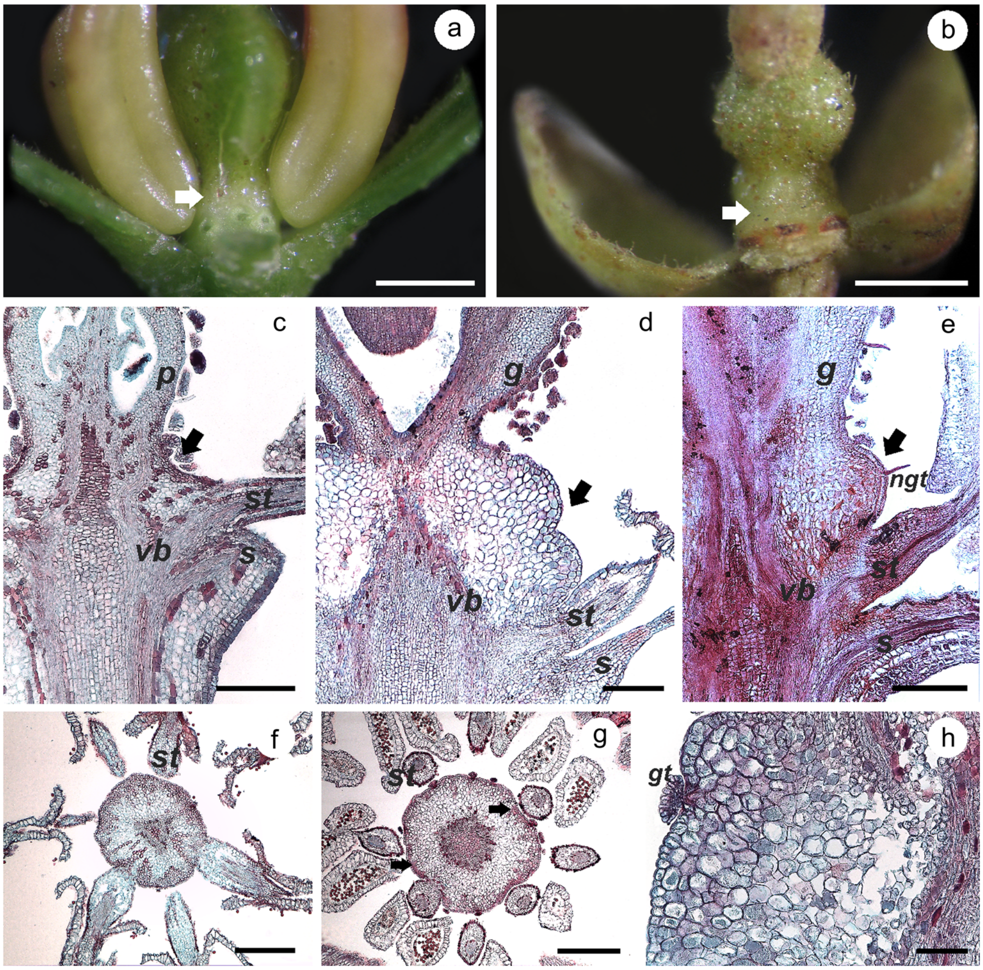

Fig. 6 Receptacular outgrowth in *Dodonaea viscosa*. **a**, **b** Details of gynophore tissue (*arrows*) in hermaphrodite flowers at anthesis (**a**) and at post-anthesis (**b**). **c** Staminate flower. **d** Hermaphrodite flower. **e** Pistillate flower. **c**, **d** Longitudinal sections showing the region between the androecium and gynoecium (*arrows*) at anthesis; gynoecium (*g*), pistillode (*p*), sepal (*s*), stamen (*st*), and vascular bundle (*vb*), non-glandular trichome (*ngt*). **f**, **g** Transverse sections of the gynophore in staminate and hermaphrodite flowers, respectively. Note tannic idioblasts in **g** (*arrows*). **h** Detail of non-secretory tissue in the hermaphrodite flower, showing a glandular trichome (*gt*). *Scale bars*: **a**, **b** = 1 mm; **c**–**e** = 250 μm; **f**, **g** = 500 μm; and **h** = 100 μm

parenchyma of *D. floribunda* is partly degraded and shows ultrastructural features characteristic of programmed cell death in plants (Mosti et al. 2001, 2013; Papini et al. 2013).

The red color of the nectary in individual flowers of *D. floribunda* would be associated with progressive accumulation of anthocyanins in vacuoles of the epidermal and subepidermal layers of the secretory parenchyma. The energetic source for anthocyanin synthesis can be provided by the starch grains present in the red phase. In diverse plant tissues, exposure to light induces the synthesis of anthocyanins (Lee et al. 1987; Mohr and Schopfer 1995); these compounds protect organs from low temperatures and photo-oxidative damage (Mohr and Schopfer 1995; Steyn et al. 2002). The accumulation of anthocianins in nectaries is a scarcely reported phenomenon; it was detected in *Hedera helix* L. (Araliaceae; Vezza et al. 2006; Konarska 2014) and *B. sacra* (Burseraceae; Lippi et al. 2011) in the functional phase and in *Cornus alba* L. (Cornaceae) at the end of the secretory phase (Konarska 2011). *D. floribunda* showed nectar production during the color change process, even when it reached the red color, although it was in a programmed cell death phase. Moreover, these flowers were briefly visited by bees and wasps. Red nectaries are generally linked with visitation of pollinators, with flowers becoming unattractive to them or providing a signal to animals (e.g., bees) that rewards are no longer accessible. In either situation, the insects ignore flowers with red nectaries and do not land on them (Belmonte et al. 1994; O'Brien et al. 1996; Thornburg 2007; Konarska 2011; Lippi et al. 2011). The possible role of these nectaries and of nectar quality in pollination biology of *D. floribunda* will be addressed in a future research paper.

Morphology of the floral nectaries in a systematic context

By integrating our results with data from the literature, we can provide an overview of morphological diversity of nectaries for the family. The subfamily Dodonaeoideae seems to be heterogeneous in relation to nectary presence, morphology, and position. Unilateral nectary evolved in *Llagunoa* possibly as a result of the change in floral symmetry. *Diplokeleba* and *Magonia* are currently placed with *Averrhoidium* Baill. in a clade, within the Dodonaea group (Buerki et al. 2010), and the three genera share the presence of completely annular, extrastaminal nectary, although they differ in overall

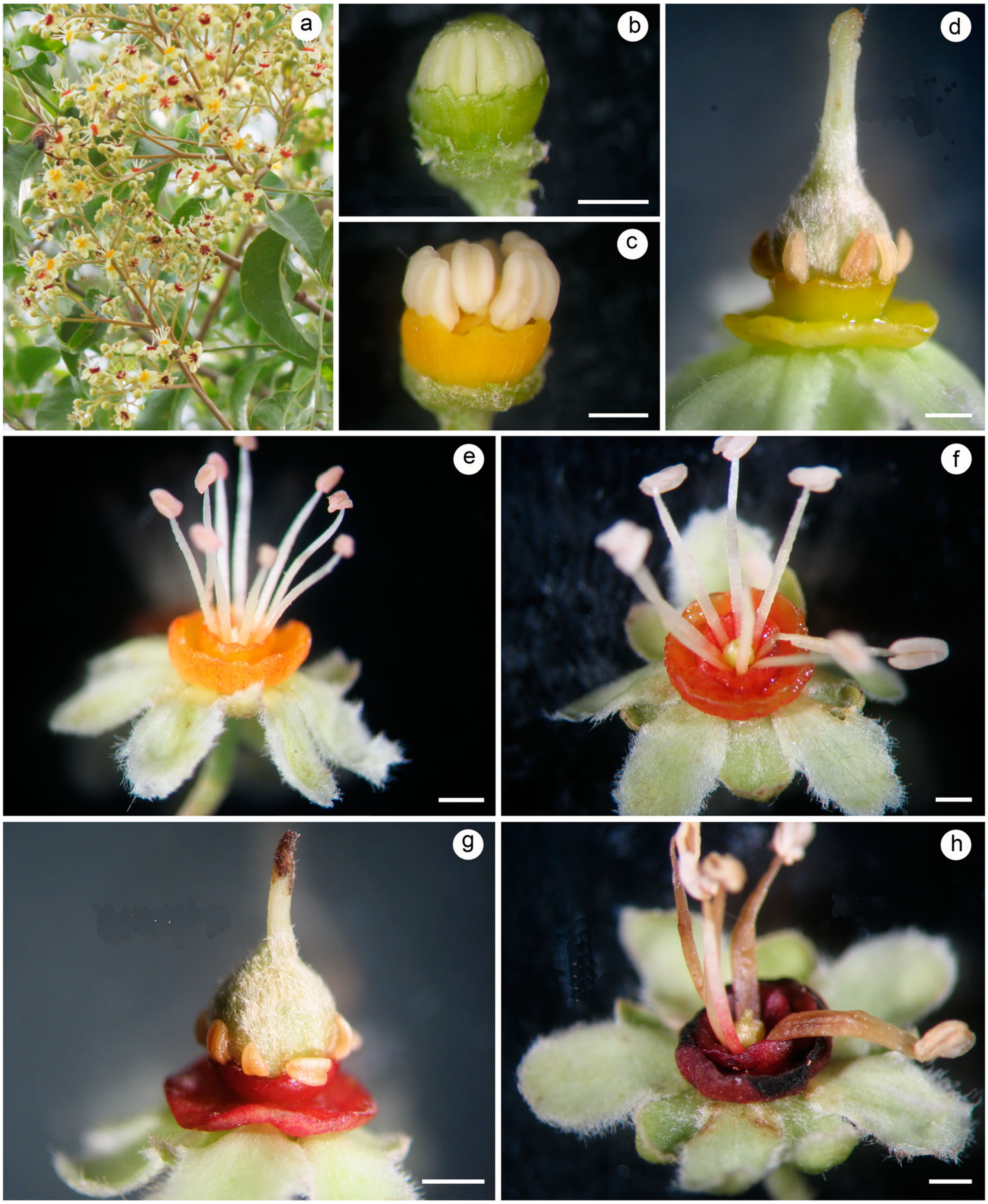

Fig. 7 Floral nectary of *Diplokeleba floribunda* at different stages. **a** Branches with inflorescences, showing flowers with nectaries at different stages. **b** Young floral bud devoid of perianth with green nectary. **c** Floral bud devoid of perianth with yellow nectary. **d** Pistillate flower with developed yellow nectary. **e** Staminate flower with orange nectary. **f** Staminate flower with red nectary. **g** Pistillate flower with dark red nectary. **h** Staminate flower in senescence with blackish red nectary. *Scale bars*: **b**–**h** = 1 mm

morphology (Weckerle and Rutishauser 2003; present study). The genus *Dodonaea s.l.* (includes *Distichostemon* F. Muell.; Harrington and Gadek 2010) deserves special attention because the nectary is largely interpreted to be annular, intrastaminal, rudimentary, or small in hermaphrodite and functionally pistillate flowers but is completely absent in staminate flowers (Muller and Leenhouts 1976; West 1984; Salywon 1999; Acevedo-Rodríguez et al. 2011). Karkare-Khushalani and Mulay (1964) describe that in *D. viscosa*, the nectary arises from the receptacle in the form of a ring in

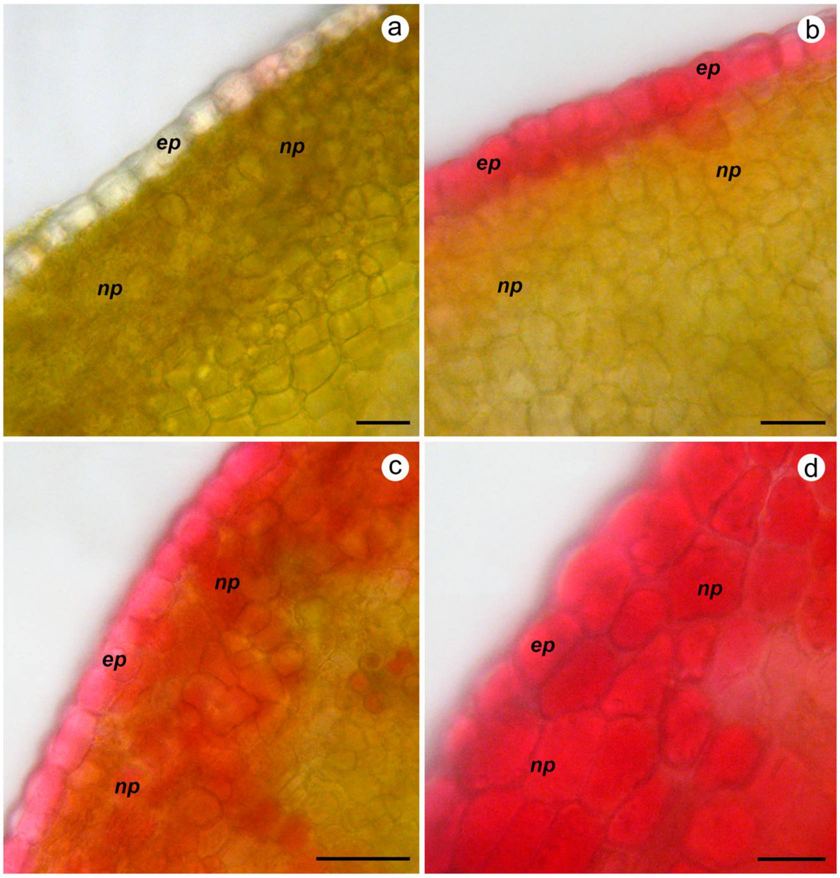

Fig. 8 Nectary tissue in *Diplokeleba floribunda.* **a**–**d** Hand cross sections. **a** Yellow nectary in flower bud. **b** Orange nectary in developed flower. **c** Red nectary. **d** Dark red nectary. Epidermis (*ep*) and nectary parenchyma (*np*). *Scale bars*: **a**–**d** = 50 μm

the female flowers. Later, West (1980) mentions that the nectary is part of a gynophore and that no nectar is produced. In this study, there was no evidence of the presence of a structural nectary on the gynophore of any of the flower morphs of *D. viscosa*. *Dodonaea* and *Diplopeltis* Endl. are considered a paradigm of wind pollination because they constitute relatively recent radiations derived from a larger predominantly animal-pollinated group of genera within Sapindaceae (Soltis et al. 2005). Accordingly, the present study confirms the assumption that the loss of floral nectary in *Dodonaea* can be considered indicative of an evolutionary shift from biotic pollination to wind pollination (West 1984; Harrington 2008).

Regarding the subfamily Sapindoideae (sensu Acevedo-Rodríguez et al. 2011), the annular and lobed nectary observed in *M. lepidopetalus* is rather constant within the *Melicoccus* group (*Melicoccus* and *Talisia* Aubl.; Buerki et al. 2009). This morphology is also present in *Castanospora* F. Muell., *Tristira* Radlk., and *Tristiropsis* Radlk. of the ex tribe Melicocceae (Radlkofer 1931–1934). However, Acevedo-Rodríguez (2003) and in part Buerki et al. (2009) determine that those genera are not closely related phylogenetically to the *Melicoccus* group; Melicocceae includes only *Melicoccus* and *Talisia* (Acevedo-Rodríguez et al. 2011). Completely annular nectaries are widely present in about 99 of 126 genera (Acevedo-Rodríguez et al. 2011); therefore, this form can be considered the ancestral state at the family level.

The Paullinieae tribe is a late-diverging lineage within the family that displays derived character states, such as the climbing habit and oblique zygomorphic flowers. This tribe has been recently recognized as part of the supertribe Paulliniodae, which contains other four subclades designated as tribes Athyaneae, Bridgesieae, and Thouinieae (Acevedo-Rodríguez et al. 2017). The presence of nectaries divided into four lobes (two anterior and two posterior ones that may be equal or unequal in shape and dimensions) is common in the genera *Allophylus*, *Cardiospermum*, *Paullinia*, *Serjania*, and *Urvillea*. The clade consisting of *Lophostigma*, *Cardiospermum*, *Paullinia*, *Urvillea*, and *Serjania* (including *Balsas* J. Jiménez Ram. & K. Vega and *Houssayanthus* Radlk. sensu Acevedo-Rodríguez et al. 2017) is exclusively characterized by the presence of obliquely zygomorphic flowers. Therefore, the occurrence of one-side nectaries in this clade is evidently linked with that floral symmetry. Repeated evolutionary transformations within *Cardiospermum*, *Paullinia*, and *Serjania* proceeded from unilateral nectaries with four equal nectary lobes toward nectaries with only two posterior lobes. Nectaries consisting exclusively of two conspicuous horn-like lobes are present in the three species of the section Ceratococcus of *Cardiospermum* (Solís and Ferrucci 2009;

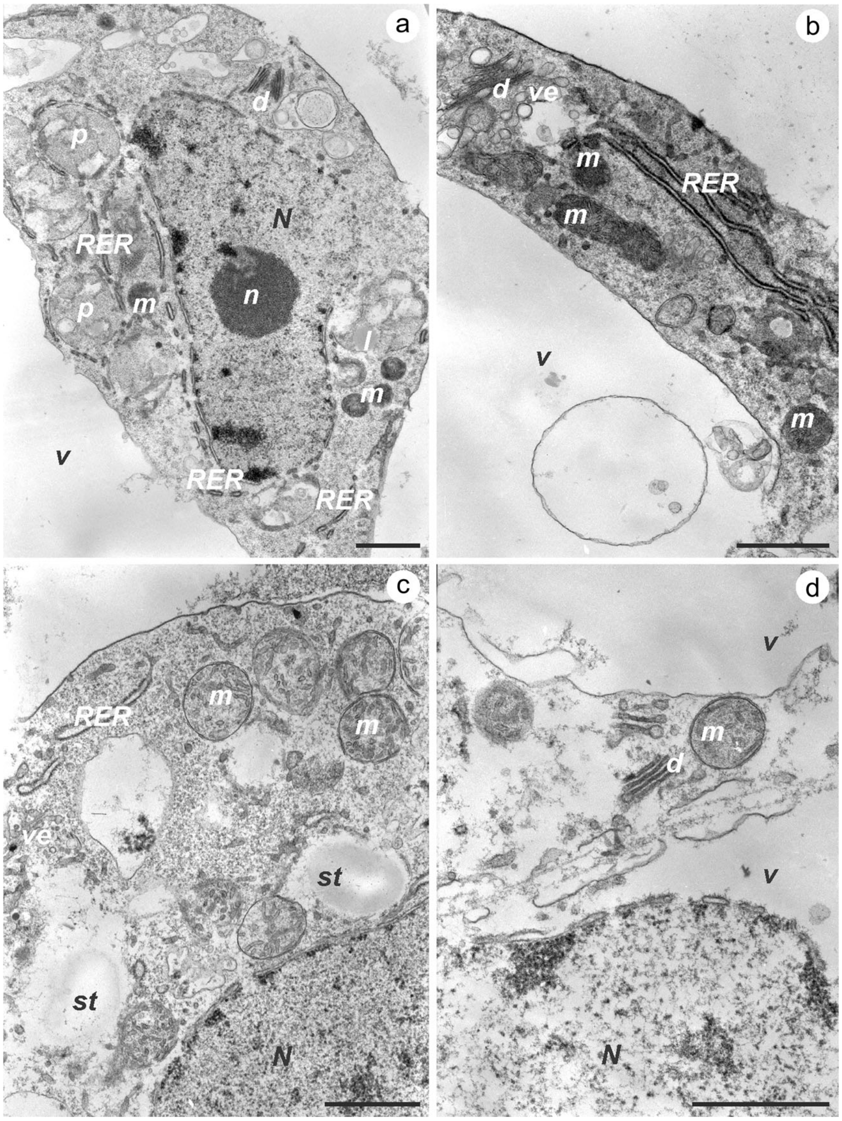

Fig. 9 TEM micrographs of the nectary parenchyma of *Diplokeleba floribunda* at post-anthesis. **a**, **b** Details of cytoplasm of cells in a yellow nectary. **c**, **d** Details of cytoplasm in cells of a red nectary, note degraded cytoplasm in **d**. Dictyosome (*d*), mitochondria (*m*), nucleus (*N*), nucleoli (*n*), plastid (*p*), rough endoplasmic reticulum (*RER*), starch grain (*st*), vacuole (*v*), vesicle (*ve*). *Scale bars*: **a**–**d** = 1 μm

Zini et al. 2014). However, the number of evolutionary transformations is unknown because of still unresolved problems with *Cardiospermum* delimitation (Acevedo-Rodríguez et al. 2017). The four unequal nectary lobes observed in *H. incanus*, *S. meridionalis*, and *P. elegans* can be regarded as intermediate forms. The nectary morphology of *L. plumosum*, which is sister to the clade consisting of *Cardiospermum*, *Paullinia*, *Serjania*, and *Urvillea*, supports the evolutionary tendency proposed for the supertribe because it was incompletely annular, with nectary tissue interrupted at the anterior region of the flower. These events of loss or suppression of the anterior part of the nectary were previously reported in some Grevilleoideae of the Proteaceae and have been associated with the horizontal position of the flower (Rao 1967).

Thinouia is sister to the remaining genera of Paullinieae; however, the completely annular nectary is a conserved trait and consistent with the overall actinomorphic symmetry of the flower. On the other hand, the annular nectary of *A. edulis* contrasts with the floral symmetry, and consequently, the nectary has remained in the evolution toward floral zygomorphy. Nevertheless, this situation is exceptional in *Allophylus*, a genus with over 200 species that generally possess unilateral nectaries fragmented in lobes (Acevedo-Rodríguez et al. 2011). Interestingly, nectaries with two halves have also been described in *A. edulis* (Ferrucci 1991), indicating the existence of developmental lability in this species. It is important to highlight that flower zygomorphy of the Paullinieae species is mainly due to the arrangement of sepals (when five sepals, two outer and three inner or when four sepals, the third and fifth ones are fused) and to the eccentric position of the gynoecium and androecium. Although these two characteristics are present in *Allophylus*, the zygomorphy in the clade comprising *Lophostigma*, *Cardiospermum*, *Paullinia*, *Serjania*, and *Urvillea* is additionally reinforced by the corolla, which consists of two anterior petals and two unequal posterior ones (subequal in *Lophostigma*).

Another important feature observed in nectaries of Sapindaceae is the degree of exposure at anthesis. In this study, the presence of a hidden nectary was linked with the occurrence of ventral petal appendages of basal position, which may show elaborated morphology and may also have a hairy indumentum. Except for Sapindaceae, there are few petal elaborations in Sapindales (Endress and Matthews 2006a, 2006b). Endress and Matthews (2006b) state that petal elaborations in this family serve as nectar covers, protecting against non-pollinating nectarivorous insects and preventing the evaporation of nectar. This advantageous floral feature arises in many lineages of the family (i.e., the *Paullinia* group, *Koelreuteria*, and *Melicoccus* group). In *K. elegans*, the fleshy crest at the base of the petal blade and the stamen filament trichomes offset the absence of the appendages (Avalos et al. 2016). However, exposed nectaries are mostly observed in genera lacking petal appendages, such as *Diplokeleba* and *Magonia* of the subfamily Dodonaeoideae, and were also noted in the apetalous flowers of *Averrhoidium*, *Diplopeltis*, *Llagunoa* (Dodonaeoideae) (Weckerle and Rutishauser 2003; present study), and *Allophylastrum* Acev-Rodr. (Paullinioideae) (Acevedo-Rodríguez et al. 2011). In *Diplokeleba*, *Magonia*, and *Allophylastrum*, the cup-shaped nectaries can be regarded as morphological modifications for holding nectar and minimizing rapid evaporation.

Conclusions

The present approach to the floral nectaries of Sapindaceae *s.s.* (actually subfam. Dodonaeoideae + Sapindoideae) reveals a broad morphological variability and a relatively conserved anatomy. Nectarostomata are present in many lineages of the family and can be considered the main pathway of nectar secretion; their distribution on the nectary surface is related to the morphology of the secretory structure. The nectary tissue varies histologically in terms of presence/absence and distribution of both tanniferous and cristaliferous (druses) idioblasts, and these traits seem to be species-specific. The vascular bundles that supply the nectaries consist mostly of phloem, but phloem and xylem may also be present. In *D. floribunda*, the remarkable color change of the nectary is related to progressive accumulation of anthocyanins during its functional phase. This process initiates at floral anthesis and continues until just before senescence of the nectary. We hypothesize that the two basic known morphologies of the nectary in the family (i.e., completely annular or unilateral) can be linked with developmental constraints due to evolutionary changes in flower symmetry, whereas the diversity in shape could be partly due to selective forces that favor protection strategies for the nectary tissue and the secreted nectar. In Paullinieae, the unilateral nectaries have resulted from reductive events from complete to fractioned secretory structures into four and even only two nectary lobes, with the latter condition being a derived character state for the family. Finally, in *D. viscosa*, a nectary tissue is absent; the floral nectary of intrastaminal position probably has represented a basal state shared with the other families of Sapindales, although it was subsequently lost as a consequence of the evolution toward anemophilous pollination. A better understanding of floral nectary evolution in Sapindaceae will depend on further comparative analyses including additional taxa and a more detailed phylogeny within the family.

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Compliance with ethical standards

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

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