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Review

Nectary structure and ultrastructure in two floral morphs of *Koelreuteria elegans* subsp. *formosana* (Sapindaceae)

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ABSTRACT

The structure and ultrastructure of the floral nectary of staminate and pistillate flowers of *Koelreuteria elegans* subsp. *formosana* (Sapindaceae, Koelreuterieae) were studied. In both floral morphs, the floral nectary is extrastaminal, receptacular, thimble-shaped and persistent. The anatomical analysis revealed a differentiated secretory parenchyma and a less developed, inner non-secretory parenchyma. These anatomical features reveal that the nectary is structured, and is supplied by phloem traces derived from the central stele. In the middle of the floral nectary there are few modified stomata. The nectarostomata were of anomocytic type; they may occur at the level of or sunken below the adjacent epidermal cells. Statistically significant differences were observed between both floral morphs in relation to nectar volume and the different moments of day, whereas the sugar concentration showed no significant differences. Ultrastructural studies showed no difference between staminate and pistillate flowers. At anthesis, amyloplasts and lipid globules were observed in the secretory parenchyma cells, whereas at post-anthesis the secretory parenchyma cells were degenerated. Floral nectar is secreted through nectarostomata and the outer epidermal cell walls. These results are discussed in relation to other species belonging to different tribes within Sapindaceae.

Contents

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1. Introduction

Sapindaceae *s.s.* is a family with a cosmopolitan distribution that is mainly found in tropical and subtropical regions; it comprises about 1800 species distributed in ca. 140 genera. The genera include monoecious or less frequently dioecious or polygamous species. Radlkofer (1931–1934) recognized 14 tribes segregated into two subfamilies, Sapindoideae and Dodonaeoideae. Recent studies based on molecular data recognized four subfamilies for Sapindaceae *s.l.* (Buerki et al., 2009): Sapindoideae, Hippocastanoideae, Dodonaeoideae and Xanthoceroideae. However, a year later, based on molecular evidence, the same authors (Buerki et al., 2010) supported the concept of Sapindaceae *s.s.* proposed by Radlkofer (1931–1934). Based on these results, in this work we maintain the criteria of tribes proposed by the latter.

A receptacular nectary is typical of the order Sapindales (Cronquist, 1981). In Sapindaceae, the floral nectaries are extrastaminal, an apomorphic character that differentiates it from other families in the order (Judd et al., 1999; Ronse Decraene et al., 2000). In the orderís remaining families, the nectaries are usually intrastaminal, by exception may be absent (Bernardello (2007) provides an update of the available literature). Floral nectaries in the Sapindaceae have systematic value at generic or specific levels (Radlkofer, 1931–1934; Ferrucci 1993, 2000).

The anatomical studies of the floral nectaries in this family are scarce, with most of the analyses focusing on species of tribe Paullinieae. Solís and Ferrucci (2009) studied the morpho-anatomy and ontogeny of the floral nectaries in *Cardiospermum grandiflorum* Sw. and *Urvillea chacoensis* Hunz. Unpublished results on nectary structure in other genera of Paullinieae are in Solís (2011). Recently, Zini et al. (2014) conducted studies on the development of floral nectaries in species of *Cardiospermum* L. and indicated the evolutionary trend within the Paullinieae.

Koelreuteria Laxm., belonging to the tribe Koelreuterieae, is a small genus of monoecious, medium-sized trees, native to eastern Asia. The number of species of the genus varies according to the criteria used by the different authors. Radlkofer (1897) recognizes *K. paniculata* Laxm. and *K. bipinnata* Franch.; Mabberley (1987) records *K. elegans* (Seem.) A. C. Sm., *K. bipinnata* and *K. paniculata*; and Melchior (1964) and Krüssman (1985) mention seven species. The latter contribution was not taken into account because it exists in a manual of cultivated plants and shrubs in which genera do not have a taxonomic treatment. In this paper, we adopted the criteria of Meyer (1976), who performed the taxonomic study of the genus and recognized three species, *K. bipinnata*, *K. paniculata* and *K. elegans*, the latter with two subspecies, *K. elegans* subsp. *elegans* and *K. elegans* subsp. *formosana* (Hayata) F. G. Mey. The species of this genus are cultivated in Europe, Africa, Australia and United States (Meyer, 1976), being widespread for their ornamental value.

The aim of this work was to study the morpho-anatomy and the ultrastructure of the nectary in staminate and pistillate flowers of *K. elegans* subsp. *formosana*, as well as to analyze the sugar concentration in both floral morphs. These results would help to characterize the genus and the tribe Koelreuterieae, and provide information for comparative analyses with data known for the family.

2. Materials and methods

2.1. Plant material

Koelreuteria elegans (Seem.) A. C. Sm. subsp. *formosana* (Hayata) F. G. Mey.: ARGENTINA. Corrientes province, Corrientes department, locality of Corrientes, Stella Gutiérrez & Dominicana street, cultivated on sidewalk, tree 7 m in height, with abundant yellow flowers and pale pink fruits, *Avalos & Ramírez 1* (CTES); ib., tree 8 m, with abundant yellow flowers and red fruits, *Avalos & Ramírez 2* (CTES); cultivated in the garden of IBONE, Tree 12 m, abundant yellow flowers *lattar* 25 (CTES); cultivated in the garden of IBONE, tree 6 m, *Avalos 17* (CTES).

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

2.2. Light microscopy

Open flowers and floral buds at different developmental stages: pre-anthesis (24 buds of 3–5 mm length), anthesis (64 flowers of 6-8 mm) and post-anthesis stages (64 flowers of 10 mm) were fixed in formalin, acetic acid and alcohol (FAA) for anatomical and scanning electron microscopy (SEM) examination. For preparing permanent slides, the fixed 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 paraffin Histoplast® (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). Anatomical and morphological analyses were performed under a Leica DM LB2 compound microscope (Leica, Wetzlar, Germany) and a Leica MZ6 stereomicroscope, respectively, both equipped with a digital camera.

2.3. Scanning electron microscopy (SEM)

The preserved material was 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. Micrographs were obtained with a JEOL 5800LV at 10 Kv and JEOL 100c.

2.4. Transmission electron microscopy (TEM)

Fresh floral nectaries were prefixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 2 h and post-fixed in osmium tetroxide (OsO_4) at 2 °C in the same buffer for 3 h. Then, the material was dehydrated in an ascending ethanol series and embedded in Spurr's resin. Ultrathin sections (750–900 nm) were made on a Reichert ultramicrotome and stained with uranyl acetate and lead citrate (O'Brien and McCully, 1981). The samples were observed and photographed using the Electron Microscope Unit CICVy A, INTA-Castelar (JEOL-JEM 1200 EXII at 85 Kv).

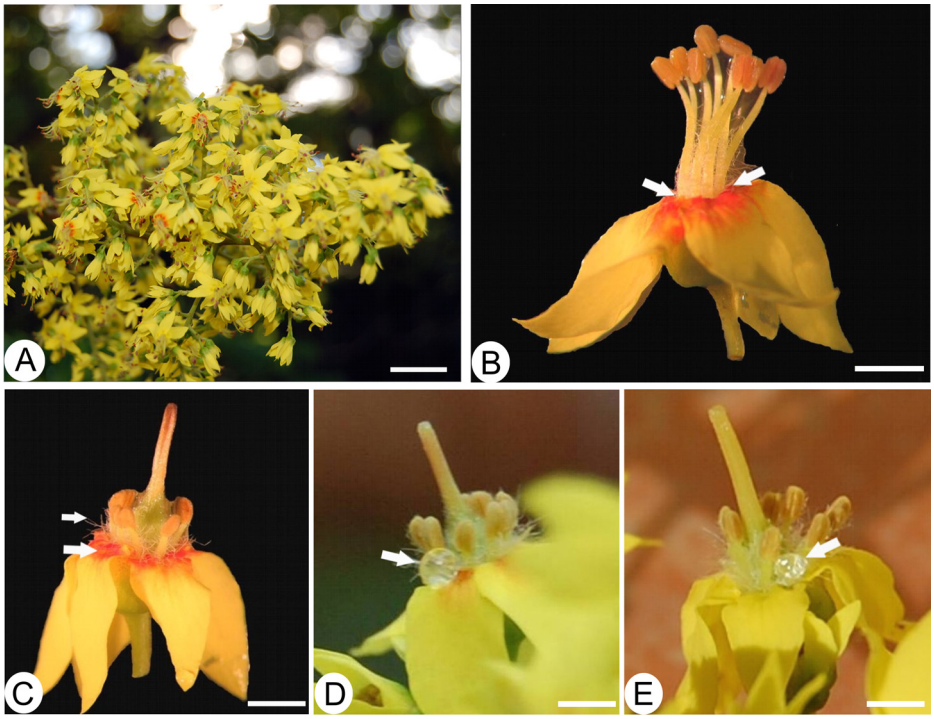

Fig. 1. *Koelreuteria elegans* subsp. *formosana*. A: Inflorescence at the beginning of staminate phase. B: Staminate flower on the second day of anthesis showing long exerted stamens, petals with the crest and the blade base reddish (arrows). C: Pistillate flower on the second day of anthesis showing part of gynoecium surrounded by short stamens, petals with the crest and the blade base reddish (arrows) and trichomes of stamen filaments (arrows) also protect evaporation of nectar. D: Pistillate morph on the first day of anthesis at noon, a drop of nectar is visible (arrow). E: Pistillate morph on the second day of anthesis at afternoon, a drop of nectar is visible (arrow). Scales: A: 10 mm; B-E: 0.5 mm.

2.5. Analysis of nectar

The mean duration of the flower lifespan was calculated from eight individuals. Five flowers per plant were marked and checked every 3 h from anthesis to flower senescence. Nectar was extracted using glass capillaries from open flowers of both morphs in two consecutive days. Flowers were covered with cloth bags to prevent visitors from having access to the nectar. Four tree were sampled; the nectar was collected from anthesis to floral senescence during different time of day: in the morning from 7:00 to 9:00 a.m.; at noon from 12:00 to 2:00 p.m.; and in the afternoon from 6:00 to 8:00 p.m. The nectar volume was calculated with capillary tube of 75 mm length and of 80 μl, a total of 60 samples per morph was collected for this analysis, each sample contained nectar gathered from 1 flower.

The sugar concentration (% sucrose w/w) was measured with a hand refractometer Arcano, REF 103 (range 0–32%). A total of 24 samples per morph was measured, each sample contained nectar gathered from 20 flowers. Dilutions were performed as necessary to keep the concentration readings within the range of the refractometer.

2.6. Statistical analysis

The statistical differences corresponding to nectar volume and sugar concentration in both pistillate and staminate flower at the same time of the day (morning, noon and afternoon) and at different moments within each time of the day were estimated using an analysis of variance (ANOVA), at significance level of 5% ($P \leq 0.05$). Whenever the ANOVA test indicated a significant difference, a pairwise comparison of means by Fisher's least significant different (LSD) (Sokal and Rohlf, 1995) was performed. All statistical analysis was performed using software Infostat (Di Rienzo et al., 2009).

3. Results

3.1. Floral morphology

Koelreuteria elegans subsp. *formosana* has yellow flowers, 5-6 mm in length, grouped in a paniculiform inflorescence (Fig. 1A). The species has two types of flowers in the same inflorescence: staminate flower and another type that is morphologically perfect but functionally pistillate with indehiscent anthers and non-functional pollen grains, (Fig. 1B–D); both morphs last for two days. The inflorescence showed a duodichogamic sequence of flowering (pistillate-staminate-pistillate). Though rarely, two flower morphs overlapped within the same individual. The flowers are actinomorphic, nectariferous and scented; however, staminate flowers are considered secondary zygomorphic after curvature of the stamens, an event occurring on the first day of anthesis. The calyx consists of 5 sepals partially fused at the base. The corolla is choripetalous, 5-merous, with clawed petals exhibiting a fleshy crest at the base of the blade; the crest is yellow and then becomes red at the second day of anthesis. The flower has a thimble-shaped and lobed nectary disc of receptacular origin, which hinders the differentiation of the androgynophore (Fig. 2A–C). The calyx together with the connivent pilose petal claws delimit a pseudo-tube where nectar accumulates. The arrangement of the fleshy crests together with the petal claws and stamen filament trichomes avoid evaporation of nectar. The main differences between the two types of floral morphs are the long exerted stamens and gynoecium reduced to a pistillode in staminate flowers (Fig. 1B), whereas in the pistillate

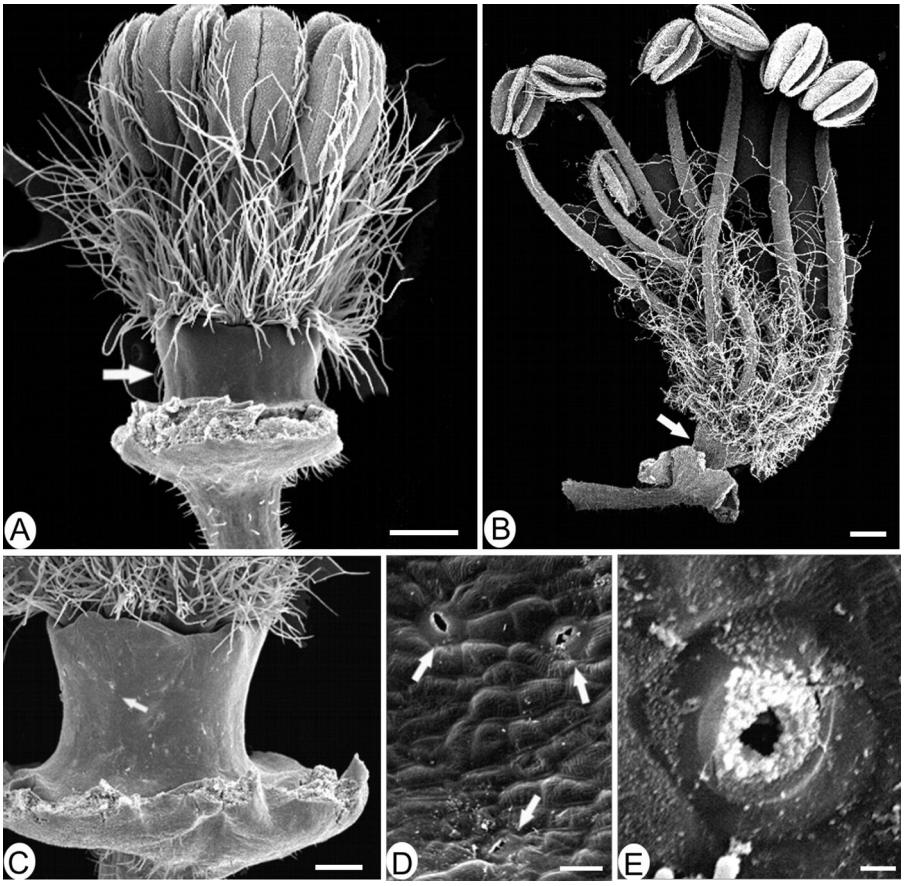

Fig. 2. *Koelreuteria elegans* subsp. *formosana*. Photomicrographs of floral nectary obtained with scanning electronic microscope. *Pre-anthesis stage*. A: Bud pistillate morph (3.8 mm length) devoid of perianth; nectary (arrow). *Anthesis stage*. B: Staminate flower devoid of perianth; nectary (arrow). C: Floral nectary in staminate flower, a nectarostoma (arrow). D: Epidermis showing nectarostomata in staminate floral nectary (arrows). E: Detail of nectarostoma with residual secretion in pistillate floral nectary. Scales: A–B: 0.5 mm; C: 5 μm; D–E: 10 μm.

flowers (Fig. 1C), the stamens are short, the anthers indehiscent, and the gynoecium is 3-carpelar with 2 ovules per locule.

3.2. Floral nectary

3.2.1. Morphology

In both floral morphs, the nectary is extrastaminal, receptacular and persistent (Fig. 2A–C); the yellow-green color is maintained throughout pre-anthesis, anthesis and post-anthesis stages.

The SEM observations revealed sub-rectangular epidermal cells with striated cuticle, and few anomocytic nectarostomata, i.e., non-functional stomata secreting nectar, located in the middle of the nectary (Fig. 2D–E).

3.3. Analysis of nectar

During the first day of anthesis the presence of nectar was observed in both morphs at different moments of day. However, on the second day the nectar was observed only at noon (Fig. 1D) and in the afternoon (Fig. 1E). Nectar was secreted by all individual flowers in an inflorescence simultaneously. The analysis of variance corresponding to total nectar volume in both floral morphs at different moments of the day showed significant difference between ($P > 0.05$) (Table 1). The pistillate morph produced an average of 2.73 μl ± 0.68 and the staminate morph 1.64 μl ± 0.62. The nectar volume was significantly lower in the morning (1.98 μl ± 0.75) than those in the noon (2.28 μl ± 0.97) and afternoon (2.30 μl ± 0.79) (Fig. 3).

Table 1
Analysis of Variance for Nectar volume (μl). Abbreviations: SS: Sum of squares; gl: Degree of freedom; MS: Mean squares; F: Value F-ratio.

Sources of variation	SS	gl	MS	F	p-value
Flower type	35.21	1	35.21	85.73	<0.0001
Moment	2.62	2	1.31	3.19	0.0450

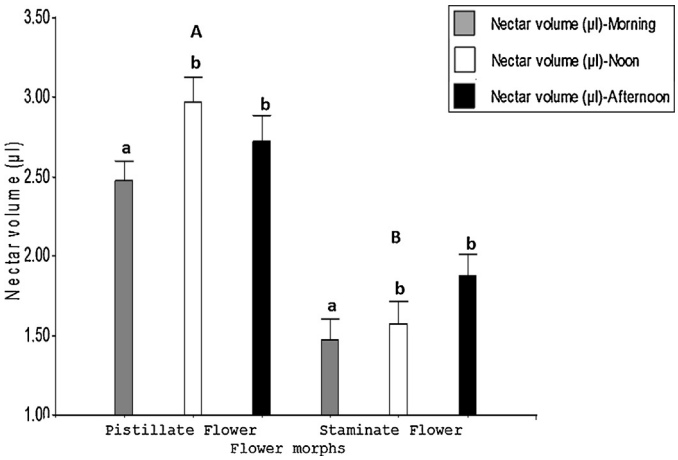

Fig. 3. Bar graph corresponding to total nectar volume (μl) in both floral morphs produced at different moments (morning, noon and afternoon). Different letters indicate statistically significant differences ($p \leq 0.05$). Abbreviations: A–B: flower morph (pistillate and staminate flower); a–b: different moments.

Table 2
Analysis of Variance for Sucrose concentration (w/w). Abbreviations: SS: Sum of squares; gl: Degree of freedom; MS: Mean squares; F: Value F-ratio.

Sources of variation	SS	gl	MS	F	p-value
Flower type	1.69	1	1.69	0.34	0.5630
Moment	1.17	2	0.58	0.12	0.8895

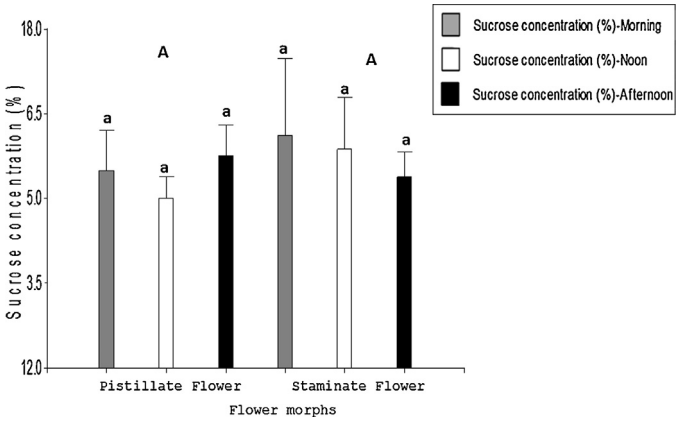

Fig. 4. Bar graph corresponding to total sucrose concentration (%) in both floral morphs produced at different moments (morning, noon and afternoon). Equal letters indicate no significant differences ($p \leq 0.05$). Abbreviations: A–B (capital letters): flower morph (pistillate and staminate flower); a–b (lowercase letters): different moments.

The sugar concentration (% sucrose w/w) no showed significant difference between both pistillate (15.42% ± 1.56) and staminate morphs (15.79% ± 2.66) (Table 2). Moreover, there were no significant differences between the sugar concentrations at the different moments: morning (15.81% ± 2.97); noon (15.44% ± 1.97) and afternoon (15.56% ± 1.41) (Fig. 4).

3.4. Anatomy

3.4.1. Epidermis

The unistratified epidermis has quadrangular cells, with dense cytoplasm and nucleus in parietal position, as evident in longitudinal (Fig. 5A–D) and transverse sections (Fig. 6A). The cuticle is thin and the nectarostomata may occur at the level of or sunken below the adjacent epidermal cells (Fig. 6E–F).

3.4.2. Secretory parenchyma

Longitudinal sections revealed that the secretory parenchyma prevails over the underlying non-secretory parenchyma (Fig. 5B). In transverse section, at anthesis, the secretory cells are isodiametric, small, and compact; the walls are thin, with dense granular cytoplasm and conspicuous nucleus; and intercellular spaces were observed. At post-anthesis, secretory cells become disorganized and the secretory parenchyma was subsequently degraded in both types of flowers (Fig. 5F). The nectary corresponds to a structured type because it presents a differentiated nectary parenchyma.

3.4.3. Non-secretory parenchyma

This parenchyma is underlying the secretory parenchyma; presents larger cells of polygonal contour, less dense cytoplasm and fewer parietal nuclei than the secretory parenchyma. The cells delimit small intercellular spaces (Fig. 5B-C).

3.4.4. Vascularization

The nectary is innervated only by phloem traces deriving from the central stele; the phloem is composed of short sieve-tube elements which may be branched, smaller companion cells and large parenchyma cells (Fig. 5D–E).

3.4.5. Ultrastructure

Ultrastructure studies revealed that both pistillate and staminate flowers have a floral nectary with similar characteristics. At the pre-anthesis and anthesis stages, secretory parenchyma cells have a very dense cytoplasm with few amyloplasts (Fig. 6B). Abundant mitochondria, RER and numerous plasmodesmata are observed. Many of the intercellular spaces of this secretory parenchyma are occupied by an electron-dense content (Fig. 6C). In the epidermal cells, subcuticular cavities of the outer tangential cell walls show similar electron-dense (Fig. 6B). At post-anthesis, the presence of a large vacuole in cells of nectary tissue is observed (Fig. 6D). A slow cell degradation process begins (Figs. 5F and 6E-F).

4. Discussion

The nectaries can have different structures; they may consist of a secretory parenchyma secreting the nectar via intercellular spaces and then exuded through modified stomata or nectarostomas (Fahn and Shimony, 2001), or they may secrete via a modified epidermis with or without secretory trichomes (Cristóbal and Arbo, 1971; Arbo, 1972; Nepi, 2007).

The floral nectary in the staminate and pistillate flowers of *K. elegans* subsp. *formosana* is structured and consists of three histological components: epidermis, secretory parenchyma and vascular system (Zimmermann, 1932; Nepi, 2007). This feature is also shared with other species of Sapindaceae (Solís and Ferrucci, 2009; Zini et al., 2014). According to Smets (1986), the floral nectary in the species here studied is persistent; this character is shared among the species of Sapindaceae and is considered an apomorphy in Eudicots. According to the classification system proposed by Fahn (1988), the floral nectary of *K. elegans* is of thalamic type because it develops on the thalamus or receptacle. In the order Sapindales, the receptacular nectary would be interpreted as a synapomorphy (Gadek et al., 1996).

Davis and Gunning (1993) reported the presence of stomata in the floral nectary in more than 75 families of angiosperms. Fahn (1979) mentioned that the nectaries exuding nectar through stomata generally possess a differentiated nectariferous tissue. Ronse Decraene et al. (2000) studied floral ontogeny and anatomy in *Koelreuteria paniculata* and indicated the absence of the nectarostomata in the floral nectary. These authors analyzed preanthetic buds, possibly did not observe the nectarostomata because of their reduced number. However, the floral nectary of *K. elegans* subsp. *formosana* presents few nectarostomata, suggesting that the nectar is exuded through the modified stomata which are of anomocytic. The presence of nectarostomata is a character shared with other species of different tribes of Sapindaceae, such as: *Litchi chinensis* Sonn. (Ning and Wu, 2005); *Cardiospermum grandiflorum* Sw. and *Urvillea chacoensis* Hunz. (Solís and Ferrucci, 2009); and *Cardiospermum heringeri* Ferrucci and *C. integerrimum* Radlk. (Zini et al., 2014). We observed that in *K. elegans* subsp. *formosana* the nectarostomata may be sunken below or at the level of the epidermal cells. The other species analyzed fit in the subfamily Sapindoideae (Solís and Ferrucci, 2009; Zini et al., 2014). Moreover, the distribution of nectarostomata in *K. elegans* subsp. *formosana* is restricted only to the middle of the floral nectary, where they are scarce, whereas in Paullinieae species, the nectarostomata occur mostly on the top of two horn-like lobes, with the number decreasing towards the base, and may be located at the base of the androgynophore, as in *C. grandiflorum*, or on the adaxial face of the posterior lobe, as in *U. chacoensis* (Solís and Ferrucci, 2009). Otherwise, in *L. chinensis*, tribe Nephelieae, sporadic nectarostomata are distributed on the

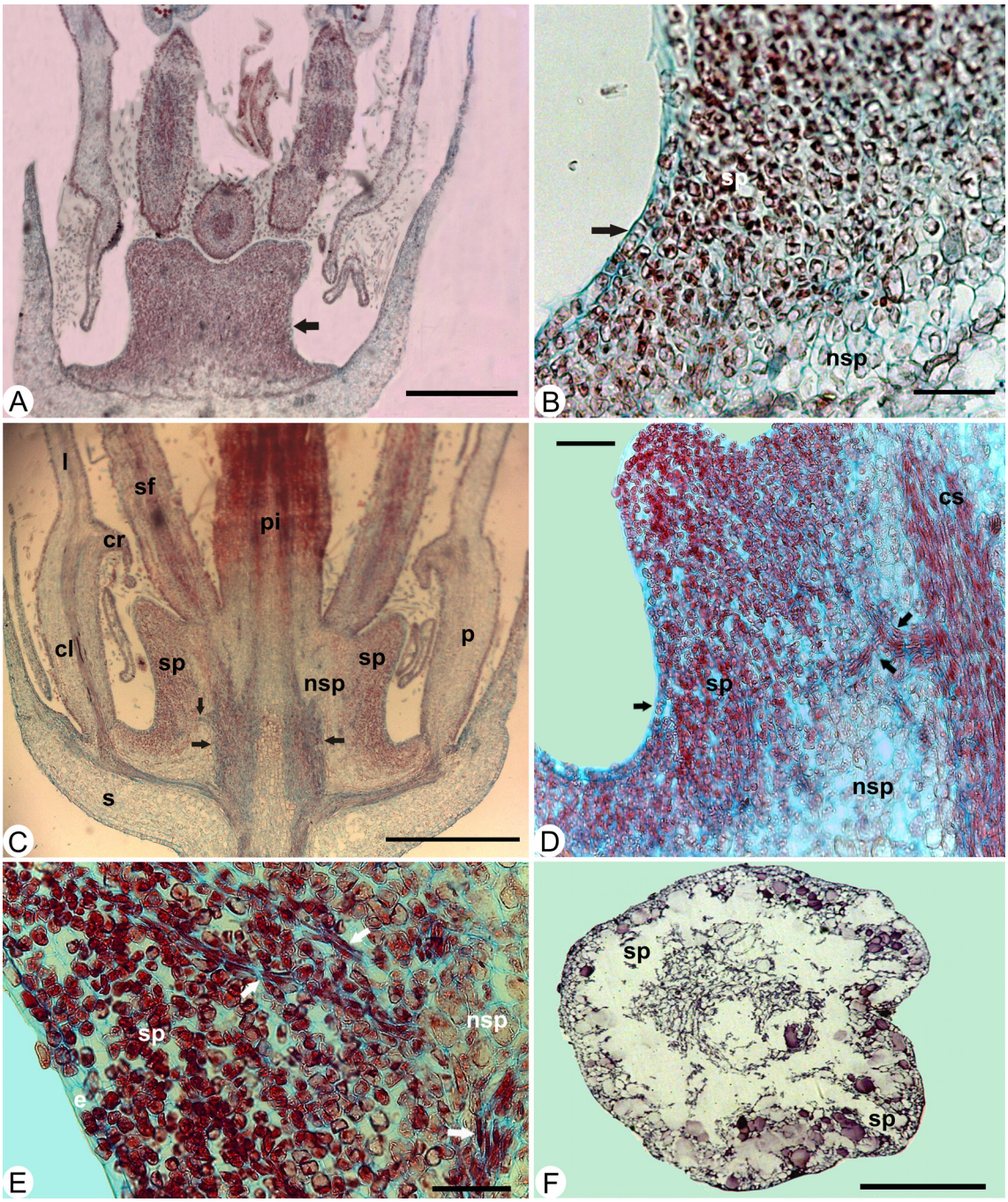

Fig. 5. *Koelreuteria elegans* subsp. *formosana*. Anatomy of floral nectary. *Anthesis stage.* A-E: Longitudinal sections; *Post-anthesis stage*. F: Transverse section. A: Tangential longitudinal secretion of floral nectary (arrow) in pistillate flower. B: Tissues of the basal part of the nectary in pistillate flower: uniestratificate epidermis (arrow), secretory parenchyma (sp) and non-secretory parenchyma (nsp). C: Floral nectary in staminate flower, showing secretory parenchyma (sp), the vascularization (arrows), non-secretory parenchyma (nsp), sepals (s), petals (p) formed by claw (cl), crest (cr) and limb (l), stamen filaments (sf) and part of the pistillode (pi). D: Floral nectary in the staminate flower, shows a central stele (cs) with a branch (arrows), secretory parenchyma (sp) and non-secretory parenchyma (nsp). E: Detail of phloem in staminate morph, the phloem is composed of short sieve elements which may be branched (arrows) innervating the secretory parenchyma (sp). F: Floral nectary of pistillate flower in post-anthesis stage showing the secretory parenchyma cells (sp). Scales: A, C: 500 μm; B, D, F: 100 μm; E: 500 μm.

upper surface of nectary disc (Ning and Wu, 2005). The variability observed in the location of the nectarostomas in the analyzed species would be an inherent adaptive trait of them that could be related to pollinators.

According to Frey-Wyssling (1955), the floral nectary of *K. elegans* subsp. *formosana* would be advanced because it has its own vascular tissue, a character that might be related to the nectary size. The floral nectary of the studied species is supplied by phloem traces of receptacle origin, a trait shared by all the Sapindaceae species analyzed (Ning and Wu, 2005; Solís and Ferrucci, 2009; Zini et al., 2014). According to Antoń and Kamińska (2015), who analyzed the structure and ultrastructure organization of the nectary spurs of four species of Ranunculaceae, the sugar concentration is positively correlated with the amount of phloem elements present in the nectaries, and this is in accordance with the views expressed by earlier researchers (Frey-Wyssling, 1955;

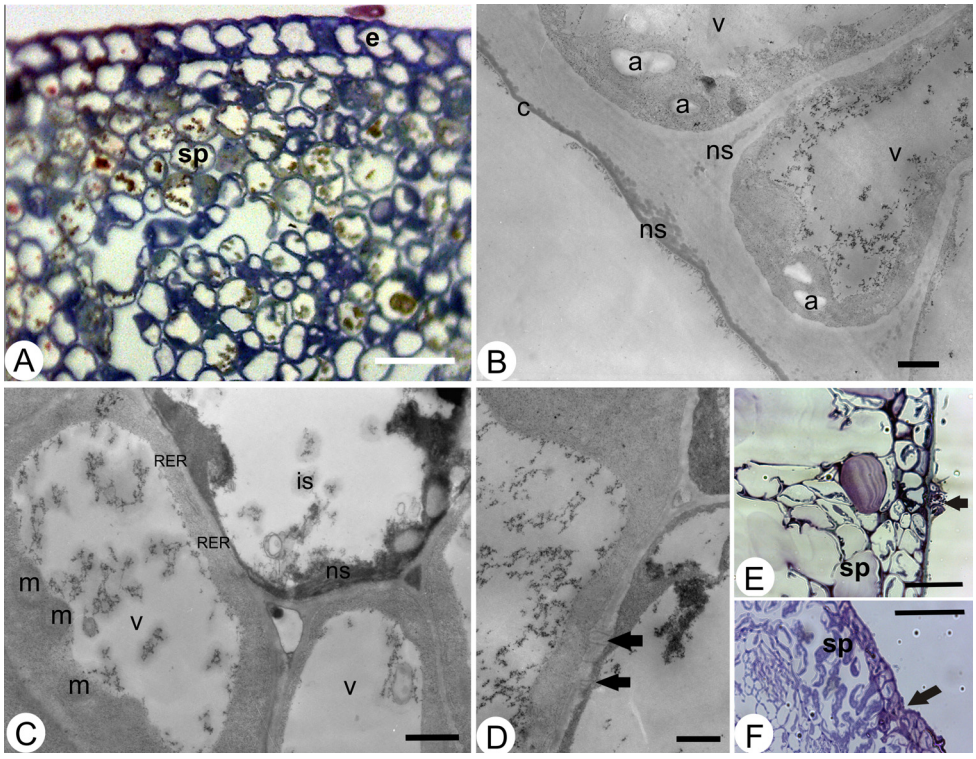

Fig. 6. *Koelreuteria elegans* subsp. *formosana*. Photomicrographs of floral nectary obtained with bright-field microscope and transmission electron microscope (TEM). *Antesis stage.* A: Bright-field microscope. Detail of the secretory parenchyma (sp) in staminate flower, and the uniestratificate epidermis (ep). *Anthesis stage.* B: TEM. Detail of epidermal cells of the pistillate floral nectary, showing the nectar secretion (ns) below the cuticle (c) and presence of amyloplast (a). *Post-anthesis stage*. C–D: Detail of the secretory parenchyma cells in pistillate floral nectary, showing large intercellular spaces (is) with nectar secretion (ns) and presence of mitochondria (m), vacuoles (v) and rough endoplasmic reticulum (RER) in the cytoplasm. D: Presence of numerous plasmodesmata (arrows) in the cells walls of the staminate floral nectary. *Post-anthesis stage*. E–F: Detail of a nectarostoma (arrow) in the staminate flower nectary, the secretory parenchyma cells (sp) are partly degraded. F: Detail of a nectarostoma (arrow) in the pistillate floral nectary, the secretory parenchyma cells (sp) are partly degraded. Scales: A, E–F: 50 μm; B–D: 1 μm.

Fahn, 1979). Moreover, Frey-Wyssling (1955) noted that vascularized nectaries present elements of relatively short sieve tubes, a character that matches the present observations.

In the regarding to nectar secretion in the species analyzed, in the first day of anthesis was observed at the different moments of day. Whereas in the second day was detected nectar only at noon and in the afternoon. Nectar volume was significantly higher in pistillate flower than staminate. Moreover, nectar volume was higher at noon and in the afternoon than in the morning. Concerning to sucrose concentration, it did not show significant differences between both floral morphs as well as different moments of day. However, Appanah (1982) who analyzed the nectar composition in the androdioecious *Xerospermun intermedium* Radlk. (Sapindaceae) found differences in sucrose concentration between pistillate and staminate flowers. This author suggested that these differences between the two sexes in dioecious species may promote inter-tree movement of pollinators. Moreover, Plowright (1987) suggested that evaporation may play part in increasing nectar concentration but the pseudo-tube formed in *K. elegans* subsp. *formosana* together with the arrangement of fleshy crest and stamen filament trichomes prevents the nectar evaporation. Therefore the nectar concentration remains constant during the two days of anthesis. These results constitute the first comprehensive contribution on nectar secretion in Sapindaceae.

The studies on the ultrastructure of floral nectary in staminate and pistillate flowers of *K. elegans* subsp. *formosana* revealed that ultrastructural characteristics are similar in both floral types. This is reflected in the similar sucrose concentration between two floral morphs, coinciding with Mosti et al. (2013) who propose that the ultrastructural features are linked to the nectar composition. At the pre-anthesis stage the secretory parenchyma cells present numerous plasmodesmata in the specie examined here, according to Mosti et al. (2001) who analyzed the ultrastructure of the hypanthial epithelium in *Selenicereus grandiflorus* (L.) Britton & Rose (Cactaceae), the plasmodesmata connecting the epithelial cells assure a uniform development of these cells, indicating the necessity of an exchange of substances among the epidermis and secretory cells. The secretory parenchyma cells at the anthesis stage contain amyloplasts; then at the post-anthesis stage these organelles gradually disappear as occur in three species of *Tillandsia* L. (Bromeliaceae) because of apoptosis or programmed cell death (PCD) (Mosti et al., 2013). These findings agree with observations in *Prunus persica* (L.) Batsch (Radice and Galati, 2003). According to Nepi et al. (1996), the presence of amyloplasts at the pre-secretory stage is a feature of many floral nectaries. Some authors, such as Durkee et al. (1981), Zer and Fahn (1992), Fahn and Shimony (2001), suggest that the source of pre-nectar is the phloem sap and the carbohydrate component is stored in the amyloplasts of nectary parenchyma. This phenomenon might be present in *K. elegans* subsp. *formosana*, as observed in the floral nectary of staminate and pistillate flowers. On the other hand, studies in *Swietenia macrophylla* King showed that starch reserves for the production of nectar are stored outside the nectary, the latter being responsible for changing nectar composition (Paiva, 2012). Moreover, the mechanism of nectar exudation is related to the origin of the secretory cells, which may be parenchymal or epidermal cells (Konarska, 2015). In *K. elegans* subsp. *formosana*, the nectar would diffuse through the intercellular spaces of secretory parenchyma cells; then it would be exuded through nectarostomata present in the middle of the floral nectary, or through the outer tangential wall of epidermal cells in which has been observed subcuticular cavities with nectar secretion. Regarding the cytological features of

the secretory cells, the presence of abundant mitochondria suggests a high level of cellular respiration, which would be related to the secretion of nectar (Fahn and Rachmilevitz, 1970). At anthesis, the cytoplasm of the secretory cells in *K. elegans* subsp. *formosana* is dense and has abundant amyloplasts and mitochondria; these cytological features are associated with high metabolic activity, which is reflected in the sugar concentration above 15% in both floral morphs. This is the first study of nectaries within Sapindaceae involving transmission electron microscopy observations.

The structure of the floral nectary in the species here examined is essentially similar to the others species investigated in Sapindaceae, in contrast with the broad variation in floral morphology. This finding is consistent with that reported by Nores et al. (2013), which found that the basic structure of the nectary is a relatively conservative trait in Nyctaginaceae, independent of the primary group of flower visitors and other floral traits. These authors suggested that relatively low variation in nectary traits compared with the relatively broad variation in flower morphology, shape and color, indicate that selective pressures are not uniform among floral characteristics. Moreover, Baker and Baker (1982, 1983) propose that nectar traits are sometimes associated with taxonomic families, suggesting phylogenetic constraints had a role in nectar evolution. However, more study of nectar and nectary as well as a more-resolved phylogeny are necessary to determine the likelihood that the species of Sapindaceae exhibit phylogenetic constraints in nectar traits.

The results of this study allow us to conclude that morpho-anatomical and ultrastructural characteristics of the floral nectary in *K. elegans* subsp. *formosana* help to characterize this species and would be of diagnostic value at generic or higher taxonomic hierarchies within the family.

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