



# Four o'clock pollination biology: nectaries, nectar and flower visitors in Nyctaginaceae from southern South America

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Floral nectary structure and nectar sugar composition were investigated in relation to other floral traits and flower visitors in contrasting species of Nyctaginaceae from southern South America, representing four tribes (Bougainvilleae, Colignoniae, Nyctagineae, Pisoneae). Our comparative data will aid in the understanding of plant–pollinator interactions and in the development of hypotheses on the origin of floral and reproductive characters in this family. The nectaries are located on the inner side of the staminal tube. The nectariferous tissue is composed of an epidermis and three to ten layers of secretory parenchymal cells, supplied indirectly by the filament vascular bundles. Stomata appear to be associated with nectar secretion. For the first time in Nyctaginaceae, nectary ultrastructure is described in *Boerhavia diffusa* var. *leiocarpa*. Nectary parenchyma cells are densely cytoplasmic and contain numerous starch grains. Plasmodesmata connect the nectariferous cells. Flowers of Nyctaginaceae secrete a small volume of nectar of variable concentration (10–47%). Nectar is dominated by hexoses, but *Mirabilis jalapa* showed a balanced proportion of sucrose and hexoses. Hymenoptera are the most common visitors for most species; nocturnal Lepidoptera are the most common visitors for *M. jalapa* and *Bougainvillea stipitata*. We found relatively low variation in the nectary characteristics of Nyctaginaceae compared with broad variation in flower structure, shape, colour and nectar traits. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **171**, 551–567.

**ADDITIONAL KEYWORDS:** insect pollination – nectar composition – nectary structure – nectary ultrastructure – reproductive biology.

## INTRODUCTION

Plant species that depend on animal pollinators for their reproduction have evolved many complex phenotypes determined by traits such as floral display, flower architecture, colour, scent and nectar. Such sets of morphological and functional traits constitute pollination syndromes and can serve as general hypotheses for the prediction of different groups of pollinators visiting flowers (Proctor, Yeo & Lack, 1996). Pollination syndromes can differ even between closely related species, raising questions as to how

evolutionary change can occur in a group of plants that interact with a particular guild of pollinators, and which traits are more resilient to change. Floral characters appear to change relatively easily during evolutionary time compared with nectar traits, which are comparatively resilient to change (Agostini, Sazima & Galetto, 2011).

Floral nectaries can be located on a wide range of floral organs and can display a variety of forms and structures (Zandonella, 1977; Bernardello, 2007). The diversity of nectaries is, to some extent, associated with the varying morphology and behaviour of pollinators (Bernardello, 2007; Nepi, 2007). Conversely, nectary structure can be conserved in a lineage on account of phylogenetic constraints (Galetto, 1995;

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Galetto & Bernardello, 2004). Nectar-secreting tissues appear to be conservative in their generalized traits in different groups of plants (e.g. Smets, 1988; Bernardello, Galetto & Juliani, 1991; Galetto, 1995; Bernardello, Galetto & Anderson, 2000; Galetto & Bernardello, 2004).

Variation in nectar quantity and quality can influence the behaviour of pollinators in their visits to flowers (e.g. Real & Rathcke, 1988; Mitchell & Waser, 1992). In turn, this variation can contribute differentially to plant fitness. Based on this trade-off, differences in nectar characteristics, such as sugar composition and concentration, can occur in related species with different pollinators (e.g. Baker & Baker, 1983; Cruden, Hermann & Peterson, 1983; Freeman, Worthington & Corral, 1985; Nicolson & Thornburg, 2007). For example, nectar that attracts butterflies and nocturnal hawkmoths is rich in sucrose, whereas high proportions of glucose and fructose are characteristic of species pollinated by flies and short-tongued bees (e.g. Baker & Baker, 1983; Galetto & Bernardello, 2003). In broad terms, insect-pollinated flowers produce concentrated nectar, whereas flowers pollinated by birds generally produce relatively dilute nectar; dilute nectars are also characteristic of hawkmoth-pollinated species (Pyke & Waser, 1981; Baker & Baker, 1983). Nevertheless, in some plant groups (e.g. Verbenaceae, Onagraceae and Fabaceae), the available data indicate that sugar composition is a conservative character that reflects phylogenetic constraints (e.g. Galetto & Bernardello, 2003; Nicolson, 2007). In other cases, nectar characteristics are not associated with pollinators or taxonomic affiliation, but are associated with historical or environmental factors (Forcone, Galetto & Bernardello, 1997).

The 'four o'clock' family Nyctaginaceae (Caryophyllales) displays a wide range of floral traits to attract pollinators, including different fragrances, visual cues and diurnal or nocturnal anthesis (Valla & Ancibor, 1978; Levin, Raguso & McDade, 2001; López & Galetto, 2002; Fenster *et al.*, 2004). Nyctaginaceae includes 28–31 genera and 300–400 species (Bittrich & Kühn, 1993; Mabberley, 1997) in tropical and subtropical regions worldwide, with two major centres of distribution in the Americas (the Neotropics and Caribbean; arid western North America). Based on morphology, the family was classified by Bittrich & Kühn (1993) into six tribes. However, molecular phylogenetic analyses (Levin, 2000; Douglas & Manos, 2007) have led to a new monophyly-based classification with seven recircumscribed tribes (Nyctagineae, Pisonieae, Bougainvilleae, Colignonieae, Boldoeae, Leucastereae and Caribeae) (Douglas & Spellenberg, 2010).

Data on floral traits and visitors have been documented for cultivated and North American species of Nyctaginaceae, but are less well known for species

from southern South America. The range of flower visitors recorded for the family mainly includes Hymenoptera, but also Lepidoptera (mostly Sphingidae and diurnal butterflies), Diptera and Coleoptera (Melyridae and Nitidulidae) and, exceptionally, hummingbirds (Trochilidae; e.g. Baker, 1961; Tillett, 1967; Gillis, 1976; Grant, 1983; Bohlin, 1988; Bittrich & Kühn, 1993; Spellenberg, 2000; Levin *et al.*, 2001). Wind pollination has also been proposed for members of Pisonieae (Bullock, 1994). In general terms, for the small number of species examined, the nectariferous tissue is located at both the inner side of the staminal tube and the base of the gynoecium (e.g. Bonnier, 1879; Zandonella, 1972, 1977; Rohweder & Huber, 1974; Valla & Ancibor, 1978; Vanvinckenroye *et al.*, 1993). Furthermore, nectar characteristics are known for relatively few species (e.g. Bonnier, 1879; Percival, 1961; Valla & Ancibor, 1978; Forcone *et al.*, 1997; López & Galetto, 2002). For example, nectar rich in sucrose was found in the hawkmoth-pollinated species *Mirabilis longiflora* L. (Grant, 1983; Freeman *et al.*, 1985) and the purported butterfly-pollinated species *Nyctaginia capitata* Choisy (Freeman & Worthington, 1985), suggesting a relationship between nectar composition and pollinator type.

Here, we present a detailed study of nectary structure and some data on nectar composition in relation to other floral traits and flower visitors in wild-source Nyctaginaceae from southern South America in order to improve the understanding of plant–pollinator relationships in this family. We examine species showing contrasting flower morphology representing four tribes of this diverse family. In particular, we address the following questions. Is the structure of the nectary conserved or diverse in species with contrasting flower morphology and displaying different floral traits to attract pollinators? Which are the main flower visitors of selected contrasting species? Are the nectary structure and nectar sugar composition and concentration related to flower visitors in these species? We predict similar nectary and nectar characteristics independent of flower visitors if these traits are conserved across the family. Conversely, we predict differences in nectary and nectar characteristics if these traits are related to flower visitors. We integrate novel data from a range of different sources that will aid in the understanding of plant–pollinator interactions and in the development of hypotheses on the origin of floral and reproductive characters in Nyctaginaceae.

## MATERIAL AND METHODS

### MATERIAL

The species examined are listed in Table 1. We examined 21 Argentinian taxa from seven genera and four

**Table 1.** Species analysed in this study. Taxa were arranged according to Douglas & Spellenberg (2010)

Tribe	Species	Locality (province): department: locality	Voucher no.	Data taken
Bougainvilleaceae (3)	<i>Bougainvillea campanulata</i> Heimerl	Salta: La Viña: Alemania	Galetto <i>et al.</i> 172	F, LM, V
	<i>Bo. infesta</i> Griseb.	Chaco: Almirante Brown: nd	Fortunato <i>et al.</i> 6377	S
	<i>Bo. praecox</i> Griseb.	Chaco: General Güemes: nd	Fortunato <i>et al.</i> 6435	F, LM
	<i>Bo. spinosa</i> (Cav.) Heimerl	La Rioja: Coronel Felipe Varela: Talampayá	Chiariini 223	F, S
		Chubut: Rawson: Trelew	Forcone 25	NC*
		Chubut: Rawson: Isla Escondida	Forcone 26	NC*
	<i>Bo. stipitata</i> Griseb.	Córdoba: Colón: La Calera	López 130	LM†, NC†, V†
		Catamarca: Capayán: San Pedro	Nores 66	F, SEM
Pisonieae (7)	<i>Pisonia aculeata</i> L. var. <i>aculeata</i>	Formosa: Formosa: Ea. Guaycolec	Tressens & Marunak 6322	S
	<i>P. zapallo</i> Griseb. var. <i>guaranitica</i> Toursark.	Corrientes: Empedrado: Ea. Las Tres Marias	TMP 14574	F, LM, NC, V
	<i>P. zapallo</i> Griseb. var. <i>zapallo</i>	Catamarca: Paclín: Cuesta del Totoral	Cerana & Nores 2073, 2074	F, LM, V
	<i>P. arborescens</i> (Lag. & Rodr.) Standl. var. <i>glabrata</i> (Heimerl) Heimerl	Catamarca: Capayán: San Pedro	Nores 65	F, LM, SEM, ST
Colignonieae (1)	<i>Colignonia glomerata</i> Griseb. var. <i>glomerata</i>	Jujuy: Ledesma: Calilegua	Ahumada 5338	F, S
Nyctagineae (11)	<i>Boerhavia cordobensis</i> Kuntze‡	San Luis: nd: RN 79 km 52	Fortunato <i>et al.</i> 9900	F, S, V
	<i>B. diffusa</i> L. var. <i>diffusa</i>	Córdoba: Capital: Córdoba	Anton & López 325	S
	<i>B. diffusa</i> L. var. <i>leiocarpa</i> (Heimerl) C. D. Adams	La Rioja: Capital: Las Padercitas	Cerana & Nores 2078	F, LM, SEM, ST, TEM, V
	<i>B. pulchella</i> Griseb.	Córdoba: Colón: Saldán	Anton & López 323	F, LM, NC, V
	<i>B. torreyana</i> (S. Watson) Standl.	Catamarca: Tinogasta: Carrizal	Pedersen 15318	S
	<i>Allionia choisyi</i> Standl.	San Luis: Junín: RN 20, km 244	Fortunato <i>et al.</i> 9895	F, LM, NC, SEM, V
	<i>A. incarnata</i> L. var. <i>incarnata</i>	La Rioja: nd: RN 150, km 45	Fortunato <i>et al.</i> 9924	S
	<i>Mirabilis bracteosa</i> (Griseb.) Heimerl var. <i>bracteosa</i>	Salta: La Poma: La Quesera	Barboza <i>et al.</i> 733	S
	<i>M. bracteosa</i> (Griseb.) Heimerl var. <i>micrantha</i> Toursark.	Jujuy: Capital: Volcán Cerros Oeste	Cocucci <i>et al.</i> 2650	S
	<i>M. jalapa</i> L.	Córdoba: Colón: Villa Allende	Nores 68	F, LM, NC, SEM, V
	<i>M. ovata</i> (Ruiz & Pav.) F. Meigen	Mendoza: San Rafael: RN 144	Galetto & Torres 817	F, LM, NC, SEM, V

Number of genera in parentheses. Material was collected in Argentina and the voucher specimens are deposited at the Museo Botánico de Córdoba (CORD), Argentina. F, flower and inflorescence morphology; LM, light microscopy; NC, nectar chemistry; S, stereoscopic microscopy; SEM, scanning electron microscopy; ST, light microscopy semi-thin sections; TEM, transmission electron microscopy; V, flower visitors.

\*Extracted from Forcone *et al.* (1997).

†Extracted from López & Galetto (2002).

‡*Sensu* López & Anton (2006).

tribes of Nyctaginaceae according to the classification of Douglas & Spellenberg (2010). These tribes share a common ancestor (Douglas & Manos, 2007). Most of the taxa studied are endemic to South America, except for *Mirabilis jalapa*, *Boerhavia diffusa* L., *Pisonia aculeata* L. var. *aculeata* and both species of *Allionia* L., which are more widely distributed. For most species, fresh or herbarium specimens were examined using a stereoscopic microscope (Carl Zeiss, Jena, Germany). Selected species, with contrasting flower morphology, displaying different morphological traits to attract pollinators and representing the four tribes and most genera, were analysed in more detail (Table 1). We included species possessing different-sized flowers, one- to multi-flowered inflorescences, small to enlarged bracts, different coloured flowers or bracts, diurnal or nocturnal scent or anthesis, dioecy or monoecy, and self-compatibility or self-incompatibility (see examples in Fig. 1). Data on nectar sugar composition and flower visitors were obtained when possible (i.e. allowing for available resources for fieldwork to study flower visitors and to collect nectar).

#### LIGHT MICROSCOPY (LM)

Flowers were fixed in formalin–acetic acid–alcohol (FAA) or 70% ethanol, and stored in 70% ethanol. For sectioning, flowers were embedded in Paraplast (Sigma, St. Louis, MO, USA) using standard methods of wax embedding and serially sectioned using a rotary microtome. Sections were stained in safranin and Alcian blue, dehydrated through an alcohol series to 100% ethanol and then placed in Histoclear (National Diagnostics, Atlanta, GA, USA). In a few cases, flowers were embedded in Histoplast and stained with toluidine blue. Sections were mounted in DPX (Aldrich Chemical Company, Gillingham, Dorset, UK) and examined using a Zeiss Axiolab light microscope (Carl Zeiss).

To detect the presence of stomata on the nectary, the flowers were cleared with NaOH (10% aqueous solution), washed with acetic acid–water (1 : 3), dissected and extended over a slide, and finally stained with an aqueous I<sub>2</sub>–KI solution (Johansen, 1940).

#### SCANNING ELECTRON MICROSCOPY (SEM)

Flowers and buds were fixed in FAA or 70% ethanol, dehydrated in an ethanol series and carefully dissected. Dehydrated material was critical point dried using a Balzer CPD 020 (Balzer Union, Furstentum, Liechtenstein), mounted onto SEM stubs using double-sided sellotape, sputter-coated with platinum using an Emitech K550 Sputter Coater (Emitech Limited, Ashford, Kent, UK) and examined using a

Hitachi cold-field emission scanning electron microscope S-4700-II (Hitachi Co., Tokyo, Japan) at 2–5 kV.

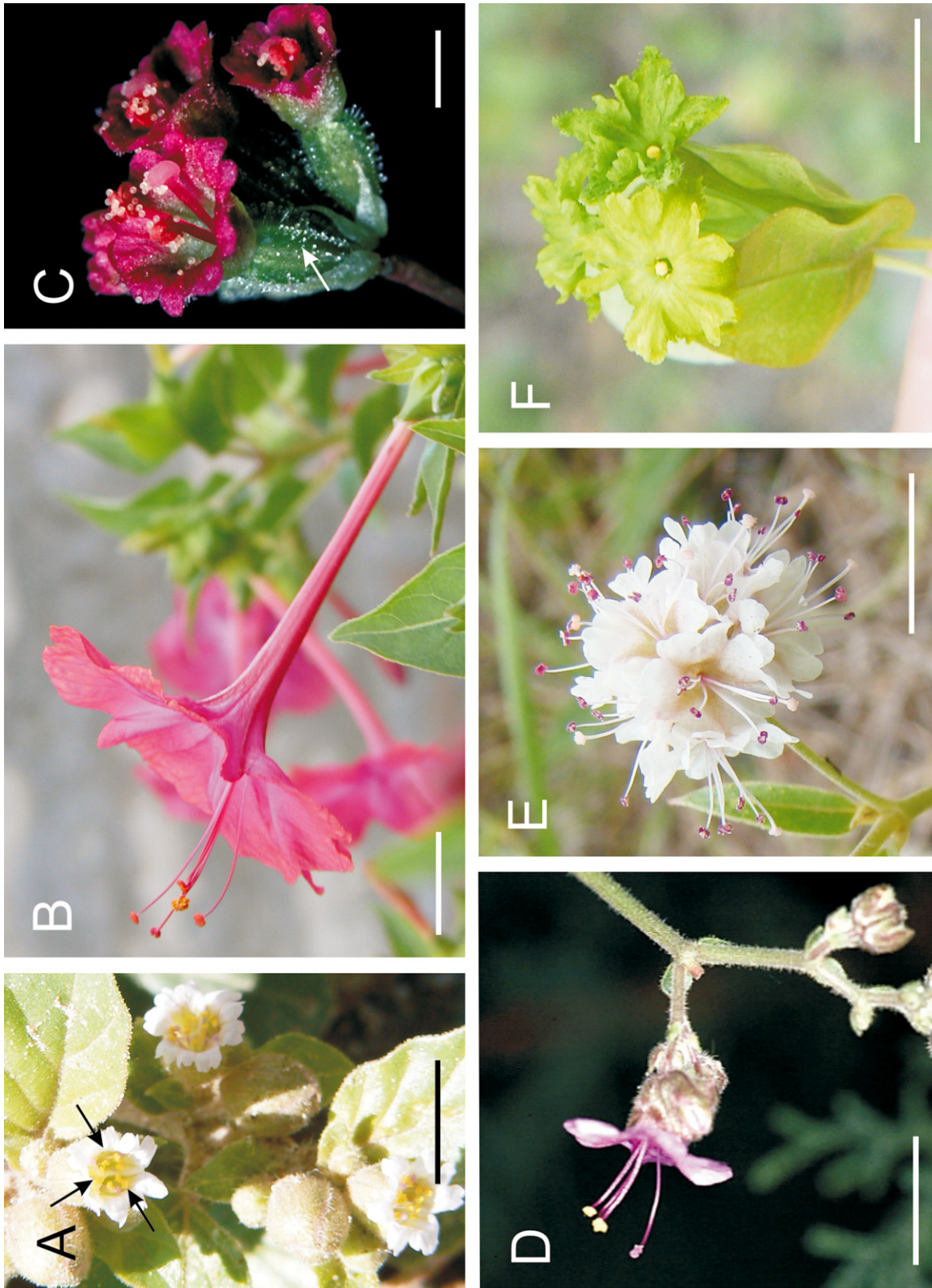
#### TRANSMISSION ELECTRON MICROSCOPY (TEM)

Flowers of *Boerhavia diffusa* L. var. *leiocarpa* (Heimerl) C.D. Adams were dissected, placed in fixative (2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0), de-aerated under vacuum and fixed overnight. They were washed in cacodylate buffer, fixed in 1% OsO<sub>4</sub>, washed again and dehydrated through a graded ethanol series. Samples were embedded in medium-grade LR white resin (London Resin Company, Reading, Berkshire, UK) in gelatine capsules. Semi-thin sections of approximately 1 µm were cut using a Reichert Ultracut (Leica, Milton Keynes, Buckinghamshire, UK) and a dry glass knife, stained with toluidine blue and mounted in DPX (Aldrich Chemical Company). They were examined using a Zeiss Axiolab light microscope (Carl Zeiss) employing normal bright-field optics. Ultrathin silver–gold interference colour serial sections were cut using a diamond knife, stained with uranyl acetate and lead citrate in an LKB Ultrastainer (LKB-Produkter AB, Bromma, Sweden) and examined using a JEOL JEM-1210 transmission electron microscope (JEOL, Welwyn Garden City, Hertfordshire, UK).

#### NECTAR SUGAR COMPOSITION

Nectar was withdrawn from the unbagged flowers using capillary glass tubes. Two variables were measured immediately: volume (µL), using graduated micropipettes, and sugar concentration (% sucrose : mass/total mass), with a pocket refractometer (Atago, Tokyo, Japan). The nectar was stored on Whatman #1 chromatography paper. Sugar separation was accomplished by gas chromatography. Nectar was lyophilized and silylated according to Sweeley *et al.* (1963). The derivatives were then injected into a Konik KNK 3000-HRGS gas chromatograph equipped with a Spectra-Physics SP 4290 data integrator, a flame ionization detector and an OV 101 3% column (length, 2 m) on Chromosorb G/AW-DMCS (mesh 100–120) (Konik, Barcelona, Spain). Nitrogen was the carrier gas (30 mL min<sup>-1</sup>) and the following temperature programme was used: 208 °C for 1 min, 1 °C min<sup>-1</sup> to 215 °C, 10 °C min<sup>-1</sup> to 280 °C for 2 min. Carbohydrate standards (Sigma) were prepared using the same method. Chromatographic sugar analyses were run at least twice for each sample. Sucrose and hexose ratios were calculated as follows: sucrose/(fructose + glucose) and glucose/fructose, respectively. For comparison, *Bougainvillea* data were obtained from previous work (Forcone *et al.*, 1997; López & Galetto, 2002).





**Figure 1.** Representative flowers and inflorescences of southern South American Nyctaginaceae with contrasting flower morphology and displaying different morphological traits to attract pollinators. A, *Allionia choisyi*, inflorescence with three zygomorphic flowers simultaneously opened (arrows indicate each flower). B, *Mirabilis jalapa*, large funnel-shaped flower. C, *Boerhavia diffusa* var. *leiocarpa*, small flowers with perianth constricted in the median portion. The lower part (arrow) persists in the fruit. D, *Mirabilis ovata*, campanulate flower. E, *Boerhavia pulchella*, partial glomerulate inflorescence. F, *Bougainvillea stipitata*, three-flowered inflorescence with yellowish-green enlarged bracts. Scale bars: C, 0.5 mm; A, B, D–F, 10 mm.

## FLOWER VISITORS

Data on visitors were obtained by diurnal and/or nocturnal observations for each species. Observations were recorded according to species and population abundances in different numbers of periods of 10 min. These observation periods were equally distributed, if possible, in the morning and afternoon on different sampling days. Floral visitors were captured and/or photographed for identification. Data for *Bougainvillea stipitata* Griseb. were taken from previous work (López & Galetto, 2002).

## RESULTS

## GENERAL MORPHOLOGY

The South American species examined are trees, shrubs, climbers or perennial herbs from different environments (e.g. semi-desert shrubland, semi-arid forest and woodland, forest). They possess either terminal or axillary cymose or racemose inflorescences (Fig. 1) with sequential flowering, although flowers of *Allionia choisyi* Standl. open simultaneously (Fig. 1A). Bracts are mostly present, sometimes small and early caducous, often enlarged, free or fused. For example, in *Mirabilis* L., the connate bracts form a calyx-like involucre (Fig. 1B); in *Bougainvillea*, the bracts are large and conspicuous, 10–25 mm long, yellowish-green, reddish or brownish, according to species (Fig. 1F). The number of flowers per inflorescence varies from one [*M. jalapa*, *Bougainvillea spinosa* (Cav.) Heimerl] to three [e.g. *A. choisyi*, *Mirabilis ovata* (Ruiz & Pav.) F.Meigen, other *Bougainvillea* spp.], three to five (*B. diffusa*) or ten to > 30 [e.g. *Boerhavia pulchella* Griseb., *Boerhavia cordobensis* Kuntze, *Colignonia glomerata* Griseb. var. *glomerata*, *Pisonia zapallo* Griseb., *Pisoniella arborescens* (Lag. & Rodr.) Standl. var. *glabrata* (Heimerl) Heimerl] (Fig. 1; Table 2). Flower size ranges from 1–15 mm in length in most species, 17–20 mm in *Bo. stipitata* and 30–60 mm in *M. jalapa* (Fig. 1; Table 2). Flowers are hermaphrodite, except for the dioecious species *Pisonia zapallo*, with unisexual flowers. The uniseriate petaloid perianth, composed of three to five fused tepals, is actinomorphic (zygomorphic in *A. choisyi*), sometimes constricted in the medial portion with the upper part often caducous after anthesis (Fig. 1C). The perianth is campanulate, funnel-shaped, tubular or salverform, yellowish-green, pink-reddish, fuchsia-purple, chestnut-brown or varied depending on the species. The lower part of the perianth encloses a superior ovary (Fig. 1C). The three to nine stamens (one to three in *B. diffusa*) are connate at the base, forming a staminal tube; in *P. zapallo*, the staminal tube is short as the filament bases are less fused. Filaments are mostly unequal and can be exerted or

included. The gynoecium is monocarpellate, unilocular, uniovulate, sessile or stipitate, with the stigma exerted or included. In *P. zapallo*, staminate flowers have fully developed stamens and the gynoecium is reduced to a pistillode; in pistillate flowers, the gynoecium is well developed and stamens are reduced to staminodes.

In *B. cordobensis*, we observed closed flowers (c. 2 mm flowers with stamens and stigma included, and fruit at maturity) in all herbarium specimens analysed and in multiple field observations. All fruits possess developed embryo and perisperm between cotyledons. Exceptionally, open flowers, 3.0–3.2 mm in length, were observed in one individual collected in Mendoza.

Most species display diurnal anthesis and *P. zapallo* emits diurnal scent. *Bo. stipitata* flowers open at sunset and last five days, emitting nocturnal fragrance during the first two days. *Mirabilis jalapa* anthesis and fragrance emission start at sunset and finish at noon.

## STRUCTURE OF FLORAL NECTARIES

To investigate nectary structure, we carried out LM or SEM (Table 1). In all species examined, the nectariferous tissue is located at the inner surface of the staminal tube (Figs 2A, F, G, J–L; 3A, C, E, H, K–R; 4A). The epidermis is composed of epithelial cells with a thin cuticle. In general, the nectary parenchyma consists of three to ten layers of secretory cells with thin walls, densely stained cytoplasm and large nuclei (Fig. 3B, D, F, G, I). The nectary parenchyma is indirectly supplied by the stamen vasculature; the arrangement of both phloem and xylem branches differs between species (Figs 3C, D, G, I, J, P, Q; 4B). Few stomata irregularly distributed over the nectary surface were observed (Figs 2B, H, L; 3B, D). In general, stomata remain open (Fig. 2C, I, M). Raphides in idioblasts are usually present (Figs 2E; 3K).

There are some differences between the species examined with respect to the development of nectary parenchyma, nectary size, shape, symmetry, relative position with respect to the gynoecium and stomata characteristics:

1. A conspicuous bowl-shaped nectary, composed of a multilayered nectary parenchyma, partially or completely surrounding the ovary, occurs in *B. pulchella*, *M. jalapa* and *M. ovata* (Figs 2G; 3H, K, L). On the upper region of the nectary of *M. jalapa*, interstaminal nectariferous bulges protrude, forming a nectariferous chamber where nectar can accumulate (Fig. 2H). Stomata are distributed at the inner and upper surfaces of the staminal tube.
2. A ring-shaped nectary is present in both flower types of *P. zapallo*. In staminate flowers, the nec-



**Table 2.** Flower visitors of Nyctaginaceae species

Species	Flower		Visitors			Visits <i>N<sub>v</sub></i> ( <i>t</i> )
	<i>N</i>	<i>L</i>	<i>N<sub>p</sub></i>	Order	Family (Genus/species)	
<i>Allionia choisyi</i>	3	3–6	36	Diptera Hymenoptera Lepidoptera	Syrphidae ( <i>Toxomerus</i> ) Apidae ( <i>Apis</i> , <i>Bombus</i> ) nd	5 (5) nd 1 (2)
<i>Boerhavia cordobensis</i>	10–30	2–3	30	–		
<i>B. diffusa</i> var. <i>leiocarpa</i>	3–5	±2	20	Hymenoptera	Apidae ( <i>Plebeia</i> ) Halictidae Vespidae [ <i>Polybia ruficeps</i> Schrott, <i>Polybia occidentalis</i> (Oliver), <i>Polistes versicolor</i> (Oliv.)]	5 (5) 1 (3) 5 (5.2)
<i>B. pulchella</i>	10–20	±8	20	Diptera Hymenoptera	Bombilidae Apidae ( <i>Apis mellifera</i> L., <i>Apis</i> , <i>Bombus</i> ) Halictidae [ <i>Augloclora phoemonoe</i> (Schrottsky), <i>Myschocyttarus</i> <i>deussenii</i> Richards] Tachinidae	6 (3) 4 (3) 9 (6) nd
<i>Bougainvillea</i> <i>campanulata</i>	3	6–7	4	Diptera Hymenoptera	nd nd	3 (4) 5 (5)
<i>Bo. stipitata</i>	3–4	17–20		Lepidoptera	Hesperiidae ( <i>Chioides</i> *) Sphingidae*	
<i>Mirabilis jalapa</i>	1	30–60	13	Lepidoptera	Sphingidae [ <i>Lintneria maura</i> (Burmeister, 1879)]	6 (4)
<i>M. ovata</i>	1–3	±10	20	Hymenoptera	Apidae ( <i>Apis mellifera</i> L., <i>Bombus</i> <i>opifex</i> Sm., <i>Bombus</i> ) Halictidae [ <i>Augloclora phoemonoe</i> (Schrottsky), <i>Dialictus</i> ] Vespidae [ <i>Polybia occidentalis</i> (Oliver)]	9 (3.3) 2 (4) 1 (7)
<i>Pisonia zapallo</i> var. <i>guaranitica</i>	Many	±3 (s) ±5 (p)	10	Hymenoptera –	Apidae ( <i>Apis mellifera</i> L.)	40 (3)
<i>P. zapallo</i> var. <i>zapallo</i>	Many	±4 (s) ±2 (p)	10	– –		

*N*, number of flowers per inflorescence; *L*, flower length (mm); *N<sub>p</sub>*, number of observation periods (10 min); *N<sub>v</sub>*, number of visits; *t*, median time (s); s, staminate flower; p, pistillate flower; –, absent.

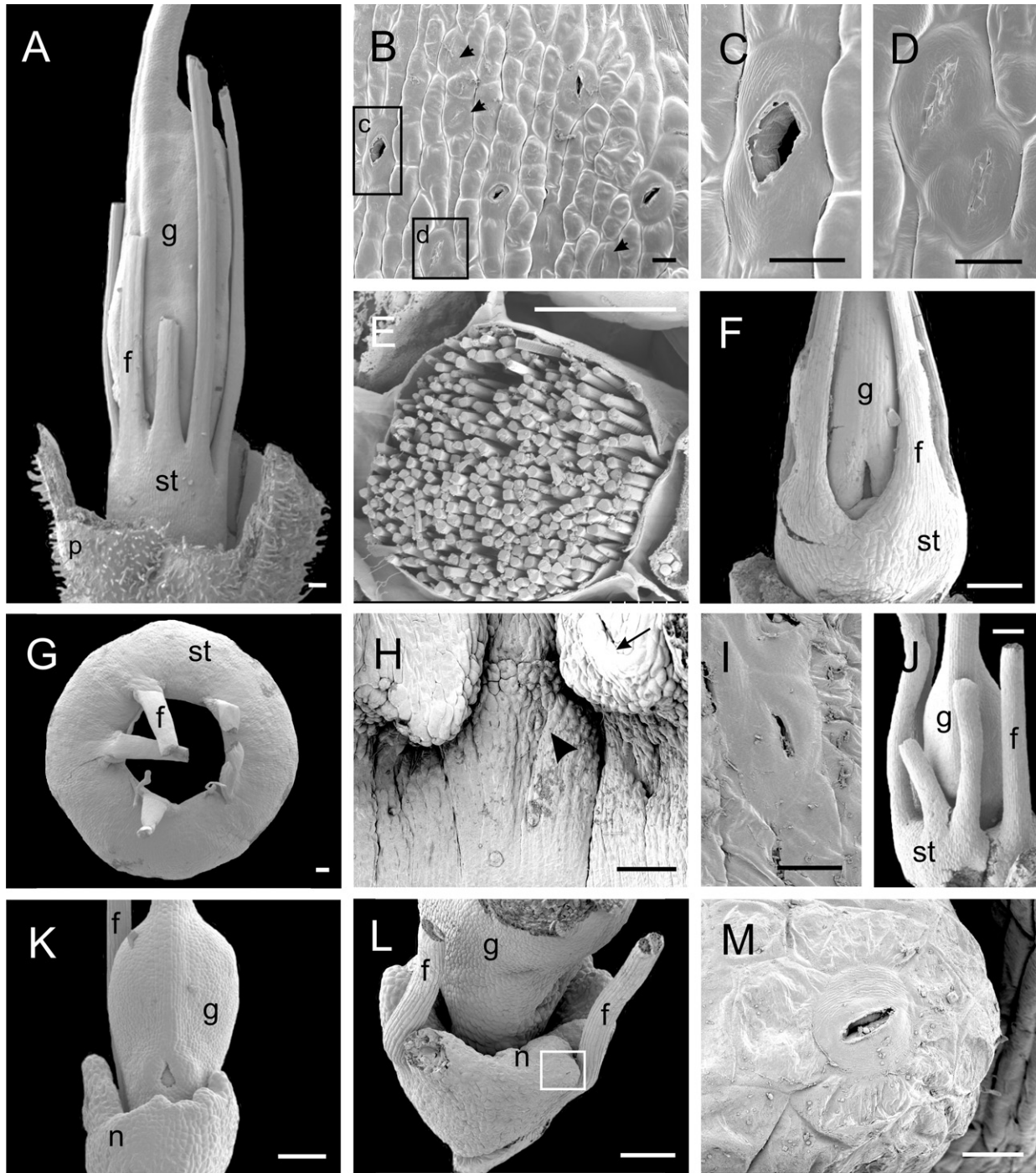
\*Extracted from López & Galetto (2002). nd, no data.

tariferous tissue occupies the inner side of the short staminal tube and the base of the pistillode; in pistillate flowers, the nectary is located at the inner side of the staminodes and the base of the gynoecium (Fig. 3P–R).

3. In the remaining species, a cup-shaped nectary is located basally at the level of the gynophore (Figs 2A, F, J, K; 3A, E, M, O).

In addition to general observations, we highlight some unusual features in the nectaries of some species. In the nectary of *Bo. stipitata*, *Bougainvillea campanulata* Heimerl and *Bougainvillea praecox* Griseb., open and closed stomata are homogeneously distributed all over the nectary surface (Figs 2B–D;

3B, D). *Pisoniella arborescens* apparently lacks stomata on the nectary surface; in this species, the size of the epidermal cells increases in cells facing the gynophore and patches of relatively large dark-staining cells are present in the epidermis and parenchyma (Fig. 3F). In *B. diffusa* var. *leiocarpa*, flowers of which have one to three stamens, the nectary is asymmetric (Figs 2K; 3O); the nectariferous tissue is continuous at the androecial base (Fig. 4A) and expands asymmetrically close to the base of the free parts of the filaments, showing the characteristic anatomical structure described above (Fig. 4C). Stomata (one per filament) are restricted to the bases of the filaments (Fig. 2L, M).



**Figure 2.** Scanning electron microscopy (SEM) photomicrographs showing nectary structure in selected species of Nyctaginaceae. A–E, *Bougainvillea stipitata*. A, Basal portion of the flower from which the perianth has been removed. The nectariferous tissue is located at the internal surface of the cup-shaped staminal tube. B, Nectary epidermis with open and closed (arrowheads) stomata. C, D, Details of the stomata outlined in (B). E, Raphides in an idioblast present in the nectary parenchyma. F, *Allionia choisyi*, cup-shaped staminal tube. G, H, *Mirabilis jalapa*. G, Top view of bowl-shaped staminal tube; the gynoecium has been removed. H, Inner and upper surface of the nectary with protruding bulges (arrow) and a stoma (arrowhead). I, *Mirabilis ovata*, detail of a stoma. J, *Pisoniella arborescens* (bud), cup-shaped staminal tube. K–M, *Boerhavia diffusa* var. *leiocarpa*. K, Asymmetrical nectary. L, Top view of nectary showing a stoma close to the base of the filament. M, Detail of stoma indicated in (L). f, filament; g, gynoecium; n, nectariferous tissue; p, perianth; st, staminal tube. Scale bars: A, F–H, J–L, 1 mm; B–E, I, M, 100  $\mu$ m.



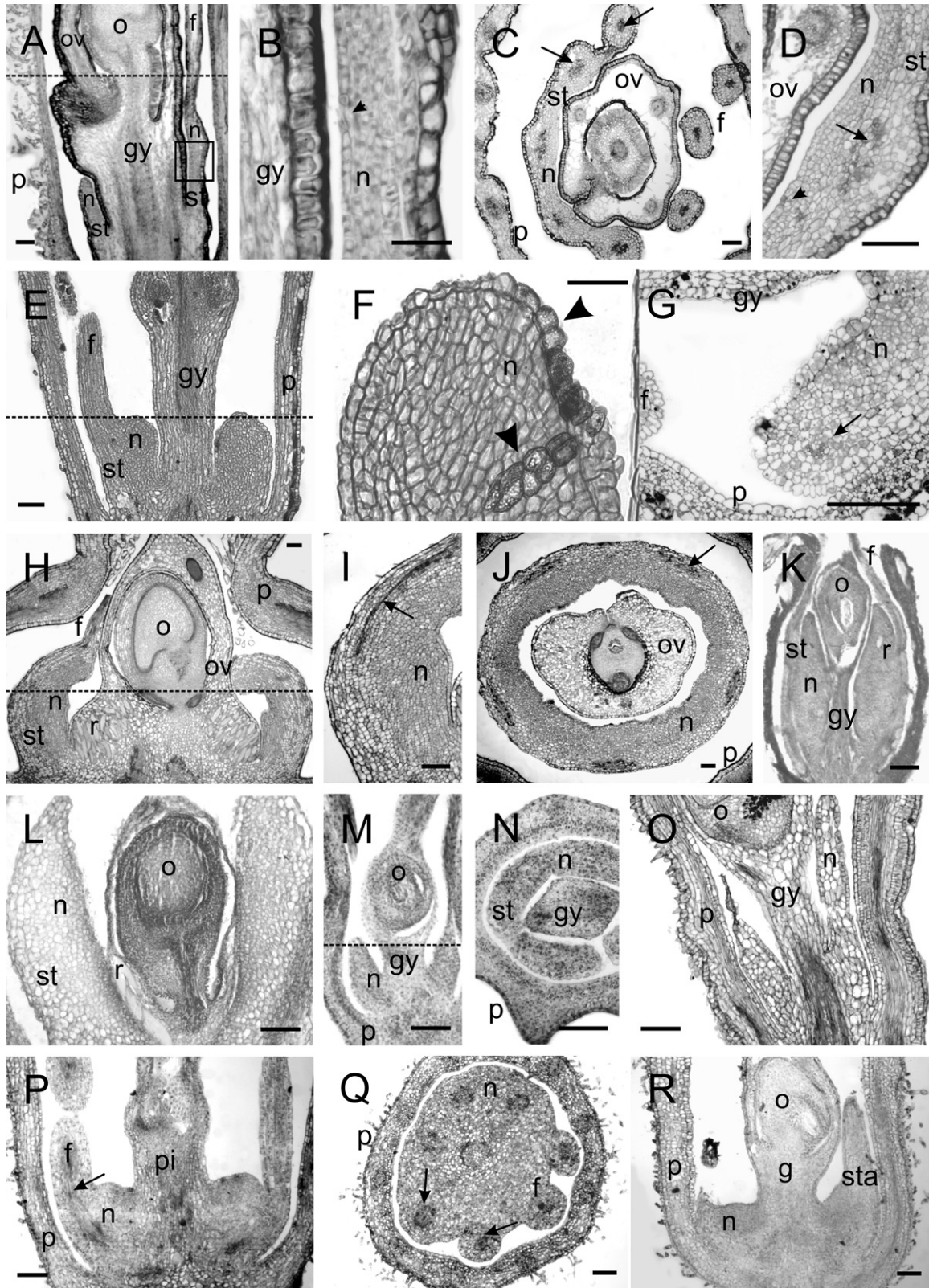


Figure 3. See caption on next page.

**Figure 3.** Light microscopy photomicrographs showing nectary structure in selected species. A,B, *Bougainvillea praecox*. A, Flower partial longisection showing nectariferous tissue at the inner surface of the staminal tube. B, Detail of nectariferous tissue shown in (A). C, D, *Bougainvillea campanulata*. C, Flower cross-section at the top of the nectariferous zone [indicated in (A) for a congeneric species], showing nectariferous tissue at the fused base of the androecium and expanding on the free parts of some filaments. The vascular traces of the stamens supply the nectary parenchyma. D, Detail of nectariferous tissue. E–G, *Pisoniella arborescens*. E, Flower longisection. F, Nectariferous zone with dark-staining cells in nectary epidermis and parenchyma (large arrowheads). G, Flower semi-thin cross-section indicated in (E). H–J, *Mirabilis jalapa*. H, Flower longisection. I, Details of nectariferous tissue. J, Flower cross-section outlined in (H). K, *Boerhavia pulchella*, flower longisection. L, *Mirabilis ovata*, flower longisection. M, N, *Allionia choisyi*. M, Flower longisection. N, Flower cross-section indicated in (M). O, *Boerhavia diffusa* var. *leiocarpa*, flower longisection showing the asymmetrical nectary. P, *Pisonia zapallo* var. *guaranitica*, staminate flower longisection. Q, R, *Pisonia zapallo* var. *zapallo*. Q, Staminate flower cross-section showing the nectariferous ring and the free parts of some filaments. R, Pistillate flower longisection. The arrows indicate vascular bundles and the small arrowheads the stomata. f, filament; gy, gynophores; n, nectariferous tissue; o, ovule; ov, ovary wall; p, perianth; pi, pistillode; r, raphides; st, staminal tube; sta, staminode. Scale bars: A, C–E, G–R, 100 µm; B, F, 50 µm.

Observations under a stereoscopic microscope of the other species generally showed a similar staminal nectary (not shown), and a ring-shaped nectary is present in *P. aculeata* flowers.

#### ULTRASTRUCTURE OF THE FLORAL NECTARY IN *B. DIFFUSA* VAR. *LEIOCARPA*

A detailed study of the floral nectary was carried out in pre-anthetic flowers of *B. diffusa* var. *leiocarpa* using TEM (Fig. 4D–K) to study the ultrastructure of secretory tissues. The epidermis is composed of one or two layers of small, tightly packed cells that are polyhedral in anticlinal orientation (Fig. 4A, C). The epidermal cells have thin walls, a relatively large nucleus, usually containing a nucleolus, densely stained granular cytoplasm and a vacuole generally oriented to the nectary internal surface (Fig. 4D). Cells often possess starch grains stored in plastids; mitochondria and components of the endomembrane system are also present. A continuous thin cuticle covers the epidermis surface.

Based on the large nuclei and the abundance of plastids with starch grains, the cell layers underneath the epidermis represent the secretory parenchyma of the nectary (Fig. 4A–C). Nectary parenchymal cells are large and irregular, more loosely packed than those of the epidermis, with thin walls, dark granular cytoplasm and a large vacuole usually oriented towards the epidermis (Fig. 4E–G). In some cases, the cytoplasm is restricted to a relatively narrow region around the large central vacuole. In general, each nucleus contains a nucleolus and deeply stained chromatin. The cytoplasm is rich in ribosomes and mitochondria; endoplasmic reticulum (ER) cisternae and other endomembrane elements are present. Golgi stacks are absent. The subnectary parenchyma is composed of large irregular cells con-

taining little or no starch (Fig. 4A, B). Although they resemble the nectary parenchymal cells, the subnectary parenchymal cells have less dense granular contents and narrower intercellular spaces. Plastids, mitochondria with well-developed cristae and portions of ER are present (Fig. 4H).

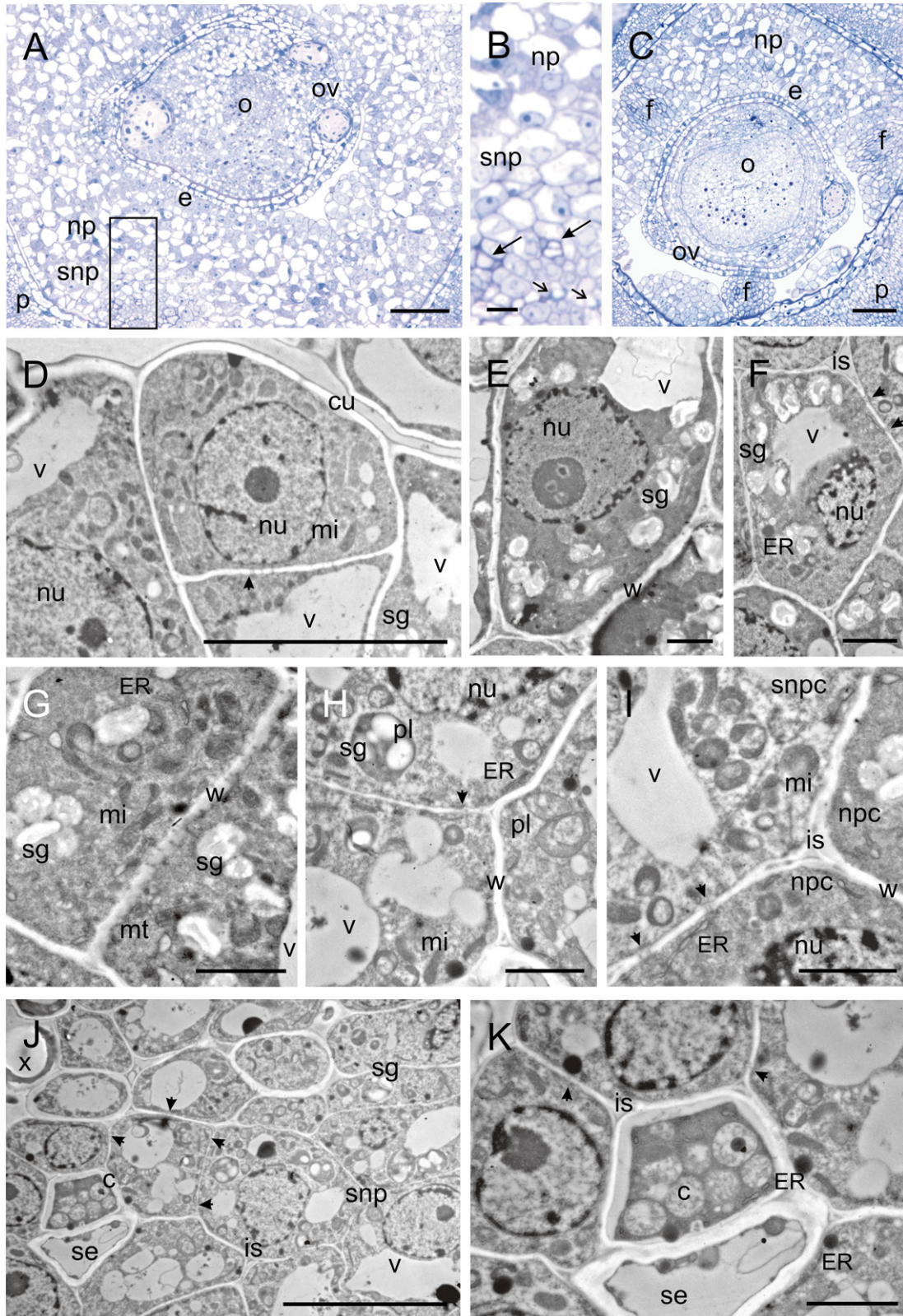
Plasmodesmata are observed both interconnecting the epidermal cells (not shown) and connecting them with the subepidermal nectariferous cells (Fig. 4D). Plasmodesmata also connect nectary parenchymal cells (Fig. 4F), subnectary parenchymal cells (Fig. 4H) and both cell types (Fig. 4I). ER cisternae are often located close to or oriented towards the plasmodesmata (Fig. 4H, I).

The phloem and xylem bundles are embedded in the subnectary parenchyma (Fig. 4A, B, J). The phloem is composed of sieve-tube elements with a peripheral cytoplasm and one to three adjacent companion cells containing dense-staining cytoplasm and organelles (Fig. 4K). Both cells possess thick walls and intercellular spaces. Plasmodesmata connect the surrounding parenchyma (Fig. 4J, K).

#### FLORAL VISITORS

Table 2 shows the floral visitors recorded for selected contrasting species of Nyctaginaceae. In general, Hymenoptera (Apidae, Vespidae, Halictidae) and Diptera are common visitors for most species; thus, these groups of species can be characterized by a generalized pollination system. *Allionia choisyi* and *Boerhavia* L. have small flowers visited by many different visitors. No visitors were recorded for *B. cordobensis*. Nocturnal Lepidoptera were the most common visitors to flowers of *M. jalapa* and *Bo. stipi-*





**Figure 4.** See caption on next page.



**Figure 4.** Light microscopy and transmission electron microscopy (TEM) photomicrographs showing the nectary ultrastructure in *Boerhavia diffusa* var. *leiocarpa*. A–C, Light micrographs of flower semi-thin cross-sections. A, The nectariferous tissue is an annular band at the base of the staminal tube. B, Details of nectary parenchyma, subnectary parenchyma and vascular bundles outlined in (A). C, The nectariferous tissue is asymmetrical at the top of the nectary. D–K, TEM ultrathin cross-sections showing details of the nectariferous tissue. D, Epidermal cells, with densely stained cytoplasm, a vacuole and a thin cuticle. E–G, Nectary parenchymal cells, with a large nucleus, dense granular cytoplasm and high content of starch grains. H, Subnectary parenchymal cells, each with a large vacuole, less dense cytoplasm and fewer starch grains. I, Nectary and subnectary parenchymal cells connected by plasmodesmata. J, General view of the vascular bundles and surrounding tissue. K, Sieve-tube element with a densely cytoplasmic companion cell. The large arrows indicate phloem elements, the small arrows xylem elements and the arrowheads the plasmodesmata. c, companion cell; cu, cuticle; e, epidermis; ER, endoplasmic reticulum; f, filament; is, intercellular space; mi, mitochondrion; mt, microtubule; np, nectary parenchyma; npc, nectary parenchymal cell; nu, nucleus; o, ovule; ov, ovary wall; p, perianth; pl, plastid; se, sieve-tube element; sg, starch grain; snp, subnectary parenchyma; snpc, subnectary parenchymal cell; v, vacuole; ve, vesicle; w, wall; x, xylem. Scale bars: A, C, 50 µm; B, D, G, J, 10 µm; E, H, I, K, 2 µm; F, 5 µm.

*tata*. The flowers of *Bo. stipitata* are also visited by tiny moths (Hesperiidae) and can be classified as phalaenophilous.

#### NECTAR SUGAR COMPOSITION

In order to compare nectar traits in some contrasting species, we analysed nectar volume, concentration and sugar composition (Table 3). Each flower secretes a small volume of nectar (0.09–3.50 µL) with variable concentration (10–47%) according to species. In most species, hexoses predominate among nectar sugars, but *M. jalapa* has higher levels of sucrose. Flowers of *B. cordobensis* do not produce detectable nectar.

### DISCUSSION

#### STRUCTURE OF FLORAL NECTARIES

In all species of Nyctaginaceae, the floral nectary is located basally on the adaxial surface of the staminal tube (or the staminodes in pistillate flowers of *P. zapallo*). Nectar is secreted through modified stomata, accumulating between the base of the stamens and the ovary. Nectary structure in the species examined here is consistent with that reported previously in *Mirabilis*, *Bougainvillea*, *Pisonia* and *Colignonia* Endl. (Bonnier, 1879; Heimerl, 1934; Zandonella, 1972, 1977; Rohweder & Huber, 1974; Valla & Ancibor, 1978; Bohlin, 1988; Vanvinckenroye *et al.*, 1993; López & Galetto, 2002), although the nectary nomenclature is nonuniform in earlier accounts. The differences observed in nectary morphology between different species are not expected to affect reproductive success.

Our data for the nectaries of *M. jalapa* and *M. ovata* resemble earlier descriptions for *M. jalapa* and *M. longiflora* (Bonnier, 1879; Valla & Ancibor, 1978; Vanvinckenroye *et al.*, 1993), except that our material lacked some features described by Valla & Ancibor (1978), such as secretory hairs and stomata on the external face of the nectar, forming masses in the internal face.

In most angiosperms, nectary parenchyma consists of small cells with dense granular cytoplasm, small vacuoles and relatively large nuclei (reviewed by Fahn, 1979, 1988; Nepi, 2007). In contrast, our TEM studies of pre-anthetic flowers of *B. diffusa* var. *leiocarpa* revealed relatively large cells with a large vacuole. However, vacuole size can vary at different stages of nectary development, increasing in volume at the time of secretion because of cellular growth (Fahn, 1988; Nepi, 2007). As is common in secretory cells, the nectary cytoplasm in *B. diffusa* is rich in ribosomes and elements of endomembrane systems. Large numbers of starch grains are present, probably because the source of nectar carbohydrates requires temporary starch storage in the parenchymal cells (Fahn, 1988; Nepi, 2007). Towards the stage of secretion, intercellular spaces are increased and mitochondria are numerous because of the energy requirements for nectar production (Nepi, 2007), which could explain our observations in *B. diffusa*. Nectary cell walls contain numerous plasmodesmata and ER cisternae close to them. Thus, the symplast represents a possible conduit for pre-nectar flow through the parenchymatous cells and secretory cells (Fahn, 1979, 1988), although transport through intercellular spaces (Vassilyev, 1969) to the one to three stomata at the free filament bases is also possible. Further experimental studies during flower development are necessary to determine possible mechanisms of pre-nectar transport and nectar secretion in this species (Vassilyev, 1969, 2010; Fahn, 1979, 1988; Heil, 2011). This first ultrastructural study of nectaries in Nyctaginaceae will not only serve as a model, but will also be useful in future comparative studies in the family.

#### FACTORS INFLUENCING VARIATION IN NECTARY AND NECTAR TRAITS

Phylogenetic and ecological constraints have been hypothesized to influence the evolution of nectary

**Table 3.** Nectar composition in selected species of Nyctaginaceae

Species	Sample (N)	Concentration (%)	Volume ( $\mu$ L)	Sucrose (%)	Glucose (%)	Fructose (%)	Sr	Hr
<i>Allionia choisyi</i>	1 (72)	25.33 $\pm$ 2.00	0.09 $\pm$ 0.01	11.81 $\pm$ 4.44	57.57 $\pm$ 0.40	30.61 $\pm$ 4.72	0.13	1.88
<i>Boerhavia pulchella</i>	1 (2)	47.14 $\pm$ 3.52	1.28 $\pm$ 0.76	1.51 $\pm$ 2.03	28.71 $\pm$ 8.28	69.77 $\pm$ 8.56	0.01	0.71
<i>Bougainvillea spinosa</i>	2 (70)	33 $\pm$ nd	nd	4.1 $\pm$ 1.60	57.9 $\pm$ 2.42	37.9 $\pm$ 1.41	0.04	1.53
<i>Bougainvillea stipitata</i>	5 (45)	30.50 $\pm$ 3.52	3.5 $\pm$ 0.76	2.66 $\pm$ 5.26	19.36 $\pm$ 11.41	79.00 $\pm$ 7.53	0.04	0.26
<i>Mirabilis jalapa</i>	11 (61)	10.35 $\pm$ 2.8	2.62 $\pm$ 0.66	41.63 $\pm$ 9.02	27.86 $\pm$ 4.19	30.04 $\pm$ 5.85	0.72	1.07
<i>Mirabilis ovata</i>	3 (14)	33.45 $\pm$ 7.83	2.14 $\pm$ 2.28	27.09 $\pm$ 4.90	28.79 $\pm$ 0.02	43.29 $\pm$ 4.87	0.42	0.81
<i>Pisonia zapallo</i> var. <i>guaranitica</i> (p)	1 (20)	45.50 $\pm$ 7.42	0.68 $\pm$ 0.56	15.03 $\pm$ 0.59	40.54 $\pm$ 1.02	44.43 $\pm$ 0.44	0.18	0.9

Values are means  $\pm$  SD. N, number of sampled plants (number of sampled flowers); Sr, sugar ratio; sucrose/(fructose + glucose); Hr, hexose ratio; glucose/fructose; nd, no data; p, pistillate flower. *Bougainvillea spinosa* and *Bo. stipitata* data were extracted from Forcone *et al.* (1997) and López & Galetto (2002), respectively.

traits. Our results for several genera from different tribes of Nyctaginaceae, with contrasting flower morphology and displaying different floral traits to attract pollinators, support the hypothesis that the basic structure of the nectary is a relatively conservative trait in the family, independent of the primary group of flower visitors and other floral traits. Relatively broad variation in flower structure, shape and colour in the different species suggests that these traits are more labile in the family and can change more rapidly, in a few generations, than the more conservative nectary traits (see also Schemske & Bradshaw, 1999; Bradshaw & Schemske, 2003; Galetto & Bernardello, 2004, 2005; Nicolson, 2007). Prior to a phylogenetic study, Zandonella (1977) suggested *Pisonia* as the best candidate for the ancestral nectar type in the family because of the limited regions of concrescence of the stamens. As nectary structure and location are exceptionally diverse among other Caryophyllales (Zandonella, 1977; Bernardello, 2007), a future detailed optimization of nectary morphology in Nyctaginaceae will help to reconstruct the evolution of this character in the order. However, more studies are needed on the tribe Leucastereae (the earliest branching tribe of Nyctaginaceae), tribe Boldoeae (which has free filaments) and the unplaced tribe Caribeae (in which the filaments are adnate to the perianth base).

We found some variation in nectar traits in the species of Nyctaginaceae examined here, which could be related to ecological specialization to different groups of flower visitor. Some of the differences are in nectar concentration, with lower values for the hawkmoth-pollinated species *M. jalapa* and higher values for the diurnal insect-pollinated species (e.g. *B. pulchella*, *M. ovata* and *P. zapallo*) and the phalaenophilous *Bo. stipitata*. The sucrose-predominant nectar found here in *M. jalapa* is usual for flowers pollinated by nocturnal hawkmoths (Galetto & Bernardello, 2003), as also reported by Grant (1983) and Freeman *et al.* (1985) for a related species, *M. longiflora*. In natural populations, *M. jalapa* is primarily pollinated by hawkmoths (Valla & Ancibor, 1978; Martínez del Río & Búrquez, 1986), but shows some variation in nectar sugar composition. For example, Bonnier (1879) found that the nectar of this species is almost entirely composed of sucrose and no glucose, and Percival (1961) and Valla & Ancibor (1978) found fructose and glucose as dominant sugars and sucrose in minor proportions. Nectar variability is not uncommon in some plant species with multiple and complex sources (Galetto & Bernardello, 2005; Herrera, Pérez & Alonso, 2006; Canto *et al.*, 2007), including nocturnal species with sphingophily and phanophily (Oliveira, Gibbs & Barbosa, 2004). Thus, the nocturnal species studied here can

be divided into two groups. The moth-pollinated *M. jalapa* has relatively large flowers with tubular scented corollas and large quantities of sucrose-predominant nectar. The other species, *Bo. stipitata*, is primarily visited by other moths (e.g. noctuids, geometrids, pyralids) that fly slowly and usually settle on the flower. The flowers present less nectar and tend to be small and aggregated.

Pollinators are by no means the only constraint governing nectar traits. Several authors have pointed out that species that are taxonomically closely related display similar patterns of nectar sugar composition because they share common ancestors, rather than because they share the same floral visitors. Moreover, historical and environmental factors, such as humidity, temperature and wind, could be involved in determining sugar composition according to the exposure of the nectar (Forcone *et al.*, 1997; Chalcoff, Aizen & Galetto, 2006; Nicolson & Thornburg, 2007). Thus, it is likely that a complex range of factors can influence nectar traits in Nyctagineae, in which taxa inhabit different environments and have different flower shapes and corolla tube lengths.

#### INSECT POLLINATION AND REPRODUCTIVE BIOLOGY

The southern South American Nyctagineae examined here that have a relatively short perianth tube (< 15 mm) and diurnal anthesis are visited by two or more insect orders and apparently show a generalized pollination system. In contrast, species that are visited by different groups of nocturnal floral visitors (*M. jalapa* and *Bo. stipitata*) possess a relatively long perianth tube (> 15 mm), nocturnal anthesis and fragrance emission, and apparently have relatively specialized flowers (Table 2), but display considerable differences in nectar traits, as outlined above (Table 3). Sphingid pollination has been described previously for *M. jalapa* (Valla & Ancibor, 1978; Martínez del Río & Búrquez, 1986) and for other *Mirabilis* spp. (especially those belonging to section *Mirabilis*), most of them emitting a strong nocturnal fragrance and sometimes possessing a long perianth tube compatible with pollinators with a long proboscis (Baker, 1961; Grant, 1983; Bittrich & Kühn, 1993; Levin *et al.*, 2001). In contrast, *M. ovata* section *Oxybaphus* (L'Hér. ex Willd.) Heimerl and other *Mirabilis* spp. are pollinated by different visitors with relatively short proboscises (e.g., Cruden, 1973; Barnes, 1996), and hummingbird pollination is also possible in this genus (Baker, 1961). Sphingid pollination has also been recorded in *Anulocaulis* Standl. and *Acleisanthes* A.Gray and some species of *Abronia* Juss., which mostly show nocturnal anthesis, fragrance emission and possess tubular or funnelliform to salverform flowers (Tillett, 1967; Bittrich & Kühn, 1993; Levin

*et al.*, 2001; Levin, 2002). In *Bo. stipitata*, the reduced availability of hexose-predominant nectar and a constriction of the perianth probably facilitate pollination by other kinds of moth, as it enforces contact between the proboscis and the stigma (López & Galetto, 2002).

Other interesting observations were found in the South American members examined here. In Bougainvilleae, our records of the pollination of *Bo. stipitata* by small moths (López & Galetto, 2002) and *Bo. campanulata* by dipterans are novel for the genus; floral fragrance appears to be the major insect attractant in these cases. In contrast, the ornamental scentless species *Bougainvillea spectabilis* Willd. and *Bougainvillea glabra* Choisy attract diurnal Lepidoptera (Vogel, 1954) and hummingbirds (Gillis, 1976), respectively, using visual cues.

Concerning members of Nyctagineae, the syrphid visitors recorded here are new for the genus *Allionia*, for which lepidopteran pollination was reported previously in *Allionia incarnata* (Phillips, 1976). Regarding *Boerhavia* species, hymenopteran and dipteran visitors are common (Chaturvedi, 1989; Spellenberg, 2000); we found neither Lepidoptera nor Coleoptera visiting South American species, as reported by other authors in *B. diffusa* and the North American *Boerhavia intermedia* M. E. Jones (Chaturvedi, 1989; Spellenberg, 2000).

In Pisonieae, few species have been studied previously. Bullock (1994) proposed wind pollination for *Pisonia* and *Guapira* Aubl., based on the small and inconspicuous flowers. However, our study clearly corroborates other records indicating that entomophily could be a pollination syndrome of this group, as found in *P. aculeata* (Chodat & Rehfoos, 1925), *Guapira noxia* (Netto) Lundell and *Neea theifera* Oerst (Oliveira & Gibbs, 2000; Amorim *et al.*, 2011). Both male and female flowers of *P. zapallo* var *guaranitica* Toursark. produce nectar, and male inflorescences attract many honeybees, apparently by sweet fragrance. Thus, native insects can be postulated as the usual pollinators in this species.

Based on phylogenetic studies and the recent tribal classification (Douglas & Manos, 2007; Douglas & Spellenberg, 2010), our data combined with a literature review (e.g. Baker, 1961; Tillett, 1967; Gillis, 1976; Grant, 1983; Bohlin, 1988; Bittrich & Kühn, 1993; Spellenberg, 2000; Levin *et al.*, 2001) allow some preliminary speculation regarding the diversification of pollination syndromes in Nyctagineae. Most species in the four tribes analysed are pollinated by Hymenoptera, Diptera and Lepidoptera. Sphingid pollination seems to have evolved separately in four genera of Nyctagineae (*Abronia*, *Acleisanthes*, *Anulocaulis*, *Mirabilis*) and in *Bougainvillea* (Bougainvilleae). Coleopteran pollination has been recorded in *Abronia* and *Boerhavia* (Nyctagineae), whereas polli-



nation by hummingbirds has evolved independently in *Mirabilis* and *Bougainvillea*, which belong to different tribes. Finally, insect and wind pollination are proposed for Pisonieae. Our study shows that several groups of floral visitors could be involved in the sexual reproduction of Nyctaginaceae and could contribute to the maintenance of plant–pollinator networks in Argentinian semi-arid environments.

Although insect pollination is essential for reproduction in the self-incompatible species *Bo. stipitata* (López & Galetto, 2002) and the dioecious species *P. zapallo*, most of the species studied here are self-compatible [A. L. López, L. Galetto & A. M. Anton, unpubl. data; no data are recorded for *Mirabilis bracteosa* (Griseb.) Heimerl, *Boerhavia torreyana* (S. Watson) Standl., *Pisoniella* and the other *Bougainvillea* spp.]. These observations agree with previous evidence of self-compatibility in most genera of the herbaceous xerophytic tribe Nyctagineae [*Allionia*, *Boerhavia*, *Commicarpus* Standl., *Tripterocalyx* (Torr.) Hook., most *Mirabilis* and some *Abronia*] and also *Colignonia* (e.g. Cruden, 1973; Bohlin, 1988; Bittrich & Kühn, 1993; Spellenberg, 2000; Douglas & Manos, 2007; Douglas, 2008). In contrast, *Mirabilis* section *Quamoclidion* (Choisy) A. Gray, some *Abronia*, some *Bougainvillea* and the dioecious *Pisonia* are self-incompatible (Cruden, 1973; Zadoo, Roy & Khoshoo, 1975; Williamson & Bazeer, 1997). Cleistogamous flowers are produced in addition to chasmogamous flowers in *Cyphomeris* Standl., *Nyctaginia* Choisy, some *Mirabilis* and *Acleisanthes* (Cruden, 1973; Douglas & Manos, 2007). In *B. cordobensis*, we found closed cleistogamous flowers and, exceptionally, a few open flowers that produced a few pollen grains but lacked nectar; no visitors were recorded visiting chasmogamous flowers. Thus, it is not plausible that pollen is offered as a reward for pollinators by chasmogamous flowers of *B. cordobensis*. A detailed auto-ecological study with this species will help to better understand its reproductive biology. Although self-compatibility seems to be common for many Nyctaginaceae, insect pollination could be essential for plant reproduction in taxa from different environments, as found for many other species in the Chaco vegetation (Morales & Galetto, 2003).

Our results reveal relatively low variation in nectary characteristics in southern South American Nyctaginaceae, compared with the relatively broad variation in flower structure, shape and colour, indicating that selective pressures are not uniform among floral features. However, some differences in nectar traits were evident, and these differences can be related to both pollinator and plant reproductive strategies. Hymenoptera are the most common visitors for most species studied here, and nocturnal Lepidoptera are the most common visitors for the

more specialized *M. jalapa* and *Bo. stipitata*. In most South American species, as in the family as a whole, reproduction would be guaranteed by insect pollination combined with self-compatibility (when resources are limited, in the absence of pollinators or under other factors that limit pollen production). Further data collection for members of the small tribes Boldoeae, Caribeeae and Leucastereae, followed by reproductive character reconstruction (A. L. López, M. J. Nores & A. M. Anton, unpubl. data), is currently underway to provide a general framework in which to discuss the evolutionary scenario for plant–pollinator interactions in this small but interesting family.

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