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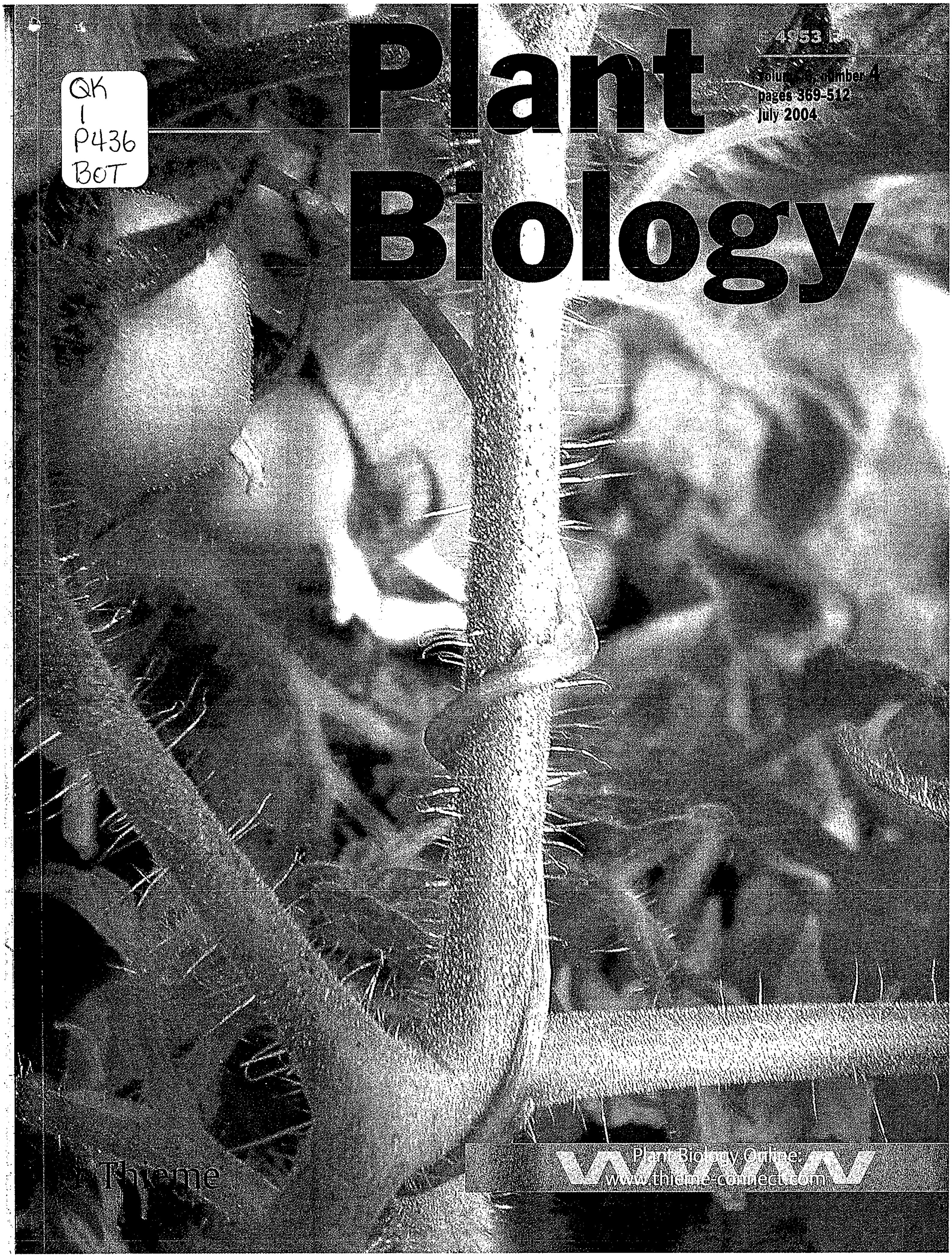

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Non-Euglossine Bees also Function as Pollinators of *Sinningia* Species (Gesneriaceae) in Southeastern Brazil

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Abstract: Pollination by male and female Euglossini bees, euglossophily, was suggested for a number of neotropical Gesneriaceae species. Information on bee species other than Euglossini as pollinators of neotropical members of this family is limited, and in the tribe Sinningieae data about bee pollination are still lacking. Here, we report on floral biology and bee pollination of four *Sinningia* species: *S. schiffneri, S. eumorpha, S. villosa,* and *Sinningia* "canastrensis". The flower features, such as corolla size, shape, and colour, are very different among the four species, but all conform to the melittophilous syndrome. The average nectar volume and sugar amount is low in *S. schiffneri, S. eumorpha,* and *Sinningia* "canastrensis", when compared to that of *S. villosa,* but low nectar amounts is a general feature of *Sinningia* species. The main pollinators of the four species are: small Tapinotaspidini *(Trigonopedia ferruginea)* of *S. schiffneri,* large Bombini *(Bombus morio)* and large Centridini *(Epicharis morio)* of *S. eumorpha,* large Euglossini (*Eulaema cingulata* and *Eufriesea surinamensis*) of *S. villosa,* and large Euglossini *(Eufriesea violascens)* and Megachilini (*Megachile* sp.) of *Sinningia* "canastrensis". Out of the four species, only *S. villosa* is exclusively Euglossini-pollinated. The marked differences in flower features and nectar production of these *Sinningia* species may reflect their pollination by distinct groups of bees. These results strengthen the idea of multiple origins for the pollination systems involving bees within this genus, which is highly supported by molecular phylogenetic analyses.

Key words: Gesneriaceae, *Sinningia,* floral biology, pollination, non-euglossine bees, nectar production, melittophily.

Introduction

The Gesneriaceae is a medium to large tropical family comprising about 3000 species in 140 genera (Burtt and Wiehler, 1995; Smith et al., 1997). The family is divided into three subfamilies: Cyrtandroideae Endl. which is primarily paleotropical, Gesnerioideae Dumort. which is neotropical and Coronantheroideae Wiehler which occurs in the South Pacific Islands, Northeast Australia and Chile (Burtt and Wiehler, 1995). In Brazil, the Gesneriaceae comprises 23 genera and about 220 species that mostly occur in rain forests in the Amazon region or on the Atlantic coast (Chautems, 1991; pers. com.).

Wiehler (1983) estimated that about 60% of the neotropical Gesneriaceae are hummingbird-pollinated, about 30% are pollinated by male and female Euglossine bees in search of nectar (gynandro-euglossophily), and 10% have other modes of pollination, including andro-euglossophily (male Euglossine bees gathering fragrances). Euglossine bees as the pollinators of Gesneriaceae was confirmed by Dressler (1968) and Wiehler (1977, 1978) for a few Central and Andean species of *Gloxinia, Nautilocalyx* and *Drymonia.* A first report of bee species other than Euglossine as pollinators of neotropical Gesneriaceae was made by Steiner (1985), who recorded *Epicharis* species (Anthophoridae) as pollinators of *Drymonia serrulata.*

The tribe Sinningieae (Gesnerioideae) comprises about 80 species which are mostly ornithophilous (Chautems, 2002). Based on a set of floral characters, Perret et al. (2001) suggest nine melittophilous species in this tribe, but, there is no information on pollination biology. This paper reports on floral biology and bee pollination of *Sinningia schiffneri* Fritsch, *S. eumorpha* H. E. Moore, *S. villosa* Lindl., and *Sinningia* "canastrensis" Chautems sp. nov. ined. (in prep.), as well as on the breeding system of *S. schiffneri* and *S. eumorpha,* and supports the view that differences in floral morphology favour different groups of bees as pollinators of these species.

Materials and Methods

All studies were located in southeast Brazil. *Sinningia schiffneri* was studied at the Parque Estadual da Serra do Mar, Reserva Biológica do Alto da Serra de Paranapiacaba (23°46′S, 46°18′W; about 890 m a.s.l.) and in Ubatuba (23°30′S, 45°09′W, at sea level), São Paulo State. *S. eumorpha* was studied at two sites, 8 km distant from each other (22°23′S, 47°53′W and 22°25′S, 47°59′W about 920 m a.s.l.) in Itirapina, São Paulo State. *S. villosa* was studied in São João de Petrópolis, Espírito Santo State (19°48′S, 40°41′W; about 180 m a.s.l.), and *Sinningia* "canastrensis" was studied at the Parque Nacional da Serra da Canastra, São Roque de Minas, Minas Gerais State (20°15′S, 46°25′W; about 820 m a.s.l.).

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Table 1 Floral features of bee-pollinated species of *Sinningia* in southeastern Brazil. Measurements in millimeters. Different letters indicate significant differences among species ($p < 0.05$) according to Scheffé *post hoc* test

Floral features	*S. schiffneri* n = 31	*S. eumorpha* n = 19	*S. villosa* n = 20	*S.* "canastrensis" n = 11
Corolla length	23 ± 1.2^{a}	51 ± 7.2^{b}	34 ± 1.8^{c}	33 ± 2.8^{c}
Corolla opening	5 ± 0.5^{a}	14 ± 1.6^{b}	8 ± 1.0^{c}	12 ± 1.2^{c}
Stamen length – male phase	13 ± 1.0^{a}	22 ± 2.1^{b}	24 ± 1.4^{c}	23 ± 0.3^{c}
Style length – female phase	13 ± 1.8^{a}	21 ± 4.8^{b}	24 ± 2.7^{c}	24 ± 0.4^{c}
Nectar volume (µl)	1.3 ± 1.2^{a}	2.3 ± 1.6^{a}	11.3 ± 6.5^{b}	2.3 ± 2.7^{a}
Nectar concentration (%w/w)	22.1 ± 5.6^{a}	29.2 ± 8.3^{b}	38.5 ± 1.3^{c}	29.0 ± 4.1^{ab}
Sugar amount (mg)	0.3 ± 0.3^{a}	0.8 ± 0.1^{a}	6.2 ± 1.5^{b}	1.3 ± 0.9^{a}

Phases of anthesis and other floral attributes (e.g., dimensions and colour) were observed *in situ*. Pollen viability was estimated by cytoplasmic staining, using the aceto-carmine technique (Radford et al., 1974). Stigmatic receptivity was tested using the H_2O_2 catalase activity method (Zeisler, 1938). Corolla measurements (effective length cf. Wolf et al., 1976) were taken from fresh flowers sampled from different individuals. Voucher specimens are deposited at the Herbarium of the Universidade Estadual de Campinas (UEC).

Flower buds were bagged at pre-anthesis and nectar availability was determined after 24 h. Two variables were measured: volume of nectar with a graduated microliter syringe (Hamilton, Nevada, USA), and sugar concentration (percent sucrose, w/w) with a pocket refractometer (Atago HSR 500, Japan). The amount of sugar was expressed in milligrams and calculated after Bolten et al. (1979). We compared total nectar volume, nectar sugar concentration and total sugar produced for *S. schiffneri*, *S. eumorpha*, *S. villosa* and *S.* "canastrensis" by ANOVA test and Scheffé *post hoc* test at the 0.05 significance level.

The breeding system of *S. schiffneri* and *S. eumorpha* was assessed by hand-pollination treatments with previously bagged flowers (self-pollination and cross-pollination), and bagged buds (spontaneous self-pollination), and by open-pollinated flowers (control), following methods outlined in Radford et al. (1974).

Direct visual observations of bee visitors were made from 7:00 h to 18:00 h and photographs were taken to analyze their visiting behaviour. Observations on *S. schiffneri* were made during September and December 2000, and November to December 2001, totalling 70 h. *S. eumorpha* was observed in February 2000 and January 2001, totalling 54 h, and *S. villosa* and *Sinningia* "canastrensis" were observed, respectively, in January 2002 (21 h of observations) and January 2003 (20 h of observations). Collected bees are deposited in the Museu de História Natural – Universidade Estadual de Campinas, Coleção de Entomologia Jesus de Santiago Moure – Universidade Federal do Paraná and Museu de Entomologia da Universidade do Norte Fluminense.

Results

Plant characteristics and flowering phenology

The studied *Sinningia* species grow in clusters of 5 to 30 individuals. *Sinningia schiffneri* is a 0.5- to 1.5-m tall shrub without developed tubers, and is common in wet forests. *S. eumorpha* is a tuberous herb with a rosette habit (*sensu* Chautems and Weber, 1999) and *S. villosa* is a tuberous shrub, 1.0- to 1.5-m tall, both species grow on rocky outcrops in semi-deciduous forests. *Sinningia* "canastrensis" is a tuberous shrub, 0.3- to 0.6-m tall, growing on montane rocky outcrops, so called "campos rupestres" (*sensu* Giulietti and Pirani, 1988). The main flowering period of these species is the wet season: *S. schiffneri* flowered mainly from October to December, however, some flowers were recorded from September to April on individuals in the coastal areas, and *S. eumorpha*, *S. villosa*, and *Sinningia* "canastrensis" flowered once a year, from December to February, with a flowering peak in January. Number of flowers open per day per plant varied from 1–3 for *S. schiffneri*, 1–5 for *S. villosa* and *Sinningia* "canastrensis", and 1–7 for *S. eumorpha*.

Floral characteristics and breeding system

Flowers of the four species are zygomorphic, have broad limbs with a large inferior petal and conspicuous nectar guides. *Sinningia schiffneri* presents trumpet-shaped flowers with a somewhat short corolla and a narrow opening (Table **1**). Its flowers are white with dark magenta dots (Fig. **1 A**) and yellow stripes on the lower corolla surface. The funnel-shaped flowers of *S. eumorpha* have the longest corolla and the widest opening (Table **1**), its colour varies from white to lilac, and greenish-yellow stripes and violet lines are on the lower surface of the corolla (Fig. **1 B**). *S. villosa* flowers are tubular and its corolla is of moderate length (Table **1**), being light yellow coloured (Fig. **1 C**) with magenta dots inside and partially outside. The funnel-shaped corolla of *Sinningia* "canastrensis" is also of moderate length (Table **1**), violet red in colour and with white and magenta stripes on the lower corolla surface (Fig. **1 D**). No odour was perceivable in the flowers of these species.

In each species, stamens and styles are equal in length (in the male and female phases, respectively), but are shorter than the corolla (Table **1**), so that anthers and stigmas are inside the flower. The four connate anthers of *S. schiffneri*, *S. eumorpha*, and *Sinningia* "canastrensis" are parallel to the longest corolla axis (Figs. **2 A**, **B**), whereas in *S. villosa* the anthers are perpen-

Fig. 1 Bee-pollinated *Sinningia* species in southeastern Brazil. Flowers of *Sinningia schiffneri* (**A**), *S. eumorpha* (**B**), *S. villosa* (**C**), and *S.* "canastrensis" (**D**). Note shape, colour, and width of the corolla. *Trigonopedia ferruginea* visiting *S. schiffneri* (**E**), *Bombus morio* on *S. eumorpha* (**F**), in both bee species the pollen is deposited on the dorsal part of the thorax. *Eufriesea surinamensis* with pollen on the forehead, visiting *S. villosa* (**G**).

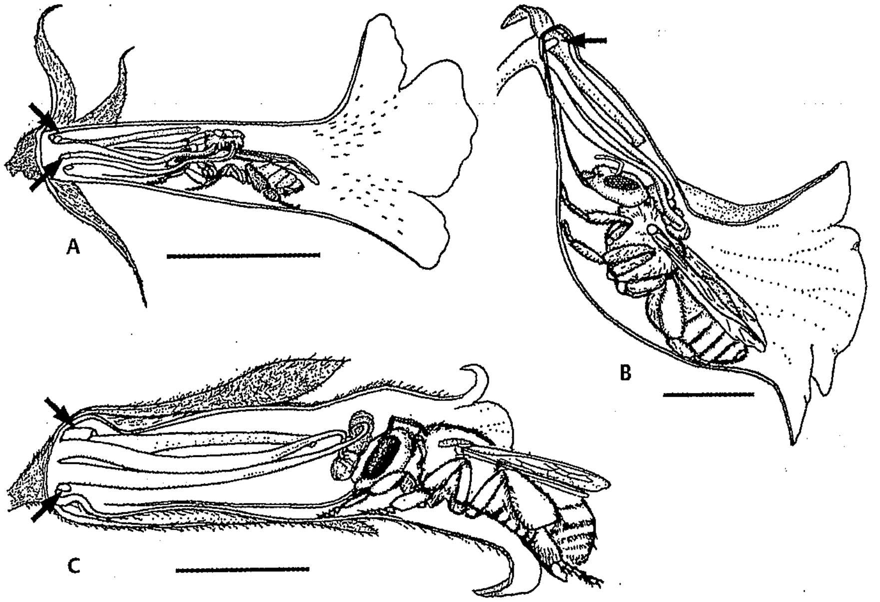

Fig. 2 Side view of *Sinningia* flowers in male phases (part of the calyx and corolla removed) in southeastern Brazil. In *Sinningia schiffneri* (**A**) and *S. eumorpha* (**B**) anthers contact *Trigonopedia ferruginea* and *Bombus morio* on the thorax. In *S. villosa* (**C**) *Eufrisea surinamensis* contacts the anthers which have a perpendicular position in the corolla. Note the nectary glands at the corolla base (arrows).

dicularly positioned to this axis, occupying the centre of the corolla tube and almost blocking the flower mouth (Fig. **2C**). The flowers open mainly in the morning and some of them throughout the day, last five to six days and show marked protandry combined with herkogamy. During the first three days, the flowers are in the male phase; the pollen is somewhat sticky and its viability is about 90%. From the fourth day on, the anthers bend down slowly, the style elongates to place the now receptive stigma in the same place that the anthers occupied earlier, characterizing the female phase. From the fifth or sixth day on, the corolla withers. An intermediate reproductive phase (hermaphrodite) can occur in flowers where the pollen is not removed (unvisited ones), and in this case self pollination is possible. Fruit set from autonomous self-pollination in *S. schiffneri* did not occur and in *S. eumorpha* it was low, whereas fruit set from both hand self- and cross-pollinations was similar to that from open pollinations in both species (Table **2**).

The nectary in *S. schiffneri* consists of five separate glands of equal size (Fig. **2A**), in *S. eumorpha* of two separate dorsal glands (Fig. **2B**), in *S. villosa* of a double-connate dorsal gland and a small ventral gland (Fig. **2C**), and in *Sinningia* "canastrensis" of five glands, one double-connate dorsal gland, two small lateral, and one ventral gland. These glands surround the base of the ovary, and access to the nectar must be gained through small openings of the filaments. The average daily nectar volume was low in *S. schiffneri, S. eumorpha,* and *Sinningia* "canastrensis", and high in *S. villosa,* which differed significantly from the former species (ANOVA, F = 40; M = 11.2; $p < 0.05$ – Table **1**). Mean nectar concentration varied from 22 to 38% and these values differed significantly among the species (ANOVA, F = 22.6; $p < 0.05$). According to this, *S. villosa* is different from the remaining species (38.5% vs. <30%), *S. schiffneri* is different from *S. eumorpha* but *S.* "canastrensis" is similar to *S. schiffneri* and *S. eumorpha* (Table **1**). The sugar amount was also higher in *S. villosa,* differing significantly from the other species (ANOVA, F = 140.8, M = 6.2; $p < 0.05$ – Table **1**).

Table 2 Percentage of fruit set from autonomous, hand self- and cross-pollination and open pollination of *Sinningia schiffneri* and *S. eumorpha* flowers. Figures in parenthesis are number of fruits/number of flowers

Treatments	*S. schiffneri*	*S. eumorpha*
Autonomous self-pollination	0 (0/43)	8 (2/24)
Hand self-pollination	57 (8/14)	56 (14/25)
Hand cross-pollination	50 (9/18)	61 (16/26)
Open pollination (control)	44 (36/81)	69 (18/26)

Flower visitors

Sinningia schiffneri flowers were visited by six species of bees (Table **3**). The most frequent was *Trigonopedia ferruginea* whose individuals are small and to take nectar, they enter in the corolla, contacting anthers and stigmas with the dorsal part of the head and thorax (Fig. **2A**), where a large load of pollen may adhere (Fig. **1E**). At each visit to *S. schiffneri,* a bee may visit 2–3 flowers of the same individual and subsequently flowers of other individuals in the population. *T. ferruginea* is the main pollinator of *S. schiffneri* due to its size, behaviour, and frequency. *Ceratinula* sp. 1 and *Ceratina* sp. 1 only collected pollen from *S. schiffneri* and, although frequent visitors (Table **3**), they were not recorded visiting flowers in the female phase, but occasionally may pollinate flowers in the hermaphrodite phase. *Bombus brasiliensis* and *Euglossa cordata* visited one to two flowers per individual in search of nectar. These bees did not contact the reproductive organs and their frequency was very low (Table **3**). *Augochlora* sp. pierced the corolla at the base to take nectar (Table **3**).

Sinningia eumorpha flowers received visits by eight bee species (Table **3**). *Bombus morio, Epicharis morio,* and *Eufriesea* sp. are large species but, when foraging for nectar, they enter the corolla, touching anthers and stigmas with the dorsal part of the thorax (Fig. **2B**), where the pollen adheres (Fig. **1F**). During

Table 3 Bee visitors in *Sinningia* species in southeastern Brazil. Abbreviations: N = nectar; P = pollen; MP = main pollinator; SP = secondary pollinator; EP = eventual pollinator; NR = nectar robber; PR = pollen robber. In parenthesis is the range in number of flowers observed on each population

Species	Sub-tribe	Genera	Visits (N)[a]	Reward	Role in pollination
S. schiffneri (20–80)	Tapinotaspidini	*Trigonopedia ferruginea*	25	N	MP
	Ceratinini	*Ceratinula* sp. 1/*Ceratina* sp. 1	20	P	PR/EP
	Bombini	*Bombus brasiliensis*	5	N	NR
	Euglossini	*Euglossa cordata*	4	N	NR
	Augochlorini	*Augochlora* sp.	14	N	NR
S. eumorpha (10–40)	Bombini	*Bombus morio*	13	N	MP
	Centridini	*Epicharis morio*	11	N	MP
	Euglossini	*Eufriesea* sp.	4	N	SP
	Tapinotaspidini	*Paratetrapedia* aff. *velutina*	4	N	NR
	Augochlorini	*Augochloropsis* sp.	4	N	NR
	Meliponini	*Plebeia* sp.	3	P	PR
	Ceratinini	*Ceratinula* sp. 2	3	P	PR
	Apini	*Apis mellifera*	13	P	PR
S. villosa (25–30)	Euglossini	*Eulaema cingulata*	13	N	MP
	Euglossini	*Eufriesea surinamensis*	11	N	MP
	Euglossini	*Euglossa* sp.	1	N	NR
	Meliponini	*Tetragonisca* sp.	5	P	PR
S. "canastrensis" (20–30)	Euglossini	*Eufriesea violascens*	5	N	MP
	Megachilini	*Megachile* sp.	2	N	MP
	Ceratinini	*Ceratina* sp. 2	4	N	NR

[a] Visits to plant populations

each visiting bout they may visit 3–8 flowers of different individuals in sequence. *B. morio* and *E. morio* are the main pollinators of *S. eumorpha,* whereas *Eufriesea* sp. is a secondary pollinator due to its low visit frequency (Table **3**). Other visitors to *S. eumorpha* were *Paratetrapedia* aff. *velutina* and *Augochloropsis* sp., which take nectar but are too small to contact stamens and stigmas, and *Plebeia* sp., *Ceratinula* sp. 2, and *Apis mellifera,* which only visit staminate flowers to collect pollen (Table **3**).

S. villosa was visited by four bee species: *Eulaema cingulata, Eufriesea surinamensis, Euglossa* sp., and *Tetragonisca* sp. (Table **3**). Male and female *Eul. cingulata* and *Euf. surinamensis* were the most frequent visitors (Table **3**). These large bees usually only inserted the head in the corolla and, when taking nectar, they contact stamens and stigmas with the forehead, where pollen is deposited (Figs. **1 G, 2 C**). Visits began early in the morning and continued throughout the day until late afternoon, with intervals of 40–60 min, visiting 2–15 flowers of different individuals in sequence. These bee species were the main pollinators of *S. villosa. Euglossa* sp. was occasionally seen on the flowers, hovering and facing several flowers, but made only one visit to probe on nectar. *Tetragonisca* sp. visited only staminate flowers to collect pollen.

Visits to *Sinningia* "canastrensis" occurred only early in the morning and the observed bee species were: *Eufriesea violascens* (females), *Megachile* sp., and *Ceratina* sp. 2 (Table **3**). The first two species entered the flower and, during nectar feeding, contacted stamens and stigmas with the dorsal part of the thorax where pollen adheres, and visited two or three flowers of different individuals in sequence. Both species were main pollinators of *Sinningia* "canastrensis". *Ceratina* sp. 2 also enters the flower, but is too small to contact stamens and stigmas during nectar intake.

Discussion

The four *Sinningia* species have an annual reproductive phenological pattern (*sensu* Newstrom et al., 1994), as is commonly found in species of the tribe Sinningieae. The long flowering period of the *S. schiffneri* population in Ubatuba is a common pattern in coastal areas with low climatic seasonality (SanMartin-Gajardo and Morellato, 2003). The flowering strategy of the four species is characteristic of the steady state type, as defined by Gentry (1974), and has been related to plant species dependent on pollinators that visit flowers in a regular sequence, such as Euglossini (Janzen, 1971) or *Bombus* species (Heinrich, 1976).

General floral features of these *Sinningia* species, such as zygomorphy, colour, corolla with a large inferior petal and nectar guides, as well as hidden nectar, correspond to the melittophilous syndrome (Faegri and van der Pijl, 1980), but special attributes to support the euglossophilous system of Wiehler (1983) are hardly evident. Size and shape of the corolla in the studied species seem to determine which bees are appropriate pollinators, while body size and tongue length of bee visitors are the features which influence their pollinating capability. Thus, in *S. schiffneri* only small bees (5–8 mm body length), and in *S. eumorpha* and *Sinningia* "canastrensis" only large bees (14–18 mm body length) have adequate dimensions to match flower size. On the other hand, in *S. villosa,* where the anthers block the mouth of the flowers, only long-tongued bees are able to act as pollinators. These features resemble those designed by Steiner (1985) who pointed out that effective pollination of *Drymonia* flowers depends on pollinator size.

Although self-compatible, *S. schiffneri* and *S. eumorpha* are basically pollinator-dependent. Self-compatibility also occurs in other *Sinningia* species (Clayberg, 1968) and in other species of the tribe Sinningieae (SanMartin-Gajardo, pers. obs.), but autonomous self-pollination is rare because protandry combined with herkogamy prevents it (SanMartin-Gajardo and Freitas, 1999). Autonomous self-pollination, however, was recorded in cultivated individuals of *S. pusilla* (Clayberg, 1996), which has very small bee-pollinated flowers.

The number of nectary glands was very diverse in the studied species and reflects the multiple origins within the tribe, as suggested by Perret et al. (2003). Differences in nectar features between *S. villosa* and the other three species seem to be related to the different groups of pollinating bees. High nectar volume, concentration, and sugar amount, as found for *S. villosa*, is expected for flowers adapted to Euglossine pollination, since these bees forage long distances along a feeding route (Janzen, 1971) and consume relatively large quantities of nectar (Dressler, 1982). However, the nectar features of *Sinningia* "canastrensis" and behaviour of the Euglossine pollinator, are not typical for trapliners (Janzen, 1971), which may be related to the adverse climatic conditions (mist, low temperatures, and strong, constant winds) of the montane rocky outcrops where this species occurs (Faria, 1994).

The mean nectar volume of the studied species is slightly lower than the mean values for tropical species pollinated by bees (Opler, 1983), but low nectar production of *Sinningia* species seems to be a general feature of the genus regardless of the pollinator agent, since the mean volume of ornithophilous *Sinningia* species is also very low (6.0 µl for *S. douglasii* [Lindl.] Chautems, Sazima et al., 1996; 2 – 3 µl for *S. allagophylla* [Mart.] Wiehler, L. Freitas pers. comm.; 2.6 µl for *S. tuberosa* [Mart.] H. E. Moore, 4.5 µl for *S. cochlearis* [Hook.] Chautems, and 10.0 µl for *S. gigantifolia* Chautems, SanMartin-Gajardo, in prep.). Nectar concentration of these species, however, conform to the average values for tropical bee-pollinated species (Opler, 1983), in contrast to that for the ornithophilous *Sinningia* species, whose nectar concentration is lower than the average value for this syndrome (I. SanMartin-Gajardo, pers. obs.). Perret et al. (2001) found great similarity on nectar volume, sugar composition and concentration for both bee and hummingbird flowers of Sinningieae species. According to Perret et al. (2001), switches between these pollination syndromes in the tribe have thus relied on characters linked to flower display and to accessibility of the reward rather than on nectar characteristics.

The pollinators of the *Sinningia* species here studied belong to several groups of bees, namely Tapinotaspidini, Bombini, Centridini, Euglossini, and Megachilini, with three of the four species pollinated by non-Euglossine bees. This is inconsistent with the proposal of Wiehler (1983), based on flower size, corolla shape, and colour, that Euglossine would be pollinators of all bee-pollinated neotropical Gesneriaceae. Previous studies, based on occasional and restricted field records for eight Gesnerioideae species (Dressler, 1968; Wiehler, 1977, 1978), had indeed supported that view. Pollination by distinct groups of bees, as found in this study, reflects the marked differences in floral morphology present in these species. Such a variability may indicate multiple origins within the genus for pollination systems involving bees, an idea highly supported by molecular phylogenetic analyses of 76 species of the Sinningieae (Perret et al., 2003). The four species here studied each belong to a different lineage of the monophyletic clades within this tribe as recognized by Perret et al. (2003). Such a scenario indicates that the approximately 20 melittophilous species, in the predominantly ornithophilous Sinningieae tribe, may correspond to a series of independent changes toward bee pollination.

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