ANNALS OF
BOTANY
Founded 1887

Mutual reproductive dependence of distylic *Cordia leucocephala* (Cordiaceae) and oligolectic *Ceblurgus longipalpis* (Halictidae, Rophitinae) in the Caatinga

Paulo Milet-Pinheiro[1],* and Clemens Schlindwein[2]

[1]*Programa de Pós-Graduação em Biologia Vegetal, Universidade Federal de Pernambuco. Av. Prof. Moraes Rego, s/n, 50670-901 Recife, PE, Brazil and* [2]*Departamento de Botânica, Universidade Federal de Pernambuco, Av. Prof. Moraes Rego, s/n, 50670-901 Recife, PE, Brazil*
* For correspondence. E-mail miletpinheiro@hotmail.com

• *Background and Aims* The close relationship between distylic *Cordia leucocephala* and the bee *Ceblurgus longipalpis*, both endemic to the Caatinga, north-east Brazil, was investigated, emphasizing reproductive dependence, morphological adaptations of the partners, and pollen flow.
• *Methods* In the municipality of Pedra, in the Caatinga of Pernambuco, the breeding system and reproductive success of *C. leucocephala*, its interaction with flower visitors and inter- and intramorph pollen flow were determined.
• *Key Results* The bee *Ceblurgus longipalpis*, the unique flower visitor and effective pollinator of self-incompatible *Cordia leucocephala*, presents morphological features adapted to exploit hidden pollen and nectar in the long and narrow corolla tubes. Pollen of low-level anthers is collected with hairs on prolonged mouthparts and pollen of high-level anthers with clypeus, mandibles, and labrum, showing pollen removal from both levels with the same effectiveness. In both morphs, this results in similar legitimate, i.e. intermorph cross-pollen flow. Illegitimate pollen flow to stigmas of pin flowers, however, was much higher than to stigmas of thrum flowers. Moreover, more illegitimate pollen was transported to stigmas of pin and less to those of thrum flowers when compared with legitimate pollen flow.
• *Conclusions* The study reveals a one-to-one reproductive inter-dependence between both partners. Data indicate that this relationship between bee species and plant species is one of the rare cases of monolecty among bees. Monotypic *Ceblurgus longipalpis*, the only rophitine species of Brazil, evolved prolonged mouthparts rare among short-tongued bees that enable them to access pollen from flowers with short-level anthers hidden for bees of other species, and nectar at the base of the flower tube.

Key words: Caatinga, *Ceblurgus longipalpis*, *Cordia leucocephala*, Cordiaceae, heterostyly, north-east Brazil, oligolectic bees, pollen flow, pollination, Rophitinae.

INTRODUCTION

Plant–pollinator associations range from apparently unspecialized relationships, where the flowers of a plant species attract and are pollinated by animals of numerous interchangeable species, to highly specialized associations that involve only a few species of animal pollinators, which often show morphological or behavioural adaptations enabling or optimizing resource collection in these specialists. Topics of specialization versus generalization and analyses of webs of interactions between flowers and pollinators have received considerable attention recently (Vásquez and Aizen, 2003, 2006; Armbruster, 2006; Jordano *et al.*, 2006; Minckley and Roulston, 2006; Ollerton *et al.*, 2006; Petanidou and Potts, 2006; Stang *et al.*, 2009; Vásquez *et al.*, 2009). A group of such specialist flower visitors are oligolectic bees, which are pollen specialists that feed their larvae with pollen only from species of the same genus or plant family (Robertson, 1925; Wcislo and Cane, 1996; Cane and Sipes, 2006). Recent analyses showed that in lineages where oligolectic bee species are common, oligolecty was basal and polylectic species derived (Müller, 1996*a*; Sipes and Tepedino, 2005; Danforth *et al.*,

2006; Patiny *et al.*, 2007; Larkin *et al.*, 2008; Sedivy *et al.*, 2008). Several pollination studies involving oligolectic pollinators showed that the oligolectic species were the most effective pollinators (Cane *et al.*, 1985; Neff and Rozen, 1995; Schlindwein and Wittmann, 1995, 1997*a*, *b*; Schlindwein and Martins, 2000; Larsson, 2005; Schlindwein *et al.*, 2005), but in a few cases oligolectic species did not contribute to cross pollination (Linsley *et al.*, 1963*a*, *b*, 1964; Barrows *et al.*, 1976; Grant and Grant, 1979; Grant and Hurd, 1979).

Ceblurgus longipalpis, the only species of the halictid subfamily Rophitinae in Brazil, was recorded exclusively in flowers of *Cordia leucocephala* (Cordiaceae) in the Caatinga of north-east Brazil (Aguiar and Martins, 1995). Rophitinae comprises 208 species, mainly distributed in the holarctic and Africa. Existing information on host plants indicates that rophitine bees are oligolectic (Michener, 2007; Patiny *et al.*, 2007). Special features to collect pollen, such as specialized facial pilosity, as well as unbranched setae and long plumose hairs in the basal segments of mouthparts (prementum, galeae, first and second labial palpi) were observed in other rophitine species (see, for example, Eickwort *et al.*, 1986; Müller, 1996*b*; Rozen, 1997).

The cosmopolitan genus *Cordia* (Cordiaceae, formerly Boraginaceae, recently given family status) comprises about 350 species (Judd *et al.*, 1999; Miller, 2001), which show, in general, distyly with pin (long-styled) and thrum (short-styled) flowers (Gibbs and Taroda, 1983; Taroda and Gibbs, 1986, 1987; Machado, 1990). Heterostylic species are predominantly pollinated by insects (Ganders, 1979) and flowers are frequently related to several pollinator species (Barrett, 1978; Schlindwein and Medeiros, 2006; Kuriakose *et al.*, 2009), but recent studies showed that interactions with pollinators can be very close (Alves-dos-Santos and Wittmann, 1999, 2000).

The pollination of *Cordia leucocephala* was investigated by asking the following questions. (*a*) Which features differ in pin and thrum morphs? (*b*) Are bees of *Ceblurgus longipalpis* effective pollinators adapted to collect low- and high-level pollen in flowers of *C. leucocephala*? (*c*) Are both partners reproductively interdependent? (*d*) What is the magnitude of intra- and intermorph pollen flow in the *Cordia–Ceblurgus* association?

MATERIALS AND METHODS

Study site

The field study was carried out from March to June 2007 and 2008 at the Farm 'Gentio' (8°65′35·54″S, 36°87′30·2″W; 550 m a.s.l.), located in the municipality of Pedra, Pernambuco, north-east Brazil. The farm is situated in the region of Caatinga, a tropical dry forest dominated by xerophytic thorny trees and shrubs and abundant succulent plants. A dense stratum of ephemeral herbs occurs during the short, irregular rainy season. The area studied is used by extensive cattle and caprine livestock farming. The meteorological station at Pedra has recorded annual averages for temperature of 22·9 °C and precipitation of 550 mm. The rainy season is from February to June (CPTEC, 2009).

Species studied

Cordia leucocephala is a distylic shrub endemic to the Caatinga and flowers during the rainy season, mostly from February to April (Machado, 1990). Globose inflorescences bear up to 20 white funnel-shaped flowers and a stigma with four appendices (Melo and Sales, 2005). Due to rapid unfolding of petals from flower buds, inconspicuous pre-flowering inflorescences develop in characteristic showy white flower balls in up to 30 min in the morning. In a population of Alagoinha, Pernambuco, Brazil, Machado (1990) found differences in style and stamen length, as well as pollen size between pin and thrum morphs of *C. leucocephala*. A controlled pollination experiment, however, did not provide an explanation of the breeding system. Legitimate hand cross-pollination resulted in only one fruit, while flowers accessible for pollinators did not set fruits.

Flower morphology and anthesis

Fifty flower buds of each morph were bagged to accompany and describe the anthesis from opening to abscission. The time of flower opening, anther dehiscence, stigma receptivity,

pollen viability, nectar availability and blooming patterns were recorded. Stigma receptivity was determined using H_2O_2 (10%) and potassium permanganate (0·1%). To determine pollen viability, grains from ten thrum and ten pin flowers were removed from anthers fixed in FAA, and transferred to microscope slides with acetocarmine solution. Three hundred pollen grains per flower were counted under the microscope to determine the rate of stained viable grains (Dafni *et al.*, 2005). Microscope slides were prepared with fresh pollen grains from each of ten flowers of each morph. Pollen grains were picked up with a small piece of glycerin gelatine, mounted with a cover glass and sealed with paraffin wax. The grains were measured, and ornamentation of the exine was analysed under the microscope. The diameter of 15 grains was measured in equatorial view in each of ten flowers per morph. Unviable empty pollen grains were not considered in the measurements of size. Moreover floral parts were analysed by scanning electron microscope LEO VP 438 (SEM). Material was dehydrated in a graded ethanol series, and critical point dried following the standard procedure and sputter coated with gold.

Volume and concentration of nectar were measured with 0·5-µL microcapillars (Brand) and a pocket refractometer (Atago), respectively. Measurements were made at the end of anthesis, using 38 previously bagged flowers from each flower morph of 38 individuals. In 15 flowers of thrum and pin individuals ($n = 15$ for each morph), the diameter of the flower entrance, and length of the corolla, calyx, stamens, style and stigma lobes, were measured using a digital caliper (Stainless Digimess). Differences in stigmatic papillae between both morphs were analysed in SEM. The ratio of thrum and pin individuals in the population was determined in 100 individual plants.

Breeding system

To determine the breeding system of *C. leucocephala* the following controlled pollination experiments were performed in both morphs: (*a*) spontaneous self-pollination, bagged flowers were maintained closed; (*b*) hand self-pollination, bagged flowers were pollinated with self-pollen; (*c*) inter- and intra-morph hand cross-pollination, flowers were pollinated with different pollen donors, at least 1 km distant; and (*d*) pollination of open flowers accessible for flower visitors in natural conditions (control). For each treatment in each morph, 40 flowers bagged before anthesis, excepting 60 marked control flowers per morph, were used. Mature fruits were collected to count the seeds.

The pollen : ovule ratio was determined from ten flower buds collected from ten random plant individuals in the field. All pollen grains were removed from the anthers, stained with fuchsin, and counted under the stereo-microscope (Zeiss Stemi SV8). The ovaries of each flower were dissected and the ovules counted under a stereo-microscope. Average and standard deviation of the pollen : ovule ratio of *C. leucocephala* were determined.

Flower visitors and effective pollinators

The flower-visiting insects were captured in thrum and pin morphs with entomological nets, mounted, identified and stored in the Entomological Collection of the Federal

University of Pernambuco (UFPE). Collection data were included in the database of the working group. Plant vouchers were stored in the Herbarium Geraldo Mariz UPE, Recife.

The frequency of visitors was determined in three thrum and three pin individuals for three non-consecutive days. In each individual, ten flowers were monitored from 0800 h to 1400 h, corresponding to a total of 18 observation hours. During flower visits, the behaviour of visitors was observed and the floral resource collected noted. To determine effective pollinators, their frequency, contacts to stigmas and anthers and flights performed among conspecific plant individuals were considered.

The relative frequency of pollen from thrum and pin morphs of *Cordia leucocephala* in metasomal and hind leg scopae of flower visitors was taken to determine their flower fidelity, considering uniform scopa loads of females as indicative of flower constancy. The body surface of six individuals was screened for pollen, and samples were removed from the different body parts of the visitors with small pieces of glycerin gelatine, transferred to a microscope slide, mounted with a cover glass and sealed with paraffin wax. Pollen loads were analysed by counting 300 pollen grains per sample. The ratio of pollen grains from thrum and pin morphs of *C. leucocephala*, as well as those of other plant species adhered to each body part of the visitors was counted under the microscope. Pollen grains adhering to specific body parts were also analysed and documented with SEM.

The number of pollen grains was counted from thrum and pin flowers of *C. leucocephala* adhering to stigmas as a measure of legitimate and illegitimate pollen flow per floral morph, as well as the non-*Cordia* pollen grains deposited on the stigma surface. The stigmas of ten thrum and ten pin flowers were removed at the end of anthesis in still-open flowers.

Moreover, only one flower visit of males and females ($n = 10$ for each morph per sex of bee) of *Ceblurgus longipalpis* was permitted to recently unbagged flowers to measure the pollen flow per single-flower visits. After the flower visit, the stigma was placed on a microscope slide containing fuchsin-stained glycerine gelatine. Pollen grains from thrum and pin morphs of *C. leucocephala* and other plants were counted under the microscope.

Statistical analyses

The *t*-test (independent samples) was used to verify differences between the following means in distinct morphs: stigma and stamen length, nectar volume, pollen number, and flow of legitimate and illegitimate pollen grains. The *t*-tests (dependent samples) was used to compare mean flow of legitimate and illegitimate pollen grains in flowers of the same morph and to verify if pollen grains of thrum and pin flowers were deposited on specific body parts of the pollinators. Normality of the samples was taken by Shapiro−Wilk's tests (Zar, 1999).

RESULTS

Flower morphology and anthesis

Cordia leucocephala has distylic flowers, which varied in at least seven features. Pollen grains of thrum flowers were larger (mean diameter $62 \cdot 2 \pm 2 \cdot 1$ μm, $n = 150$) than those

of pin flowers (mean diameter $50 \cdot 1 \pm 1 \cdot 7$ μm, $n = 150$), and there was no overlap in size variation of pollen grains between both morphs. Thrum flowers produced much fewer pollen grains per flower (mean $= 3361 \pm 211$, $n = 10$) than pin flowers (5223 ± 339, $n = 10$) ($t = 14 \cdot 74$; d.f. $= 18$; $P = 0 \cdot 0001$). Styles of thrum flowers were shorter ($6 \pm 0 \cdot 3$ mm, $n = 10$) than those of pin flowers ($14 \cdot 2 \pm 1 \cdot 1$ mm, $n = 10$), as well as stigma lobes ($1 \cdot 33 \pm 0 \cdot 16$ mm, $n = 15$ and $1 \cdot 81 \pm 0 \cdot 2$ mm, $n = 15$, respectively). The stigmatic papilla from thrum flowers were smaller (from $5 \cdot 5$ to $10 \cdot 1$ μm) than those from pin flowers (from 13 to $20 \cdot 2$ μm) (Fig. 1A, B).
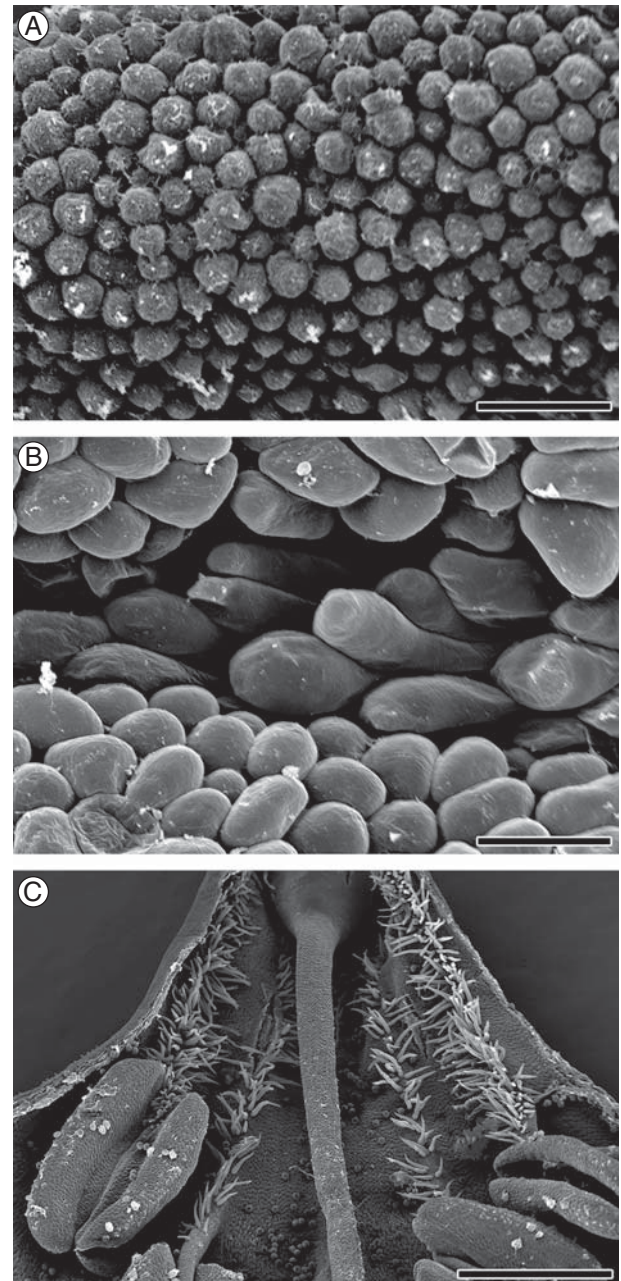

FIG. 1. (A, B) Scanning electron microscopy of the stigmatic papillae of thrum (A) and pin flowers (B). (C) Hairs at the basis of filaments of pin flowers of *Cordia leucocephala*, forming five channels where the bees insert their tongues. Scale bars: (A, B) = 25 μm; (C) = 1 mm.

Filaments were disposed in three levels that were shorter in pin flowers ($6·1 \pm 1·1$; $8·2 \pm 1·4$; $9·7 \pm 1·3$ mm, $n = 15$) than in thrum flowers ($9·4 \pm 0·8$; $11·6 \pm 1·1$; $13·4 \pm 1·1$ mm, $n = 15$). The base of the filaments of both morphs, and its continuation downward with the fused petals bears dense trichomes and delimits five channels that lead to the nectar disc at the base of the ovary (Fig. 1C). The flowers of *C. leucocephala* opened simultaneously, depending upon weather conditions between 0710 h and 0830 h, and had stigmas already receptive and anthers dehiscing at the beginning of anthesis. The flowers wilted and corollas fell between 1445 h and 1700 h. Pollen viability was similar and high in thrum (mean and standard deviation, $96·5\% \pm 1·5$) and pin flowers ($95·2\% \pm 1·9$). Flowers of both morphs produced small amounts of nectar, but thrum flowers on average produced more ($0·16 \pm 0·05$ µL, $n = 38$) than pin flowers ($0·09 \pm 0·03$ µL, $n = 38$) ($t = 6·83$; d.f. $= 74$; $P < 0·0001$).

Breeding system

Controlled pollination showed that the flowers of *Cordia leucocephala* were self-incompatible (Table 1). Hand self-pollinated as well as intra-morph hand cross-pollinated flowers set only one fruit each. Only open-pollinated and inter-morph hand cross-pollinated flowers showed substantial fruit set. Fruit set in open-ollinated flowers was similar to that of intermorph hand cross-pollinated flowers (Table 1). The ovaries of *Cordia leucocephala* contained four ovules ($n = 10$ for each morph) but all fruits set only one seed. The pollen : ovule ratio was $840·2 \pm 52·8$ in thrum flowers ($n = 10$) and $1305·7 \pm 84·7$ in pin flowers ($n = 10$).

Flower visitors and effective pollinators

Males and females of *Ceblurgus longipalpis* were almost exclusive flower visitors of *Cordia leucocephala*. During the whole period of fieldwork, only sporadic visits of workers of

Apis mellifera and one flower visit of the butterfly *Phoebes sennae* (Pieridae) were observed. Visits to flowers of *Cordia leucocephala* began as soon as the flowers opened but were more frequent between 1000 h and 1200 h (Fig. 2). Females were more frequent flower visitors (on average 9·5 visits per flower, $n = 60$ flowers) than males (6·4 visits per flower, $n = 60$ flowers). Males visited the flowers about 30 min before females (Fig. 2).

Screening for pollen on the body surface of females of *Ceblurgus longipalpis* after flower visits showed that pollen grains adhered to (*a*) mouthparts (the ventral parts of first and second labial palpal segments and prementum), (*b*) anterior part of clypeus, labrum and mandibles, (*c*) ventral metasomal scopa and (*d*) scopa on femur and tibia of hind legs. Labial palpi and prementum carried on average 77·4 % pollen grains from pin flowers and only 22·6 % from thrum flowers ($t = 4·09$; d.f. $= 5$; $P = 0·009$; Fig. 3A). Clypeus, labrum and mandibles, on the other hand, carried pollen grains from pin and thrum flowers in similar numbers (41 % and 59 %, respectively; $P = 0·5$; $t = 0·7$; d.f. $= 5$; Fig. 3B). The first and second segment of labial palpi carried long, plumose setae (length 300–400 µm), sparsely distributed on the ventral surface (distance between hairs 100–200 µm) (Fig. 4A). The surface between the setae was dotted with short (length about 10 µm) and dense bristles, separated by approx. 10 µm from each other. Pollen grains found on both basal segments were located in the space between the long setae and the short bristles (Fig. 4B). The ventral surface of the prementum was covered only with short bristles. Clypeus and mandibles contained long plumose hairs forming a fringe (Fig. 4C). Males of *Ceblurgus longipalpis* showed no setae on the ventral surface of both basal segments of the labial palpi and the specimens collected carried no or only sporadically a few grains at the mouthparts.

The scopae of females were formed by long and dense plumose setae, both those on the hind femur and tibia (Fig. 5A) and on metasomal sterna (Fig. 5B). Scopal pollen loads were exclusively composed of pollen from *Cordia leucocephala* (Fig. 5C, D). Scopal hairs on metasoma and on hind legs contained more pollen grains from pin than from thrum flowers and both in similar proportions (62·4 % and 60 %, respectively, from pin flowers at metasomal sterna and hind legs, and 37·6 % and 40 %, respectively, from thrum flowers; $n = 6$; Fig. 6A, B).

TABLE 1. *Controlled pollination experiments in* Cordia leucocephala

Pollination treatment	Floral morph	No. of flowers	Fruit set (*n*)	Fruit set (%)
Spontaneous self-pollination	S	40	1	2·5
	L	40	0	0
Hand self-pollination	S	40	1	2·5
	L	40	0	0
Hand cross-pollination	L* × L[†]	40	0	0
	S* × S[†]	40	1	2·5
	S* × L[†]	40	14	35
	L* × S[†]	40	9	22·5
Open-pollinated flowers (control)	S	60	25	41
	L	60	12	20

In the treatments, hand self-pollination, spontaneous self-pollination and hand cross-pollination the flower buds were bagged before anthesis. Open pollinated flowers (control) were marked and received flower visitors.

S, short-styled morph; L, long-styled morph.

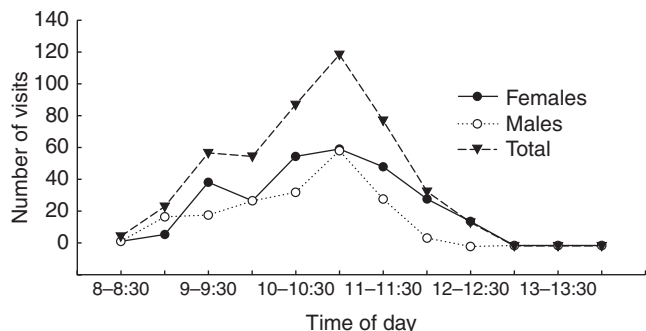* Pollen receptor; †pollen donor.

FIG. 2. Frequency of flower visits of males and females of *Ceblurgus longipalpis* in *Cordia leucocephala*. Observations were made on three non-consecutive days from flower opening to flower abscission.
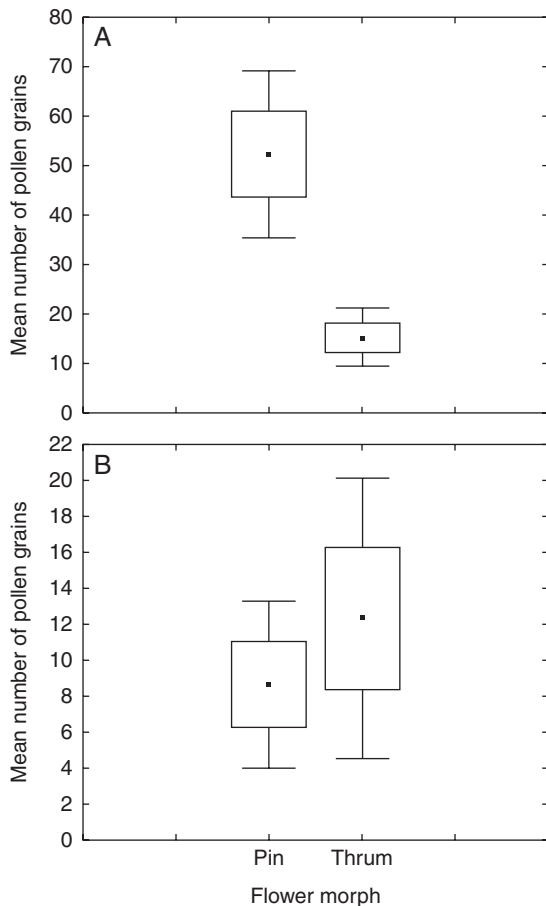
FIG. 3. Mean numbers of pollen grains from pin and thrum flowers of *Cordia leucocephala* in the mouth parts of *Ceblurgus longipalpis*: (A) ventral parts of first and second labial palpal segments and prementum and (B) anterior part of clypeus, labrum and mandibles. Square dots indicate the mean, boxes indicate the mean ± s.e., and error bars are the mean ± 1·96 × s.e.

Pollen flow

During a single flower visit, *Ceblurgus longipalpis* deposited more pollen grains from pin flowers on stigmas of both floral morphs. Females deposited, on average, almost twice the number of pollen grains on the stigma of thrum flowers and pin flowers than males (Fig. 7A, B). A lower amount of pollen from high-level anthers (thrum flowers) was deposited on stigmas of pin flowers (legitimate pollination) than vice versa (i.e. legitimate pollination, low-level anthers).

At the end of anthesis only conspecific pollen grains adhered to stigmas of thrum and pin flowers of *Cordia leucocephala*. Pollen from thrum and pin flowers was present on stigmas of both morphs. Stigmas of pin flowers received more pollen grains than those of thrum flowers (Fig. 8). In both morphs, intermorph pollen flow was similar ($t = 0.3$; d.f. $= 18$; $P = 0.75$). Intramorph pollen flow in pin flowers, however, was higher than intermorph pollen flow ($t = 2.2$; d.f. $= 9$; $P = 0.05$) and in thrum flowers lower than intermorph pollen flow ($t = 3.7$; d.f. $= 9$; $P = 0.005$). Intramorph pollen flow in pin flowers was much higher than that of short-styled flower ($t = 4.7$; d.f. $= 18$; $P = 0.0001$).

Females of *Ceblurgus longipalpis* foraged in flowers for nectar and pollen (Fig. 9A, B). During nectar uptake, abdominal segments retracted and extended repeatedly. Pollen was collected extending the mouthparts. After several visits, females cleaned face and mouthparts with the anterior legs, and then transferred the pollen grains from forelegs to the scopae. Tibia and femur of forelegs show a fringe of extraordinarily long plumose setae along the ventral border (Fig. 10A). Several females carried pollen grains in the hairs of the tibial fringe near the strigilis (Fig. 10B).

Patrolling flowers in the search of females, males generally visited the flowers to take up nectar. Near the end of anthesis, they looked for pin flowers in which to sleep. Attached to the long styles with their mandibles, they avoided the falling abscised corolla.

DISCUSSION

Reciprocal reproductive dependence of Cordia leucocephala *and* Ceblurgus longipalpis

This study strongly indicates that both partners are reproductively interdependent. The flowers of *Cordia leucocephala* were visited exclusively by females and males of *Ceblurgus longipalpis*, aside from the sporadic visits of introduced honeybees. This can be inferred also from bee-plant surveys at other sites in the Caatinga, like Sertânia, Buique (Pernambuco) (C. Schlindwein *et al.*, unpubl. res.), Serra Branca (C. Schlindwein, unpubl. res.), São João do Cariri (Paraíba) (Aguiar and Martins, 1994; Aguiar *et al.*, 1995) and Casa Nova (Bahia) (Martins, 1994), where these bees were also the unique flower visitors. Thus, self-incompatible *C. leucocephala* flowers may set no fruits in the absence of this bee species. All information on host–plant relationships of *Ceblurgus longipalpis* points exclusively to *C. leucocephala* as the only food source even in the presence of congeneric individuals of *C. globosa*, indicating that this is one of the rare cases of monolecty among bees. Thus, bees of *Ceblurgus longipalpis* do not survive in the absence of this plant species. Such a one-to-one reproductive interdependence among pollinators and plants is rare, and it would be interesting to determine if this bee is the sole pollinator of *C. leucocephala* in other parts of the distribution of the plant.

The white, showy, funnel-shaped flowers, however, should attract several flower visitors. An outstanding feature that excludes other pollen-searching bees as flower visitors is concealment of anthers in the basal tubular part of the funnel-shaped flowers. Only bees with prolonged, specialized mouthparts can collect this hidden pollen. Pollen collection from narrow flower tubes with mouthparts is rare, but evolved several times in different lineages and always involves specific adaptations of the bee species (Eickwort *et al.*, 1986; Müller, 1995, 1996b, 2006; Alves-dos-Santos, 2003; Müller and Kuhlmann, 2003; Krenn *et al.*, 2005). In *Ceblurgus longipalpis*, the long, densely plumose hairs on both basal segments of the labial palpi are adaptations for pollen removal from the low-level anthers. Pollen analyses show that thrum flowers, thus, are pollinated exclusively by grains adhering to this labial palpi pollen brush.
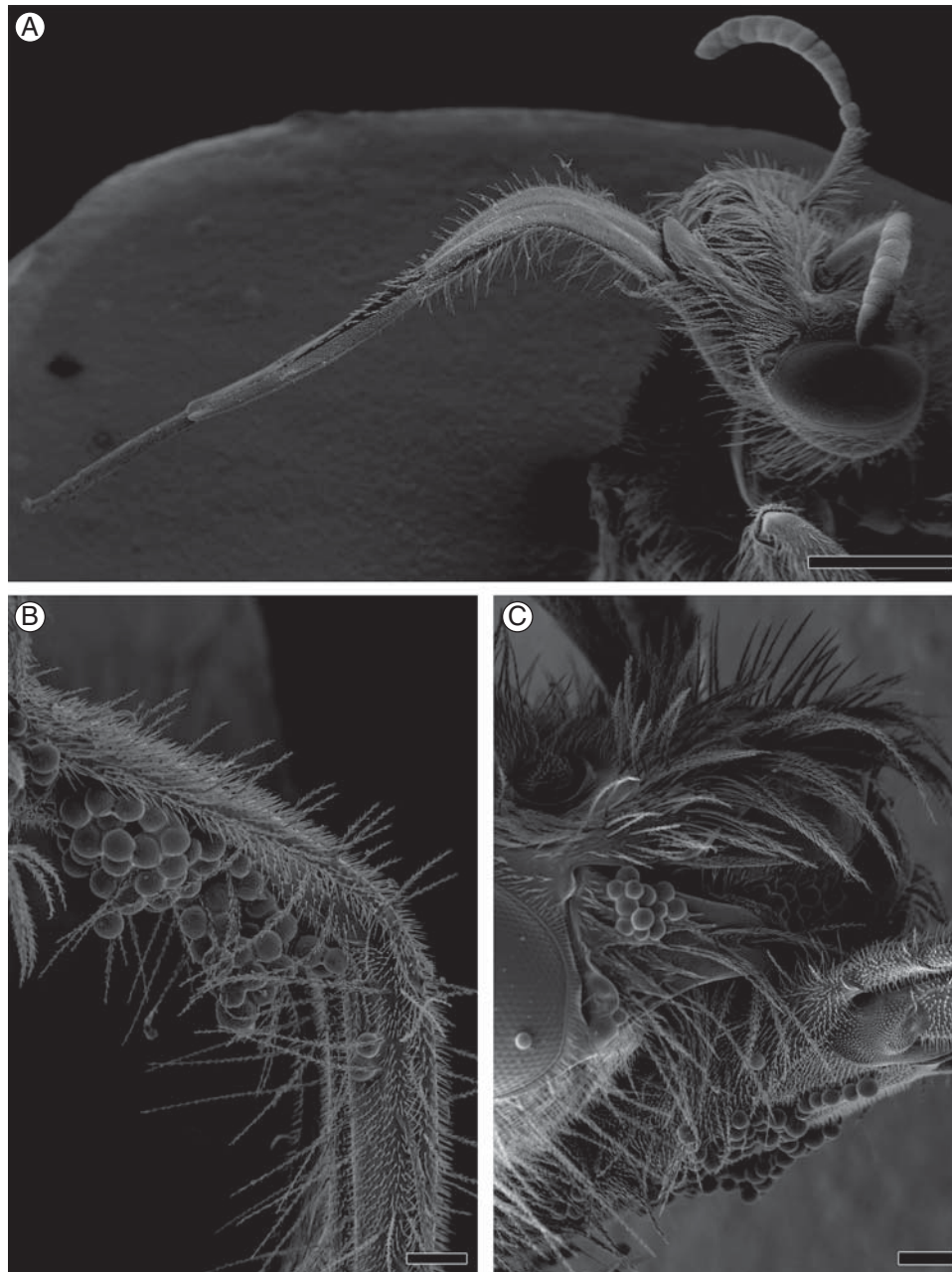
Fıg. 4. Hairs of *Ceblurgus longipalpis* specialized to collect pollen grains from flowers of *Cordia leucocephala*. (A) General view of the head and tongue of *Ceblurgus longipalpis*. (B, C) Pollen grains adhered to the plumose setae and bristles of the basal segments of the labial palpis (B) and to the long plumose hairs in the clypeus and mandibles forming a fringe (C). Scale bars: (A) = 1 mm; (B, C) = 100 μm.

Various features characterizing the *Ceblurgus longipalpis–Cordia leucocephala* association resemble those of *Ancyloscelis gigas–Eichhornia azurea* association (Alves-dos-Santos and Wittmann, 1999, 2000; Alves-dos-Santos, 2002, 2003). In both cases, the plant species involved show heteromorphic self-incompatibility (tristyly in the case of *Eichornia*) and funnel-shaped flowers with a long narrow corolla tube that bears concealed anthers. In both associations, there is only one highly specialized effective bee pollinator, which is narrowly oligolectic (probably monolectic in both cases) and shows extraordinarily long mouthparts with an unusual pollen brush to remove pollen from morphs with low-level anthers.

In both cases, competing flower visitors are almost absent. In places where *Ancyloscelis gigas* did not occur, the populations of *E. azurea* showed a breakdown of heterostyly (Alves-dos-Santos, 2002). This is also to be expected, in the *Ceblurgus longipalpis–Cordia leucocephala* association, if *C. longipalpis* is not substituted by another specialized pollinator appropriately equipped to guarantee pollination of the thrum flowers.

Besides collecting hidden pollen grains, long mouthparts of *C. longipalpis* are suitable for collecting nectar from flowers of *C. leucocephala*. Other flower-visiting insects with long mouthparts like butterflies and bees of Euglossini, Centridini
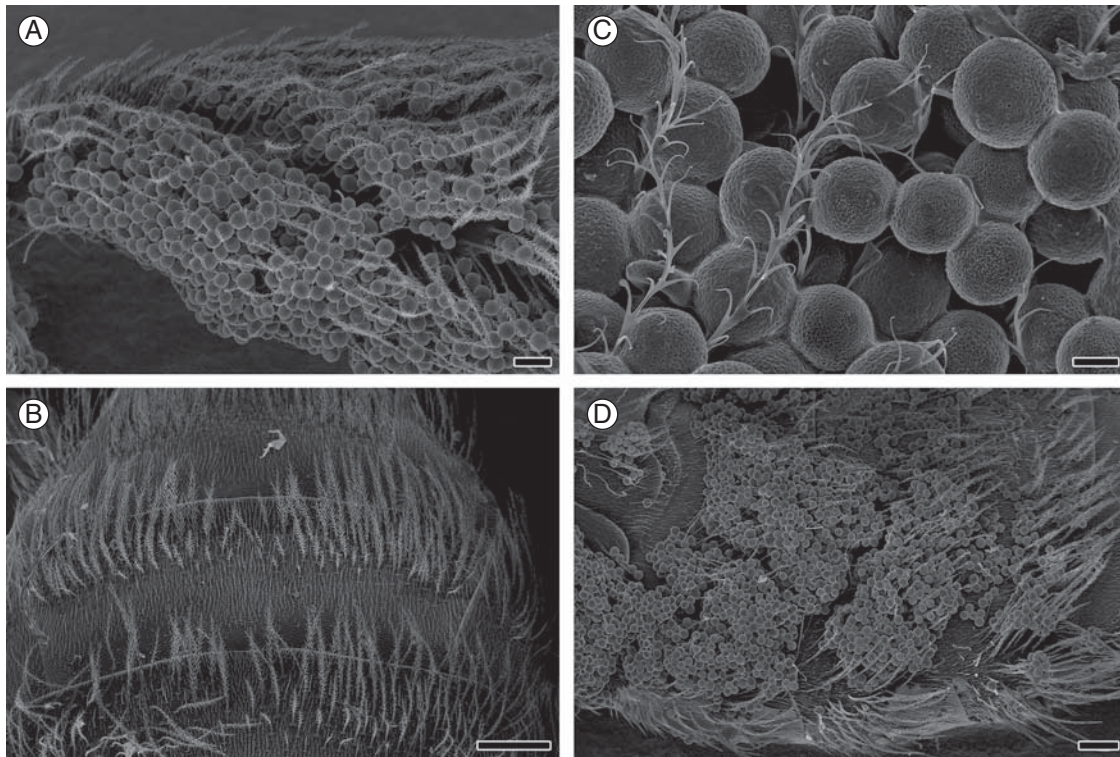
FIG. 5. Hairs of *Ceblurgus longipalpis* specialized to store and transport pollen grains of flowers of *Cordia leucocephala*. (A, B) Long and dense plumose setae on hind femur and tibia (A) and on metasomal sternits (B). (C, D) Detail of the scopae on hind femur and tibia (C) and on metasomal sternits (D) full of pollen grains from pin and thrum flowers. Scale bars: (A) = 100 μm; (B) = 300 μm; (C) = 20 μm; (D) = 200 μm.

and Eucerini or hummingbirds would also reach the nectaries at the base of the flowers. The very low nectar amounts, together with the narrow flower tube, however, do not make the flowers of *C. leucocephala* attractive to these medium- to large-sized pollinators. The low availability of nectar per flower, however, seems to be enough to fulfil the energy requirements of *Ceblurgus longipalpis* and to nourish their larvae, no visits to nectar-rich flowers being observed.

The obligate association of *Ceblurgus longipalpis* and *Cordia leucocephala* is unknown to occur elsewhere among close relatives of either of the two partners. *Cordia* is a huge pantropical genus, comprising about 350 species (Judd *et al.*, 1999; Miller, 2001). Available information points to several plant–pollinator systems including pollination by flies, butterflies, sphingids, bees, hummingbirds and bats (Opler *et al.*, 1975; Machado and Loiola, 2000; Souza-Silva *et al.*, 2001; Darrault and Schlindwein, 2002).

The monotypic *Ceblurgus longipalpis* is the unique, geographically isolated Brazilian species of Rophitinae. Rophitine species found most closely to the Caatinga-resident *Ceblurgus* belong to the genera *Goeletapis* and *Penapis* and occur about 5000 km apart, west of the Andean Cordillera under desert climates in Peru and Chile (Rozen, 1997). With *Ceblurgus* they constitute the South American tribe Penapini, with a total of only five species, the unique Rophitinae in South America (Engel, 2001; Michener, 2007; Patiny *et al.*, 2007). Bees of this tribe differ from most other Halictidae by their long mouthparts formed by the extraordinarily elongated glossae, labial palpi and galeal blades (Rozen, 1997; Engel, 2001; Michener, 2007). However, in *Goeletapis*

and *Penapis*, the setae on labial palpi are fine, nonplumose, short and inconspicuous, different from the very long plumose setae that form the pollen brush on the two basal segments of the labial palpi in *Ceblurgus* (Rozen, 1997). Like other species of Rophitinae, the Penapini are also oligolectic. The host plants of *Goeletapis peruensis* are *Exodeconus maritimus* and *E. prostratus* (Solanaceae) and those of *Penapis penai*, *P. moldenkei* and probably of *P. toroi* as well, are two species of *Argylya* (Bignoniaceae) (Rozen, 1997). All three families of the host plants involved are phylogenetically not closely related to each other and belong to different plant orders (Stevens, 2001), but the plant species have narrow corolla tubes in common. The long, modified mouthparts of Penapini were probably the precondition for specializing on concealed food resources which are not available to bees with short mouthparts. Host plant switches of ancestral bee species, to unrelated plant species, thus, might have occurred. The unusual prolonged glossa, enclosed in a tube formed by the four elongated and flattened segments of the labial palpi in the species of Penapini, are suitable for taking up nectar from long flower tubes. The structures can be interpreted as a convergent adaptation to that found in the numerous long-tongued bees in the sense of Michener (2007). Structural components similar to those of long-tongued bees found in the mouthparts of short-tongued bees were also described for *Niltonia virgilii* (Colletidae) (Laroca and Almeida, 1985; Laroca *et al.*, 1989) and *Dufourea novaeangliae* (Eickwort *et al.*, 1986). These bees collect nectar in deep corolla tubes of *Jacaranda puberula* (Bignoniaceae) and *Pontederia cordata* (Pontederiaceae), respectively.
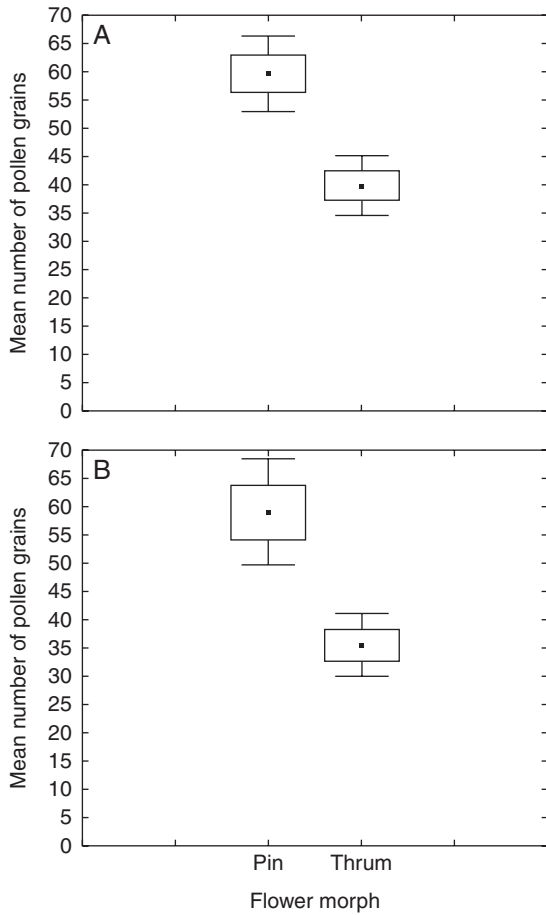
FIG. 6. Means of pollen grains from pin and thrum flowers of *Cordia leucocephala* in the hind legs scopa (A) and metasomal scopa (B) of *Ceblurgus longipalpis*. The difference between mean of pollen grains from pin and thrum flowers in metasomal scopa ($t = 5.01$; d.f. = 5; $P = 0.004$) and hind leg scopae ($t = 5.11$; d.f. = 5; $P = 0.0037$) was significant. Dots indicate the mean, boxes indicate the mean $\pm$ s.e., and error bars are the mean $\pm 1.96 \times$ s.e.
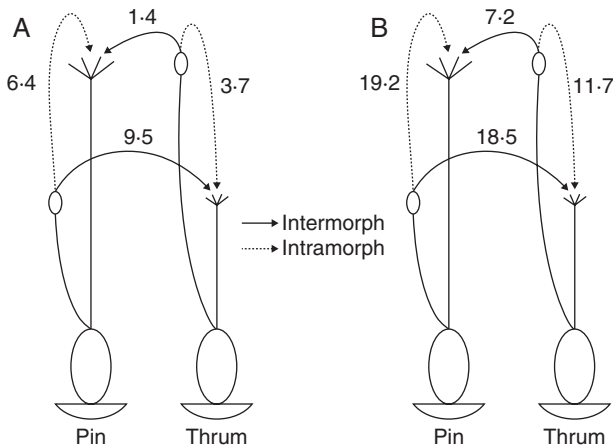

FIG. 7. Mean number of pollen grains of *Cordia leucocephala* deposited on stigmas in a single flower visit of *Ceblurgus longipalpis*; (A) males ($n = 10$), (B) females ($n = 10$). Continuous lines, intermorph (legitimate) pollen flow; dotted lines, intramorph (illegitimate) pollen flow.
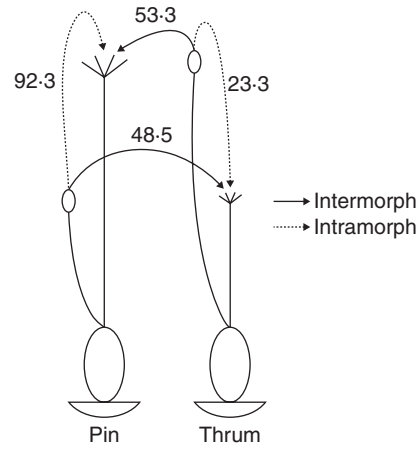

FIG. 8. Mean number of pollen grains of *Cordia leucocephala* deposited by flower visitors on the stigma of pin and thrum flowers at the end of the anthesis Continuous lines, intermorph (legitimate) pollen flow; dotted lines, intramorph (illegitimate) pollen flow.

FIG. 9. (A) Female of *Ceblurgus longipalpis* collecting pollen of *Cordia leucocephala* and transferring to the hind legs and metasomal scopae. (B) Scopae of a female of *C. longipalpis* full of pollen of *Cordia leucocephala*.

Pollen flow

Pin and thrum morphs of *C. leucocephala* differ in several features (three of the seven have already been mentioned in Machado, 1990), a characteristic also found in other species of this genus (Nowicke and Ridgway, 1973; Percival, 1974; Opler *et al.*, 1975; Gibbs and Taroda, 1983; Taroda and
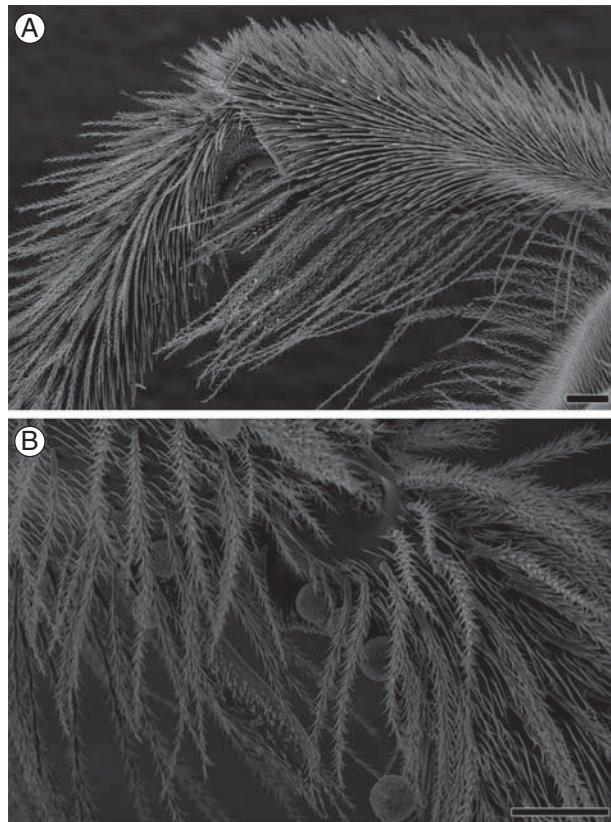
Fig. 10. Forelegs of females of *Ceblurgus longipalpis* with a fringe of extraordinarily long plumose setae along the ventral border (A) and pollen grains of *Cordia leucocephala* adhered to setae around the strigilis (B). Scale bars = 100 μm.

Gibbs, 1986, 1987; Nowicke and Miller, 1990). According to Darwin (1877), reciprocal positions of anthers and stigmas in distylic species lead to deposition of pollen grains of low- and high-level anthers to distinct body parts of effective pollinators. This would enhance cross-pollination because flower visitors would touch the stigmas at the level corresponding to these body parts. This means distyly would optimize intermorph pollen flow. In *Cordia leucocephala*, the clearly separated size classes of pollen grains from thrum and pin flowers enabled an analysis of the origin of pollen grains that adhere to different body parts of the bees and an evaluation of intra- and intermorph pollen flow in the *Ceblurgus longipalpis–Cordia leucocephala* association.

Pollen analyses clearly revealed that the pollen brush on labial palpi of females of *C. longipalpis* is a specific tool for extracting pollen from low-level anthers, while pollen from high-level anthers adhered mainly to clypeus, mandibles and labrum, mixed with pollen from low-level anthers. As expected, pollen-transporting scopae contained a mixture of pollen from thrum and pin morphs in their natural ratio. This shows that females of *Ceblurgus longipalpis* collected pollen from both levels with the same effectiveness. The evaluation of pollen flow, however, revealed unexpected results: while there was no quantitative difference in legitimate pollen flow between pin and thrum morphs, i.e. intermorph cross-pollination, illegitimate (intramorph) pollen flow from low-level anthers to stigmas of pin flowers was surprisingly much higher than pollen flow from high-level anthers to thrum stigmas. Moreover, more illegitimate pollen was transferred to stigmas of pin flowers and less illegitimate pollen to stigmas of thrum flowers than legitimate (intermorph) cross-pollen to the stigmas at both levels. However, it is not known if illegitimate pollen flow origins mainly from intramorph cross or self pollen. The association of *Ceblurgus longipalpis–Cordia leucocephala*, thus, is characterized by similar legitimate pollen flow in thrum and pin morphs, despite of unequal illegitimate pollen flow in both levels and a higher male investment in pin flowers when compared with thrum flowers.

The extraordinarily narrow relationship between *Cordia leucocephala* and its probable monolectic, highly specialized, effective pollinator *Ceblurgus longipalpis* appears to be the result of adaptations of both partners. However, missing relationships of related species make co-evolution unlikely. Characteristic traits, like the uncommon long mouthparts also found in other rophitine bees, are necessary to take up nectar in their specific host plants, which are not related to *Cordia*.

ACKNOWLEDGEMENTS

LITERATURE CITED

Aguiar CML, Martins CF. 1994. Fenologia e preferência alimentar de *Ceblurgus longipalpis* Urban e Moure, 1993 (Hymenoptera, Halictidae, Dufoureinae). *Revista Nordestina de Biologia* **9**: 125–131.

Aguiar CML, Martins CF, Moura ACA. 1995. Recursos florais utilizados por abelhas (Hymenoptera, Apoidea) em área de Caatinga (São João do Cariri, Paraíba). *Revista Nordestina de Biologia* **10**: 101–117.

Alves-dos-Santos I. 2002. Flower-visiting bees and the breakdown of the tristylous breeding system of *Eichornia azurea* (Swartz) Kunth. (Pontederiaceae). *Biological Journal of the Linnean Society* **77**: 499–507.

Alves-dos-Santos I. 2003. Adaptation of bee proboscides for collecting pollen from Pontederiaceae flowers. In: Melo GAR, Alves-dos-Santos I. eds. *Apoidea neotropica: Homenagem aos 90 anos de Jesus Santiago Moure*. Criciúma: Editora UNESC, 257–263.

Alves-dos-Santos I, Wittmann D. 1999. The proboscis of the long-tongued *Ancyloscelis* bees (Anthophoridae/Apoidea), with remarks on flowers visits and pollen collecting with the mouthparts. *Journal of the Kansas Entomological Society* **72**: 277–288.

Alves-dos-Santos I, Wittmann D. 2000. Legitimate pollination of the tristylous flowers of *Eichornia azurea* (Pontederiaceae) by *Ancyloscelis gigas* bees (Anthophoridae, Apoidea). *Plant Systematics and Evolution* **223**: 127–137.

Armbruster WS. 2006. Evolutionary and ecological aspects of specialized pollination: views from the artic to the tropics. In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization*. Chicago, IL: University of Chicago Press, 260–282.

Barrett SCH. 1978. Heterostyly in a tropical weed: the reproductive biology of the *Turnera ulmifolia* complex (Turneraceae). *Canadian Journal of Botany* **56**: 1715–1725.

Barrows EM, Chabot MR, Michener CD, Snyder TP. 1976. Foraging and mating behavior in *Perdita texana* (Hymenoptera: Andrenidae). *Journal of the Kansas Entomological Society* **49**: 275–279.

Cane JH, Sipes S. 2006. Characterizing floral specialization by bees: analytical methods and revised lexicon for oligolecty. In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization.* Chicago, IL: University of Chicago Press, 99–121.

Cane JH, Eickwort GC, Wesley FR, Spielholz J. 1985. Pollination ecology of *Vacinium stamineum* (Ericaceae: Vaccinioideae). *American Journal of Botany* **72**: 135–142.

CPTEC. 2009. *Centro de previsão de tempos e estudos climáticos.* http://www.cptec.inpe.br. Accessed on 26 October 2009.

Dafni A, Kevan PG, Husband BC. 2005. *Practical pollination ecology.* Cambridge: Enviroquest.

Danforth BN, Sipes SD, Fang J, Brady SG. 2006. The history of early bee diversification based on five genes plus morphology. *Proceedings of the National Academy of Sciences of the USA* **103**: 15118–15123.

Darrault RO, Schlindwein C. 2002. Esfingídeos (Lepidoptera, Sphingidae) no tabuleiro paraibano, Nordeste do Brasil: Abundância, riqueza e relação com plantas esfingófilas. *Revista Brasileira de Zoologia* **19**: 429–443.

Darwin C. 1877. *The different forms of flowers on plants of the same species.* London: John Murray.

Eickwort GC, Kukuk PF, Wesley FR. 1986. The nesting biology of *Dufourea novaeangliae* (Hymenoptera: Halictidae) and the systematic position of the Dufoureinae based on behavior and development. *Journal of the Kansas Entomological Society* **59**: 103–120.

Engel MS. 2001. A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). *Bulletin of the American Museum of Natural History* **259**: 1–192.

Ganders FR. 1979. The biology of heterostily. *New Zealand Journal of Botany* **17**: 607–635.

Gibbs PE, Taroda N. 1983. Heterostily in the *Cordia alliodora–C.trichotoma* complex in Brazil. *Revista Brasileira de Botânica* **6**: 1–10.

Grant V, Grant KA. 1979. Pollination of *Echinocereus fasciculatus* and *Ferocactus wisiizenii.* *Plant Systematics and Evolution* **132**: 85–90.

Grant V, Hurd PD. 1979. Pollination of the southwestern opuntias. *Plant Systematics and Evolution* **133**: 15–28.

Jordano P, Bascompte J, Olesen JM. 2006. The ecological consequences of complex topology and nested structure in pollination webs. In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization.* Chicago, IL: University of Chicago Press, 173–199.

Judd WS, Campbell CS, Kellogg EA, Stevens PF. 1999. *Plant systematics: a phylogenetic approach.* Sunderland, MA: Sinauer Associates.

Krenn HW, Plant JD, Szucsich NU. 2005. Mouthparts of flower-visiting insects. *Arthropod, Structure & Development* **34**: 1–40.

Kuriakose G, Sinu PA, Shivanna KR. 2009. Domestication of cardamom (*Elettaria cardamomum*) in Western Ghats, India: divergence in productive traits and a shift in major pollinators. *Annals of Botany* **103**: 727–733.

Larkin LL, Neff JL, Simpson BB. 2008. The evolution of a pollen diet: host choice and diet breadth of *Andrena* bees (Hymenoptera: Andrenidae). *Apidologie* **39**: 133–145.

Laroca S, Almeida MC. 1985. Adaptação dos palpos labiais de *Niltonia virgilii* (Hymenoptera, Apoidea, Colletidae) para coleta de néctar em *Jacaranda puberula* (Bignoniaceae), com descrição do macho. *Revista Brasileira de Entomologia* **29**: 289–297.

Laroca S, Michener CD, Hofmeister M. 1989. Long mouthparts among 'short-tongued' bees and the fine structure of the labium in *Niltonia* (Hymenoptera, Colletidae). *Journal of the Kansas Entomological Society* **62**: 400–410.

Larsson M. 2005. Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (Dipsacaceae). *Oecologia* **146**: 394–403.

Linsley EG, MacSwain JW, Raven PH. 1963a. Comparative behavior of bees and Onagraceae. I. *Oenothera* bees of the Colorado Desert. *University of California Publications in Entomology* **33**: 1–24.

Linsley EG, Macswain JW, Raven PH. 1963b. Comparative behavior of bees and Onagraceae. II. *Oenothera* bees of the Great Basin. *University of California Publications in Entomology* **33**: 25–58.

Linsley EG, Macswain JW, Raven PH. 1964. Comparative behavior of bees and Onagraceae. III. *Oenothera* bees of the Mojave Desert, California. *University of California Publications in Entomology* **33**: 59–98.

Machado IC. 1990. *Biologia floral de espécies de Caatinga em Alagoinha–PE.* PhD Thesis, Universidade Estadual de Campinas, Brazil.

Machado IC, Loiola MI. 2000. Fly pollination and pollinator sharing in two synchronopatric species: *Cordia multispicata* (Boraginaceae) and *Borreria alata* (Rubiaceae). *Revista Brasileira de Botânica* **23**: 305–311.

Martins CF. 1994. Comunidade de abelhas (Hymenoptera, Apoidea) da Caatinga e do Cerrado com elementos de campo rupestre do estado da Bahia, Brasil. *Revista Nordestina de Biologia* **9**: 225–257.

Melo JIM, Sales MF. 2005. Boraginaceae a. Juss. Na região de xingó: Alagoas e Sergipe. *Hoehnea* **32**: 369–380.

Michener CD. 2007. *The bees of the world.* Baltimore, MD: Johns Hopkins University Press.

Miller JS. 2001. Two new species of *Cordia* L. (Boraginaceae) from Madagascar. *Adansonia* **23**: 289–295.

Minckley RL, Roulston TH. 2006. Incidental mutualisms and pollen specialization among bees. In: Waser NM, Ollerton J. eds. *Plant–pollinator interaction: from specialization to generalization.* Chicago, IL: University of Chicago Press, 69–98.

Müller A. 1995. Morphological specializations in central European bees for the uptake of pollen from flowers with anthers hidden in narrow corolla tubes (Hymenoptera: Apoidea). *Entomologia Generalis* **20**: 43–57.

Müller A. 1996a. Host-plant specialization in western palearctic anthidiine bees (Hymenoptera: Apoidea: Megachilidae). *Ecological Monographs* **66**: 235–257.

Müller A. 1996b. Convergent evolution of morphological specializations in central European bee and honey wasp species as an adaptation to the uptake of pollen from nototribic flowers (Hymenoptera, Apoidea and Masaridae). *Biological Journal of the Linnean Society* **57**: 235–252.

Müller A. 2006. Unusual host plant of *Hoplitis pici,* a bee with hooked bristles on its mouthparts (Hymenoptera: Megachilidae: Osmiini). *European Journal of Entomology* **103**: 497–500.

Müller A, Kuhlmann M. 2003. Narrow flower specialization in two European bee species of the genus *Colletes* (Hymenoptera: Apoidea: Colletidae). *European Journal of Entomology* **100**: 631–635.

Neff JL, Rozen JG. 1995. Foraging and nesting biology of the bee *Anthemurgus passiflorae* (hymenoptera: Apoidea), descriptions of its immature stages, and observations on its floral host (Passifloraceae). *American Museum Novitates* **3138**: 1–19.

Nowicke JW, Miller JS. 1990. Pollen morphology of the Cordioideae (Boraginaceae). *Auxema, Cordia,* and *Patagonula. Plant Systematics and Evolution* **5**: 103–121.

Nowicke JW, Ridgway JE. 1973. Pollen studies in the genus *Cordia* (Boraginaceae). *American Journal of Botany* **60**: 584–591.

Ollerton J, Johnson SD, Hingston AB. 2006. Geographical variation in diversity and specificity of pollination systems. In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization.* Chicago, IL: University of Chicago Press, 283–308.

Opler PA, Baker HG, Frankie GW. 1975. Reproductive biology of some Costa Rican *Cordia* species (Boraginaceae). *Biotropica* **7**: 234–247.

Patiny S, Michez D, Danforth BN. 2007. Phylogenetic relationships and host-plant evolution within the basal clade of Halictidae (Hymenoptera, Apoidea). *Cladistics* **23**: 1–15.

Percival M. 1974. Floral ecology of coastal shrub in southeast Jamaica. *Biotropica* **6**: 104–129.

Petanidou T, Potts SM. 2006. Mutual use of resources in Mediterranean plant–pollinator communities: how specialized are pollination webs? In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization.* Chicago, IL: University of Chicago Press, 220–244.

Robertson C. 1925. Heterotropic bees. *Ecology* **6**: 412–436.

Rozen JG. 1997. South American rophitine bees (Hymenoptera: Rophitinae). *American Museum Novitates* **3206**: 1–27.

Schlindwein C, Martins CF. 2000. Competition between the oligolectic bee *Ptilothrix plumata* (Anthophoridae) and the flower closing beetle *Pristimerus calcaratus* (Curculionidae) for floral resources of *Pavonia cancelata* (Malvaceae). *Plant Systematics and Evolution* **224**: 183–194.

Schlindwein C, Medeiros PCR. 2006. Pollination in *Turnera subulata* (Turneraceae): unilateral reproductive dependence of the narrowly oligolectic bee *Protomeliturga turnerae* (Hymenoptera, Andrenidae). *Flora* **201**: 178–188.

Schlindwein C, Wittmann D. 1995. Specialized solitary bees as effective pollinators of south Brazilian species of *Notocactus* and *Gymnocalycium* (Cactaceae). *Bradleya* **13**: 25–34.

Schlindwein C, Wittmann D. 1997a. Stamen movements in flowers of *Opuntia* (Cactaceae) favour oligolectic pollinators. *Plant Systematics and Evolution* **204**: 179–193.

Schlindwein C, Wittmann D. 1997b. Micro-foraging routes of *Bicolletes pampeana* (Colletidae) and bee-induced pollen presentation in *Cajophora arechavaletae* (Loasaceae). *Botanica Acta* **110**: 177–183.

Schlindwein C, Wittmann D, Martins CF, *et al.* **2005.** Pollination of *Campanula rapunculus* L. (Campanulaceae): how much pollen flows into pollination and into reproduction of oligolectic pollinators? *Plant Systematics and Evolution* **250**: 147–156.

Sedivy C, Praz CJ, Müller A, Widmer A, Dorn S. 2008. Patterns of host-plant choice in bees of the genus *Chelostoma*: the constraint hypothesis of host-range evolution in bees. *Evolution* **62**: 2487–2507.

Sipes SD, Tepedino VJ. 2005. Pollen-host specificity and evolutionary patterns of host switching in a clade of specialist bees (Apoidea: *Diadasia*). *Biological Journal of the Linnean Society* **86**: 487–505.

Souza-Silva M, Fontenelle JCR, Martins RP. 2001. Seasonal abundance and species composition of flower-visiting flies. *Neotropical Entomology* **30**: 351–359.

Stang M, Klinkhamer PGL, Waser NM, Stang I, van der Meijden E. 2009. Size-specific interaction patterns and size matching in a plant–pollinator interaction web. *Annals of Botany* **103**: 1459–1469.

Stevens PF. 2001. *Angiosperm Phylogeny Website*. Version 9. June 2008. http://www.mobot.org/MOBOT/research/APweb/.

Taroda N, Gibbs PE. 1986. Studies on the genus *Cordia* L. (Boraginaceae) in Brazil. 1. A new infrageneric classification and conspectus. *Revista Brasileira de Botânica* **9**: 31–42.

Taroda N, Gibbs PE. 1987. Studies on the genus *Cordia* L. (Boraginaceae) in Brazil. 2. An outline taxonomic revision of subgenus *Myxa* Taroda. *Hoehnea* **14**: 31–52.

Vásquez DP, Aizen MA. 2003. Null model analyses of specialization in plant–pollinator interactions. *Ecology* **84**: 2493–2501.

Vásquez DP, Aizen MA. 2006. Community-wide patterns of specialization in plant–pollinator interactions revealed by null models. In: Waser NM, Ollerton J. eds. *Plant–pollination interactions: from specialization to generalization*. Chicago, IL: University of Chicago Press, 200–219.

Vásquez DP, Blüthgen N, Cagnolo L, Chacoff NP. 2009. Uniting patterns and process in plant–animal mutualistic networks: a review. *Annals of Botany* **103**: 1445–1457.

Wcislo WT, Cane JH. 1996. Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. *Annual Review of Entomology* **41**: 257–286.

Zar JH. 1999. *Biostatistical analysis*. Upper Saddle River, NJ: Prentice Hall.