

At Daybreak – Reproductive Biology and Isolating Mechanisms of *Cirrhaea dependens* (Orchidaceae)

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Abstract: Floral biology, reproduction, pollinator specificity, and fruit set of *Cirrhaea dependens* were recorded in forest areas of Southeastern Brazil. *Cirrhaea dependens* is a lithophytic or epiphytic herb occurring very sparsely below dense canopies. Nearly all the flowers of a single plant open simultaneously before dawn. They are short-lasting and offer floral fragrances as rewards, which are collected by male euglossine bees. Observations carried out in mesophytic forests at Serra do Japi revealed that *Euglossa viridis* is their principal pollinator, with *Eufriesea violacea* a sporadic co-pollinator. Visitation started soon after flower opening, and attractiveness remained high for about 2 h, decreasing abruptly at sunrise. Flower anthesis with subsequent fragrance release seems to be correlated with bee attraction. Observations using chemical baits were carried out at Serra do Japi, and in the Atlantic Rain Forest of Picinguaba. Three euglossine species were captured with pollinaria of *C. dependens* in Picinguaba, whereas only *E. violacea* was attracted in Serra do Japi. In Picinguaba, *C. dependens* occurs sympatrically with *C. saccata* and *C. loddigesii*. Each species attracted different pollinators. The specificity and resulting reproductive isolation are due to the production of different odours by each orchid species. *Cirrhaea dependens* is self-compatible but pollinator-dependent. The reproductive success was low and appears to result from a combination of factors discussed here, such as the production of short-lived flowers, presence of floral mechanisms avoiding self-pollination, non-synchronization of flower phases among plants, and populations with few and sparsely distributed individuals.

Key words: *Cirrhaea*, Euglossini, Orchidaceae, pollination, reproductive biology, reproductive success, Stanhopeinae.

Introduction

The genus *Cirrhaea* L. belongs to the subtribe Stanhopeinae (Orchidaceae, Epidendroideae, Cymbidieae), which includes 22 genera distributed in Tropical America (Dressler, 1993). All members of the Stanhopeinae produce floral fragrances as re-

wards. These perfumes consist of volatile oils, produced by floral osmophores, which are exclusively collected by male bees of the tribe Euglossini (Hymenoptera, Apidae) (see Williams, 1982). The role of the male euglossine bees in the pollination of orchids has often been reported (e.g., Williams, 1982 for a review; Ackerman, 1983 a, b; Williams and Whitten, 1983; Whitten et al., 1986; Roubik and Ackerman, 1987; Zimmerman et al., 1989; Murren, 2002). The male euglossine bees collect fragrances using specialized front tarsi. These substances are then stored on their posterior legs, in the hind tibial organs (Dressler, 1968, 1982).

Even though certain substances may occur in many different orchids, the flowers of certain species emit a unique fragrance (Dodson, 1970; Hills et al., 1968, 1972; Williams et al., 1981; Gerlach and Schill, 1991). Some of these orchids are pollinated by several bee species, but in most cases they are more pollinator-specific. In orchids pollinated by euglossine bees, differential fragrance production is important for reproductive isolation and speciation (van der Pijl and Dodson, 1966; Dressler, 1968; Hills et al., 1972; Williams and Dodson, 1972; Williams, 1982; Williams and Whitten, 1983; Gerlach and Schill, 1991). In some cases, geographical, seasonal, and mechanical barriers, such location of the pollinarium on different parts of the insect body, also seem to be important mechanisms of isolation (Dressler, 1968; Williams, 1982). The floral fragrances of flowers pollinated by male euglossine bees are mainly composed of mono- and sesquiterpenes, and aromatic compounds (Dodson, 1970; Williams, 1982; Williams and Whitten, 1983). Purified substances are frequently used as attractants to conduct studies on male euglossine bees for a variety of purposes (see Williams, 1982 for a review). Several authors have used chemical baits to examine the day activity of euglossine bees and have reported that these insects are more active in the morning (Ricklefs et al., 1969; Braga, 1976 a; Janzen et al., 1982; Ackerman, 1983 c; Powell and Powell, 1987; Armbruster and McCormick, 1990; Armbruster and Berg, 1994). Other factors also seem to influence the activity of Euglossini bees, such as cloud cover and precipitation (Inouye, 1975; Janzen et al., 1982; Williams, 1982), wind speed (Ricklefs et al., 1969), air temperature (Braga, 1976 b; Armbruster and McCormick, 1990; Armbruster and Berg, 1994), solar radiation, and humidity (Inouye, 1975; Braga, 1976 b).

According to van der Pijl and Dodson (1966) and Dressler (1968), few male euglossine-pollinated orchids are self-sterile, so cross-pollination appears to be the rule. In the subtribe Stanhopeinae, self-pollination tends to be avoided by floral mechanisms (van der Pijl and Dodson, 1966; Dressler, 1968). Good data on the reproductive success of euglossine-mediated pollination in most Stanhopeinae species are still rare (Dressler, 1968), however, studies have revealed a low natural fruit set for *Gongora quinquenervis* Ruíz and Pavón (Dressler, 1968; Martini et al., 2003).

The genus *Cirrhaea* includes about six species endemic to Brazil (Morrison, 1997). According to van der Pijl and Dodson (1966), members of this genus are pollinated by species of *Eufriesea* (e.g., *Euplusia* and *Euglossa*) with pollinaria attached to the legs of the bees. According to Hoehne (1933), *Cirrhaea* sp. is pollinated by *Eufriesea* (also known as *Euplusia*) *violacea* and *Euglossa mandibularis* Friese. Kimsey (1982) reports Dressler's observation of *Eufriesea venusta* Moure as pollinator of *Cirrhaea viridipurpurea* Lindl. Analyses of the floral fragrance of *C. dependens* were made by Kaiser (1993) and revealed that the perfume of this species is composed of common orchid scent components, such as myrcene, eucalyptol, (E)-ocimene, and eugenol.

In this paper we report on the floral biology, breeding system, and pollination mechanism of *Cirrhaea dependens* (Lodd.) Rchb. f. The implications of pollination at daybreak (mainly before and at dawn), and factors related to low natural fruit set are discussed. We also investigate the factors that prevent self-pollination in *C. dependens*, the reproductive isolation from two other sympatric species of *Cirrhaea* (*C. saccata* Lindl. and *C. loddigesii* Lindl.), and inter-generic crosses between *Cirrhaea* and co-occurring genera at Serra do Japi and Picinguaba.

Materials and Methods

Study sites

Fieldwork was carried out at Serra do Japi and Picinguaba, both located in the State of São Paulo, and in Macaé de Cima, in the State of Rio de Janeiro. All study areas are natural reserves in Southeastern Brazil. Serra do Japi is about 350 km from Picinguaba and 700 km from Macaé de Cima.

At Serra do Japi (23°11'S, 46°52'W, 700–1300 m a.s.l.) the annual rainfall is about 1500 mm, and annual mean temperature is ca. 17.5 °C (Pinto, 1992). A semi-deciduous mesophytic forest of medium altitude with occasional rocky outcrops characterizes this region. In Picinguaba (23°33'S, 45°04'W, 0–50 m a.s.l.) the annual rainfall is approximately 2600 mm, with an average temperature of ca. 21 °C (Nimer, 1977). It is characterized by evergreen Atlantic rain forests on steep slopes and "restinga" vegetation. Macaé de Cima (22°28'S, 43°02'W, 1850 m a.s.l.) has an annual rainfall of 1500–2000 mm and an annual mean temperature of ca. 17.5 °C. This region is also characterized by evergreen Atlantic rain forest.

Voucher specimens

Cirrhaea dependens (Lodd.) Rchb. f.: Brazil, São Paulo: **Jundiaí**, XII.1999, E. R. Pansarin 95 (UEC). **Ubatuba**, I.1990, F. C. P. Garcia et al. 526 (HRCB). Rio de Janeiro: **Nova Friburgo**, II.2003, E. R. Pansarin 1057 (UEC).

Cirrhaea loddigesii Lindl.: Brazil, São Paulo: **Ubatuba**, I.2001, F. Pinheiro and M. R. Cunha 36 (SP).

Cirrhaea saccata Lindl.: Brazil, São Paulo: **Ubatuba**, X.2000, E. R. Pansarin 717 (UEC). **Piaçaguera**, I.1954, O. Handro 382 (SP).

Phenology and plant features

Phenological data were gathered in Serra do Japi and Picinguaba. Both study areas were visited monthly, from December 1997 to April 2001. Morphological features of fresh flowers were recorded and the pollinaria were drawn under a binocular stereomicroscope equipped with a camera lucida. The flowers of *C. dependens* used in the study of floral morphology were collected from Serra do Japi and Macaé de Cima populations. Flowers of *C. saccata* and *C. loddigesii* were obtained from the orchid collection of the "Instituto de Botânica de São Paulo" (State of São Paulo-Brazil). For the anatomical study, fresh flowers collected in Serra do Japi were fixed in 50% FAA. Labella were dehydrated in an ethanol series and embedded in glycol methacrylate. Cross sections were obtained using a microtome. Sections 9–12 µm thick were stained with toluidine blue (Sakai, 1973).

Measurements of the pollinia and stigmatic cavity of *Cirrhaea dependens* (n = 18), *C. loddigesii* (n = 7), and *C. saccata* (n = 17) were made from drawings and directly from structures using a caliper ruler. The flowers were collected in the morning (from 7:00 until 8:30 h) and on the first day of anthesis. The pollinaria were removed and kept in Petri dishes at room temperature (ca. 22 °C) and immediately measured. They were subsequently measured every 5 min during the 2 h after their removal.

Floral visitors

Pollination observations were made on four plants at Serra do Japi, on December 13–16, 1997, December 17–20, 1998, December 21–23, 2000, and December 18–21, 2002, a total of 146 h. Observations were made between 4:30–18:30 h on the first day of flower anthesis (from before dawn to late afternoon). On the second and subsequent days, the daily observation period was between 4:30–14:00 h. To detect any possible nocturnal pollination, flowers were tagged in the afternoon (18:30 h) and examined on the next day in the early morning, at about 4:30 h.

Chemical baits

Pollinators were captured using pure substances as chemical baits at both Serra do Japi and Picinguaba. At Serra do Japi, censuses were conducted from December 1997 to January 1998, December 1998 to January 1999, December 1999 to January 2000, and December 2000 to January 2001, a total of 70 h. In Picinguaba, censuses were conducted from January to February 1999, January to February 2000, and January to February

2001, a total of 68 h. In both study areas censuses were conducted between 4:30–18:30 h.

The fragrance compounds: benzyl alcohol, benzyl benzoate, eucalyptol, eugenol, indol, linalool, methyl salicylate, and vanillin were released as baits to capture bees with pollinaria of species of *Cirrhaea*. According to Kaiser (1993), eucalyptol, eugenol, linalool, and vanillin are substances present in the odour of *C. dependens*. Each substance was applied separately onto 6 × 6 cm pads of clear herbarium blotter paper and pinned on tree trunks at both study sites. The quantity and frequency of substance repositioning varied depending on evaporation rates.

The insects collected on the flowers or on the chemical baits were identified and vouchers were deposited at the Natural History Museum of the Universidade Estadual de Campinas (ZUEC).

Breeding system and fruit set

The breeding system of *Cirrhaea dependens* was investigated in the field and using plants collected in the study areas and maintained in a greenhouse. Four plants (12 inflorescences) collected at Serra do Japi and four plants (four inflorescences) collected at Macaé de Cima, maintained in a greenhouse of the Universidade Estadual de Campinas, Campinas, State of São Paulo (22°49'S, 47°06'W, 700 m a.s.l.), were used. At Serra do Japi, four plants (13 inflorescences) were used for the field treatments. In all cases, the experimental treatments included: a) flowers left unmanipulated, b) manual self-pollination, c) emasculation, and d) cross-pollination. The number of pollinations varied between treatments, depending on the availability of flowers. Treatments were randomly applied to each inflorescence, but always using flowers on their first day of anthesis.

Natural fruit set (open pollination) was recorded at Serra do Japi and in Macaé de Cima. In Serra do Japi, two plants were sampled over four years (1998–2001) and four individuals in 2002, while at Macaé de Cima five plants were sampled in 2003.

Results and Discussion

Phenology and plant features

Cirrhaea dependens flowers in the wet season: in December at Serra do Japi, and between January–February in the Picinguaba region. Nearly all the flowers of each plant open simultaneously on the same day, although a few (generally one) terminal buds of each inflorescence open only on the next day. Flower opening occurs before dawn, between about 5:00–5:30 h, and each flower lasts about 3 days.

Cirrhaea dependens is a lithophytic or, more rarely, epiphytic herb. The plants grow sympodially, producing conic, densely grouped, and unifoliate pseudo-bulbs. A single young pseudo-bulb produces one or rarely two lateral and pendulous inflorescences with up to 17 flowers (Fig. 2A). At Serra do Japi, the flowers are predominantly green-pink with several vinaceous dots and transversal lines on the sepals and lip, and small dots on the petals and column (Fig. 2B), while in Picinguaba and

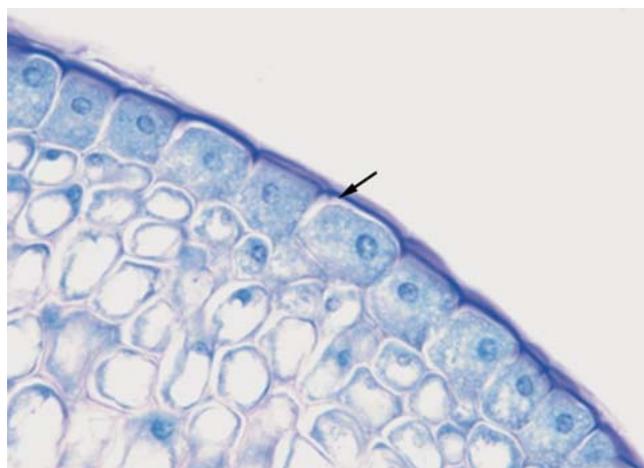


Fig. 1 Cross section of the labellar protuberance of the lip of *Cirrhaea dependens*. Note the uniseriate secretory epidermis and the cuticle (arrow), × 40.

Macaé de Cima they are mainly red-brown. The dorsal sepal is elliptic and the lateral ones are elliptic-lanceolate. All sepals are about 2 cm long and 0.7 cm wide. The petals are falcate and approximately 2 cm long and 0.2 cm wide. The 3-lobed lip is erect, about 2 cm long, and 0.6 cm wide. Lateral lobes are folded backward and parallel. Above the lateral lobes there is a single globose protuberance (ca. 2 mm in diameter). The column is erect (ca. 1 cm long) and ends in a narrow transversal stigma. Like other species of *Cirrhaea*, the inflorescences are pendulous, but the lips of flowers are turned into an erect position by torsion of the ovary (van der Pijl and Dodson, 1966). The pollinarium is composed of two yellow compressed pollinia (approx. 2.5 mm long), one large and basally spatulate stipe, and a terminal ovate and brown viscidium (Figs. 3D–F). For additional information on flower morphology and illustrations see Hoehne (1942).

The flowers of *Cirrhaea dependens* produce a sweet fragrance, which is most intense at flower opening, before dawn, and decreases throughout the day. In contrast to these observations, plants belonging to other genera of the subtribe Stanhopeinae (e.g., *Stanhopea* and *Gongora*) are reported to release a more intense scent during the warmest hours of the day, coinciding with the activity period of their pollinators (Williams, 1982). According to Williams (1982), air temperature plays an important role in the production and release of scents. The lip protuberance of *C. dependens* presents epidermal cells with dense cytoplasm, a very big nucleus, and is covered with a thick cuticle (Fig. 1). The absence of such cells in other parts of the lip suggests that the fragrances are produced only in the protuberance region. The secretory surface of the lip is smooth and lacks papillae, a structure very similar to that reported for other Stanhopeinae, such as *Sievekingia* (Curry et al., 1991).

Floral visitors and pollination mechanisms

At Serra do Japi, *Cirrhaea dependens* was pollinated by males of *Euglossa viridis* and *Eufriesea violacea*. The most frequent pollinator was *E. viridis*, whose main activity period was between about 5:00–6:30 h, i.e., before and at dawn. In this period, dozens of bees were attracted to the flowers, both on dry and

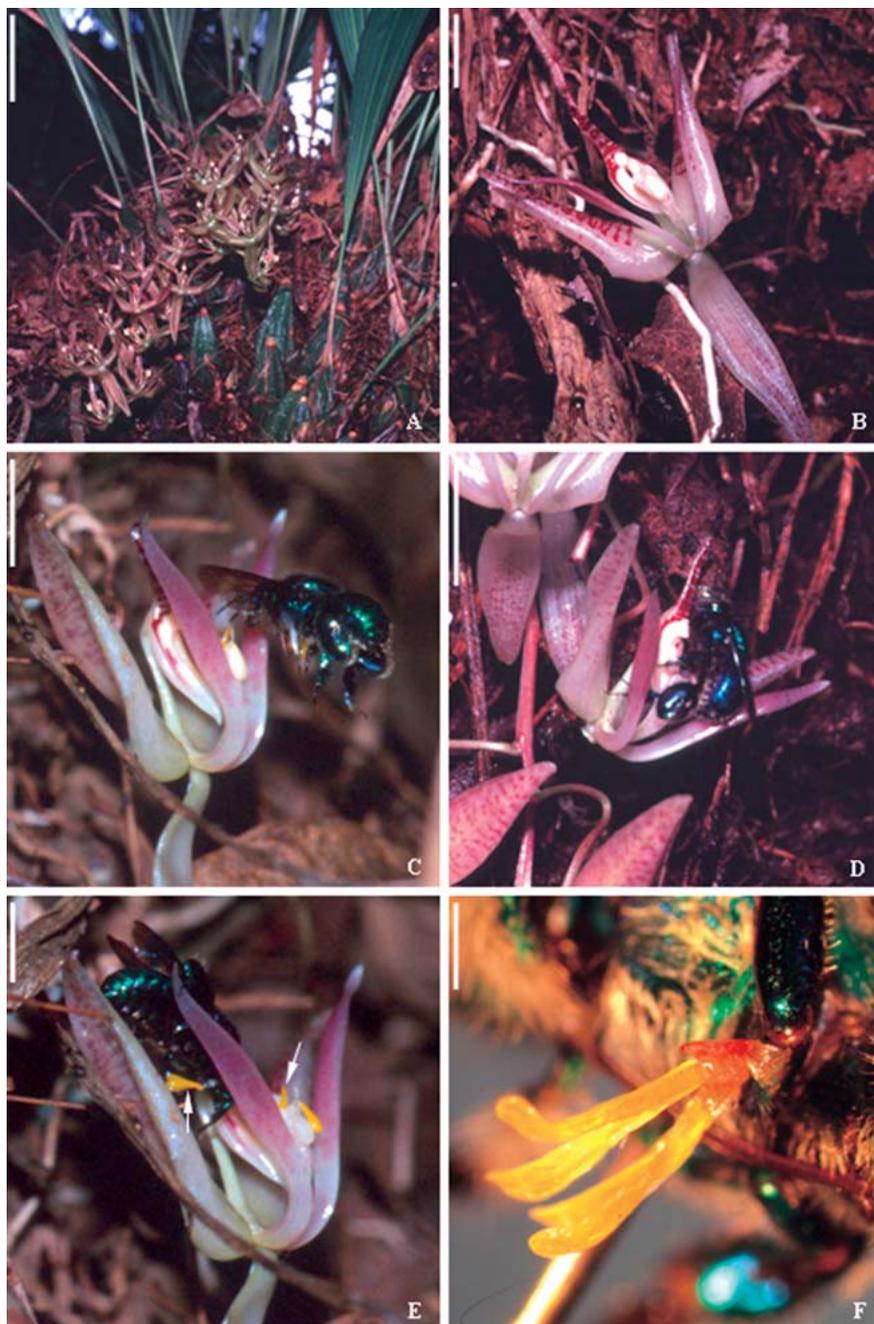


Fig. 2 *Cirrhaea dependens*. (A) Habit and inflorescence. (B) Detail of a flower. (C) *Euglossa viridis* hovering in front of flowers. (D) *Euglossa viridis* settled on the flower lip. (E) *Euglossa viridis* collecting fragrances. Note a pollinarium of *C. dependens* on one of the bee legs, and a pollinium on the stigma of the flower (arrows). (F) *Euglossa cordata* with two pollinaria fixed on the trochanter of its median leg. Scale bars: A=5 cm, B–E=1 cm, F=1 mm.

wet days. In 1998 and 2000, the flowering peak occurred during wet days. Since it is dark during the visitation peak, and the use of artificial light chased the bees away, the number of floral visitors could not be recorded unequivocally. Visits decreased abruptly after 6:30 h, at sunrise, with only a few bees visiting until about 12:00 h. Only three visits of *E. viridis* were recorded after sunrise, one in 1998 and two in 2002. *Eufriesea violacea* visited the flowers sporadically (only two visits registered), between 10:00–14:00 h. Both bee species visited the flowers only on the first day of anthesis. None of the tagged flowers had their pollinarium removed or received pollinia between 18:30 and 4:30 h. The pollination mechanisms involving *Euglossa viridis* and *Eufriesea violacea* were similar. The bees hov-

ered in front of the flower (Fig. 2C) before landing on the lip (Fig. 2D), where they brushed the protuberance region with their anterior legs to collect the fragrance. Pollinarium removal by *E. viridis* occurred when the bee hovered in order to transfer the perfumes to its posterior tibia or when it slipped during fragrance collection. This type of pollinarium removal has been well documented in *Stanhoepa* (van der Pijl and Dodson, 1966; Dressler, 1968; Braga, 1976b) and *Gongora* (van der Pijl and Dodson, 1966; Dressler, 1968; Dodson et al., 1969; Martini et al., 2003). *Eufriesea violacea* removed pollinaria when it landed on the column. On *E. viridis*, the pollinarium was fixed on the first segments of the anterior and median pair of legs, on the thigh, trochanter, and femur (Figs. 2E, F). Although

Hoehne (1933) reported the fixation of pollinaria of an unidentified species of *Cirrhaea* on the legs of *E. violacea*, we observed that the pollinaria of *C. dependens* are deposited on the first segments of the ventral portion of the abdomen. Pollinaria deposition on the legs of the pollinator has also been reported for other Stanhopeinae, like *Paphinia clausula* Dressler (van der Pijl and Dodson, 1966), *Sievekingia* (van der Pijl and Dodson, 1966; Dressler, 1968; Ackerman, 1983 a; Gerlach, 1999), and *Sotosanthus* (Gerlach, 1999). When turgid, the pollinarium has a pollinia thickness equal to the stigmatic slit (0.3 mm) and, consequently, deposition on the stigma was impossible. When dehydrated, the pollinia thickness is reduced to 0.2 mm, allowing deposition onto the stigmatic slit. The necessary time for sufficient dehydration of the pollinia, on dry days, was about 45 min. This mechanism of pollinia dehydration that prevents immediate self-pollination has been well documented in other Stanhopeinae (see van der Pijl and Dodson, 1966; Dressler, 1968). In *C. dependens* the described mechanism indeed generally prevents self-pollination. It may occur, however, that the pollinia are already sufficiently dehydrated when the bees are attracted to the flowers of the same plant. In this case, the bees sometimes pollinate the plant with their own pollinia. Nevertheless, during five years of observation we only observed *E. viridis* self-pollinating two flowers of *C. dependens* (Fig. 2E). In addition to the necessity of pollinia dehydration, certain ecological factors favour cross-pollination in *C. dependens*: most flowers of the same individual open simultaneously, and pollinators are mainly attracted during a few hours and only on the first day of anthesis. This increases the probability that the bees visit another individual presenting a flowering peak on the next or subsequent days.

Chemical baits and pollinator specificity

At Serra do Japi and Picinguaba, pure compounds used as chemical baits allowed us to capture males of several species of euglossine bees carrying pollinaria of orchids (Table 1). Although *E. viridis* was attracted to flowers of *Cirrhaea dependens* mainly before or at dawn, the activity of euglossine bees at chemical baits was more frequent during the warmer hours of the day (between 9:00–13:00 h), as has been reported in several other studies (e.g., Ricklefs et al., 1969; Braga, 1976 a; Janzen et al., 1982; Ackerman, 1983 c; Powell and Powell, 1987; Armbruster and McCormick, 1990; Armbruster and Berg, 1994). At Serra do Japi, *Eufriesea violacea* was frequently attracted to vanillin, eucalyptol, and benzyl benzoate, and sometimes captured with pollinaria of *C. dependens* (Table 1). Two of these substances (vanillin and eucalyptol) are constituents of the floral fragrance of *C. dependens* (Kaiser, 1993). *Euglossa viridis* was not attracted by the baits used at Serra do Japi.

In Picinguaba, the use of baits allowed us to capture *Eulaema nigrita*, *Euglossa cordata*, *E. sapphirina*, *E. chalybeata iopocila*, and *E. viridis* carrying pollinaria of *Cirrhaea* species (Table 1). Although *E. viridis* was not attracted by the substances used as baits at Serra do Japi, in Picinguaba, bees of this species were attracted only to eugenol, a substance present in the floral fragrance of *C. dependens* (Kaiser, 1993). Geographic variation in fragrance choice and preferences at chemical baits has been reported for male euglossine bees occurring in distinct areas in Panama (Ackerman, 1989). In the Picinguaba region, three species of *Cirrhaea* occur: *C. dependens*, *C. saccata*, and *C. loddigesii*, and the pollinaria fixed on the captured bees were com-

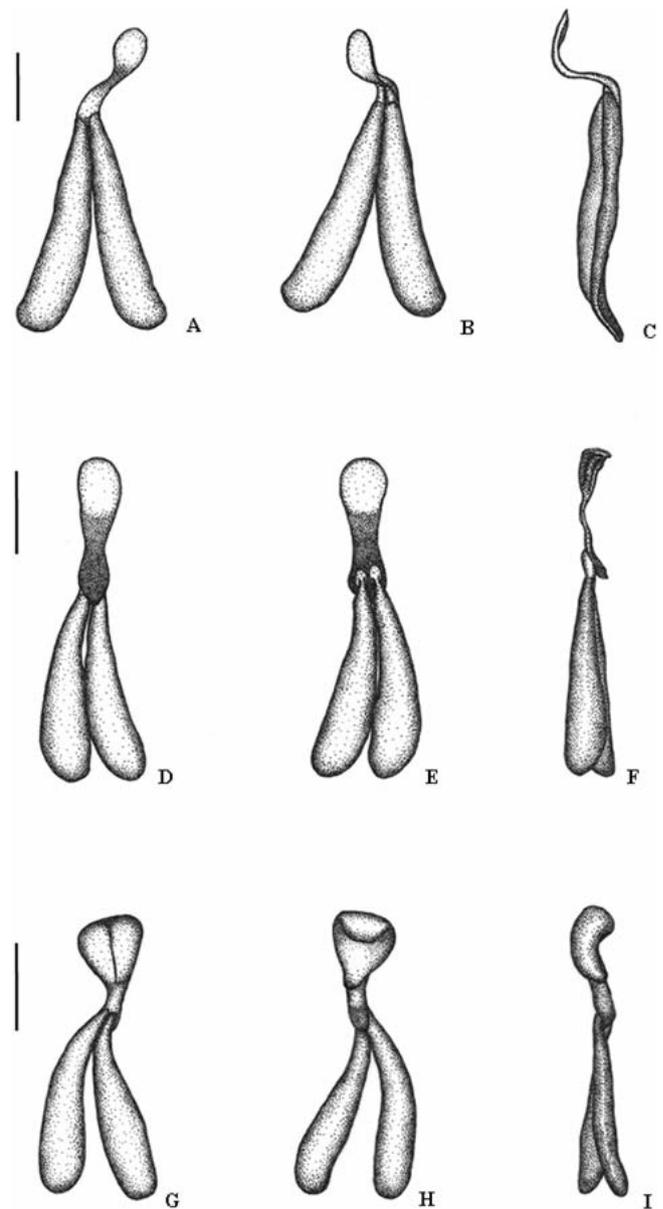


Fig. 3 Pollinaria of southeastern species of *Cirrhaea*. (A) Pollinarium of *C. saccata* in dorsal, (B) ventral, and (C) lateral views. (D) Pollinarium of *C. dependens* in dorsal, (E) ventral, and (F) lateral views. (G) Pollinarium of *C. loddigesii* in dorsal, (H) ventral, and (I) lateral views. Scale bars = 1 mm.

pared with those of the *Cirrhaea* species cultivated at “Instituto de Botânica de São Paulo”. The pollinaria of these three species of *Cirrhaea* are easily distinguished. In *C. saccata* the viscidium is rounded, membranaceous, and whitish-hyaline, while in *C. loddigesii*, it is triangular, fleshy, and brown (Figs. 3A–I). A pollinarium carrier is likely to be a legitimate pollinator of the species from which it removes pollinaria (Dressler, 1976, 1981; Ackerman, 1983 b). Thus it was possible to identify the pollinators of each species of *Cirrhaea* occurring in the region. *Eulaema nigrita* and *Euglossa chalybeata* were captured with pollinaria of *C. saccata*, and *Euglossa cordata*, *E. sapphirina*, and *E. viridis* carried pollinaria of *C. dependens* (Table 1), while none of the captured bees carried pollinaria of *C. loddigesii*. In

Table 1 Pollinators of *Cirrhaea* species attracted to chemical baits at Serra do Japi and Picinguaba, and additional orchids visited by each bee species, their flowering period and pollinaria placement on the pollinator body

Pollinator/orchid species	Flowering period	Chemical baits ³	Pollinarium placement
<i>Eufriesea violacea</i> (Blanchard, 1840) ¹			
<i>Bifrenaria tyrianthina</i> (Lodd.) Rchb. f. ^a	February ^a	Ec ^a , V ^a	scutum ^a
<i>Cirrhaea dependens</i> (Lodd.) Rchb. f. ^a	December ^a	Ec ^a , V ^a	abdomen ^a
<i>Gongora bufonia</i> Lindl. ^a	October ^a	BB ^a , Ec ^a , V ^a	scutellum ^a
<i>Catasetum cernuum</i> (Lindl.) Rchb. f. ^a	October ^a	BB ^a , Ec ^a , V ^a	scutum ^a
<i>Euglossa cordata</i> (Linnaeus, 1758) ²			
<i>Cirrhaea dependens</i> (Lodd.) Rchb. f. ^{a,b}	January–February ^a	Ec ^a , C ^b	legs ^{a,b}
<i>Catasetum hookeri</i> Lindl. ^b	December–January ^a	C ^b	scutum ^b
<i>Euglossa chalybeata iopocila</i> (Dressler, 1982) ²			
<i>Cirrhaea saccata</i> Lindl. ^{a,b}	November ^a	Ec ^a , C ^b , Eg ^b	legs ^{a,b}
<i>Catasetum hookeri</i> Lindl. ^b	December–January ^a	C ^b , Eg ^b	scutum ^b
<i>Houlletia brocklehurstiana</i> Lindl. ^b	April ^b	C ^b , Eg ^b	head ^b
<i>Notylia</i> sp. ^b	February ^b	C ^b , Eg ^b	clypeus ^b , legs ^b
<i>Euglossa sapphirina</i> (Moure, 1968) ²			
<i>Cirrhaea dependens</i> (Lodd.) Rchb. f. ^{a,b}	January–February ^a	Eg ^a , C ^b , V ^b	legs ^{a,b}
<i>Notylia</i> sp. ^b	February ^b	C ^b , V ^b	clypeus ^b
<i>Euglossa viridis</i> (Perty, 1833) ²			
<i>Cirrhaea dependens</i> (Lodd.) Rchb. f. ^{a,b}	January–February ^a	Eg ^{a,b}	legs ^{a,b}
<i>Catasetum hookeri</i> Lindl. ^b	December–January ^a	Eg ^b	scutum ^b
<i>Eulaema nigrata</i> (Lepeletier, 1841) ²			
<i>Cirrhaea saccata</i> Lindl. ^{a,b}	November ^a	Ec ^a , C ^b , V ^{a,b}	legs ^{a,b}
<i>Cattleya intermedia</i> Graham ^b	December–January ^a	C ^b , V ^b	scutum ^b

^a Data collected on the present study. ^b Data based on Singer and Sazima (2004) for Picinguaba region. ¹ Serra do Japi. ² Picinguaba. ³ BA, benzyl alcohol; BB, benzyl benzoate; C, cineole; Ec, eucalyptol; Eg, eugenol; I, indole; L, linalool; MS, methyl salicylate; V, vanillin.

contrast to other Stanhopeinae (e.g., *Stanhopea*), which are often pollinated by a single bee species (van der Pijl and Dodson, 1966; Dressler, 1968; Braga, 1976b; Williams, 1982), *Cirrhaea dependens* is not pollinator-specific. However, although *C. dependens* was visited by three bee species in Picinguaba, the sympatric *Cirrhaea* species occurring in this region attracted different pollinators (Table 1). A chemical fragrance analysis of the flowers of the three species has not yet been carried out, but they are olfactorily different from each other (Pansarin, pers. obs.). This may be the main factor that impedes pollen transfer between the three sympatric species in Picinguaba. In orchid species that only offer floral fragrances as a reward and are exclusively pollinated by male euglossine bees, the production of different fragrances can be responsible for pollinator specificity and consequently represent an important isolating mechanism (see Williams, 1982; Williams and Whitten, 1983 for reviews). In many cases, a single euglossine bee species visits plants belonging to more than one orchid genus in the same region. During the flowering period of *Cirrhaea dependens* we captured males of *E. violacea* carrying pollinaria of *Gongora bufonia* (on their scutellum) and *Catasetum cernuum* (on their scutum). The pollination of these two orchid species by *E. violacea* had already been recorded by Hoehne (1933). As in *C. cernuum*, the pollinaria of *Bifrenaria tyrianthina* also are deposited on the scutum of *E. violacea*, but these two species have different flowering periods (Table 1). As described above, pollinaria fixation of *C. dependens* occurs on the ventral abdo-

men of *E. violacea*. Similarly, in the Picinguaba region, the pollinators of *Cirrhaea* species also pollinate other orchid genera, with the pollinaria being placed on different parts of the bee's body (Table 1). Thus, crosses between sympatric genera at Serra do Japi and Picinguaba tend to be prevented by differences in flower morphology, flowering period, and the placement of the pollinaria on the insect body, as suggested for other taxa pollinated by male euglossine bees (Dressler, 1968; Williams, 1982).

Implications of pollination before and at dawn

Rarely recorded in orchids (van der Pijl and Dodson, 1966), pollination before or at dawn has never been observed in species pollinated by euglossine bees. As reported here, the main activity period of *E. viridis* on flowers of *C. dependens* occurs before and at dawn. Armbruster and McCormick (1990) reported that male euglossine bees began foraging for chemical baits one to two hours after sunrise in Costa Rica. Furthermore, several studies revealed that male euglossine bees are more active in the morning (Dodson et al., 1969; Ricklefs et al., 1969; Braga, 1976a; Janzen et al., 1982; Ackerman, 1983c; Powell and Powell, 1987; Zimmermann et al., 1989; Armbruster and McCormick, 1990; Armbruster and Berg, 1994). Probably partly based on these reports, some investigators did not even make observations during other periods of the day (Braga, 1976b; Ackerman, 1983c; Whitten et al., 1986; Powell and Powell, 1987; Becker et

Table 2 Reproductive system and natural fruit set (open pollination) of *Cirrhaea dependens* in plants from two localities, Serra do Japi and Macaé de Cima. Characters between parentheses indicate the number of flowers/fruits, respectively. Empty cell = absence of data

Location	Untouched	Self-pollination	Emasculated	Cross-pollination	Open
Serra do Japi	(13/0) ^a	(28/18) 64% ^a	(14/0) ^a	(27/19) 70% ^a	(670/6) 0.9%
Serra do Japi	(21/0) ^b	(23/17) 74% ^b	(17/0) ^b	(22/15) 68% ^b	
Macaé de Cima	(8/0) ^a	(13/9) 69% ^a	(11/0) ^a	(12/8) 67% ^a	(57/1) 1.7%
Total	(42/0)	(64/44) 69%	(42/0)	(61/42) 69%	(727/7) 0.96%

^a Experiments in greenhouse. ^b Experiments in natural habitat.

al., 1991; Murren, 2002). Thus, although our observations on the relationship between *E. viridis* and *C. dependens* seem to represent a rare case, the lack of observations on the local flora or during certain periods of the day can compromise the results and interpretations about male euglossine behaviour and plant-pollinator relationships.

The flowering period of *Cirrhaea dependens* occurs during the Brazilian summer and, in Serra do Japi, the temperature between 12:00 and 14:00 h reaches approximately 35 °C (Pinto, 1992). In this region, before or at dawn, the air temperature is rather low, about 20 °C or less (Pansarin, pers. obs.). Additionally, we recorded visits on both dry and wet days. Thus, low air temperature and precipitation do not seem to influence the attraction of *Euglossa viridis* to flowers of *C. dependens*. Attraction, in this case, appears to be only determined by the flower opening with subsequent fragrance release. These findings contrast with several previous studies on the behaviour of male euglossine bees. Most of them state that, during the first hours of the morning, low air temperature and cloud cover affect bee activity negatively (May and Casey, 1983; Armbruster and McCormic, 1990; Armbruster and Berg, 1994). Furthermore, other authors argued that an early morning warm-up may exert significant energetic costs to the bees (Heinrich, 1975, 1979; May and Casey, 1983; Casey et al., 1985), and that the reduced bee activity in cloudy weather is primarily the result of the absence of direct solar radiation (Inouye, 1975). Why the pollination of *C. dependens* occurs even in the early morning hours remains enigmatic.

Breeding system and fruit set

Conclusive data on the reproductive biology of Stanhopeinae species are scarce in the literature. Martini et al. (2003) reported self-pollination for *Gongora quinquenervis* in Northeastern Brazil. According to van der Pijl and Dodson (1966) and Dressler (1968), few species pollinated by euglossine bees are self-sterile, and self-pollination tends to be avoided basically by floral mechanisms. Although it requires a pollinator to guarantee fruit set, *Cirrhaea dependens* is self-compatible. The fruit set of *C. dependens* was similar in plants from Macaé de Cima and Serra do Japi for both manual self- and cross-pollinations. No fruits were formed in untouched or emasculated flowers. *Cirrhaea dependens* showed about 30–35% of fruit abortion in both self- and cross-pollinated flowers. Martini et al. (2003) also demonstrated an elevated rate of fruit abortion (mainly in self-pollinations) for *Gongora quinquenervis* in Pernambuco, Northeastern Brazil. The results of the experimental treatments are summarized in Table 2.

Under natural conditions (open pollination), the fruit set of *Cirrhaea dependens* was very low: 1% and 1.7% at Serra do Japi and Macaé de Cima, respectively (Table 2). For Macaé de Cima, Miller and Warren (1996) reported a fruit set of about 100%, with 40 capsules distributed on five inflorescences. On the other hand, on the same plants sampled by Miller and Warren (1996), we recorded the production of 57 flowers in 2003, with only one fruit being formed (Table 2). At Serra do Japi, the fruit set on the studied plants (between 1998 and 2002) was constantly low (Table 2). Although good data on the efficiency of pollination in Stanhopeinae are very rare (Dressler, 1968), as was the case with our observations for *C. dependens*, the natural fruit set of *Gongora quinquenervis* was reported to be very low (Dressler, 1968; Martini et al., 2003), as was the case with our observations for *C. dependens*. We are unable to explain the causes leading to significant fruit set variation of *Cirrhaea dependens* in Macaé de Cima. Therefore, at Serra do Japi, as Martini et al. (2003) have reported for *Gongora quinquenervis*, the low fruit set appears to be the result of a combination of factors, such as: production of short-lived flowers, presence of floral mechanisms avoiding self-pollination, non-synchronization of flower phases among plants, and low number of sparsely distributed individuals within populations. Differences in population structure may cause low reproductive success in tropical orchids (Neiland and Wilcock, 1998), mainly when these populations are small and the plants are widely dispersed (Ackerman, 1986).

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