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Reproductive biology and pollination of southeastern Brazilian *Stanhopea* Frost *ex* Hook. (Orchidaceae)

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Abstract

Reproductive biology and pollination of *Stanhopea lietzei* and *Stanhopea insignis* were studied in a semi-deciduous mesophytic forest in the Serra do Japi (SJ), and in the coastal plain of Picinguaba, both in the State of São Paulo, Brazil. Floral morphology, pollination, breeding system and fruit set of both species were investigated. *S. lietzei* and *S. insignis* are pollinator-specific, being pollinated by male bees of *Eufriesea* (Apidae, Euglossini), which collect the fragrance produced by pluricellular osmophores at the base of the saccate hypochile. *S. lietzei* and *S. insignis* were pollinated by *Eufriesea pulchra* and *Eufriesea purpurata*, respectively. Observations using substances present in the floral fragrance of both studied species as chemical baits were also performed. *E. purpurata* was attracted by benzyl alcohol, the major compound of the perfume of *S. insignis*, while *E. pulchra* was attracted by none of the compounds used. Both studied *Stanhopea* are self-compatible but pollinator dependent. Self-pollination, however, tends to be avoided by floral mechanisms. In experimental self- and cross-pollinations the proportion of fruit abortion was high and related to resource limitation. The reproductive success of *S. lietzei* and *S. insignis* was low as a consequence of deficient pollen transference while pollinator scarcity was the main factor.

Introduction

The subtribe Stanhopeinae (Epidendroideae, Cymbidieae) presents 22 genera of orchids distributed among tropical America, and includes *Stanhopea* Frost *ex* Hook. (Dressler, 1993), a genus with about 55 species that occurs from Mexico to Brazil (Morrison, 1997). As all the other genera of Stanhopeinae, the members of *Stanhopea* offer floral fragrances as rewards. These are produced by floral osmophores and exclusively collected by male euglossine bees (Hymenoptera, Apidae, Euglossini) (see Arditti, 1992; Vogel, 1990; Williams, 1982; Williams and Whitten, 1983 for reviews). The relation of the male euglossine bees to the pollination of orchid flowers has often been reported (see Williams, 1982 for a review). Although several hypotheses have been published (see Peruquetti, 2000), the reason why these bees collect fragrances is still unknown.

The pollination mechanisms of species of *Stanhopea* are well known (Dressler, 1968; van der Pijl and Dodson,

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1966; Williams, 1982). The most derived species of *Stanhopea* (i.e., "*insignis* complex"), in which the lip is distinctly divided into hypochile, mesochile and epichile (Curry et al., 1991), has been called "drop flower" or "fall-through" flower (see Williams, 1982). The bee enters from the side and brushes against the saccate base of the lip hypochile. Since the surface of the lip is very smooth, the bee may slip and fall when withdrawing from the flower, hooking the viscidium under its scutellum or depositing pollinia in the stigma (Braga, 1976; Dressler, 1968; Williams, 1982). Species of *Stanhopea* are pollinated by male bees of the genera *Eufriesea* (*Euplusia*), *Euglossa* and *Eulaema* (e.g., van der Pijl and Dodson, 1966; Williams, 1982; Williams and Whitten, 1983).

The relationship between orchids and euglossine bees is often highly specific. This pollinator specificity is based upon a combination of floral fragrance compounds (Williams and Whitten, 1983), although certain substances are present in the odor of many different species (Dodson, 1970; Gerlach and Schill, 1991; Hills et al., 1968, 1972; Whitten and Williams, 1992; Williams and Whitten, 1983; Williams et al., 1981). Although some of these orchids are pollinated by several bee species, in most cases they are pollinator-specific. Species of *Stanhopea* can present sympatric and inter-fertile species (Dressler, 1968). The odor produced by each species, however, is critical in attracting different pollinators (Dressler, 1968; van der Pijl and Dodson, 1966). This specificity has a great importance in the mechanisms of isolation and speciation within Stanhopeinae (e.g., Dressler, 1968; Hills et al., 1972; van der Pijl and Dodson, 1966; Williams, 1982; Williams and Dodson, 1972). The floral fragrances of *Stanhopea* species are composed mainly of several mono- and sesquiterpenes, and aromatic compounds (Dodson, 1970; Whitten and Williams, 1992; Williams, 1982; Williams and Whitten, 1983) produced by secretory tissue (papillate osmophores) located in the basal portion of the saccate hypochile (Stern et al., 1987). The fragrance of *Stanhopea lietzei* is composed mainly by indole, linalool and benzyl benzoate, while the odor of *Stanhopea insignis* presents benzyl alcohol as its principal component (Reis et al., 2004). As present in the floral fragrances of Stanhopeinae species (Dodson, 1970; Kaiser, 1993; Whitten and Williams, 1992; Williams, 1982; Williams and Whitten, 1983), these substances are frequently used as baits to conduct studies on male euglossine bees for a variety of purposes (see Williams, 1982, for a review).

According to Dressler (1968), a few euglossine-pollinated orchids are self-sterile, and cross-pollination appears to be the rule. In the subtribe Stanhopeinae, self-pollination tends to be avoided basically by floral mechanisms (Dressler, 1968; van der Pijl and Dodson, 1966). In *Stanhopea*, the stigmatic cavity is too narrow to admit pollinia when the bee first visits a flower since, before drying, the pollinia are too thick to be inserted (van der Pijl and Dodson, 1966). Dehydration reduces the pollinia thickness, making its deposition in the stigmatic cavity easier (Dressler, 1968; van der Pijl and Dodson, 1966).

Observations of pollinators and pollination mechanisms of *Stanhopea* have been performed for many Central American species (see Dressler, 1968; van der Pijl and Dodson, 1966). For South-American *Stanhopea*, data on pollination concern the Amazonian *S. candida* Barb. Rodr. (Braga, 1976). These works, however, give little emphasis to the reproductive biology and natural fruit set of these orchids. According to Dressler (1968), good data on the reproductive success of euglossine pollination in Stanhopeinae species are still missing. However, more conclusive studies have revealed a low natural fruit set for *Gongora quinquenervis* Ruíz and Pavón (Dressler, 1968; Martini et al., 2003) and *Cirrhaea dependens* (Lodd.) Rchb.f. (Pansarin et al., 2006). Data on reproductive biology and natural fruit set of *Stanhopea* are lacking in literature.

In the State of São Paulo, southeastern Brazil, two species of *Stanhopea* occur: *S. lietzei* (Regel) Schltr., in inland semi-deciduous and gallery forests, and *S. insignis* Frost *ex* Hook., on the coast, in "restinga" vegetation. This paper aims to report the floral biology and reproduction of South-American *S. lietzei* and *S. insignis*, based on records of the floral morphology, pollination mechanisms, breeding system and fruit set in natural conditions. Some aspects of pollinator specificity are also discussed.

Materials and methods

Study sites

The floral and reproductive biology of *S. lietzei* was recorded in a mesophytic semi-deciduous forest in the Serra do Japi (SJ), district of Jundiaí (approx. 23°11′S, 46°52′W; 700–1300 m asl), while *S. insignis* was studied in the Atlantic rain forest of Picinguaba (approx. 23°33′S, 45°04′W; 0–50 m asl), district of Ubatuba. Both studied areas are natural reserves of the State of São Paulo, Brazil (see also Pansarin and Amaral, 2008). The SJ is located inland and Picinguaba on the coast. The SJ is about 250 km away from Picinguaba. In the SJ, the annual rainfall is about 1500 mm, and annual average temperature circa 17.5 °C (Pinto, 1992). This region is mainly characterized by semi-deciduous mesophytic forests of medium altitude with occasional rocky outcrops (Leitão-Filho, 1992). In Picinguaba, the annual rainfall is about 2624 mm, and the average temperature circa 21 °C (Nimer, 1977). This region is characterized by evergreen Atlantic rain forests on steep slopes and "restinga" vegetation.

Phenology, plant features and floral morphology

Features of flower anthesis, its duration, and fruit dehiscence of *S. lietzei* (44 plants) and *S. insignis* (27 plants) were gathered by visiting both study areas monthly, from January 1998 to February 2000.

Morphological features of fresh flowering plants of *S. lietzei* and *S. insignis* collected in the field were recorded and drawn. Floral details of *S. lietzei* ($n = 8$) and *S. insignis* ($n = 5$) were studied and drawn under a binocular stereomicroscope equipped with a ''camera lucida''. The measurements were made from drawings and directly from floral structures using a caliper rule. For the anatomical study fresh flowers of *S. lietzei* and *S. insignis* were collected at both study sites. Longitudinal sections of the basal portion of the hypochile were manually obtained from fresh flowers and stained with safranin 1% and astra blue.

The measurements of the pollinia and stigmatic cavity of *S. lietzei* ($n = 17$) and *S. insignis* ($n = 13$) were made from drawings and directly from structures using a caliper rule. The flowers used were obtained from plants collected in the field and maintained in the greenhouse of the Universidade Estadual de Campinas, Campinas, State of São Paulo (approx. 22°49′S, 47°06′W; 700 m asl). The flowers were collected in the morning hours (from 7:30 to 8:30 h) and on the first day of flower opening. The pollinaria were removed and kept in Petri dishes at room temperature (about 22–24 °C) and immediately measured. Measurements were made each 5 min during 2 h after removal.

Pollinators and pollination mechanisms

For both studied species field visits were undertaken in the studied sites to observe and record the pollination process and the visitation frequencies, and to capture pollinators for later identification. In the SJ, the observations of flowers of *S. lietzei* were carried out from 23 to 27 November 1998, 22 to 25 November 1999, 17 to 19 November 2000, and 24 to 26 November 2003, totaling 127.5 h. In Picinguaba, the observations of *S. insignis* flowers took place from 22 to 26 February 2000 and from 18 to 21 February 2002, totaling 76.5 h. In both sites, the daily period of observation was from 7:30 to 16:00 h, and, flowers were tagged in the afternoon and examined in the early morning, at about 7:30 h, to detect possible nocturnal pollination.

Chemical baits

The floral fragrance of *S. lietzei* and *S. insignis* was analyzed by Reis et al. (2004). Compounds present in the fragrance of *S. lietzei* and *S. insignis* (linalool, indole, benzyl alcohol and benzyl benzoate) were commercially obtained and used in both study areas to attract euglossine bees carrying pollinaria of orchid species. In the SJ, a mixture of linalool, indole and benzyl alcohol was applied in the same proportion as the floral fragrance of *S. lietzei* (Reis et al., 2004). Each substance and their mixtures were applied independently, onto separate 6 × 6 cm pads of herbarium blotter paper and pinned on tree trunks, in both (SJ and Picinguaba) study sites. The quantity and frequency of substance reposition to maintain attractiveness varied from 30 min to 2 h and depended on the evaporation rates.

In the SJ, the censuses were carried out from October to December, the flowering period of *S. lietzei,* between 1998 and 2000, totaling about 90 h. In Picinguaba, they were undertaken from February to March, the flowering period of *S. insignis*, between 1999 and 2001, totaling about 70 h. In both sites, the daily period of census was from 8:00 to 16:00 h. The censuses were undertaken in alternate days along the flowering period of each *Stanhopea* species and mainly in sunny days, because cloud cover and precipitation seem to influence the activity of euglossine bees on chemical baits (Inouye, 1975; Janzen et al., 1982; Williams, 1982).

The insects collected either when visiting the flowers or attracted with chemical baits were identified and vouchers were deposited at the Natural History Museum of the Universidade Estadual de Campinas (ZUEC). The Euglossini bees were identified using Kimsey (1982) and Rebêlo and Moure (1995).

Breeding system and natural fruit set

In *S. lietzei* and *S. insignis*, the treatments to investigate the breeding system were performed in their natural habitat. The experimental treatments included untouched flowers, manual self-pollination, emasculation and cross-pollination. The number of pollinations varied between treatments depending on the availability of flowers. For *S. lietzei* 14 (33 flowers) and 17 (41 flowers) plants were used in the 1998 and 2003 flowering period, respectively. For *S. insignis*, 11 (24 flowers) and 16 (46 flowers) plants were used in the 1999 and 2002 flowering period, respectively. Treatments were randomly applied to each inflorescence, using flowers on the first day of anthesis.

Natural fruit set (open pollination) was recorded for both studied species. In the SJ, 22 plants of *S. lietzei* were sampled during 3 years (1999, 2000 and 2002) while, in Picinguaba, 13 plants of *S. insignis* were recorded in 2000 and 19 in 2003. Fruit set was recorded when fruits were dehiscent. The number of flowers sampled varied and depended on the total production of each year.

Results and discussion

Phenology, plant features and floral morphology

S. lietzei flowers in spring, from October to December, and S. insignis in summer, between February and March. The fruits of S. lietzei are dehiscent from July to August, and those of S. insignis between October and November. All the flowers of S. lietzei and S. insignis open simultaneously in the morning hours (between 6:30 and 7:30 h), and each flower lasts 3–4 days.

S. lietzei is a lithophytic herb occurring on rocks in mesophytic semi-deciduous forest in the SJ, while S. insignis occurs as epiphyte in the costal plain ("restinga") of the Atlantic rain forest of Picinguaba.

Since the vegetative and floral morphology of both studied Stanhopea species are similar, we will only present the description of the plant and floral morphology of S. lietzei. The characteristics of S. insignis that differ from those of S. lietzei are shown between brackets.

Both Stanhopea species present clusters of conic and ribbed pseudobulbs, each bearing a single and apical leaf. The leaves are elliptic, plicate and pseudopetiolate. The inflorescence is a pendant raceme, emerging from the base of pseudobulb, with up to five resupinate flowers (Figs. 1A and 2A). The dorsal sepal is about 6×2.5 cm, and symmetrically elliptic. Lateral sepals are 6×3 (6.5×4) cm, and asymmetrically elliptic. The petals are 5×1.5 (5.5×2) cm and generally lanceolate. Sepals and petals are reflexed (Figs. 1B–D and 2B and D). The fleshy and waxy lip measures about 5.5 (6.5) cm and is divided into a proximal hypochile, a mesochile and a distal epichile (Figs. 1B–D and 2B–D). The hypochile is saccate and presents multicellular and papillate osmophores at its basis. The osmophores of S. lietzei and S. insignis present a structure similar to that reported for S. tigrina Batem. ex Lindl. (Curry et al., 1991). The mesochile presents a central and longitudinally disposed furrow and a pair of forward-projecting horns (Figs. 1D and 2D), reported as the most specialized labella within Stanhopea (i.e., "insignis" complex; Curry et al., 1991). The distal epichile is flattened and triangular shaped (Figs. 1D and 2D). The arcuate, winged column is about 5 (5.5) cm, and presents a narrow transversal stigma that measures about 3.5×0.8 (5.2×0.9) mm (Figs. 1E, 2E, 4E, 5E). The ovate and white anther is about 6.5×4.5 (7.5×6) mm (Figs. 1F, 2F). The pollinarium is about 9×3 (10×4) mm and formed by two yellow, compressed pollinia, an elongate, white stipe, and a terminal, cordiform viscidium (Figs. 1G, 2G, 3A–D). Both Stanhopea species produce floral fragrances that are more vivid in the warmer hours of the day, from 10:00 to 14:00 h. S. lietzei produces an olfactively intense fragrance, while that of S. insignis is very sweet.

Fragrance is perceptible mainly in the first day of flower anthesis. The floral fragrance of S. lietzei presents benzyl benzoate, indole and linalool as its main compounds, while that of S. insignis is predominantly composed (97%) by benzyl alcohol (Reis et al., 2004). The coloration of flowers of S. lietzei varies from white to orange and, sometimes, presents vine-like dots concentrated mainly on the epichile. The color of S. insignis flowers varies from white to cream with vine-like dots (Fig. 3B–F). Further details about morphology and illustrations of S. lietzei (as S. graveolens Lindl.) and S. insignis can be found in Hoehne (1942), and in Jenny (2003).

Pollinators and pollination mechanisms

S. lietzei and S. insignis are pollinator-specific, being pollinated by Eufriesea pulchra F. Smith, and E. purpurata Mocsáry, in the SJ and Picinguaba, respectively. Fourteen and 47 visits to flowering plants of S. lietzei and S. insignis, respectively, were recorded. Observations recorded only one visit of Eulaema cingulata Fabricius to a flower of S. insignis, so that only Eufriesea purpurata served as pollinators of this species. Similarly, only one visit of Euglossa cordata Linnaeus and one of E. annectans Dressler were observed on flowers of S. lietzei, confirming that only E. pulchra acted as a pollinator. In S. lietzei, visits occurred from 10:30 to 15:00 h, while in S. insignis visits were recorded between 8:30 and 17:00 h. In both species, visit peak occurred in the warmest hours of the day, from 12:30 to 14:00 h for S. lietzei, and from 10:00 to 13:00 h for S. insignis.

In both S. lietzei and S. insignis, the pollination mechanism was similar to that reported for other Stanhopea (Braga, 1976; Dressler, 1968; van der Pijl and Dodson, 1966; Williams, 1982). Fig. 3 shows the sequence of the pollination process of S. lietzei and S. insignis. Initially, the bee landed laterally on the hypochile, on the column, or on both structures (Fig. 3A), and entered into the saccate hypochile (Fig. 3A and B) which contains the pluricellular and papillate osmophores. The bees brushed against the osmophores at the base of the saccate hypochile with their anterior legs collecting the fragrance. At regular intervals the bees left the hypochile to hover and transfer the perfumes to their posterior legs. For further details on fragrance collection mechanisms by male euglossine bees see Dressler (1968, 1982). Since the surface of the waxy lip is very smooth, the bee could slip and fall when withdrawing from the hypochile. The mesochile horns prevent the bee from slipping, directing it down through the chute past the apex of the epichile and column. During its fall, the bee could contact the projecting viscidium with its scutellum, either fixing the pollinarium (Figs. 3C and 4F) or
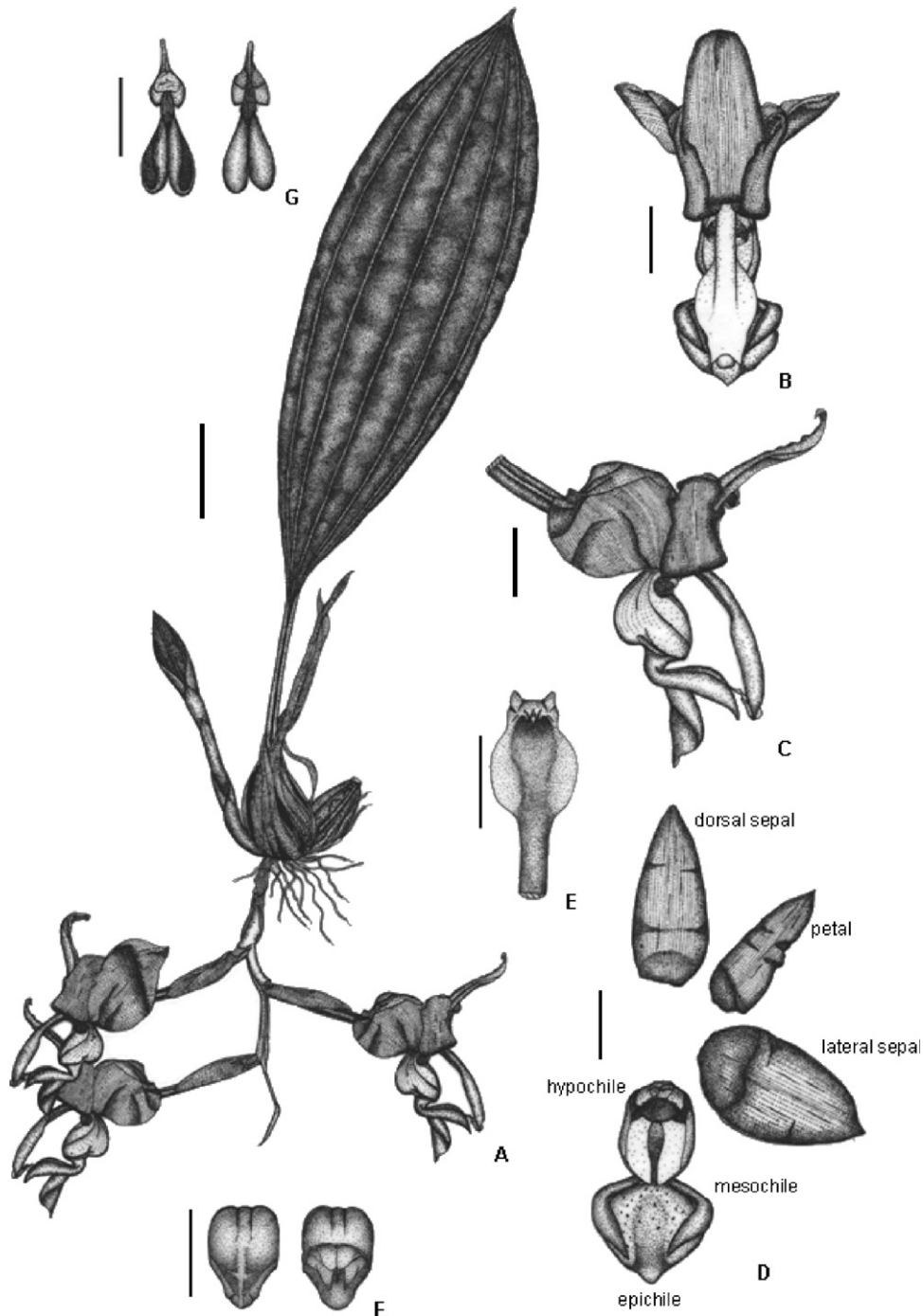
Fig. 1. *Stanhopea lietzei* (Regel) Schltr.: (A) flowering plant, (B) flower in frontal view; (C) flower in lateral view; (D) perianth parts; (E) detail of the column; (F) anther in dorsal and ventral views; (G) pollinarium in ventral and dorsal views. Scale bars: A = 5 cm; B–E = 2 cm; F–G = 5 mm.

depositing pollinia into the narrow stigma. A single bee can visit until 16 times consecutively the same flower and each visit lasts up to 120 s. For further details about pollination mechanisms of *Stanhopea* species and illustrations, see Braga (1976) and Dressler (1968).

When turgid, the pollinaria of *S. lietzei* presented pollinia with a thickness of 1.4 mm (1.2 mm for *S. insignis*) and, consequently, their deposition into the

stigmatic slit (0.8–0.9 mm) was not possible. When dehydrated, the pollinia thickness of both species was reduced to 0.6 mm, allowing a deposition into the stigmatic slit. Figs. 4 and 5 show pollinaria dimension with turgid (Figs. 4A, B and 5A, B) and dried (Figs. 4C, D and 5C, D) pollinia, 2 h after their removal. On dry days, the necessary time for sufficient dehydration of pollinia was about 30–45 min, more than any bee
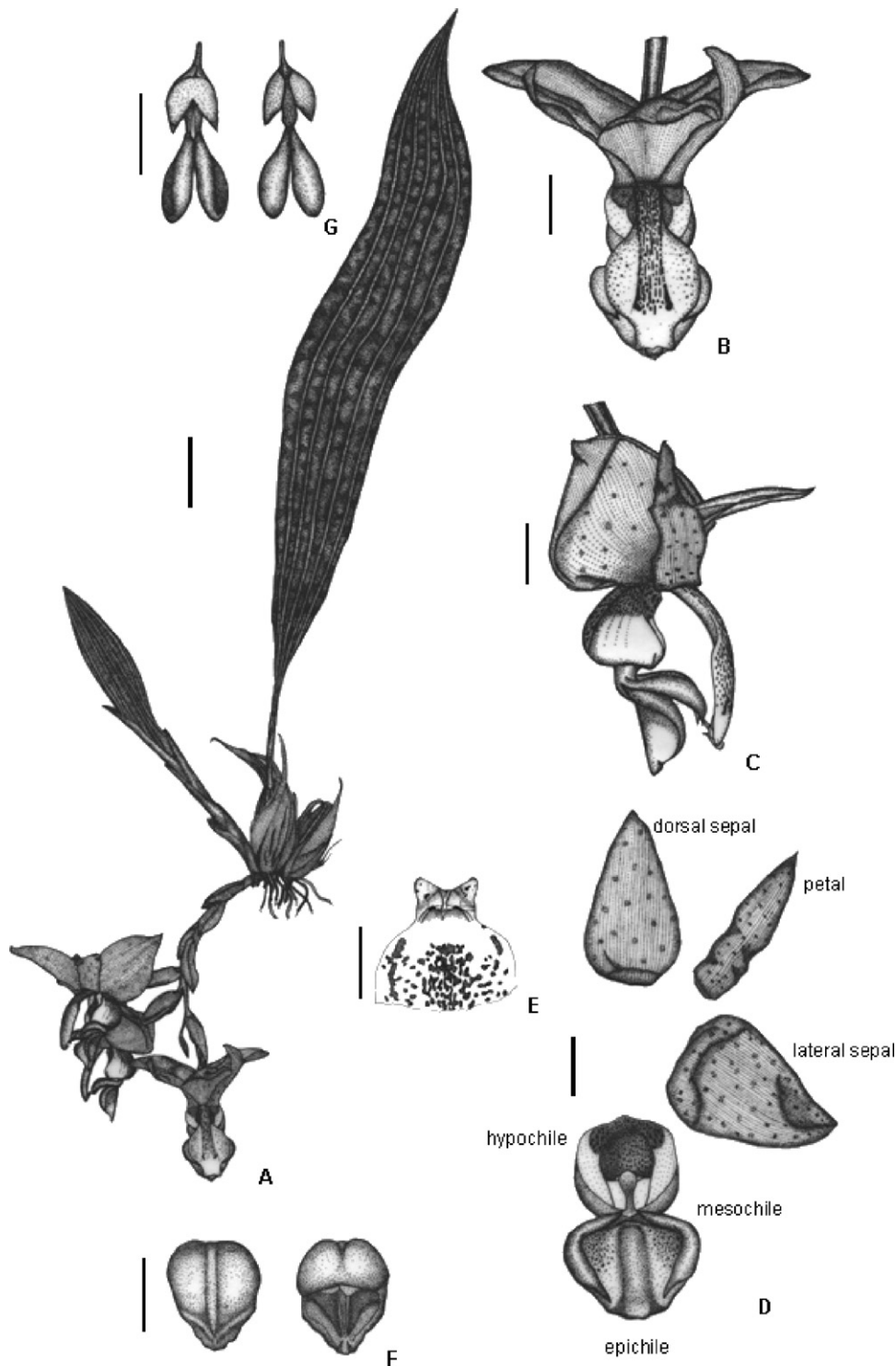
Fig. 2. *Stanhopea insignis* Frost *ex* Hook.: (A) flowering plant; (B) flower in frontal view; (C) flower in lateral view; (D) perianth parts; (E) detail of the column; (F) anther in dorsal and ventral views; (G) pollinarium in ventral and dorsal views. Scale bars: A = 5 cm; B–D = 2 cm; E = 1 cm; F–G = 5 mm.

remained in a single plant (max. 20 min). The mechanism of pollinia dehydration thus avoided self-pollination in both studied southeastern Brazilian *Stanhopea*. The mechanism of pollinia dehydration is an important strategy that prevents self-pollination in Stanhopeinae

(Dressler, 1968; Martini et al., 2003; van der Cingel, 2001; van der Pijl and Dodson, 1966) and was well documented for other species of *Stanhopea* (see Dressler, 1968; van der Pijl and Dodson, 1966). According to Dressler (1968, 1981), in the case of species pollinated by
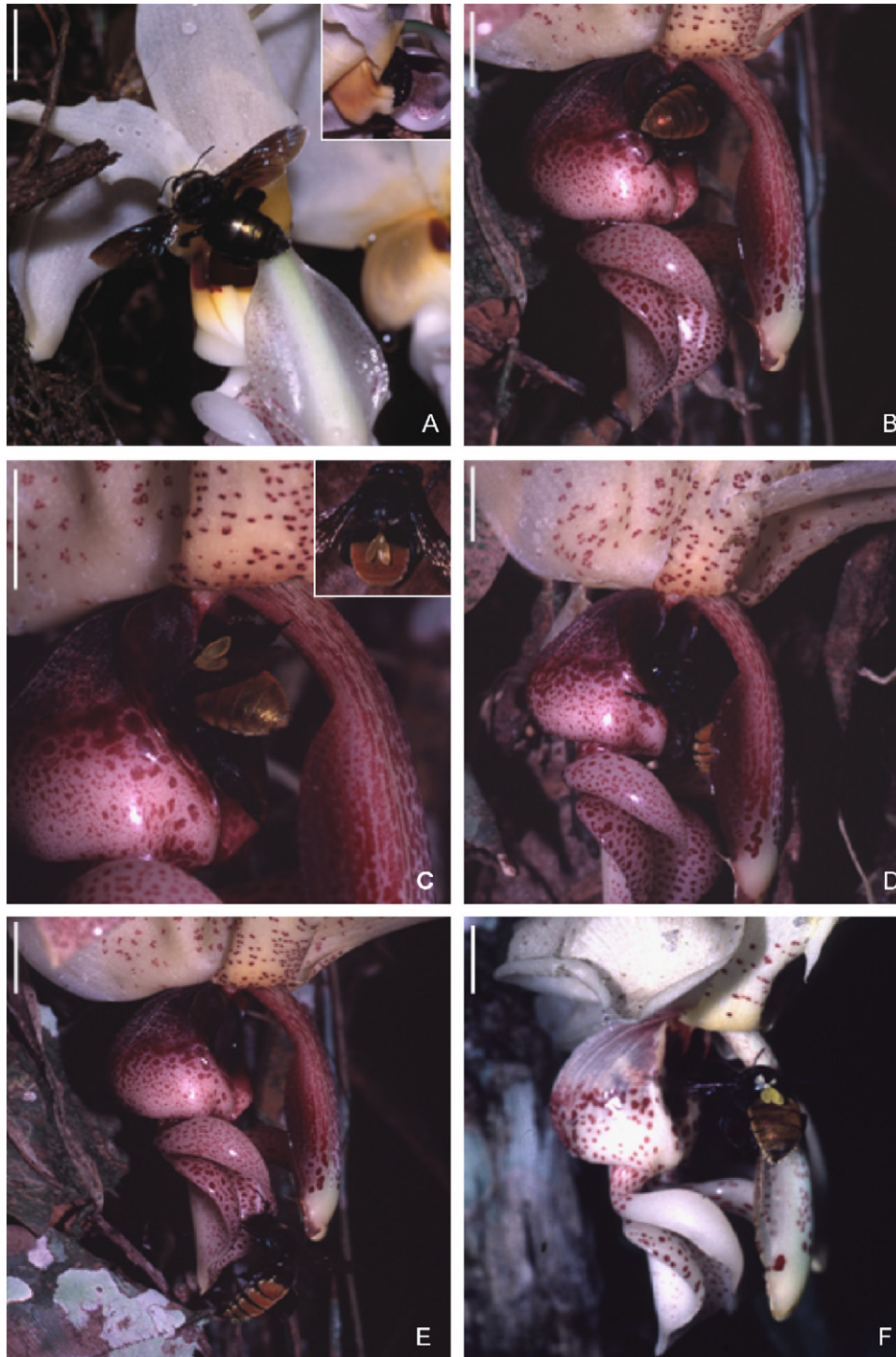
Fig. 3. Pollinators and pollination mechanism of *Stanhopea lietzei* (A) and *S. insignis* (B–F): (A) *Eufriesea pulchra* landing on the lip and column. Detail shows the bee entering into the saccate hypochile; (B) *Eufriesea purpurata* entering in the hypochile of *S. insignis*; (C) *Eufriesea purpurata* collecting fragrance at the basis of the saccate hypochile. Note the dry pollinarium of *S. insignis* on the scutellum of the bee. Detail shows a dorsal view of *E. purpurata* with a dried pollinarium of *S. insignis* on its scutellum; (D) *Eufriesea purpurata* slipping after leaving the saccate hypochile; (E) *Eufriesea purpurata* falling through and out from the epichile; (F) an individual of *E. purpurata* hovering with a turgid pollinarium of *S. insignis* on its scutellum, immediately after removal. Scale bars = 1 cm.
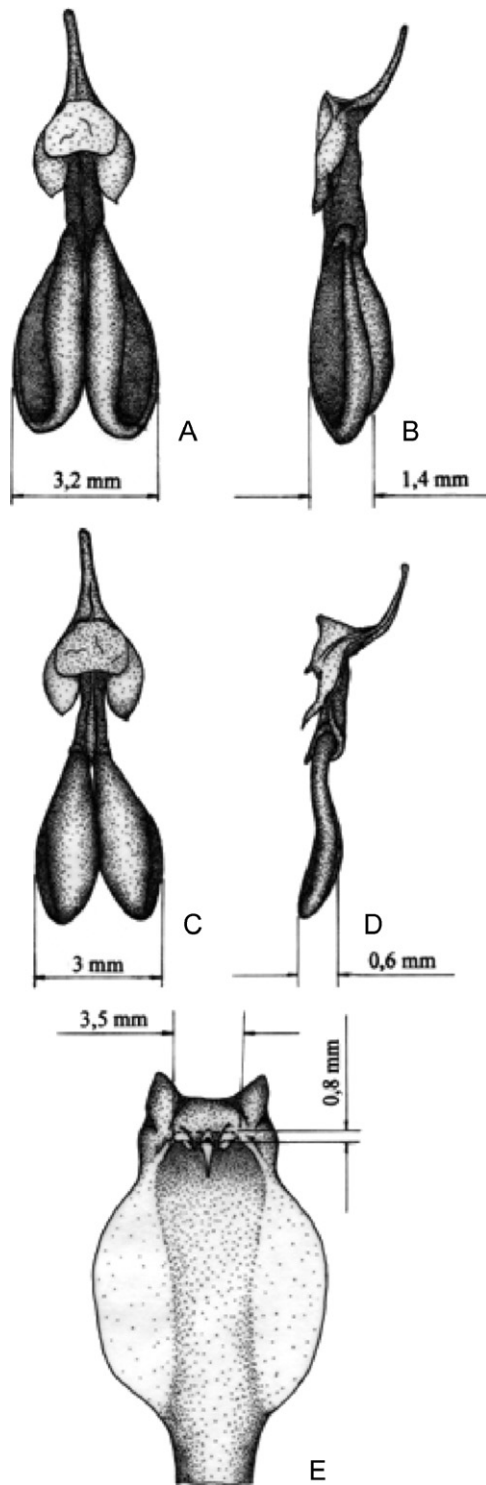
Fig. 4. *Stanhopea lietzei*: Ventral (A) and lateral (B) views of a pollinarium with turgid pollinia, immediately after removal from flower. Ventral (C) and lateral (D) views of a pollinarium with dehydrated pollinia, 120 min after removal from flower; (E) apical portion of column in frontal view, showing the narrow and transversal stigma.
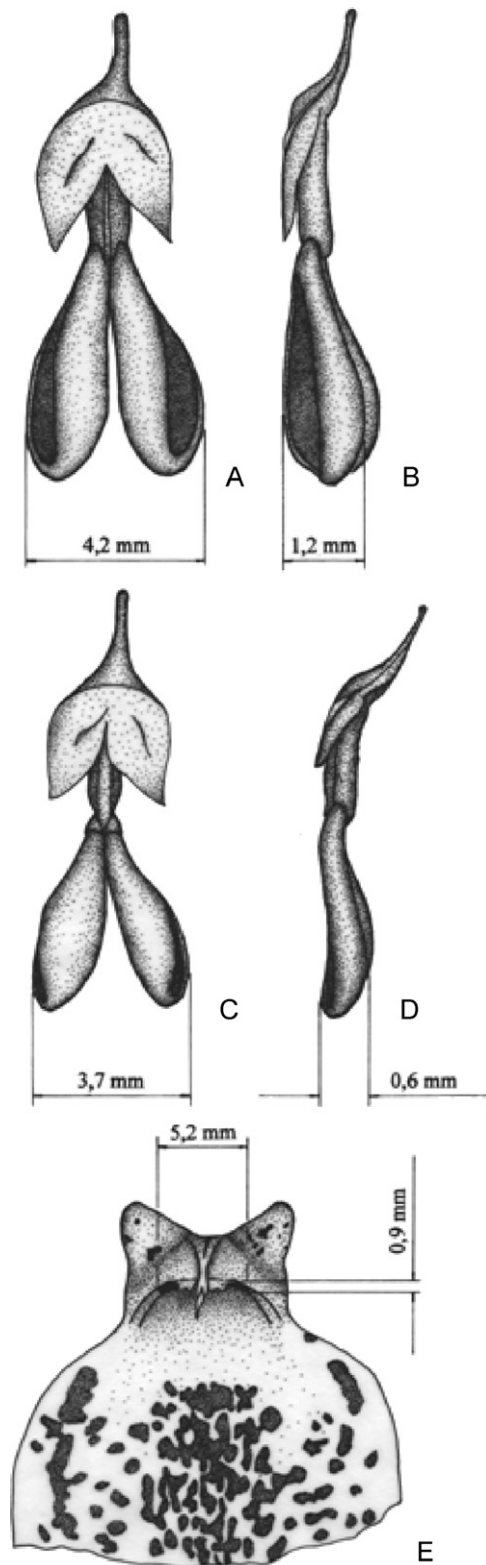
Fig. 5. *Stanhopea insignis*: Ventral (A) and lateral (B) views of a pollinarium with turgid pollinia, immediately after removal from flower. Ventral (C) and lateral (D) views of a pollinarium with dehydrated pollinia, 120 min after removal from flower; (E) apical portion of column in frontal view, showing the narrow and transversal stigma.

male euglossines in which the bees tend to visit the same flower many times, as was reported here for *S. lietzei* and *S. insignis*, this mechanism decreases the occurrence of self-pollination and of geitonogamy. Between a few minutes and some hours are necessary before the pollinaria are appropriately sized to enter the stigma (Johnson and Edwards, 2000). The mechanism of pollinia dehydration preventing self-pollination in Stanhopeinae has been characterized as a form of protandry (e.g., Dressler, 1968; van der Pijl and Dodson, 1966). The selection of characters that prevent geitonogamy usually occurs when pollinators are abundant (Johnson and Nilsson, 1999), or when the same flower tends to be visited repeatedly as occurs with species of Stanhopeinae (Johnson and Edwards, 2000; Martini et al., 2003).

Chemical baits

The fragrance of *S. lietzei* is mainly composed by indole, linalool and benzyl benzoate (Reis et al., 2004). Benzyl benzoate, the major compound of the floral fragrance of *S. lietzei* (Reis et al., 2004), is also the principal constituent of the perfume of *S. panamensis* Dodson (Whitten and Williams, 1992; Williams and Whitten, 1983). Using benzyl benzoate as chemical bait, Ackerman (1989a) and Williams and Whitten (1983) captured *Eufriesea ornata* (Mocsáry 1896) carrying pollinaria of *S. panamensis*. Although *E. ornata* occurs from Central America to North and Northeastern Brazil (Kimsey, 1982), and has sometimes been recorded in the State of Minas Gerais, southeastern Brazil (Peruquetti et al., 1999), this species has never been observed on the chemical baits of either study sites. Linalool, a substance present in the floral fragrance of *S. lietzei* (Reis et al., 2004), is also present in other species of *Stanhopea* (Hills et al., 1968; Kaiser, 1993; Whitten and Williams, 1992). According to Ackerman (1989a), a study using several substances as baits recorded *E. pulchra* as attracted to linalool, in Panama. Although linalool is present in the floral fragrance of *S. lietzei*, *E. pulchra* was never attracted by this substance in the SJ. Male euglossine bees show variation in choice and preference for fragrance, and these variations seem to be related to the combination of season, age and geography (Ackerman, 1983, 1989a; Peruquetti et al., 1999). The odor of *S. insignis* presents benzyl alcohol as its principal constituent (Reis et al., 2004). Benzyl alcohol is also present in the floral fragrance of other species of *Stanhopea* (Whitten and Williams, 1992; Williams and Whitten, 1983) and *Catasetum* (Williams and Whitten, 1983). The use of benzyl alcohol in Picinguaba attracted several males of *E. purpurata*, some of them carrying pollinaria of *S. insignis*. *Eufriesea pulchra* was attracted by no major compound, either pure or mixed in the same proportions as the floral fragrance of *S. lietzei*,

used at the SJ. *Euglossa viridis* Perty, the main pollinator of *Cirrhaea dependens* (Lodd.) Rchb.f. in the SJ, was never attracted by substances present in their fragrances and used as chemical baits in this region (E.R. Pansarin, unpubl. data). Similarly, chemical baits never attracted the pollinators of *Catasetum maculatum* Kunth in Costa Rica, and apparently the bees show a preference for the floral fragrance emitted by the flowers of this species (Janzen et al., 1982). Although certain substances are present in the odor of many different species (Dodson, 1970; Gerlach and Schill, 1991; Hills et al., 1968, 1972; Whitten and Williams, 1992; Williams and Whitten, 1983; Williams et al., 1981), each singular orchid emits a unique fragrance. Species of *Stanhopea* generally are pollinator-specific, and this specificity is based upon the combination of floral fragrance compounds (Williams and Whitten, 1983). In orchid species that only offer floral fragrances as a reward and are exclusively pollinated by male euglossine bees, as occurs in all members of Stanhopeinae (Dressler, 1993; Whitten and Williams, 1992; Williams, 1982; Williams and Whitten, 1983), the production of different fragrances can be responsible for pollinator specificity and consequently represents an important isolating mechanism (see Williams, 1982; Williams and Whitten, 1983).

Breeding system and natural fruit set

Both studied species of *Stanhopea* are self-compatible but pollinator dependent. Fruit set of *S. lietzei* was 63.6% and 65%, and that of *S. insignis*, was 92% and 90.5%, with self-pollination and cross-pollination, respectively. In both studied species, no fruits formed on untouched or emasculated flowers. The results of the experimental treatments are summarized in Table 1. According to Dressler (1968), a few euglossine-pollinated orchids are self-sterile, and cross-pollination appears to be the rule. The self-pollination in the subtribe Stanhopeinae tends to be avoided basically by floral mechanisms (Dressler, 1968; van der Pijl and Dodson, 1966). Conclusive data on reproductive biology is presented for *Gongora quinquenervis* (Martini et al., 2003). *S. lietzei* and *S. insignis* showed a high rate of fruit abortion in both self- and cross-pollinated flowers (Table 1). This abortion rate in both treatments may indicate resource limitation, which has been reported for several other orchid groups (Ackerman, 1989b; Borba and Semir, 1998; Montalvo and Ackerman, 1987; Zimmerman and Aide, 1989).

The production of fruits per inflorescence is still the most used parameter to determine reproductive success in Orchidaceae (e.g., Ackerman, 1989b; Ackerman and Montalvo, 1990; Humaña et al., 2008; Montalvo and Ackerman, 1987; Zimmerman and Aide, 1989). Under natural conditions (open pollination), the fruit set of

Table 1. Reproductive system and natural fruit set (open pollination) of *Stanhopea lietzei* and *S. insignis* in the Serra do Japi and Picinguaba, respectively

Species	Untouched	Self-pollination	Emasculated	Cross-pollination	Open-pollination
Stanhopea lietzei	(10/0)	(33/21) 63.6%	(11/0)	(20/13) 65%	(89/15) 16.8%
Stanhopea insignis	(11/0)	(25/23) 92%	(13/0)	(21/19) 90.5%	(67/17) 25.3%

Characters between brackets indicate the number of flowers/fruits.

S. lietzei and *S. insignis* was low, with 16.8% and 25.3%, respectively (Table 1). Although good data on the efficiency of pollination in Stanhopeinae are lacking (Dressler, 1968), similarly to what is reported for *Cirrhaea dependens* (Pansarin et al., 2006) and *Gongora quinquenervis* (Dressler, 1968; Martini et al., 2003), the natural fruit set of both studied *Stanhopea* contrasts with the number of fruits that can be produced in experimental pollinations (Table 1). In *Gongora quinquenervis* and *Cirrhaea dependens*, low fruit set is associated with differences in population structures, mainly because the individuals are scarce and very widely dispersed and their flowering phases do not always synchronize (Martini et al., 2003; Pansarin et al., 2006). The low fruit set of southeastern Brazilian *S. lietzei* and *S. insignis* is probably related to insufficient pollen transference resulting from pollinator scarcity as the main factor. This fact can be corroborated by the low visit frequencies documented mainly for *S. lietzei* in the SJ. Some studies show that, in experimental conditions, an increase of fruit set occurs when compared with the natural condition in several orchid species (Ackerman and Montalvo, 1990; Ackerman and Oliver, 1985; Montalvo and Ackerman, 1987; Zimmerman and Aide, 1989). The fruit set of some non-autogamous orchids is low as a consequence of a deficient pollen transference between plants (Ackerman and Montalvo, 1990; Calvo, 1990; Janzen et al., 1980; Schemske, 1980), although other features, as inflorescence size, population density, flowering synchronization and flower duration, also can exert strong influence on the reproductive success of animal-pollinated plants (e.g., Augspurger, 1983; Flores-Palacios and García-Franco, 2003; House, 1992; Humaña et al., 2008; Kunin, 1997; Marquis, 1988; Pansarin and Amaral, 2008; Schmitt et al., 1987). Furthermore, fruit production may depend on several reproductive characteristics as flower size, shape, color, reward production and breeding systems (Rulik et al., 2008; Zimmerman and Pyke, 1988).

In conclusion, *S. lietzei* and *S. insignis* are pollinator-specific, being pollinated by male bees of *Eufriesea* (Apidae, Euglossini), which collect the fragrance produced by pluricellular osmophores at the base of the hypochile. *E. purpurata* was attracted by benzyl alcohol, the major compound of the perfume of *S. insignis*, while *E. pulchra* was attracted by none of the compounds used. Both studied *Stanhopea* are self-compatible but pollinator dependent. Self-pollination, however, tends to be avoided by floral mechanisms. In experimental self- and cross-pollinations the proportion of fruit abortion was high and related to resource limitation. Low capsule production under natural conditions is a result of deficient pollen transfer due to scarcity of efficient pollinators.

Acknowledgments

References

Ackerman, J.D., 1983. Diversity and seasonality of male euglossine bees (Hymenoptera: Apidae) in Central Panama. Ecology 64, 274–283.

Ackerman, J.D., 1989a. Geographic and seasonal variation in fragrance choices and preferences of male euglossine bees. Biotropica 21, 340–347.

Ackerman, J.D., 1989b. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). Syst. Bot. 14, 101–109.

Ackerman, J.D., Montalvo, A.M., 1990. Short- and long-term limitations to fruit production in a tropical orchid. Ecology 71, 263–272.

Ackerman, J.D., Oliver, J.C., 1985. Reproductive biology of *Oncidium variegatum*: moon phases, pollination, and fruit set. Am. Orchid Soc. Bull. 54, 326–329.

Arditti, J., 1992. Fundamentals of Orchid Biology. Wiley, New York.

Augspurger, C.K., 1983. Phenology, flowering synchrony, and fruit set of six neotropical shrubs. Biotropica 15, 257–267.

Borba, E.L., Semir, J., 1998. Wind-assisted fly pollination in three *Bulbophyllum* (Orchidaceae) species occurring in the Brazilian campos rupestres. Lindleyana 13, 203–218.

Braga, P.I.S., 1976. Estudos da flora orquidológica do Amazonas. I—Descrição e observação da biologia floral de *Stanhopea candida* Barb. Rodr. Acta Amazon. 6, 433–438.

Calvo, R.N., 1990. Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in South Florida. Am. J. Bot. 77, 736–741.

Curry, K.J., McDowell, L.M., Judd, W.S., Stern, W.L., 1991. Osmophores, floral features, and systematics of *Stanhopea* (Orchidaceae). Am. J. Bot. 78, 610–623.

Dodson, C.H., 1970. The role of chemical attractants in orchid pollination. In: Chambers, K.L. (Ed.), Biochemical Coevolution. Oregon State University Press, Oregon, pp. 83–107.

Dressler, R.L., 1968. Pollination by euglossine bees. Evolution 22, 202–210.

Dressler, R.L., 1981. The Orchids: Natural History and Classification. Harvard University Press, Cambridge.

Dressler, R.L., 1982. Biology of the orchid bees (Euglossini). Ann. Rev. Ecol. Syst. 13, 373–394.

Dressler, R.L., 1993. Phylogeny and Classification of the Orchid Family. Dioscorides Press, Portland.

Flores-Palacios, A., García-Franco, J.G., 2003. Effects of floral display and plant abundance on fruit production of *Ryncholaelia glauca* (Orchidaceae). Rev. Biol. Trop. 51, 71–78.

Gerlach, G., Schill, R., 1991. Composition of orchid scents attracting euglossine bees. Bot. Acta 104, 379–391.

Hills, H.G., Williams, N.H., Dodson, C.H., 1968. Identification of some orchid fragrance. Am. Orchid Soc. Bull. 37, 967–971.

Hills, H.G., Williams, N.H., Dodson, C.H., 1972. Floral fragrances and isolating mechanisms in the genus *Catasetum* (Orchidaceae). Biotropica 4, 61–76.

Hoehne, F.C., 1942. Iconografia de Orquidaceas do Brasil. S.A. Indústrias "Graphicars" F. Lanzara," São Paulo.

House, S.M., 1992. Population density and fruit set in three dioecious tree species in Australian tropical rain forest. J. Ecol. 80, 57–69.

Humaña, A.M., Cisternas, M.A., Valdivia, C.E., 2008. Breeding system and pollination of selected orchids of the genus *Chloraea* (Orchidaceae) from central Chile. Flora 203 (6), 469–473.

Inouye, D.W., 1975. Flight temperatures of male euglossine bees (Hymenoptera: Apidae: Euglossini). J. Kans. Entomol. Soc. 48, 366–377.

Janzen, D.H., DeVries, P.J., Gladstone, D.E., Higgins, M.L., Lewinsohn, T.M., 1980. Self- and cross-pollination of *Encyclia cordigera* (Orchidaceae) in Santa Rosa National Park, Costa Rica. Biotropica 12, 72–74.

Janzen, D.H., DeVries, P.J., Higgins, M.L., Kimsey, L.S., 1982. Seasonal and site variation in Costa Rican euglossine bees at chemical baits in lowland deciduous and evergreen forests. Ecology 63, 66–74.

Jenny, R., 2003. The genus *Stanhopea*. Part 1: *S. anfracta* to *S. napoensis*. Caesiana 21, 1–160.

Johnson, S.D., Edwards, T.J., 2000. The structure and function of orchid pollinaria. Plant Syst. Evol. 222, 243–269.

Johnson, S.D., Nilsson, L.A., 1999. Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. Ecology 80, 2607–2619.

Kaiser, R., 1993. The Scent of the Orchids. Olfactory and Chemical Investigations. Elsevier, Amsterdam.

Kimsey, L.S., 1982. Systematics of Bees of the Genus *Eufriesea* (Hymenoptera, Apidae). University of California Press, Berkeley.

Kunin, W.E., 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. J. Ecol. 85, 225–234.

Leitão-Filho, H.F., 1992. A flora arbórea da Serra do Japi. In: Morellato, L.P.C. (Ed.), História natural da Serra do Japi. Editora da Unicamp/Fapesp. Campinas, pp. 40–62.

Marquis, R.J., 1988. Phenological variation in the neotropical understory shrub *Piper arieianum*: causes and consequences. Ecology 69, 1552–1565.

Martini, P., Schlindwein, C., Montenegro, A., 2003. Pollination, flower longevity, and reproductive biology of *Gongora quinquenervis* Ruíz and Pavón (Orchidaceae) in an Atlantic forest fragment of Pernambuco, Brazil. Plant Biol. 5, 495–503.

Montalvo, A.M., Ackerman, J.D., 1987. Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). Biotropica 19, 24–31.

Morrison, A., 1997. *Stanhopea*. In: Pridgeon, A. (Ed.), The Illustrated Encyclopedia of Orchids. Timer Press, Oregon, pp. 267–271.

Nimer, E., 1977. Climatologia da região sudeste do Brasil. Rev. bras. Geogr. 34, 3–48.

Pansarin, E.R., Amaral, M.C.E., 2008. Pollen and nectar as a reward in the basal epidendroid *Psilochilus modestus* (Orchidaceae: Triphoreae): a study of floral morphology, reproductive biology and pollination strategy. Flora 203 (6), 474–483.

Pansarin, E.R., Bittrich, V., Amaral, M.C.E., 2006. At daybreak – reproductive biology and isolating mechanisms of *Cirrhaea dependens* (Orchidaceae). Plant Biol. 8, 494–502.

Peruquetti, R.C., 2000. Function of fragrances collected by euglossine males (Hymenoptera: Apidae). Entomol. Gen. 25, 33–37.

Peruquetti, R.C., Campos, L.A.O., Coelho, C.D.P., Abrantes, C.V.M., Lisboa, L.C.O., 1999. Abelhas euglossini (Apidae) de áreas de mata atlântica: abundância, riqueza e aspectos biológicos. Revta bras. Zool. 16, 101–118.

Pinto, H.S., 1992. O clima da Serra do Japi. In: Morellato, L.P.C. (Ed.), História natural da Serra do Japi. Editora da Unicamp/Fapesp. Campinas, pp. 30–38.

Rebêlo, J.M.M., Moure, J.S., 1995. As espécies de *Euglossa* latreille do nordeste de São Paulo (Apidae, Euglossinae). Revta bras. Zool. 12, 445–466.

Reis, M.G., Pansarin, E.R., Silva, U.F., Amaral, M.C.E., Marsaioli, A.J., 2004. Pollinator attraction devices (floral fragrances) of some Brazilian orchids. ARKIVOC 6, 89–97.

Rulik, B., Wanke, S., Nuss, M., Neinhuis, C., 2008. Pollination of *Aristolochia pallida* Willd. (Aristolochiaceae) in the Mediterranean. Flora 203 (2), 175–184.

Schemske, D.W., 1980. Evolution of floral display in the orchid *Brassavola nodosa*. Evolution 34, 489–493.

Schmitt, J., Eccleston, J., Ehrhardt, D.W., 1987. Density-dependent flowering phenology, outcrossing, and reproduction in *Impatiens capensis*. Oecologia 72, 341–347.

Stern, W.L., Curry, K.J., Pridgeon, A.M., 1987. Osmophores of *Stanhopea* (Orchidaceae). Am. J. Bot. 74, 1323–1331.

van der Cingel, N.A., 2001. An Atlas of Orchid Pollination. America, Africa, Asia and Australia. Balkema Publishers, Rotterdam.

van der Pijl, L., Dodson, C.H., 1966. Orchid Flowers: Their Pollination and Evolution. University of Miami Press, Coral Gables.

Vogel, S., 1990. The role of scent glands in pollination: on the structure and function of osmophores. Smithsonian Institution Libraries, and The National Science Foundation, Washington, DC.

Whitten, W.M., Williams, N.H., 1992. Floral fragrances of *Stanhopea* (Orchidaceae). Lindleyana 7, 130–153.

Williams, N.H., 1982. The biology of orchids and euglossine bees. In: Arditti, J. (Ed.), Orchid Biology and Perspectives. Cornell University Press, Ithaca, pp. 119–171.

Williams, N.H., Dodson, C.H., 1972. Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. Evolution 28, 84–95.

Williams, N.H., Whitten, W.M., 1983. Orchid floral fragrances and male euglossine bees: methods and advances in the last sesquidecade. Biol. Bull. 164, 355–395.

Williams, N.H., Atwood, J.T., Dodson, C.H., 1981. Floral fragrance analysis in *Anguloa, Lycaste*, and *Mendoncella* (Orchidaceae). Selbyana 5, 291–295.

Zimmerman, J.K., Aide, T.M., 1989. Patterns of fruit production in a neotropical orchid: pollinator vs. resource limitation. Am. J. Bot. 76, 67–73.

Zimmerman, M., Pyke, G.H., 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. Am. Nat. 131, 723–738.