

**Hummingbird pollination in *Besleria longimucronata* Hoehne
(Gesneriaceae) in southeastern Brazil.**

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HUMMINGBIRD POLLINATION IN *Besleria longimucronata* HOEHNE (GESNERIACEAE) IN SOUTH-EASTERN BRAZIL

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ABSTRACT

The floral biology, breeding system and floral visitors of *Besleria longimucronata* were studied in a submontane rainforest in southeastern Brazil. This shrub bears typical ornithophilous features: the showy flowers are tubular shaped with horizontal to pendent position and secrete dilute nectar. The flowers usually last eight days and showed a marked protandry combined with herkogamy. During the first four days, flowers were in male phase and the anthers occupied the upper side of the corolla opening, hiding the stigma. In the female phase, the anthers were found in the lower surface of the corolla tube, and the stigma showed up. Nectar was secreted in small amounts from the pre-anthesis until the corolla was dropped. *Besleria longimucronata* is self-compatible, but spontaneous self-pollination did not occur. The hummingbirds *Thalurania glaucopsis* and *Phaethornis ruber pygmaeus* were common visitors and the major pollinators of the flowers of *B. longimucronata*. The hermit *Ramphodon naevius* rarely visited this plant species. During the visits to each single flower the hummingbirds contacted the anthers or the stigma with the culmen. Hummingbirds generally exhibited trapline foraging strategy. However, on one day the small hermit *P. ruber* showed an unusual behaviour, setting up a feeding territory.

Key words: floral biology, hummingbird pollination, breeding system, Gesneriaceae.

RESUMO

Polinização por beija-flores em *Besleria longimucronata* Hochne (Gesneriaceae) no sudeste brasileiro.

A biologia floral, o sistema reprodutivo e os visitantes florais de *Besleria longimucronata* foram estudados em um trecho de floresta chuvosa submontana no sudeste brasileiro. Esse arbusto possui

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características ornitófilas típicas, tais como: flores vistosas, tubulares, em posição horizontal a pendente e néctar diluído. Em geral, as flores duram oito dias e apresentam protandria combinada a hercogamia. Durante os quatro primeiros dias, as anteras ocupam a porção superior da abertura da corola, ocultando o estigma (fase masculina). Na fase feminina, as anteras são encontradas na superfície inferior do tubo da corola, e deste modo o estigma fica exposto. O néctar é secretado em quantidades pequenas desde a pré-antese até a queda da corola. *Besleria longimucronata* é auto-compatível, porém autopolinizações espontâneas não ocorrem. Os beija-flores *Thalurania glaucopis* e *Phaethornis ruber pygmaeus* foram os visitantes mais frequentes e os polinizadores principais das flores de *B. longimucronata*. O beija-flor grande-da-mata *Ramphodon naevius* visitou essa espécie de planta apenas eventualmente. Durante as visitas a cada flor, os beija-flores contactam as anteras ou o estigma com o culmem. Normalmente, os beija-flores exibiram a estratégia de forrageamento em linhas de captura. Durante um dia de observação, o hermitão pequeno *P. ruber* apresentou um comportamento incomum, estabelecendo um território de alimentação em um agregado de plantas de *Besleria*.

Palavras chaves: biologia floral, polinização por beija-flores, sistema reprodutivo, Gesneriaceae.

INTRODUCTION

Gesneriaceae is a mainly tropical group of shrubs, herbs and epiphytes, with 126 genera and ca. 3000 species (Wiehler, 1983). The gesneriads are extremely versatile in their floral modes, which may show exceptionally broad ranges or frequent switches within genera (Endress, 1994); and speciation and evolution in the group are intimately linked with changes in the mode of pollination (Wiehler, 1983). In addition, an understanding of the pollination ecology in the neotropical Gesneriaceae is an essential prerequisite for any modern solution to the vexing problems of generic delimitations in this family (Wiehler, 1983).

Wiehler (1983) estimated that about 60% of the Neotropical Gesneriaceae are hummingbird pollinated, in opposition to ca. 30% pollinated by euglossine bees in search of nectar, and 10% pollinated by bats, butterflies, moths, flies or male euglossine bees in search of aromatic oils. In addition, Gesneriaceae is one of the most important sources of nectar for hummingbirds in southeastern Brazil (Snow, Teixeira, 1982). However, pollinators were inferred for most of species and detailed information about pollination ecology and breeding system in this family is scarce (Endress, 1994; Franco, Buzato, 1992).

Besleria longimucronata is a common shrub in Ubatuba, southeastern Brazil, where it occurs in clusters in both glades and dense places of the submontane rainforest. In this work, we present aspects of the floral biology, the breeding system and the floral visitors of this species.

MATERIAL AND METHODS

Study Site

Fieldwork was carried out in the Parque Estadual da Serra do Mar, Núcleo Picinguaba, Ubatuba, southeastern Brazil (23°22' S, 44°50' W). We studied *Besleria longimucronata* growing at two sites located at 5 m and 50 m elevation (sites 1 and 2, respectively), in a stretch of the atlantic coastal range (Sanchez, 1994). The climate is tropical wet (Af, Köppen, 1948) and annual rainfall is up to 2600 mm, average annual temperature is ca. 21°C and there is no well defined dry-cold season, even during the so called dry months (May-September) (data source: Instituto Agronômico de Campinas, IAC-SP).

Procedure

Observations were made during two subsequent flowering seasons of *B. longimucronata* (September-November, 1996 and July-September, 1997). The flowers were observed *in situ* to determine flower longevity and phases of anthesis. Osmophore presence was verified by staining flowers with neutral red (Kearns, Inouye, 1993). Pollen viability was estimated by its cytoplasm stainability, using the aceto-carmin technique (Radford et al., 1974). Stigmatic receptivity was tested using the H₂O₂ catalase activity method (Zeisler, 1938). Sugar concentration in the nectar was measured with a pocket refractometer and its volume with calibrated microcapillaries (Dafni, 1992). Voucher specimen is in the Herbário da Universidade Estadual de Campinas (UEC 67937).

The breeding system was assessed by hand-pollination treatments on previously bagged flowers, by bagged flower-buds (spontaneous self-pollination treatment) and by open pollinated flowers (control). Fruit set for all treatments was determined approximately three months after first pollinations. Some pistils were fixed in FAA at 12, 24, 36, 48 and 60 hours after pollination and analyzed under fluorescence microscopy to observe pollen tube growth (Martin, 1959).

Observations of floral visitors were made directly or with binoculars, and photographs of their visits were taken. Along eleven days, a total of 52 hours of visitor observation was spent during daylight hours.

RESULTS

The flowers of *Besleria longimucronata* are densely congested in the axils of the subtending leaves. They are solitary and tubular-shaped (Figs. 1 to 5). Flower pedicels (ca. 20 mm long) are horizontally to obliquely oriented and the flower is kept up horizontal to pendent. The calyx and pedicel are vivid yellow and the corolla, stamens and pistil are white. The mean length of the corolla was 22.7 mm (SD = 3.14, N = 60) and the mean diameter of the corolla tube was 5.3 mm (SD = 0.76, N = 60). The androecium consists of four epipetalous stamens and one staminodium. Anthers are connate in pairs (Fig. 1). The gynoecium is bicarpelar and unilocular with very high number of ovules of parietal placentation. The relatively short style (ca. 10 mm) is cylindrical, ending in a glandular unilobed stigma. Flowers present an annular nectary located at the base of ovary (Fig. 1).

Besleria longimucronata individuals showed three to eleven open flowers per day (mean = 6.8, SD = 2.39, N = 10). Flower opening is a gradual process that can take several hours to complete and it was quite variable in time of initiation. Flowers usually last eight days and show marked protandry combined with herkogamy. For the first four days they were in the male phase. In this stage, anthers occupied the upper rim of the corolla opening, hiding the stigma (Fig. 1 A and B), pollen viability inferred from cytoplasm stainability was near to 100% and the stigma was not receptive. From fifth day on anthers bent down slowly (intermediate phase, Fig. 1 C and D). During the seventh and eighth days flowers were in female phase. At this moment, anthers were found in the lower surface of the corolla tube and the stigma was exhibited (Fig. 1 E and F). Pollen grains were scarce and the stigma was receptive during this phase. Nectar was secreted in small amounts from pre-anthesis until the corolla is dropped (one-day production – male phase: range 4-5 μ l, N = 5; female phase: range 2-3 μ l, N = 5). The mean sugar concentration in nectar was 19.9% (SD = 2.5, range 16-24%, N = 10). Osmophores were not detected.

The Violet-fronted Woodnymph, *Thalurania glaucopis* (Gmelin) and the Reddish Hermit, *Phaethornis ruber pygmaeus* (Spix) were common visitors to *B. longimucronata* at the two study sites (Figs. 2 to 4). The Saw-billed Hermit, *Ramphodon naevius* (Dumont) visited this plant species a few times at the site 2 (Fig. 5). The culmen length of the hummingbirds is presented in the Table 1. During the visits to each single flower hummingbirds

contacted the anthers with the culmen, which became dusted with pollen (Figs. 4 and 5) or contacted the stigma (flowers at female phase).

On the *Besleria* plants, hummingbirds *T. glaucopsis* and *P. ruber* usually act as low-reward trapliners (in the sense of Feinsinger, Colwell, 1978). After the first visit, these birds frequently went forward to the open flowers of the same individual and subsequently to flowers of other individuals in the cluster, and then went away from the cluster. Visits of hummingbirds began early in the morning and continued throughout the day until late afternoon, at intervals of 15 to 120 min. The interval between successive visits usually lengthened through the day.

One male individual of *P. ruber* showed an unusual territorial behaviour during one morning of observation at site two. At this morning, this bird exploited continuously flowers in the *Besleria* cluster and behaved aggressively around it, excluding flower-visiting hummingbird intruders (*R. naevius* and *T. glaucopsis*). In the same day at afternoon, the hermit *R. naevius* was observed twice exploiting *Heliconia* sp. plants. *Heliconia* flowers were located in front of the *Besleria* cluster, which was not visited by this bird.

Other legitimate visitors of *B. longimucronata* are lepidoptera and a bee (*Euglossa* aff. *saphirina*), but the collected specimens did not carry *Besleria* pollen after the visits. In addition, *Trigona* bees regularly act as nectar robbers, piercing at the calice. *Phaethornis ruber* and *Thalurania glaucopsis* robbed nectar in the flowers once in a while, puncturing the base of the corolla with their bills.

The results of hand-pollination treatments are presented in the Table 2. *Besleria longimucronata* is self-compatible, but spontaneous self-pollination do not occur, due the protandry and the herkogamy. Pollen grains germinated readily following cross- and self-pollinations and after 36 h pollen tubes had reached the base of the style or the ovary. Sixteen hours after pollination ovule fertilization had occurred.

DISCUSSION

The floral features of *Besleria longimucronata* pointed to the ornithophilous syndrome (cf. Faegri, van der Pijl, 1979): tubular shape, horizontal to pendent position, flashy colour, no perceptible odour and diluted nectar. In fact hummingbirds were effective and frequent pollinators of this species.

Several species of Gesneriaceae are pollinated by hummingbirds in southeastern Brazil. Among these bird-visited species, *Nematanthus fissus* is pollinated by *Phaethornis ruber* (Buzato, 1995), and *N. gregarius* is pollinated exclusively by Trochilinae, including *Thalurania glaucopsis* (Snow, Snow, 1986). These two plant species have a short corolla, similar those observed in *B. longimucronata*. Gesneriaceae with large corolla in southeastern Brazil (e.g., *N. fritschii*, *N. fluminensis* and *Sinningia cooperi*) are preferably visited and pollinated by large hermits with large bills (high-reward trapliners), as *Ramphodon naevius* and *Phaethornis eurynome* (Franco, Buzato, 1992; Buzato, 1995).

The small hermit *Phaethornis ruber* (Phaethorninae) and the medium-sized and short-billed *Thalurania glaucopsis* (Trochilinae) were the major pollinators of *B. longimucronata*. These two hummingbird species were associated to the same floral subgroup in a lowland forest in southeastern Brazil (Buzato, 1995 cf. Stiles, 1985). The mean corolla length of the flowers in this subgroup was 21.1 mm, which comprises *Mendocia velloziana* (Acanthaceae), *Psycotria nuda* (Rubiaceae) and *Nematanthus fissus* (Gesneriaceae), among others (Buzato, 1995). Values of corolla length, nectar volume and sugar concentration in nectar of flowers of *B. longimucronata* approach those found in that floral subgroup (see Buzato, 1995).

On the other hand, the large-sized *Ramphodon naevius* was only an occasional pollinator of *B. longimucronata*. This hermit is a specialist on long-tubed flowers in southeastern Brazil, as *Nematanthus fritschii*, Gesneriaceae (Franco, Buzato, 1992) and species of Bromeliaceae and Heliconiaceae (Araujo et al., 1994; Sazima et al., 1995). Buzato (1995) observed on hummingbird community dominated by *R. naevius* (located in a coastal lowland rainforest in Caraguatatuba, near to Ubatuba), that during September and October it occurs a reduction of floral resources to hummingbirds. *Besleria longimucronata* is flowering during these months, and the utilization of its short-tube flowers with scarce nectar by *R. naevius* may be related with this period of low rewards.

Females of *Thalurania glaucopsis* act as low-reward trapliners, and the males usually act as territorial, but can fit the role of generalist (Buzato, 1995; Passos, Sazima, 1995). In the studied plant both males and females of *T. glaucopsis* exhibited a traplining behaviour. In the same way, *Phaethornis ruber* usually act as low-reward trapliner in *B. longimucronata*, as previously observed on other plants (Buzato, 1995). However, this hermit also exhibited a territorial conduct. It is the first time that this unexpected

behaviour is reported for *P. ruber*. These unpredictable strategies presented by the *B. longimucronata* pollinators reinforce the idea that patterns of hummingbird foraging derive ultimately from individual responses to food availability (Feinsinger, 1976; see also Sazima et al., 1995; 1996).

Besleria longimucronata is self-compatible, but their flowers are protandric, hindering self-pollination at the same flower. However, different flowers at the same plant can be in male and female phases, respectively, at given moment. This fact can promote geitonogamic fertilizations. The seed set in the naturally pollinated flowers had an intermediate value between the values of self- and cross-pollination treatments. Then, in spite of the foraging behaviour of hummingbirds, which may visit a single flower per plant (mainly *T. glaucopsis*), this plant seems to present a balance between allogamy and geitonogamy.

Wiehler (1983) divided the hummingbird corollas of Gesneriaceae, typically colored red, orange or strong yellow, in four different types: (1) the tubular corolla with a narrow tube and a small limb; (2) the columneoid corolla with a narrow but expanding tube and a strongly zygomorphic limb divided into a prominent galea and a long and narrow ventral lobe; (3) the urceolate corolla with an inflated tube, a very constricted throat and a very narrow regular limb and (4) the converted euglossine corolla with a moderately wide tube, a wide subregular limb and orange or red colour. Commonly, species of *Besleria* present the urceolate corolla (third type) (Wiehler, 1983). The fourth type apparently represents a "recent" adaptation from euglossophily to ornithophily and it is present in species of *Episcia*, *Nautilicalix*, *Paradrymonia* and *Drymonia* (Wiehler, 1983). The white corolla of *B. longimucronata* has a shape similar to the converted euglossine corolla (fourth type), instead of urceolate corolla. This fact, as well as the observation of *Euglossa* aff. *saphirina* visiting the *B. longimucronata* flowers, may indicate a switch from euglossophily to ornithophily among *Besleria* species.

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TABLE 1 – Culmen length of the hummingbirds that visited *Besleria longimucronata* flowers.

Hummingbird Species	Culmen (mm)*
<i>Thalurania glaucopsis</i> (female and male)	19
<i>Phaethornis ruber pygmaeus</i> (female and male)	21
<i>Ramphodon naevius</i> (male)	35
Mean corolla length of <i>Besleria longimucronata</i>	22.75 mm

* Data source: Grantsau, 1989.

TABLE 2 – Percentage of fruit-set from hand-pollinated treatments, spontaneous self-pollination and open pollinated flowers (control) in *Besleria longimucronata*. Figures in brackets are numbers of flowers/numbers of fruits, respectively.

Treatments	Percentage of fruit-set
Spontaneous self-pollination	0 (10/0)
Self-pollination – same flower	36.7 (11/4)
Self-pollination – same plant	22.2 (09/2)
Cross-pollination	45.4 (11/5)
Open-pollination (control)	30.0 (10/3)

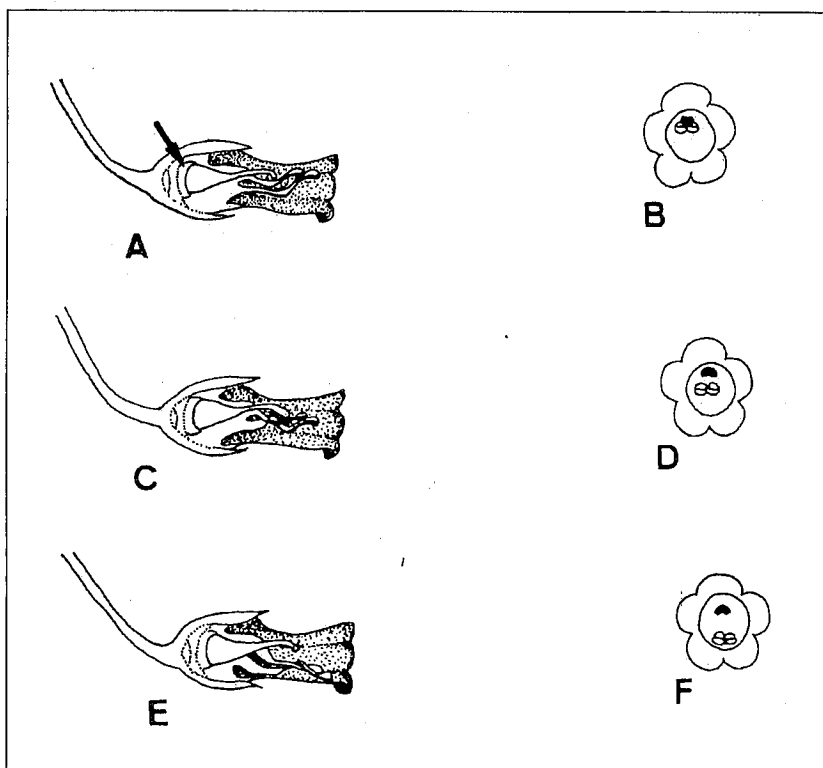
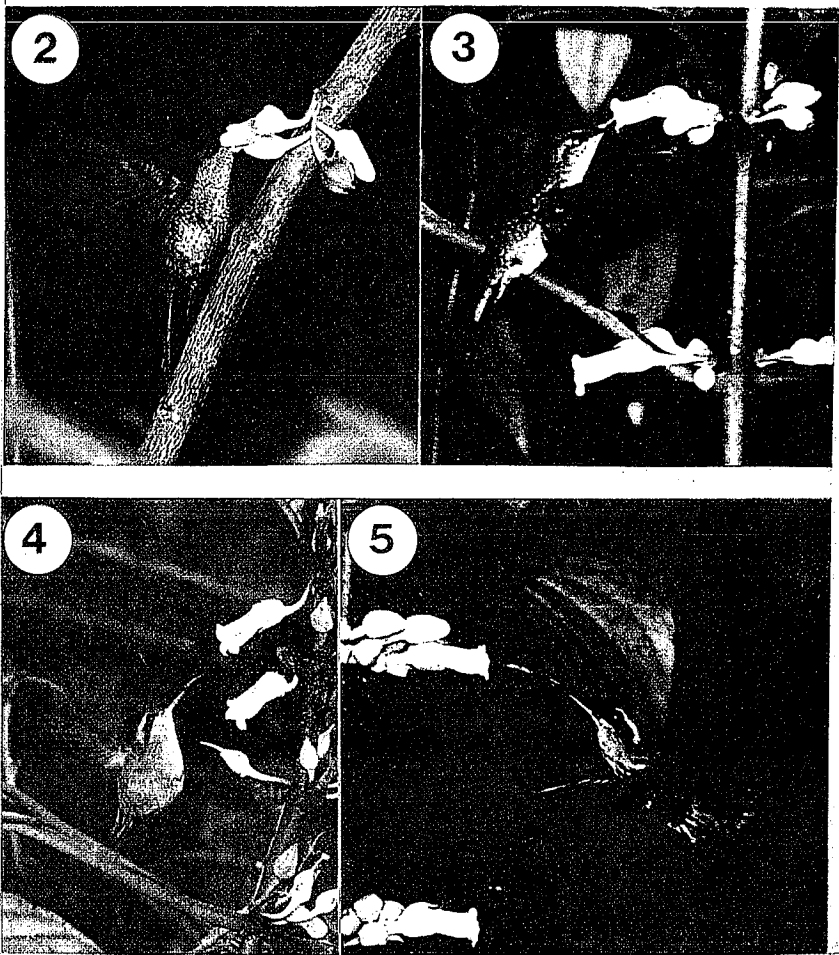


Fig. 1. Schematic representation of the displacement of reproductive structures at different sexual phases (male, intermediate and female), in the *Besleria longimucronata* flowers. A, C, E - Longitudinal view of the flower (calice and corolla portions were removed); B, D, F - Frontal view of the corolla opening. The arrow shows the annular nectary.



Figs. 2-5. Hummingbirds visiting *Besleria longimucronata* flowers. Male (2) and female (3) of *Thalurania glaucopsis* (Trochilinae) probing nectar in flowers. Males of *Phaethornis ruber* (4) and *Ramphodon naevius* (5) (Phaethorninae) approach the flowers immediately before a visit, note the pollen deposition on the hummingbird culmen.

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