INTRODUCTION

The cosmopolitan orchid genus Habenaria Willd. contains about 600 species, 170 of which have been referred to the Brazilian orchid flora (Hoehne, 1940). Habenaria parviflora Lindl. has a noteworthy wide distribution, occurring from central Argentina to southeastern Brazil (Cocucci, 1954; Correa, 1996; Singer & Cocucci, 1997).

Most pollination reports for Habenaria involve different kinds of moths or butterflies (Lepidoptera) (Dressler, 1993; Singer & Cocucci, 1997), although H. obtusata is reportedly pollinated by mosquitoes (Culicidae) and moths as well (Stoutamire, 1968; Thien, 1969; Voss & Riefner, 1983). In a recent report of flower morphology and pollination
biology of some *Habenaria* species occurring in central Argentina, Singer & Cocucci (1997) suggested that *Habenaria parviflora* (as *H. monteviendensis*) should be pollinated by both, mosquitoes and moths. Owing to its particular viscidium shape, it was suggested (Singer & Cocucci, 1997) that the pollinaria of *H. parviflora* should be fixed on the proboscis of pollinators and not on the eyes like in other studied South American species. During our studies on the reproductive biology of southern Brazilian orchids, we had the opportunity of testing both ideas. The aims of the present contribution are: 1) to broaden our knowledge about the pollination biology of South American *Habenaria* and 2) to highlight the functional morphology of this noteworthy orchid species.

**METHODS**

Studies were conducted in Picinguaba (Parque Estadual Serra do Mar, Ubatuba, São Paulo, ca. 23° 15´, 23° 55´S and 44° 45´, 44° 55´W; altitude at a study site ca. 30 m.a.s.l.). *Habenaria parviflora* occurs in natural or man-made grasslands. The studied population occurs in the neighbourhood of the Park research base. The study site is surrounded by Atlantic Rain Forest (“Mata Atlântica”) vegetation. The annual rainfall is about 2600 mm and the annual average temperature is about 22 °C. Observations were made through 17 field visits, from October 18-25, 1998, September 28 to October 10, 1999 and October 25-31, 2000. As a whole, about 35 hours and 25 minutes were spent in observations. The observation period ranged from 19 to 24 hours, in agreement with scent production and pollinator’s activity at flowers. In 1998, 24 plants (totalling about 570 flowers) were available. Only six plants (totalling about 135 flowers) were available in 1999 and 14 plants (totalling about 520 flowers) in 2000. Flower morphology was studied using flowers preserved in ethanol 70 %. Plant vouchers are deposited at UEC (voucher number: *Habenaria parviflora*, R. B. Singer 98/ 101). Drawings were made using a stereomicroscope with a camera lucida attachment. In 2000, the breeding system was studied with six bagged, inflorescence-bearing plants totalling 277 flowers. Five flowers of each inflorescence were emasculated and the same number of flowers per inflorescence were self and cross-pollinated. A total of 187 remaining flowers were left untreated and were considered controls. All treatments were performed in each inflorescence. Given the small size of flower parts, pollinaria were removed with the help of entomological (“000” size) pins. Insect behaviour at flowers was recorded through field notes and photographs. Insect behaviour was traced with the help of a flashlight; a device which apparently did not disturb normal behaviour. Some insects were captured for later identification. Insect vouchers are deposited at ZUEC (Museu de História Natural da Universidade Estadual de Campinas). Throughout this paper, the taxonomic and morphological concepts of Dressler (1993) are followed.

**RESULTS AND DISCUSSION**

**Plant features:** owing to a combination of vegetative and floral features (well-developed leaves along the stem, plant size and inconspicuous flowers), *Habenaria parviflora* was included in an artificial and heterogeneous grouping called “the Leptoceras group” by Hoehne (1940). In fact, plants of *H. parviflora* are quite variable in size, reaching 15-100 cm in length. Plants occurring in well-exposed areas tend to be smaller and yellowish in colour. Plants surrounded by grasses tend to be higher and greener. Inflorescences are terminal spikes bearing up to 60 flowers. Flowers are resupinate and green or yellowish-green and bear a noticeable spur (Fig. 1A) about 6.6 mm in length (Singer & Cocucci 1997). Readers interested in the size and shape of the perianth parts are referred to Hoehne (1940) and Cocucci (1954). The sepals are sturdier and fleshier than the petals and the lateral petals are partially held by the concave dorsal sepal. The dorsal sepal plus the lateral petals build a hood-like structure, which partially hides the column. The lip is distinctly trilobed and posteriorly prolonged in the spur (Fig. 1A) (Singer & Cocucci 1997). Nectar is clearly noticed at the bottom of the spur. Nectar secretion and nectary structure are beyond the scope of this contribution, see Galetto et al. (1997) for fine details on the nectary structure and nectar secretion in some related *Habenaria* species. The rostellum is trilobed, the two lateral lobes being inconspicuous and each one presenting a viscidium at its end. The median rostellar lobe is tooth-like (Fig. 1C) and is placed behind the spur entrance. Two small auricles (Fig. 1D), one at each side of the column, are hidden by the lateral petals (Singer &
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Cocucci 1997). The anther is erect and holds two pollinaria (until Dressler 1993 such structures were referred as “hemipollinarium”). Each pollinarium consists of a pollinium made up by ca. 70 massulae (Singer & Cocucci 1997), a hard, hyaline caudicle and a terminal, involute, glove-like viscidium (Fig. 1 E-F). The involute viscidium is, as far as we know, a unique and distinctive feature among South American *Habenaria* species (Hoehne 1940). Most *Habenaria* flowers show pad-like subcircular, slightly convex viscidia (Cocucci 1954, Hoehne 1940, Singer & Cocucci 1997) which usually attach
to the surface of pollinator’s eyes (Singer & Cocucci, 1997; Stoutamire, 1968; Thien, 1969; Voss & Riefner, 1983). There are two short, convex stigmatic surfaces (Fig. 1 C) just below the pollen sacs, surrounding the spur entrance.

Just after dusk (ca. 18 hours) the flowers emit a sweet, spicy fragrance which can be perceived up to 23-24 hours. Nocturnal scent production, together with the inconspicuous flower coloration fit very well the moth-pollination syndrome (Van der Pijl & Dodson, 1966).

Pollination biology: flowers were visited and pollinated by females of two mosquito-like, unidentified species of Tipulidae (Diptera) (probably of the genus Leptotarsus Guérin-Meneville) (Fig. 2 A-B) and several species of Pyralidae moths (Fig. 2 C-D). Insects often displayed a quite passive behaviour, staying for 2-45 minutes on a given inflorescence. Insects often probed several flowers, but also perched on the inflorescences without exploring the flowers. Crane-flies (Tipulidae) were always seen carrying and dislodging pollinaria. The crane-flies carried 1-5 pollinaria each. Pyralid moths were only sighted carrying one pollinarium each. The pollination mechanism is the same for both pollinators: when an insect probes the flowers searching for nectar, its proboscis enters the spur at such an angle that viscidia are not disturbed. The straight and rigid proboscis of the crane-flies (Tipulidae) seems better suited for pollinarium removal. Crane-flies are rarely recorded as plant pollinators, but many genera show long proboscides which suggest that these insects may probe flowers for nectar (Grimaldi, 1999). There are a few records of Tipulidae pollinating European orchids of the genera Listera (Listeriinae) and Coeloglossum (Orchidinae) (Van der Pijl & Dodson, 1966). We have occasionally observed crane-flies (apparently the same morphospecies which visit H. parviflora) visiting flowers of Prescottia plantaginacea Lindl. (Orchidaceae: Prescottinae), thought they apparently did not remove pollinaria. Unidentified Geometridae moths were frequently sighted at flowers of H. parviflora, but in any event these moths removed pollinaria. Moth pollination has recently been confirmed for some Habenaria spp. in central Argentina (Singer & Cocucci, 1997). Habenaria hexaperta (as H. hyeronimii) is pollinated by the noctuid moth Rachiplasia na (Noctuidae) and Habenaria gourlieana is pollinated by moths of Agrius cingulatus and Manduca sexta (both, Spingidae) (Singer & Cocucci 1997). Indirect evidence of moth-pollination, in the form of moth scales on the stigmatic surfaces was presented for H. rupicola and H. pauciflora (this last species from Chile) (Singer & Cocucci, 1997).

Mosquito pollination has been reported for the boreal H. obtusata (Stoutamire, 1969; Thien, 1968). These reports involved “true” mosquitoes of genus Aedes (Culicidae) and Geometridae moths of genus Xanthorhoe as well (Thien, 1968; Stoutamire, 1969). Noteworthy, Voss & Riefner (1983) more recently reported that moths of genus Anageshna (Pyralidae) also pollinate H. obtusata. In all the aforementioned reports, the pollinaria were reported to be fixed on the insect eyes. Habenaria decaryana from Madagascar does also attach its pollinaria in this way (Nilsson & Jonsson, 1985). However, some African Habenaria species have been reported to fix their pollinaria on the venter (sternothyri) or in the forelegs (Knuth & al., 1905, Vogel, 1954) of their moth pollinators. Pollinarium fixation on the proboscis is, as far as we know, reported for the first time for Habenaria.

Reproductive success (number of fruits over total flowers) could not be calculated, since unbagged inflorescences were cut by the Park inhabitants (the study area is locally used as a car-parking area). In Central Argentina, a population of H. parviflora (reported as H. montevdenseis) had 61% of its flowers pollinated (Singer & Cocucci, 1997). The same population had a male efficiency factor (the ratio between the percentage of pollinated flowers over the percentage of flowers acting as pollen...
Fig. 2.- The flowers and their pollinators. A-B: Tipulidae (Diptera) females. A: visiting an inflorescence with a pollinarium attached to its proboscis. B: specimen with three pollinaria attached to its proboscis. C-D: Pyralid moths. C: specimen visiting an inflorescence. D: individual with a pollinarium attached to its proboscis.
donors) of 0.6 (Singer & Cocucci, 1997). This means that 100 pollinaria were removed to pollinate 60 flowers, or, in other words, that more pollinaria were removed than flowers were pollinated. The fixed pollinaria may in some way disturb the insects since Tipulidae females were often seen trying to clean the pollinaria from their proboscides with the forelegs.

Remarkably, Neiland & Wilcock (1998) indicated that tropical orchids (either nectarless or nectariferous) display low fruit productivities (less than 50%). The data obtained for the Argentinian *H. parviflora* population (Singer & Cocucci, 1997) apparently contradict Neiland & Wilcox (1998) statement.

**Breeding system:** Plants are self-compatible but pollinator-dependent. This means that pollinators are needed for the plants to set fruits. Neither control (untreated) flowers (0 fruits/187 flowers) nor the emasculated ones (0 fruits/30 flowers) set fruits. Either self-pollinated or cross-pollinated flowers displayed very high and similar fruit sets, with 93.3 % (28/30) and 96.7 % (29/30) of fruiting success, respectively. Self-compatibility seems to be quite widespread among Orchidaceae (Dressler, 1981; Van der Pijl & Dodson, 1966). Since the plants are self-compatible and the pollinators are often quite passive, frequently staying several minutes and visiting several flowers of the same inflorescence, part of the fruit set may be due to geitonogamous self-pollination. Self-pollination in the strictest sense (pollen of the same flower deposited in its own stigmas) may be a rare event since the pollinarium is removed when the insect leaves the flower. Still, a combination of flower features may increase the chances of cross-pollination: the texture of the pollinarium, which is made up by several subunits (“massulae”) and the fact that clumps of massulae (instead of whole pollinia) are left on the stigmatic surfaces. This means that several flowers can be pollinated with the pollen from one pollinarium (Singer & Cocucci, 1997). In addition, the stigmatic surfaces are broad and convex which increases the chances of receiving mixed pollen loads (of the same and other flowers) and, consequently of cross-pollination. Finally, the sectile condition of the pollinia per se guarantees a considerable degree of genetic diversity (Freudenstein & Rasmussen, 1997). According to Freudenstein & Rasmussen (1997) each massula derives from a single pollen mother cell. So, each massula represents a different genotype. Since massulae are left in clumps and a single stigmatic surface could receive many pollen loads, the chances of multi-pollen genotype contributions to a single capsule are increased (Freudenstein & Rasmussen, 1997).

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**LITERATURE CITED**


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