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BIOLOGIA DA POLINIZAÇÃO EM ORQUÍDEAS NATIVAS DA REGIÃO SUDESTE DO BRASIL

Rodrigo Bustos Singer

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Orientadora: Profa. Dra. Marlies Sazima

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Rodrigo Bustos Singer
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Marlies Sazima

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BANCA EXAMINADORA

Data __/__/____

Profa. Dra. Marlies Sazima (Orientadora) Marlies Sazima

Profa. Dra. Eliana Regina Forni Martins Eliana R Forni Martins

Profa. Dra. Isabel Alves dos Santos Isabel A. Santos

Profa. Dra. Silvana Buzato ____________________

Profa. Dra. Maria do Carmo Estanislau Amaral ____________________

Prof. Dr. Fábio de Barros ____________________

Prof. Rodolfo Antônio de Figueiredo ____________________

RESUMO

Estudou-se a biologia da polinização em vários grupos de orquídeas nativas da região sudeste brasileira. De um modo geral, as orquídeas terrestres da tribo Cranichideae (localmente representada pelas subtribos Goodyerinae, Prescottinae e Spiranthinae) mostraram-se autocompatíveis, mas polinizador-dependentes. Relata-se pela primeira vez a ocorrência de protandria na subtribo Prescottinae (*Prescottia stachyodes*), bem como em algumas espécies nativas das subtribos Spiranthinae (*Sauroglossum elatum*) e Goodyerinae (*Erythrodes arietina*). Foi verificada a polinização por beija-flores em *Stenorrhynchos lanceolatus* (Spiranthinae). A polinização por mariposas foi documentada em *Sauroglossum elatum* (Spiranthinae), bem como em *Prescottia plantaginea* e *Prescottia stachyodes* (Prescottinae). A polinização por abelhas foi documentada para algumas espécies dos gêneros *Cyclopogon*, *Pelexia* e *Sarcoglottis* (Spiranthinae), bem como para *Aspigogyne longicornu*, Erythrodes arietina (Goodyerinae) e *Prescottia densiflora* (Prescottinae). Documentou-se a polinização por mariposas e Tipulideos (Diptera) em *Habenaria parviflora* (Habenarinae). Esta ultima espécie mostrou-se também autocompatível, mas polinizador-dependente. Tanto em *Habenaria parviflora* quanto nas orquídeas da tribo Cranichideae estudadas, um conjunto de caracteres morfológicos (textura friável do polinário, superfícies estigmáticas amplas) favorece a polinização cruzada. O estudo da interação entre abelhas Euglossini (Apidae) e orquídeas da subfamília Epidendroideae na região de Picinguaba (Litoral Norte do Estado de São Paulo), mostrou que as abelhas *Euglossa* (em particular do subgênero *Glossura*) são localmente mais importantes no que diz respeito a polinização de oprquídeas nativas.

Palavras chave: orquídeas, polinização, morfologia floral, abelhas, beija-flores, mariposas, Tipulidae

ABSTRACT

The pollination biology of diverse orchid groups native in southeastern Brazil was studied. In general, the species belonging to tribe Cranichidae (locally represented by subtribes Goodyerinae, Prescottinae and Spiranthinae) showed to be self-compatible, but pollinator-dependent. Protandry is reported for the first time in subtribe Prescottinae (*Prescottia stachyodes*), and in a few species in subtribes Spiranthinae (*Sauroglossum elatum*) and Goodyerinae (*Erythrodes arietina*). Hummingbird pollination was verified in *Stenorrhynchos lanceolatus* (Spiranthinae). Moth-pollination was documented in *Sauroglossum elatum* (Spiranthinae), *Prescottia plantaginea* and *Prescottia stachyodes* (Prescottinae). Bee pollination was reported in some species of the genera *Cyclopogon*, *Pelexia* , *Sarcoglottis* (Spiranthinae), *Aspigogyne longicornu*, *Erythrodes arietina* (both, Goodyerinae) and *Prescottia densiflora* (Prescottinae). Pollination by moths and Tipulid crane-flies (Diptera) was reported in *Habenaria parviflora* (Habenarinae). The latter orchid species was also recorded as self-compatible but pollinator-dependent. Either in *Habenaria parviflora* or in the species belnging to the tribe Cranichideae, a set of morphological characters (pollinarium granular/sectile texture, wide stigmatic surfaces) favour cross-pollination. The study of the interaction between Euglossini (Apidae) bees and the Epidendroideae orchids they pollinate in the region of Picinguaba (Northern Coastal São Paulo State), showed that the bees of the genus *Euglossa* (particularly these of subgenus *Glossura*) are locally more relevant in orchid pollination.

Palavras chave: orchids, pollination, flower morphology, bees, hummingbirds, moths, Tipulidae

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INTRODUÇÃO

As orquídeas, devido à sua peculiar morfologia floral, são objetos de estudo particularmente apropriados para pesquisas de biologia floral e ecologia em geral. A grande maioria das orquídeas apresenta o pólen aglutinado em pacotes discretos chamados de polínias. Por sua vez, as polínias podem estar unidas com estruturas ou secreções adicionais (geralmente originadas a partir do gineceu) que promovem a retirada do conteúdo polinico como um todo (Dressler 1993). O conjunto de tais estruturas ou secreções juntamente com as polínias denomina-se polinário. Os polinários podem então ser retirados das flores pelos polinizadores como unidades discretas, facilmente visualizáveis. Esta característica permite que o processo de polinização possa ser visualmente acompanhado. A capacidade real de um dado animal para atuar como polinizador de uma dada espécie de orquídea pode então ser avaliada por sua capacidade para fixar polinários e promover a deposição de pólen em visitas florais subseqüentes. Por sua vez, a maioria das orquídeas apresenta superfícies estigmáticas amplas (Dressler 1993), e com freqüência é muito simples verificar se a deposição de pólen ocorreu ou não.

Embora diversas linhas de pesquisa em biologia da polinização em orquídeas estejam em andamento tanto nos Paleo quanto nos Neotrópicos (Van der Cingel 2001), alguns grupos de orquídeas neotropicais são notórios pela escassez deste tipo de dados. Este é o caso das orquídeas terrestres das subfamílias Orchidoideae e Spiranthoideae (Dressler 1993). Existem alguns poucos relatos para algumas espécies de *Cyclopogon* (Singer & Cocucci 1999), *Habenaria* (Singer & Cocucci 1997), *Myrosmodes* (Berry & Calvo 1991) e *Prescottia* (Singer & Cocucci 1999). A ocorrência de ornitofilia (polinização por aves) em *Stenorrhynchos lanceolatus* (Aublet) L. C. Rich., embora observada em condições experimentais (Catling 1987), não havia sido, até recentemente, devidamente comprovada em condições naturais (Singer & Sazima 2000). A diversificada morfologia floral deste grande grupo de orquídeas sugere a evolução de diversos sitemas e síndromes de polinização. Por outro lado, os poucos dados conhecidos referentes ao sistema reprodutivo destas orquídeas provêm de especies do Hemisfério Norte (Catling & Catling 1991).

Existe ainda um tipo de interação orquídea-polinizador abundantemente documentada nos Neotrópicos (Van der Cingel 2001): a interação entre os machos de abelhas Euglossini

(Apidae) e as orquídeas da subfamília Epidendroideae (sensu Dressler 1993).

Os machos de abelhas Euglossini coletam ativamente compostos aromáticos em porções especializadas do labelo (pétala mediana) das flores. No processo, fixam e deslocam os polinários que poderão depositar em visitas florais subseqüentes, polinizando as flores visitadas.

A descoberta e a síntese química de numerosos compostos atrativos para os machos de abelhas Euglossini possibilitaram a utilização destes compostos como 'iscas" para atrair estas machos destas espécies de abelhas. Se estas abelhas estiverem carregando polinários de orquídeas, estes podem ser identificados, com base na sua morfologia, ao nível de gênero ou até de espécie (Dressler 1993). Esse metodo de estudo de polinização tem a desvantagem de não possibilitar a obtenção de dados em relação à interação entre o polinizador e a flor. No entanto, a deposição do polinário em regiões específicas do corpo do inseto permite inferir a interação morfológica entre o inseto e a flor. Estudos a médio e longo prazo foram efetuados no Peru e América Central (Van der Cingel 2001) e permitiram conhecer a interação de comunidades inteiras de abelhas Euglossini e as orquídeas que estas abelhas polinizam. No entanto, este tipo de estudo ainda falta no Brasil. Apenas algumas informações fragmentárias nestes assuntos foram dadas por Braga (1977). Este tipo de estudo apresenta também a desvantagem de não fornecer dados sobre o sistema reprodutivo das orquídeas polinizadas pelos Euglossini. Pode-se presumir, portanto, que estas abelhas atuam como polinizadores das orquídeas cujos polinários carregam. No entanto, este tipo de estudo permite obter em tempo relativamente curto de trabalho, uma visão clara da interrelação entre comunidades inteiras de orquídeas e seus polinizadores.

OBJETIVOS

A presente contribuição pode ser divida então em duas partes. Na primeira (capítulos 1-6), procura-se

1) Verificar a biologia reprodutiva de 12 espécies de orquídeas terrestres das subfamílias Orchidoideae e Spiranthoideae (sensu Dressler 1993), procurando demonstrar a importância da morfologia floral no processo da polinização;
2) documentar a interação entre os polinizadores e as flores
3) com base em plantas cultivadas em casa de vegetação, elucidar o sistema reprodutivo

das espécies estudadas. Isto permite inferir as conseqüências da conduta dos polinizadores no sucesso reprodutivo.

Na segunda parte (capítulo 7), procura-se:

1) evidenciar a interrelação entre uma comunidade de abelhas Euglossini e as orquídeas (Epidendroideae sensu Dressler 1993) polinizadas por estas abelhas em um ambiente de Mata Atlântica de encosta, no litoral norte paulista;
2) inferir se algum grupo de abelhas Euglossini é localmente mais importante como polinizador;
3) verificar o grau de especificidade das interações entre as abelhas Euglossini e as orquídeas locais.

REFERÊNCIAS

Berry P. E. & R. N. Calvo 1991. Pollinator limitation and position-dependent fruit set in the high Andean orchid *Myrosmodes cochleare*. Plant Systematics and Evolution 174: 93-101.

Braga, P. 1977. Aspectos biológicos das Orchidaceae da Amazônia Central. Acta Amazonica 7(2) (suplemento): 1-89.

Catling, P. M. & V. R. Catling. 1991. A synopsis of brreding systems and pollination in North American orchids. Lindleyana 6: 187-210.

Dressler, R. L. 1993. Phylogeny and classification of the orchid family. Portland, Dioscorides Press.

Singer R. B. & A. A. Cocucci 1997. Eye attached hemipollinaria in the hawkmoth and settling moth. Pollination of *Habenaria* (*Orchidaceae*): a study on functional morphology in five species from subtropical South America. Bot. Acta 110: 338-342.

Singer R. B. & A. A. Cocucci. 1999. Pollination mechanisms in Southern Brazilian orchids which are only or mainly pollinated by halictid bees. Plant Systematics and Evolution 217: 101-117.

Singer R. B. & M. Sazima. 2000. The pollination of Stenorrhynchos lanceolatus (Aublet) L. C. Rich. (Orchidaceae: Spiranthinae) by Hummingbirds in southeastrn Brazil. Plant Systematics and Evolution 223: 221-227.

Van der Cingel, N. 2001. An atlas of Orchid pollination. America, Africa, Asia and Australia. Rotterdam, Balkema publishers

CAPÍTULO 1:

THE POLLINATION MECHANISM IN THE PELEXIA ALLIANCE (ORCHIDACEAE: SPIRANTHINAE)

Rodrigo B. Singer & Marlies Sazima

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ABSTRACT

The biological function of the wedge-like, dorsally adhesive viscidium of the neotropical orchid genera *Cyclopogon*, *Pelexia* and *Sarcoglottis* is elucidated by the study of the pollination biology of three species occuring in São Paulo State, southeastern Brazil. *Cyclopogon congestus* is pollinated by bees of *Pseudoaugochloropsis graminea* (Halictidae), *Pelexia oestrifera* by workers of *Bombus* (*Fervidobombus) atratus* (Apidae) and *Sarcoglottis fasciculata* by males and females of *Euglossa cordata* (Apidae: Euglossini). These three orchid species offer nectar as a reward and are self-compatible, though they need pollinators to set fruits. In spite of the difference in flower sizes and in their pollinators taxonomic groups, the pollination mechanism is essentially the same for these species. The pollinarium adheres to the ventral surface of the bee labrum. The viscidium needs to be dorsally pressed in order to liberate a glue which fixes the pollinarium to a bee. Pollination is achieved by the interaction of the orchid column and the mouth parts of the bees. The fixation to the ventral surface of the labrum is advantageous for the orchid, since it is a difficult place for the bees to clean. Another advantage is that, since the labrum is articulated, when the bees fold and close their mouth parts, the pollinarium remains protected under the bee head, thus reducing the risks of pollen loss. Since the wedge-like, dorsally adhesive viscidium is a characteristic feature of *Cyclopogon*, *Pelexia* and *Sarcoglottis*, it is suggested that some kind of phylogenetic constraint may exist impeding the occurence of pollinators other than bees in these orchid genera. All other flower-visiting animals lack the labrum-like structure needed to fix the pollinarium.

ADDITIONAL KEY WORDS: flower morphology - pollination - orchids – bees – *Cyclopogon* –*Pelexia*- *Sarcoglottis*-bees-labrum-*Halictidae*- *Bombus*-*Euglossa*

INTRODUCTION

Most Spiranthinae orchids share a common and very characteristic floral groundplan (Dressler, 1981, 1993). The flowers are more or less tubular-like in shape, their column is elongated, horizontally positioned and acute. The stigmatic surface may be either entire or bilobed, plane or slightly convex. The anther is erect and holds a pollinarium made up by four friable, powdery pollinia, caudicles and a terminal viscidium. In most Spiranthinae, the viscidium is plate-like and ventrally adherent. This structure fixes the pollinarium to the pollen vectors. The pollinarium usually fixes on broad, plane surfaces on the pollinator, e. g. bee proboscices and hummingbird bills (Catling 1983, 1987; Cocucci, 1981). Among the Spiranthinae orchids, the neotropical genera *Cyclopogon* Presl., *Pelexia* Poit. and *Sarcoglottis* Presl. are distinctive because of their rostellum structure. In the afore mentioned genera, the rostellar portion differentiated in viscidium is not ventral, as in the other Spiranthinae, but dorsal and not adhesive by itself. The viscidium is covered by a membrane which ruptures when pressed, thus liberating a rostellar glue which fixes the pollinarium to the pollinator (Balogh, 1982; Burns-Balogh & Robinson, 1983; Dressler, 1993). Because of these features the genera *Cyclopogon, Pelexia* and *Sarcoglottis* have been grouped in the so-called "Pelexia alliance" (Burns-Balogh & Robinson, 1983). However, the importance in reproductive biology of such a distinctive feature has not been elucidated. A few published reports concerning the pollination biology of the *Pelexia* alliance exist, and are mostly fragmentary. Pollinaria of *Sarcoglottis* have been found in Euglossini bees, attached "behind their mouth parts" (Burns-Balogh & Robinson, 1983; Dressler, 1993). Bumble-bees have been observed pollinating *Pelexia ekmanii* in southern Brazil by Dressler (1981, 1993). Unfortunately, these reports did not include further morphological details concerning the flower-pollinator interaction.

Recently, Singer & Cocucci (in press) have described the pollination mechanism in *Cyclopogon diversifolius* (Cogn.) Schltr., from southern Brazil. *Cyclopogon diversifolius* is pollinated by halictid bees which dorsally press the orchid viscidium with their labrum while trying to get nectar at the base of the orchid column. The pollinarium is then fixed to the ventral surface of the labrum and remains protected under the head of the flying insect. As part of our studies concerning the pollination biology of southeastern brazilian

Spiranthinae orchids, we studied three orchid species, representing the three orchid genera of the "Pelexia alliance". Our findings support the observations of Singer & Cocucci (in press) and indicate the same pollination mechanism for the whole "Pelexia alliance", in spite of the differences in their pollen vectors taxonomic groups of flower sizes. The aim of the present contribution is to highlight the sophisticated pollination mechanism of the "Pelexia alliance" and to discuss the possible consequences of some flower features as well as the pollinator behaviour on the reproductive biology of this group.

METHODS

The pollination process of *Cyclopogon congestus* (Vell.) Hoehne and *Sarcoglottis fasciculata* (Vell.) Schltr. was followed in the field from August 28 to September 13, 1998, in a total of seven field visits. The study place was Serra do Japi (São Paulo State, 23° 11' S, 46° 52' W; altitude of about 700 m). The vegetation is a semideciduous forest. Annual rainfall is about 1500 mm. *Cyclopogon congestus* was studied following a population of 11 individuals, occuring on rocky slopes on the side of a road. This population produced a total of 277 flowers. During the observation period, *Sarcoglottis fasciculata* was studied following a population of eight individuals occuring in the forest understory, some 800 m. away from the *Cyclopogon* population. The population produced a total of 162 flowers during the observation period. Observations were made from 9:00 hs to 15:00 hs. Aproximately 34 man-hours were dedicated to the observation of each species.

The pollination process in *Pelexia oestrifera* (Rchb. f. & Warm.) Schltr. was followed by means of four field visits from march 20 to 28, 1998. The study place was Pedra Grande (São Paulo State, 46° 25' W, 23° 10' S; altitude about 1450 m.). The area consists of rocky, well exposed, slopes with a rupicolous kind of vegetation. Annual rainfall is about 1450 mm. The total population consisted of hundreds of flowering individuals, but only 20 of them, which produced 280 flowers, were monitored. The daily observation period was from 9:00 hs to 14:00 hs. Approximately 20 man-hours were spent observing this species.

Fresh flowers of the three species were drawn with the help of a stereomicroscope equipped with a camera lucida. Plant vouchers are deposited at the UEC herbarium. Voucher numbers: *C. congestus*, R.B. Singer 98/ 84; *P. oestrifera*, R. B. Singer 98/ 43; *S. fasciculata*, R. B. Singer 98/ 85.

Pollinators were captured, photographed and pinned for later identification. Insect vouchers are deposited at the entomological collections of the Universidade de São Paulo (MZUSP) and of the Universidade Estadual de Campinas (ZUEC).

The morphological interaction between the flower and the pollinator was drawn after manipulating fresh longitudinal sections of the flowers and fresh insects with pollinaria under the camera lucida.

Throughout this work, the taxonomic and morphological concepts of Balogh (1982) and Dressler (1993) are followed.

The breeding system of the three species was studied by means of cultivated individuals deposited at the living orchid collection of the Universidade Estadual de Campinas (UNICAMP). Treatments included untouched flowers (control), emasculation, self-pollination and cross-pollination. For each species, the treatments yielding fruits were compared by means of a χ^2 test (1 d. f., $\alpha = 0,05$).

RESULTS

GENERAL FLOWER FEATURES:

The inflorescences in the three studied species are spikes (Fig. 1 A, C, E). The flowers are resupinate, tubular-like and narrow. The lateral petals tend to adhere to the dorsal sepal, so the flowers have a bilabiate appearance (Fig. 2 A, F, K). The column is erect and elongated (Fig. 2, B-C, G-H, L-M). Some degree of adnation between the sides of the column and the edges of the labellum occurs in *Pelexia oestrifera* as well as in *Sarcoglottis fasciculata*. The anther is erect and holds a pollinarium made up by four clavate, friable, powdery pollinia, caudicles and a terminal, wedge-like viscidium (Fig. 2, D-E, I-J, N-O). The stigma is 2-lobed, plane to slightly convex (Fig. 2, C, H, M). The flowers offer nectar, which accumulates under the base of the column.

SPECIFIC FLOWER FEATURES AND POLLINATION MECHANISM

Cyclopogon congestus: the inflorescences bear 10-50 flowers. The flowers are greenish-white, about 16 mm long (Fig. 1 A, Fig. 2 A) and produce a sweet, somewhat musky fragrance, which was perceived from the morning hours to the evening.

Pollination mechanism: pollinator activity began at aproximately 9:45 hs, but became noticeably more frequent after 12:00 hs. The pollinators were females of *Pseudoaugochloropsis graminea* (Fabricius, 1804) (*Halictidae*) (Fig 1 A-B). Consecutive visits at flowers by pollinators were observed at 5-160 min intervals. The bees hover in front of the inflorescences for some seconds before landing. As the bees are larger than these flowers, they can only introduce their mouth parts. Since the flowers are narrow, the bees must open the flower entrance by pushing the dorsal sepal and the lateral petals with their heads. The flower is thus worked as if it were bilabiate. When trying to reach the nectar at the column's base, the bees press the dorsal surface of the viscidium with their labrum. The membrane covering the viscidium ruptures and releases a rostellar glue by means of which the viscidium is fixed on the labrum ventral surface (Fig. 1 B). After a few seconds (5-7), the bee leaves the flower removing the pollinarium, its mouth parts are folded and the pollinarium remains protected under the head of the bee. A single bee visits 1-4 flowers per inflorescence, and 1-2 inflorescences per visit to the population. In order to reach the next fresh flower, the bees visiting an inflorescence walk on its surface. When a bee carrying a pollinarium visits another flower, it completelly extends its mouth parts in order to reach the nectar. In doing so, the pollinarium is pushed against the stigmatic surface, and pads of pollen are deposited. The pollinarium is actively pressed by the bee proboscis, since the space between the two pairs of pollinia is not broad enough to allow the proboscis to completely pass in between. The size and shape of the pollinarium which is being carried prevent the bees from contacting the rostellum and fix another pollinarium. Only after the pollinarium has lost a considerable amount of pollen and has consequently diminished, can a second pollinarium be fixed. During the whole observation period only one bee was observed with two pollinaria.

Pelexia oestrifera: the inflorescences bear 20-50 flowers. The flowers are pilose, greenish-white, with a yolk-yellow lip and about 33 mm long (Fig. 1 C, Fig. 2 F). The flowers are markedly helmet-like in shape and bear a notable spur made up by the proximal parts of the lateral sepals (Fig. 2 F). The flowers produce a slight musky fragrance, which becomes stronger when enclosed into a vial.

Pollination mechanism: pollinator activity was noted from 9:30 hs to 12:15 hs. The pollinators were workers of *Bombus* (*Fervidobombus*) *atratus* (Franklin, 1913) (Fig. 1 D). Consecutive visits to the flowers by pollinators were observed at 45–105 min intervals. The bees hover for a few seconds in front of the inflorescences before alighting. In order to reach the next fresh flower, the bees visiting an inflorescence walk on its surface. The bees are larger than these flowers, so they can only introduce their mouth parts (Fig. 3 A) while perching on the lip and the external surface of the lateral sepals. Since the flowers are helmet-like and not so narrow, the bees do not press the dorsal sepal and lateral petals. While trying to reach the nectar in the spur, the bee presses the dorsal surface of the viscidium with its labrum. The membrane covering the viscidium ruptures and liberates a rostellar glue which fixes the viscidium to the labrum ventral surface (Fig. 1 D; Fig. 3 B). When the bee leaves the flower the pollinarium is removed (Fig. 3 C), its mouth parts are folded and the pollinarium remains protected under its head (Fig. 3 D). When a bee carrying a pollinarium visits another flower, it completely extends its proboscis and introduces its head into the flower (Fig. 3 E). In doing so, the pollinarium touches the stigmatic surface (Fig. 3 F) and pads of pollen are there deposited (Fig. 3 G). As in *Cyclopogon*, the size and shape of the fixed pollinarium hinder the fixation of a second one. In fact, only bees with one or two pollinaria were sighted. The bees spent some 3-5 seconds in each flower, visiting 1-3 flowers per inflorescence and rarely more than one inflorescence per visit to the population

Sarcoglottis fasciculata: the inflorescences bear 10-30 flowers. The flowers are pilose, brownish to salmon pink and about 33 mm long (Fig. 1 E, Fig. 2 K). The flowers have a long nectariferous cavity, which is not externally apparent. This "cuniculus-like" (Dressler, 1993) kind of nectariferous cavity is made up by the very elongated proximal parts of the lateral sepals. The flowers emit a notorious yeast-like fragrance.

Pollination mechanism: pollinator activity began after 10:00 hs. The pollinators were males and females of *Euglossa cordata* L. (Fig. 1 F). Consecutive visits at flowers by pollinators were observed at 3-70 min intervals. The bees hover in front of the inflorescences for a few seconds, then land and finally enter the flowers. Since the bees are smaller than the flower, they use the lateral sepals as a landing platform (Fig.1 E). After alighting, the bee extends its mouth parts and enters the flower by pushing the petals and dorsal sepal with its body. Trying to reach the nectar, the bee presses the viscidium with its labrum and, as in the latter cases, the membrane ruptures and liberates a glue which fixes the viscidium to the ventral surface of the labrum (Fig. 1 F). When the bee leaves the flower the pollinarium is removed, its mouth parts are folded and the pollinarium remains protected under the head of the bee. When a bee carrying a pollinarium visits another flower, the interactions occur as above described, and the pollinarium touches the stigmatic surface, leaving part of its pollen content. Once again, the shape and size of the fixed pollinarium prevent the total approximation of the bee to the rostellum and the fixation of a second pollinarium. No bees were sighted carrying more than one pollinarium.

The bees visited 1-2 inflorescences in the population, spending 3 to 12 seconds in a single flower and visiting 1-4 flowers per inflorescence, sometimes returning to a recently visited flower. The bees hovered in order to reach another fresh flower in an inflorescence. Individuals of *Euglossa annectans* Dressler (males and females alike) (Fig.1 E) were recorded at flowers of *S. fasciculata* without removing pollinaria. The bee mouth parts are apparently longer than the distance between the rostellum and the base of the orchid column, and the bees thus visit the flowers without pressing the viscidium or removing pollinaria.

BREEDING SYSTEMS

The studied species are self-compatible, though pollinator services are required so that the plants can set fruits and seeds. *Cyclopogon congestus* and *Pelexia oestrifera* produced better fruiting by cross-pollination, although none of the three studied species scored significant differences favouring cross over self-pollination. Ovarium enlargement occured in some untouched flowers of the three species. Anyhow, no seeds were produced. The results for the three species are summarized in Table I.

TABLE I: percentage of fruiting per treatment and species. Figures in brackets are number of fruits/number of flowers, respectively. N: number of individuals per species.

	N	Control	Emasc	Self-pol	Cross-pol
Cycl. Com	10	(0/ 48)	(0/ 50)	(43/49), 88 %	(46/47), 98%
Pelex. oest	7	(0/ 34)	(0/ 36)	(34/42), 81 %	(34/34), 100%
Sarc. fascic	8	(0/ 43)	(0/ 42)	(42/43), 98 %	(40/42), 95%

χ^2 values (1 d. f., $\alpha = 0,05$): C. cong. = 0, 2648; P. oest. = 0,7619; S. fascic. = 0, 0130. The results do not indicate significative differences between treatments yielding fruits.

DISCUSSION

In all the previous reports concerning the pollination mechanism of Spiranthinae orchids, the authors documented the fixation of the pollinarium on exposed surfaces as bee clipeum, prementum or galeae, or hummingbird bills (Catling, 1983, 1987; Catling & Catling, 1991; Darwin, 1904). This happens because most species of the Spiranthinae have pollinaria with terminal, ventrally adhesive viscidia (Balogh, 1982). In cladistic terms, the dorsally adhesive, wedge-like viscidium has been considered as a synapomorphy of the whole Pelexia alliance, supporting this group as a natural clade among the Spiranthinae (Balogh, 1982; Burns-Balogh & Robinson, 1983; Dressler, 1993). Since the three species studied conform very well to the morphological flower groundplan of the "Pelexia alliance" (Balogh, 1982; Burns-Balogh & Robinson, 1983; Dressler, 1993), we support the idea that the recorded pollination mechanism is the rule for this orchid group (with the obvious exception of the autogamous species).

By now, we cannot ascertain how the dorsally adhesive viscidium of the "Pelexia alliance" evolved. Nonetheless, we can understand the selective pressures through which this kind of viscidium was favoured. For example, ventrally adhesive pollinaria fixed on the dorsal surface of a bee galeae are exposed and could be removed. A hummingbird with a *Stenorrhynchos lanceolatus* pollinarium on the bill (Catling, 1987; Cocucci, 1981) could clean it by crasping it on branches. The fixation of the viscidium to the ventral surface of a bee labrum has some ecologically favourable consequences for the orchid. First, because it is a very difficult place for the bee to clean. Second, because the labrum is a flexible piece, bent when the bee closes its mouth parts, so the pollinarium remains protected under the head. In all the Spiranthinae the pollinia are friable and granular (Balogh, 1982; Dressler, 1981, 1993). A single pollinarium thus could pollinate many flowers. We observed that the pollen of a single *Pelexia oestrifera* pollinarium can completely cover the stigmatic surface of 18 flowers. Because of the pollinarium shape and size, the fixation of a second one is hindered until the fixed pollinarium has lost a considerable amount of pollen. All the afore mentioned characteristics may reduce the chances of pollen loss and increase the chances of cross pollination, particularly considering bees which fly over large foraging areas.

While studying the pollination of *Pelexia oestrifera*, we observed many individuals of *Bombus atratus* carrying pollinaria while foraging on species of *Melastomataceae* and

Solanaceae. Some bees captured at flowers of *Pelexia oestrifera* had pollen loads in their corbiculae. This, together with the relative rareness of the visits, suggests that *Bombus* visit the *Pelexia* flowers while foraging mainly in other plants, presumably over a large area.

The pollination process here described for *Cyclopogon congestus* is essentially the same as that reported for *C. diversifolius* (Singer & Cocucci, in press). Schlindwein (1995) reported halictids as flower visitors of *Cyclopogon apricus* in Rio Grande do Sul, southern Brazil, without indicating if these insects acted as pollinators. Calvo (1990) in a four-year study concerning the reproductive biology of *Cyclopogon cranichoides* did not see any potential pollinator at the flowers. We have short observations (unpublished) of halictid bees visiting and pollinating *Cyclopogon warmingii* in central Argentina. We support the idea that most (if not all) the non-autogamous *Cyclopogon* species may be pollinated by Halictid bees.

The present report agrees with Dressler's brief observations (1993) indicating *Bombus* as pollinators for *Pelexia ekmanii*, in southern Brazil. Based on flower morphological features and habitat (many species occuring in mountain environments, at high altitudes) it is likely that a number of *Pelexias* may be pollinated by bumble-bees. Curiously, Burns-Balogh & Robinson (1983) suggested the occurence of sphingophily (moth pollination) in this genus.

Our observations on *Sarcoglottis fasciculata* support those of Balogh (1982), Dressler (1981) and Roubik & Ackermann (1987). These authors obtained indirect evidence (male bees carrying pollinaria attracted to chemichal baits) indicating pollination by *Euglossa* bees in *Sarcoglottis*. Roubik & Ackermann (1987) captured an *Euglossa* male with a pollinarium attributable to *Sarcoglottis acaule* and suggested that this orchid is a nectar flower, instead of a fragrance flower. Our observations comply with this affirmation, since nectar is the only reward. The fact that males and females visited and pollinated the flowers also supports that the flowers of *Sarcoglottis* are visited to forage nectar, since females do not perform fragrance-collecting activities (Roubik, 1989).

As the three studied species are self-compatible, it is to be expected that in natural conditions, some of the fruits set are due to self-pollination, mostly by geitonogamy. Autogamy in the strictest sense may be more infrequent since the pollinarium is removed when the insect leaves the flower. However, the fact that the bees visit few flowers at each visit, together with the powdery nature of the pollinarium may increase the chances of cross pollination. The only previous report concerning the breeding system in the genus

Cyclopogon is Calvo's (1990); however, because of the metodology that compares "open" and "hand" pollination, without specifying the kind of treatment, it is difficult to compare his and our data. However, Calvo (1990) clearly established that *Cyclopogon cranichoides* is pollinator-dependent. Ackermann (1995) reported autogamy for *C. elatus* and *C. miradorensis* in Puerto Rico and the Virgin Islands.

There are no previously published reports concerning the breeding system of *Pelexia* and *Sarcoglottis*. However, previous reports on other Spiranthinae genera as *Spiranthes* and *Stenorrhynchos* indicated self-compatibility as common (Catling, 1982, 1987; Catling & Catling, 1991). The fact that no fruits were produced by untouched or emasculated flowers indicate that pollinators services are needed so that the plants set fruits. Ovarium enlargement without seed production in untouched flowers has previously been reported in species of the genus *Spiranthes* (Catling, 1982).

As a conclusion, we would like to highlight that the morphological rostellum structure in the "Pelexia alliance" exclusively requires bees in order to achieve pollination. Moths, butterflies, flies and birds are not morphologically suited for these flowers, since they lack the labrum-like structures to fix the pollinarium. Therefore, some kind of phylogenetical constraint preventing the occurence of pollinators other than bees in the "Pelexia alliance" should be expected.

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REFERENCES

Ackermann, J. D. 1995. An orchid flora of Puerto Rico and the Virgin Islands. Memoirs of the New York Botanical Garden. Bronx 73, New York.

Balogh, P. 1982. Generic redefinitions in the subtribe Spiranthinae (Orchidaceae). American Journal of Botany 69: 1119-1132.

Burns-Balogh, P. & H. Robinson 1983. Evolution and phylogeny of the Pelexia alliance (Orchidaceae: Spiranthoideae: Spiranthinae). Systematic Botany 8: 263-268.

Calvo, R. 1990. Four-year growth and reproduction of *Cyclopogon cranichoides* (Orchidaceae) in South Florida. American Journal of Botany 77: 736-741

Catling, R. M. 1982. Breeding systems of northeastern North American *Spiranthes* (Orchidaceae). Canadian Journal of Botany 60: 3017-3039

Catling, R. M. 1983 Pollination of northeastern North American *Spiranthes* (Orchidaceae). Canadian Journal of Botany 61: 1080-1093

Catling, R. M. 1987. Notes on the breeding system of *Sacoila lanceolata* (Aublet) Garay (Orchidaceae). Annals of The Missouri Botanical Garden 74: 58-68.

Catling, P. M. & V. R. Catling 1991. A synopsis of breeding systems and pollination in north american orchids. Lindleyana 6 (4): 187-210

Cocucci, C.A. 1981. Fertilización en orquídeas. Anales de la Academia Nacional de Ciencias Exactas. Buenos Aires. 33: 1-47

Darwin, C. R. 1904. The various contrivances by which orchids are fertilized by insects. 2nd ed. London, Murray.

Dressler, R. L. 1981. The orchids: natural history and classification. Cambridge, Harvard University Press.

Dressler, R. L. 1993. Phylogeny and classification of the orchid family. Portland, Dioscorides Press.

Roubik, D. W. 1989. Ecology and natural history of tropical bees. Cambridge, Cambridge University Press.

Roubik, D. W. & J. D. Ackermann 1987. Long-term ecology of Euglossini orchid-bees in Panamá. Oecologia 73: 321-333.

Schlindwein, C. 1995. Wildbienen und ihre Trachtpflanzen in einer suedbrasilianischen

Buschlandschaft: Fallstudie Guaritas, Bestaubung bei Kakteen und Loasaceen. Stuttgart, Grauer.

Singer, B. R. & A A Cocucci. (in press) Pollination mechanism in southern brazilian orchids which are exclusively or mainly pollinated by halictid bees. Plant Systematics and Evolution

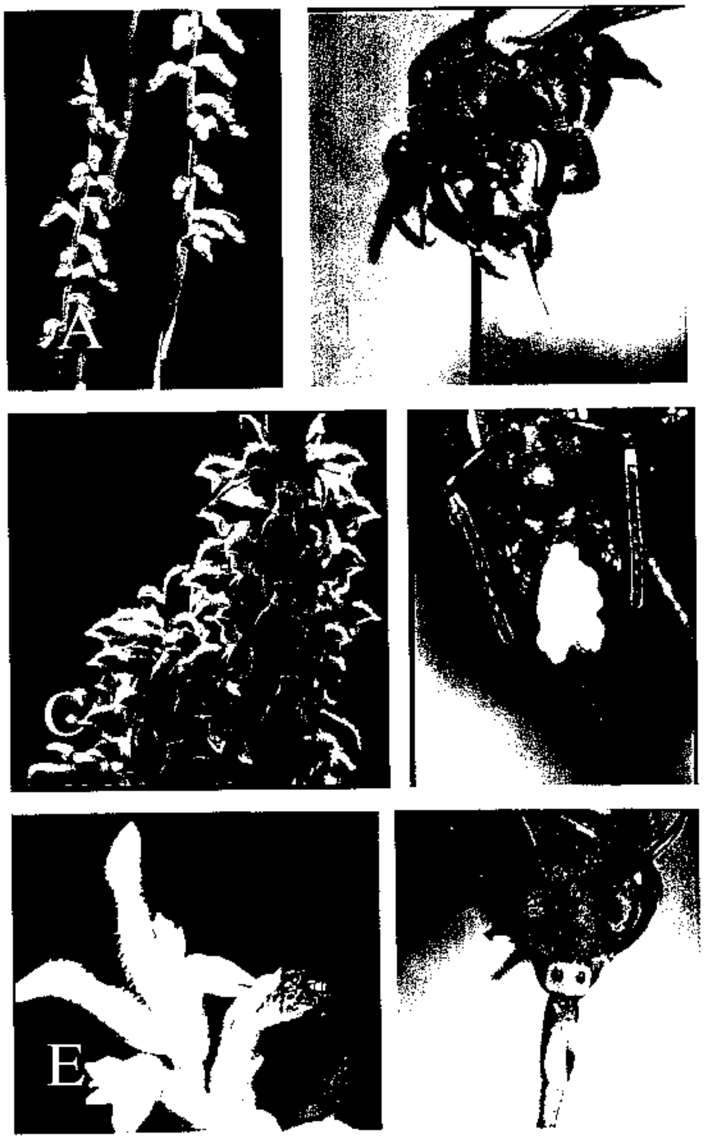

Figure1. A. *Cyclopogon congestus* being visited by *Pseudoaugochloropsis graminea* (Halictidae). B. Bee of *P. graminea* with pollinarium fixed to its labrum. C. Inflorescence of *Pelexia oestrifera.* D. Bumble-bee with a pollinarium attached to its labrum. E. *Sarcoglottis fasciculata* being visited by *Euglossa annectans* (Euglossini). F. *Euglossa* female with pollinarium fixed to its labrum.

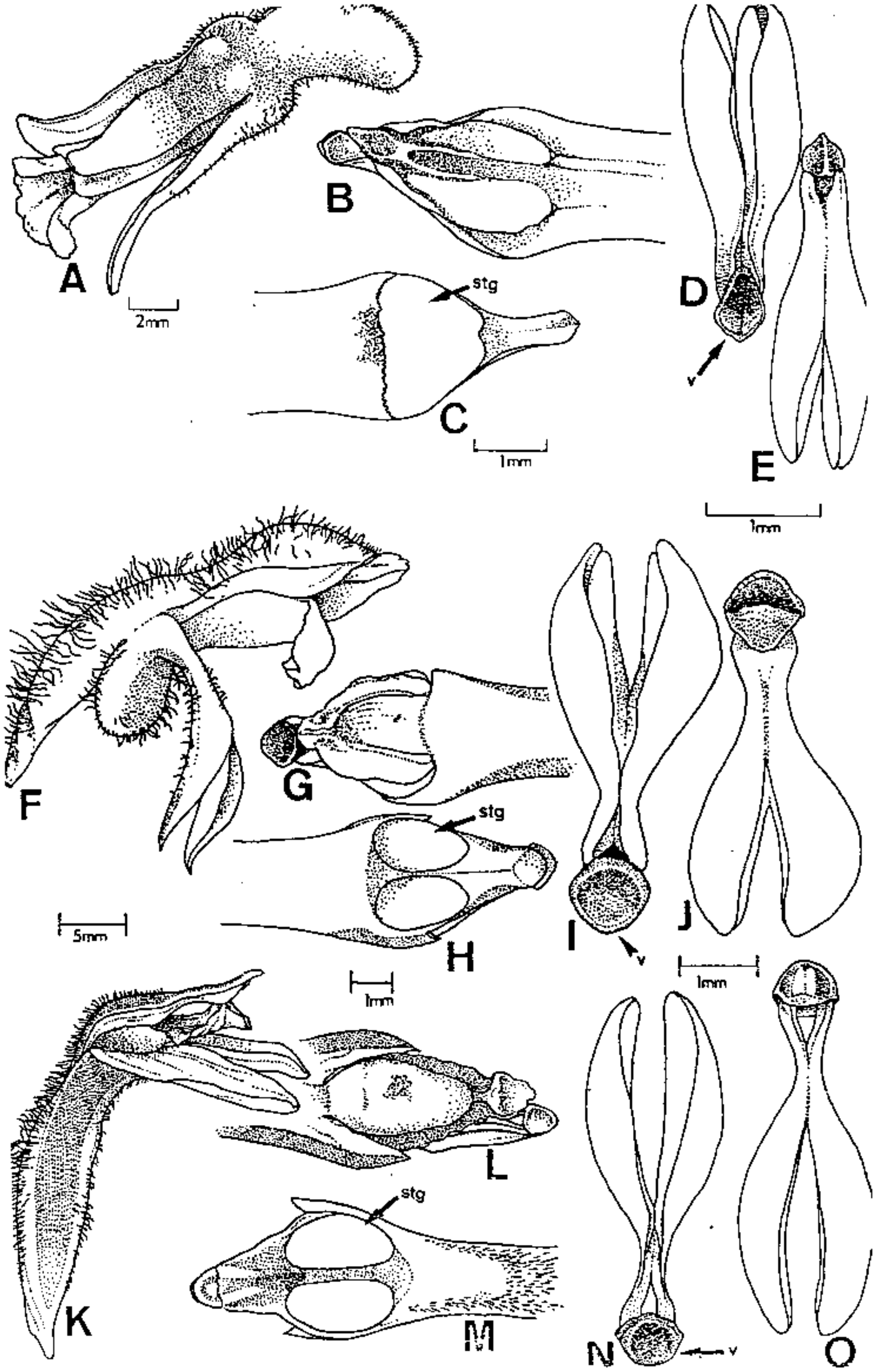

Figure 2: flower features in the studied species. A-E: *Cyclopogon congestus*. A. Flower. B-C: Column. B. Dorsal view. C. Ventral view. D-E: Pollinarium. D. Dorsal view. E. Ventral view. F-J: *Pelexia oestrifera*. F. Flower. G-H: Column. G. Dorsal view. H. Ventral view. I-J: Pollinarium. I. Dorsal view. J. Ventral view. K-O: *Sarcoglottis fasciculata*. K. Flower. L-M: column. L. Dorsal view. M. Ventral view. N-O: Pollinarium. N. Dorsal view. O. Ventral view. stg: stigmatic surface. V: viscidium

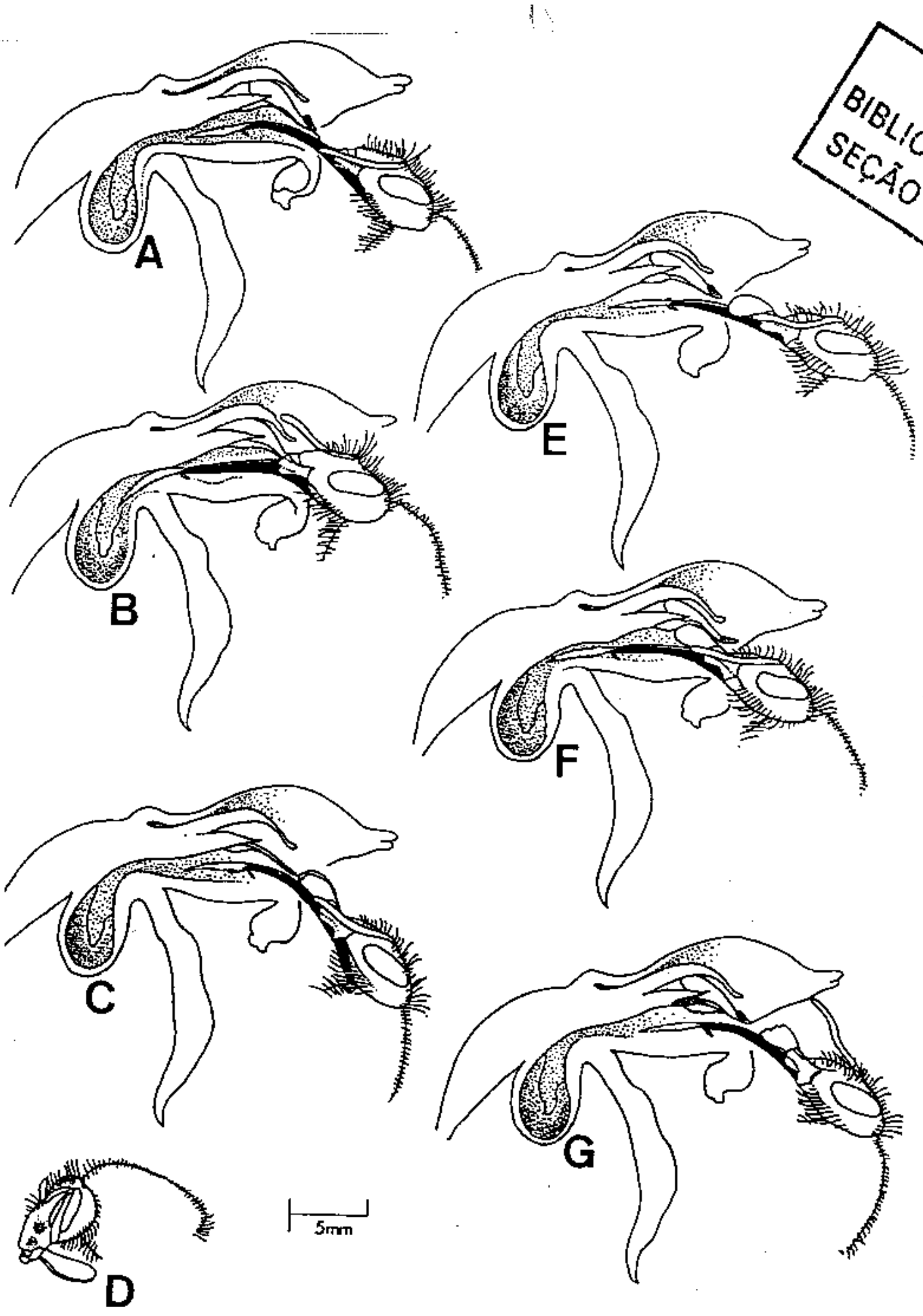

Figure 3: pollination of *Pelexia oestrifera* by *Bombus* (*Fervidobombus*) *atratus*. A. The bee inserts its mouth parts in the flower in order to reach the nectar found in the spur. B. The bee dorsally presses its labrum against the viscidium and the pollinarium is fixed. C. The bee leaves the flower, removing the pollinarium. D. The bee folds its mouth parts and the pollinarium remains protected under its head. E. A bumble-bee carrying a pollinarium visits a flower. F. While trying to take some nectar, the bee touches the stigmatic surface with the pollinarium, depositing pads of pollen. G. the bee leaves the flower.

CAPÍTULO 2:

THE POLLINATION OF *STENORRHYNCHOS LANCEOLATUS* (AUBLET) L. C. RICH. (ORCHIDACEAE: SPIRANTHINAE) BY HUMMINGBIRS IN SOUTHEASTERN BRAZIL

R. B. Singer and M. Sazima

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ABSTRACT. Hummingbird pollination is documented for a natural population of *Stenorrhynchos lanceolatus* (Aublet) L.C. Rich. occurring in Rio de Janeiro State, southeastern Brazil. At the study site the plants are pollinated by *Phaethornis eurynome* (Phaethorninae), *Thalurania glaucopis* (females only) and *Leucochloris albicollis* (Trochilinae). The plants offer nectar as a reward and the pollinaria become stuck to the surface of the hummingbird´s bill while it is probing the flowers. The orchid population received a few (0-4) hummingbird visits per day, with about 83 % of the flowers being pollinated. In spite of the low frequency of visits, the granular texture of the pollinarium plus the behaviour of the most frequent pollinators, which tend to visit all the fresh-looking flowers of each inflorescence, a very high fruiting success was promoted. Experimental evidence suggests that the pollinaria may remain up to 6:30 hs on the hummingbird´s bill, enhancing the chances of cross-pollination and long-distance pollen flow.

Key words: Orchids, Spiranthinae, pollination, hummingbirds, *Stenorrhynchos lanceolatus*

INTRODUCTION

Bird pollination has been suggested to occur in about 3 % of the known orchid species (Van der Pijl & Dodson 1966). However, it has been well documented in relatively few cases (Johnson 1996, Catling 1987, Cocucci 1981, Van der Pijl & Dodson 1966 and references therein). While in the Old World tropics birds of the family Nectarinidae are pollinators of three *Satyrium* (Orchidoideae) species (Johnson 1996), the hummingbirds (Trochilidae) apparently are the only bird group involved in orchid pollination in the Neotropics (Van der Pijl & Dodson 1966). Van der Pijl & Dodson (1966) reviewed most hummingbird-pollinated orchids, all the cases involving orchid genera of the subfamily Epidendroidae (sensu Dressler 1993). More recently, Ackermann et al. (1994) reported hummingbird pollination for *Comparettia falcata* (Epidendroideae: Oncidiinae). Among the Spiranthinae orchids, some *Stenorrhynchos* L.C. Rich. species show flower traits such as non-fragrant, reddish to pink, tubular, nectar-bearing flowers, which agree with the syndrome of ornitophily (Dressler 1981, Van der Pijl & Dodson 1966). Cocucci (1981) provided detailed illustrations of the pollination of *Stenorrhynchos orchioides* (from Central Argentina) by hummingbirds, with the purpose of illustrating the mechanics of orchid pollination in the context of an orchid biology review. No data concerning either study site or the hummingbird species involved were provided. Catling (1987) obtained experimental evidence of hummingbird pollination in cultivated plants of *Stenorrhynchos lanceolatus* (quoted as *Sacoila lanceolata*) in Ontario, Canada, outside the range of this orchid´s distribution. Galetto et al. (1997) reported visits of *Chlorostilbon aureoventris* (Trochilinae) to plants of *S. orchioides* in Central Argentina, but did not ascertain if these birds were pollinators or not. As part of our studies on the pollination biology of the Spiranthinae orchids from southeastern Brazil, we had the opportunity of documenting the pollination of *Stenorrhynchos lanceolatus* by hummingbirds in Rio de Janeiro State. The aim of the present contribution is twofold; 1) to confirm the occurrence of hummingbird pollination for this orchid under natural conditions and 2) to discuss the consequences of both, flower features and pollinator behaviour for the orchid´s reproductive success.

METHODS

The pollination biology of *Stenorrhynchos lanceolatus* was studied by means of seven field visits, from September 13 to 21, 1999; totalling about 36 man-hours of observation. The study site was a roadside bank, mostly covered by grasses at Macaé de Cima, Rio de Janeiro State, southeastern Brazil (ca. 22 21´& 22 28´S – 42 27´& 42 35´W, altitude: 900 m). Annual rainfall reaches about 1500-2000 mm and the annual mean temperature is about 17.5 °C. Daily observation periods were made from 06:00 to 18:00 hs. This study was performed with a population of 17 plants, growing in an area of ca. 36 square meters, totalling 438 flowers. Plant vouchers are deposited at the herbarium UEC (Universidade Estadual de Campinas, SP). Voucher number : R. B. Singer 16/ 99. The pollinators behaviour was recorded by means of field notes and photographs. In order to check the dynamics and efficiency of the pollination, the fresh-looking flowers of 10 selected plants were checked at three days intervals. Most flowers of the other seven inflorescences were either developing fruits or withering. The number of fruits, pollinated stigmas and the number of removed pollinaria were recorded. The male efficiency factor was calculated after Nilsson et al. (1992) as the ratio between the percentage of pollinated flowers divided by the percentage of flowers acting as pollen donors. To estimate how long a pollinarium could remain fixed to the pollinator, a brief experiment was performed. At a local residence (some 4 km. from the study site), the inhabitants offer feeders with 20 % diluted sucrose to the hummingbirds. We replaced such feeders with 5 inflorescences of *S. lanceolatus* bearing a total of 18 intact flowers. The pollinaria of these flowers were coloured in orange with water-resistant marker. These inflorescences were offered from 09:00 to 10:00 h. After this period, the bottles were relocated and the occurrence of pollinarium-bearing hummingbirds at the feeders was recorded until the dusk (18:00 h) Additional observations were performed the next day, from 08:00 to 14:30 h.

In the studied plant population, the fruiting success (dehiscent fruits divided by the number of produced flowers), was recorded. A sample of one near-dehiscing fruit of each of the twelve remaining inflorescences was taken in October 13, 1999. The seed viability was calculated as follows: a random sample of seeds from each fruit was taken and the number of embryo-bearing seeds in the first 500 units seen under the microscope was recorded. Flower features were drawn using 70 % alcohol-preserved flowers.

RESULTS AND DISCUSSION

Plant and flower features

Stenorrhynchos lanceolatus is a terrestrial herb, with fasciculated thick roots, laminar, erect, light green rosulate leaves (normally absent during anthesis) and one single, lateral inflorescence up to 60 cm tall. The inflorescence is a spike bearing up to 30 pink-salmon, tubular-like (Fig. 1A), 20-25 mm long flowers. It is noteworthy that, in the studied population, the flower bracts and the inflorescence axis are also pinkish in colour. Details on perianth part measurements can be found in Hoehne (1942) and Miller & Warren (1996). The lateral petals adhere by their dorsal surface to the dorsal sepal giving the flower a somewhat bilabiate appearance. Some degree of adnation was verified between the lip and the sides of the column. The column is erect (Fig. 1B) and holds a pollinarium made up by four friable, granular, clavate pollinia, short caudicles and a terminal, long, oblong viscidium (Fig. 1F-G). Remarkably, the viscidium sheats an acicular, needle-like, rigid rostellar remnant (Fig. 1E), which becomes visible when the pollinarium is removed. The viscidium is adherent by its ventral surface, which is light gray in colour and liberates a whitish glue when pressed. The stigmatic surface is entire, flat to slightly convex (Fig. 1B, D) and reddish coloured. The flowers lack any detectable fragrance and offer nectar as a reward. Two retrorse thick lobes, at the lip´s base secrete the nectar, which accumulates under the base of the column. All these flower features comply with previous reports (Balogh 1982, Catling 1987, Cocucci 1981, Galetto et. al. 1997 and Rasmussen 1982). We did not record rostellar narrowing or reduction as Catling (1987) reported for autogamous populations from Florida. In the studied plants, the pollinarium and the stigmatic surface remain separated by a wide rostellum, supporting that these plants are pollinator-dependent. The fruit is a capsule bearing several dust-like monoembryonic seeds. Some plants of *S. lanceolatus* in Florida (U.S.A.) were reported as apomictic by Catling (1987). These plants produce polyembryonic seeds, as a result of the proliferation of cells of the embryo sac (Catling 1987). Since the plants we studied produce only monoembryonic seeds and the plants are actively pollinated (see below), apomixis seems unlikely.

POLLINATOR BEHAVIOUR AND POLLINATION EFFICIENCY

Three hummingbird species were recorded as flower visitors and pollinators: *Phaethornis eurynome* (Phaethorninae) (Fig. 2A), *Leucochloris albicollis* (Fig. 2B) and *Thalurania glaucopis* (Trochilinae). *Phaethornis eurynome* was the most frequent flower visitor, however *T. glaucopis* was also frequent. *Leucochloris albicollis* was recorded only twice at flowers. However, the three hummingbird species carried pollinaria and pollinated the flowers. The pollination process is the same regardless of which hummingbird visits the flowers. The hummingbirds hover in front of the inflorescences and introduce their bills into the flower tube to reach the nectar accumulated at the column´s base. While probing the flowers, they press the ventral surface of the viscidium with the bill and the pollinarium becomes fixed to the upper surface of the bill´s tip (Fig. 2A, B). The pollinarium is removed when the hummingbird leaves the flower. A single hummingbird may carry 1-4 superimposed pollinaria. When a pollinarium carrying hummingbird probes another flower, the distal parts of the pollinia touch the stigmatic surface and pads of pollen are deposited. These observations agree with the reports of Catling (1987) and Cocucci (1981). Only a few (0-4) visits occurred per day. Pollinator activity was noticed from 10:00 to 16:35 h. There are some differences concerning the visiting behaviour of the three hummingbird species at the flowers. *Phaethornis euynome* systematically visited all the fresh-looking flowers of several or most of the inflorescences, spending up to a minute in each visite to the plant population. *Thalurania glaucopis* exhibited a similar behaviour, spending up to 40 seconds per visit to the population. The two visits of *L. albicollis* lasted 5 and 50 seconds, respectively. In the first visit, this hummingbird visited some flowers of two inflorescences, perched in a shrub and flew away. In the second visit, the bird probed all the available fresh-looking flowers of the population. *Phaethornis eurynome* and *L. albicollis* have been reported as very important pollinators in montane Atlantic rain forest flower assemblages in São Paulo state (Sazima et al. 1996). Both species share a number of plant species as nectar sources and have also been reported as trapliners (Sazima et al. 1996). The low frequency of visits by both hummingbird species at *S. lanceolatus* may be related to the trapliner habit (Sazima et al. 1996).

Since the pollinarium is granular in texture, several stigmas can be pollinated by a single pollinarium. Based on experimental observations, the pollen content of a single pollinarium may cover the stigmatic surface of approximately 16 flowers. Since a single bird may carry more than one pollinarium, a single stigma can receive mixed pollen loads (from pollinaria from other and the same inflorescence). Self-pollination in the strictest sense may be rare, since the pollinarium is removed when the bird leaves the flower. However, some degree of geitonogamy is to be expected owing to pollinator's behaviour at the inflorescences. Since pollinator-dependent plant of this and closely related species are self-compatible (Catling 1987, Galetto et al. 1997), part of the fruit set may be due to self-pollination (in the broad sense).

Flower features such as the granular texture of the pollinarium plus the fact that most of the pollinators visit several inflorescences, enhance the chances of cross-pollination; which, in our oppinion, may account for most part of the fruit set. The population produced a total of

368 fruits (ca. 83.2 % of fruiting success), the fruiting success per inflorescence varied from 54.2 % to 100 %. Such high percentages apparently contradict Neiland and Wilcock (1998), who suggested that tropical orchids, both nectarless and nectariferous have fruit productivities lower than 50 %. The ten monitored inflorescences produced 170 flowers, 160 (94.1 %) of which acted as pollen receivers, and 127 (74.7 %) as pollen donors. The donor (male) efficiency factor was 1.26. This value indicates that 100 pollinaria were necessary to pollinate 126 flowers. In other words, a slightly higher number of flowers were pollinated than pollinaria were removed. The seed viability was also very high, ranging from 98.2 % to 100 % (x: 99.52%; sd: 0.55).

EXPERIMENTAL OBSERVATIONS

After one hour of exposition, 27.7% (5/18) of the flowers had their pollinaria removed, and 38.8% (7/18) of them had their stigmas pollinated. The donor (male) efficiency factor was 1.4, similar to the naturally obtained value (1.26). During the exposition of the marked inflorescences, the hummingbirds *P. eurynome*, *L. albicollis*, *T. glaucopis* and *Clytolaema rubricauda* (this latter, was only observed in experimental conditions) visited the flowers and removed pollinaria. The small *Calliphlox amethystina* was sighted at flowers, but did not remove pollinaria. This hummingbird species apparently avoided contacting the rostellum by laterally accessing the flower tube. Other hummingbirds, such as *Melanothrochilus fuscus* and *Stephanoxis lalandi*, though common and frequently seen in the area, showed no interest in the flowers. Curiously, *S. lalandi* was sighted at flowers of *S. lanceolatus* in Campos do Jordão, São Paulo State (I. Sazima, pers. comm.). Hummingbirds with marked pollinaria were frequently seen at the feeders during the evening hours. A *C. rubricauda* individual, baring marked pollinaria was sighted for the last time at 14:50 h (about 4 hours, 50 min after the end of the experiment). *Leucochloris albicollis* was sighted bearing marked pollinaria until 16:30 (about 6 hours, 30 min after the end of the experiment). So, based in our observations, a pollinarium can stay fixed on a hummingbird´s bill for a period up to 6 hours, 30 min. Since *P. eurynome* and *L. albicollis* have been reported as long-distance foragers or "trapliners" (Sazima et al. 1996), the potential for cross-pollination is high. Though *P. eurynome* was recorded removing marked pollinaria, no individuals carrying pollinaria (either marked or not) were sighted at feeders thereafter. No individuals of any hummingbird species were sighted carrying marked pollinaria during observations the following day. In Central Argentina, Singer & Cocucci (unpublished) sighted females of *Chlorostilbon aureoventris* trying to actively remove pollinaria of *S. orchioides* (a species which may prove to b nothing but a sturdier and more floriferous variety of *S. lanceolatus*) by rubbing the bill against branches. Such behaviour was also noticed in Nectaridinae birds carrying pollinaria of *Satyrium* (Johnson 1996). Contrary to our previous suppositions (Singer & Sazima 1999), the pollinaria of *S. lanceolatus* are not easily removed by the hummingbirds, being as in *Satyrium* (Johnson 1996) very firmly fixed by the broad viscidium.

During the observations at feeders, individuals of *L. albicollis* and *T. glaucopis* were sighted bearing non-marked (and thus, naturally acquired) pollinaria. This is very interesting since the closest colony of *S. lanceolatus* was about 4 km from the experimental site. Incidentally, a male of *C. rubricauda* was sighted bearing a pollinarium matching those of *Sophronitis coccinea* (Orchidaceae, Epidendroideae, Laeliinae), an orchid very common at the study site and already suggested to be hummingbird-pollinated (Sazima et al. 1996).

CONCLUSION

Hummingbird pollination is here demonstrated for a Brazilian population of *S. lanceolatus*. However, since this orchid species is so widespread (Catling 1987), studies in other regions should be interesting from an ecological point of view. Populations of this species have been reported as pollinator-independent (Catling 1987), setting fruits either by self-pollination or apomixis. Catling (1987) has suggested that in this species, such breeding systems evolved under conditions of pollinator scarcity. Studies in other pollinator-dependent populations may reveal a wider range of hummingbird pollinators. The hummingbirds recorded as pollinators during our research belong to different taxonomic groups and are very different from a morphological point of view (Sazima et al. 1996). However, at least *P. eurynome* and *L. albicollis* use many the same plants as nectar sources (Sazima et al. 1996). The fact that mophologically and ecologically different hummingbirs visit an pollinate *S. lanceolatus* is a clear indication that this orchid is not morphollogically restricted to a particular kind of hummingbird. This may be one of the reasons by which this plant has achieved such a wide distribution (Catling 1987).

Owing to their flower features, other South American *Stenorrhynchos* species such as *S. congestiflorus* Cogn., *S. hassleri* Cogn. and *S. pedicellatum* Cogn. may be hummingbird pollinated. Because of its red, tubular flowers, *S. speciosum* L. C. Rich. from Central America also suggests hummingbird pollination. However, Ackermann (1995) reports that plants from Puerto Rico may be pollinator-independent since they set fruit in the greenhouse, in the absence of potential pollinators. Clearly, much fieldwork is still needed in order to get a good panorama of the pollination biology of this orchid genus. For now, *Stenorrhynchos* remains the only Spiranthinae orchid genus for which hummingbird has been demonstrated.

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REFERENCES

Ackermann J. D. (1995) An orchid flora of Puerto Rico and Virgin Islands. Mem. Of the New York Bot. Gard. Bronx 73. New York.

Ackermann J. D., Rodríguez-Robles J. A. & E. J. Melendez (1994). A meager offering of nectar by an epiphytic orchid is better than nothing. Biotropica 26: 44-49.

Balogh P. (1982). Generic redefinitions in the subtribe Spiranthinae (Orchidaceae). Am. J. Bot. 69: 1119-1132.

Catling R. M. (1987) Notes on the breeding system of *Sacoila lanceolata* (Aublet) Garay (Orchidaceae). Ann. Miss. Bot. Gard. 74: 58-68.

Cocucci C. A. (1981) Fertilización en orquídeas. Anales de la Academia Nacional de Ciencias Exactas. Buenos Aires. 33:1-47.

Dressler R. L. (1981) The orchids; natural history and classification. Harvard Univ. Pres, Cambridge.

Dressler R. L. (1993) Phylogeny and classification of the orchid family. Dioscorides Press, Portland.

Galetto L., Bernardello G., & Rivera G. L. (1997) Nectar, nectaries, flower visitors and breeding systems in five terrestrial Orchidaceae from Central argentina. J. Plant Research. 110: 393-403.

Hoehne F. C. (1945) Orchidaceas. In: Hoehne F. C. (ed.) Flora Brasilica. Vol. XII. Instituto de Botânica, São Paulo.

Johnson S. D. (1996). Bird pollination in South African species of *Satyrium* (Orchidaceae). Plant. Syst. & Evol. 203: 91-98.

Miller D. & Warren R. (1996). Orquídeas do alto da Serra. Salamandra Editora, Rio de Janeiro.

Neiland M. R. & Wilcock C. C. (1998). Fruit set, nectar reward, and rarity in the Orchidaceae. Am. J. Bot. 85 (12): 1657-1671.

Nilsson L., Rabakonandrianina A., Razananarivo E.& Randriamanindry R. (1992) Long pollinia on eyes: hawkmoth pollination of *Cynorkis uniflora* Lindl. (Orchidaceae) in Madagascar. Bot. J. Linn. Soc. 109;145-160.

Pijl, L. Van der & Dodson C. H. (1966). Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables.

Rasmussen F. N. (1982). The gynostemium of the neottioid orchids. Opera Bot. 65: 1-96.

Sazima I., Buzato S., & Sazima M. (1996). An assemblage of hummingbird-pollinated flowers in a montane forest in southeastern Brazil. Bot. Acta. 109: 149-160.

Singer R. B. & Sazima M. (1999) The pollination mechanism in the "*Pelexia* alliance" (Orchidaceae: Spiranthinae). Bot. J. Linn. Soc. 13193): 249-262.

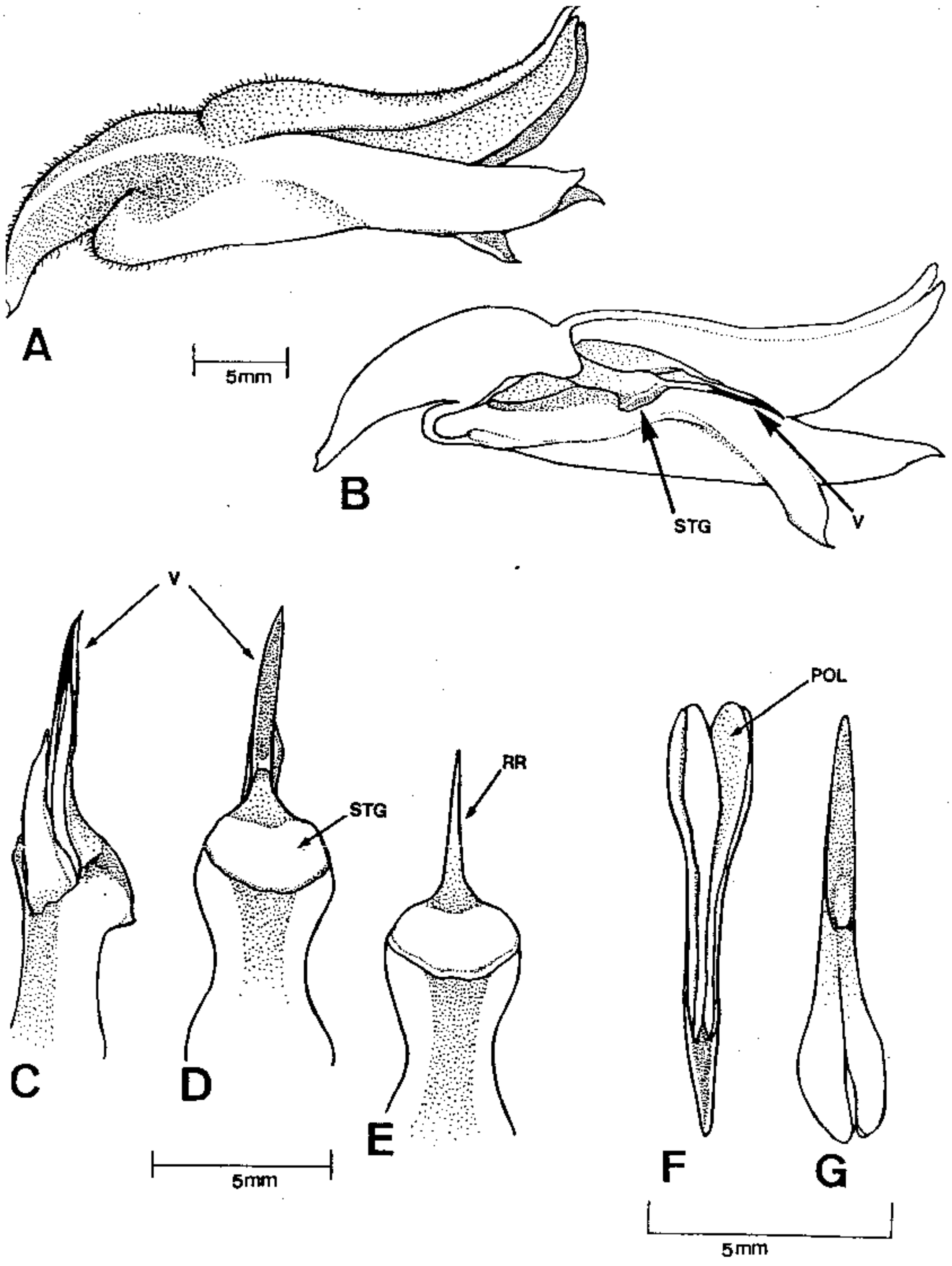

Fig. 1: Flower features of *Stenorrhynchos lanceolatus* (Aublet) L. C. Rich. A-B flower. A lateral view. B longitudinal section of the flower. C-E column. C lateral view. D-E ventral view. D with the pollinarium. E without the pollinarium. F-G pollinarium. F dorsal view. G ventral view. V viscidium ahesive surface, STG stigmatic surface, RR rostellar remnant, POL pollinia

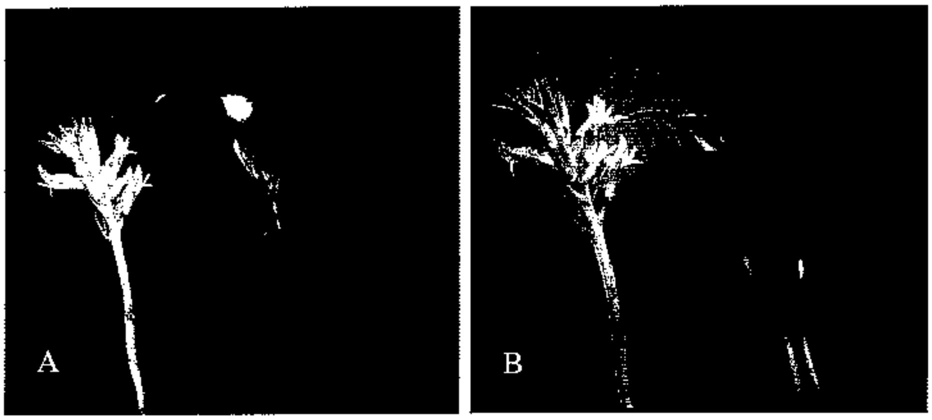

Figure 2. Hummingbird pollinators of *S. lanceolatus*. A. *Leucochloris albicollis*. B. *Phaethornis eurynome*. Notice the white pollinaria on the surface of the bill tip

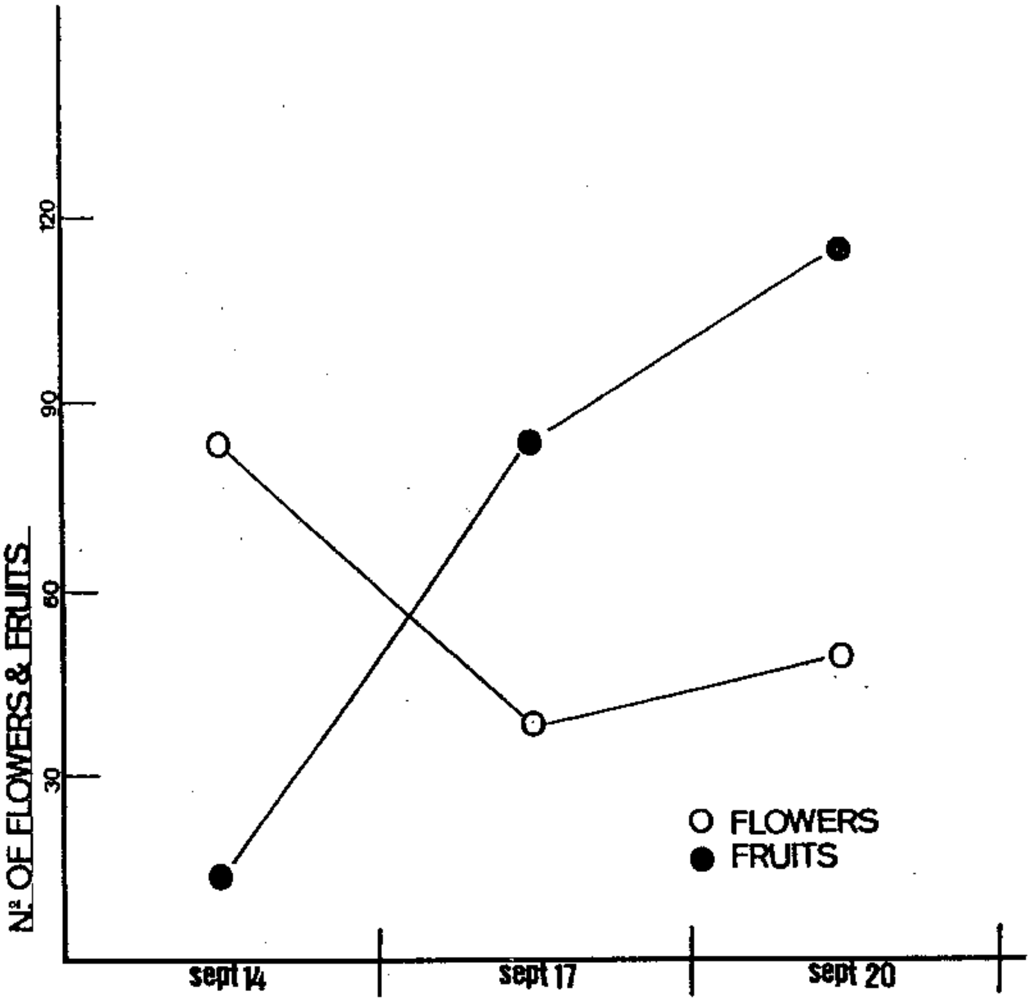

Fig. 3. Flowering and fruiting phenology of ten selected inflorescences of *S. lanceolatus* During this study, 160 flowers acted as pollen receivers and 127 as pollen donors.

CAPÍTULO 3:

THE POLLINATION BIOLOGY OF *SAUROGLOSSUM ELATUM* Lindl. (ORCHIDACEAE: SPIRANTHINAE): MOTH-POLLINATION AND PROTANDRY IN NEOTROPICAL SPIRANTHINAE

Rodrigo B. Singer

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ABSTRACT

The pollination biology of *Sauroglossum elatum* Lindl. (Orchidaceae: Spiranthinae) was studied in the State of São Paulo, southeastern Brazil. Although this orchid is protandrous and almost fully self-compatible, it is pollinator-dependent. Thus, pollinators are required for the plants to set fruits and seeds. The flowers are pollinated by moths of the family Noctuidae. Pollinia are dislodged when moths probe flowers in the male phase. At this stage the stigmatic surface is inaccessible, so that the flowers can only act as pollen-donors. Flowers in the female phase present their stigmatic surfaces well-exposed and dry viscidia; at this stage the flowers act as pollen receivers. Pollinarium-bearing moths, when visiting a flower in the female phase, will brush the pollinarium against the stigmatic surface, thus effecting the pollination. Moth-pollination is here reported for the first time for the orchid subtribe Spiranthinae. Protandry is also mentioned to occur in a few additional Brazilian Spiranthinae. Owing to the evidence presented in this work, protandry in Spiranthinae is not necessarily linked to Bumblebee pollination, as currently accepted. It is suggested that the occurrence of protandry in Spiranthinae and in the closely related subtribes Prescottinae and Goodyerinae may be a feature encompassing ecological and phylogenetical implications as well.

Key Words: *Sauroglossum*, Spiranthinae, Orchidaceae, protandry, pollination, moths.

INTRODUCTION

Many Spiranthinae orchids have been studied in their pollination biologies (Catling 1983a, 1987, Darwin 1878, Singer & Cocucci 1999, Singer & Sazima 1999, 2000). Bees of families Halictidae, Megachilidae and Apidae and hummingbirds of subfamilies Trochilinae and Phaethorninae have been recorded as pollinators (Catling 1983a, 1987, Darwin 1878, Singer & Cocucci 1999, Singer & Sazima 1999, 2000). All these pollen vectors probe the flowers in search of nectar, the only reward that Spiranthinae orchids offer to their pollinators (Catling 1983a, 1987, Catling & Catling 1991, Singer & Sazima 1999). Whereas the hummingbird-pollinated *Stenorrhynchos lanceolatus* shows pinkish, non-fragrant flowers (Singer & Sazima 2000), the bee-pollinated genera *Cyclopogon, Pelexia, Sarcoglottis* and *Spiranthes* show diurnally fragrant, often light-coloured flowers (Catling 1983a, Singer & Cocucci 1999, Singer & Sazima 1999). Several species of *Spiranthes* present protandry, a remarkable feature promoting cross-pollination (Catling 1983a). Protandry has also been recorded in some Bumblebee pollinated Goodyera species (Goodyerinae) (Ackerman 1975, Catling 1983a, Catling & Catling 1991, Darwin 1878). Catling (1982, 1983a) provided fine details on the pollination biology and breeding systems of several North American *Spiranthes* species and reviewed the pre-existing literature on this topic.

During the studies on the pollination biology of Brazilian Spiranthinae orchids moth-pollination was recorded associated with protandry in *Sauroglossum elatum* Lindl. The aims of the present contribution are: 1) broadening the knowledge on the reproductive biology of neotropical Spiranthinae orchids, 2) presenting the first evidence of protandry in neotropical Spiranthinae orchids and 3) presenting the first record of moth-pollination for the whole Spiranthinae orchid subtribe.

METHODS

Field observations were performed in the Estação Biológica do Alto da Serra, Paranapiacaba (Santo André, São Paulo State, 23° 46´S, 46° 18´W, altitude: about. 800 m.a.s.l.). Annual rainfall is about 2600 mm and the average annual temperature is about 18° C. A total of 15 field visits were performed, totalling about 68 h 50 min of observations. Field work was performed from 15 to 18 August 1998, 26 to 30 August 1999, 21 to 23

August 2000 and 14 to 19 September 2000. *Sauroglossum elatum* is common and widespread throughout this area, and for the sake of practicity, observations were focused on plant patches. During the whole observation period, attention was centered on 24 inflorescences, totalling about 1728 flowers. Fruiting success, as the number of fruits divided by the number of produced flowers was recorded. In the year 2000, ten inflorescences, totalling 728 flowers, were available. Observation period as a whole ranged from 6:00 to 23:00 h. Pollinator behaviour was recorded through field notes and photographs. Nocturnal observations were performed with the aid of a lantern. At 5-10 min intervals, inflorescences were illuminated and checked for pollinators. This procedure apparently did not affect insect behaviour at flowers. A few insects were captured and pinned for later identification.

The breeding system was studied using plants cultivated in the greenhouse at the Universidade Estadual de Campinas. The number of individuals and flowers used in each treatment are summarised in Table 1. Treatments included self-pollination, cross-pollination, emasculation and untouched flowers (control). Treatments yielding fruits were compared through a chi-square test (f. d.= 1; $\alpha = 0.05$).

Flower morphological features were drawn from fresh flowers with the help of a stereomicroscope with a camera lucida attachment. Plant vouchers are deposited at the Herbarium of the Universidade Estadual de Campinas (UEC) (Voucher number: R. B. Singer 98/83). Pots containing two flowering individuals totalling 396 flowers, were displayed in the campus of the University, near the greenhouse where breeding experiments were performed in order to record if pollinators would also appear and pollinate the flowers under ex-situ conditions.

Throughout this contribution, the taxonomic and morphological concepts of Dressler (1993) are followed.

RESULTS

PLANT FEATURES: *Sauroglossum elatum* Lindl. is a robust herb, habitually occurring as terrestrial, but circumstantially appearing as epiphyte on tree ferns (Cyatheaceae). Leaves are numerous, rosulate, atenuate and light green in colour, up to 50 cm in length (Hoehne 1945). Inflorescences are congest, almost cylindrical spikes, up to 90 cm tall. Each inflorescence may bear up to 170 flowers. Flower-opening is sequential, from the base of the inflorescence toward its apex. Flowers are resupinate, light green, with a white lip, and as a whole the flower measures about 10-12 mm in length (Hoehne 1945). Remarkably, the lateral sepals are upcurved, ascendent, giving a characteristic shape to the flowers (Fig. 1 A-B). The lip is noticeably straight and spoon-shaped (Hoehne 1945). Readers interested in the shape and size of the perianth parts are referred to Hoehne (1945). The flowers offer nectar as a reward, which is probably secreted by two retrorse lobes at lip base (Catling 1983a) before it accumulates under the base of the column. The column is notably long (Fig. 1 C), but the rostellum is short and reduced (Fig. 1 C-D). The anther holds a pollinarium made up by four granular pollinia, short caudicles and a terminal, oblong, ventrally adhesive viscidium (Fig. 1 E-F). The removal of the pollinarium leaves a "u-" shaped, foveate rostellar remnant (Balogh 1982) (Fig. 1 D). The stigmatic surface is broad, flat to slightly convex and bilobed (Fig.1 C-D). The flowers are protandrous, and column movements in the two flower phases are very evident (Fig. 2 A). Recently opened flowers present their column directed toward the lip, thus rendering the flower entrance very narrow and hiding the stigmatic surface (Fig. 2 A). At this phase, flowers can act only as pollen donors. After 3-4 days, the column acquires an uppermost position, thus exposing the stigmatic surface (Fig. 2A). At this phase, the viscidium is usually dry and the flowers act as pollen-receivers. A flower of this orchid species may keep its fresh appearance for about 16 days. Either in natural populations or cultivated plants, the flowers emit a sweet, spicy fragrance from dusk, c. 18:00 h until 23:00-24:00 hs.

POLLINATION MECHANISM

No diurnal visitors were recorded at the flowers of this orchid species, even when abundant Bumblebees, *Bombus atratus* (Franklin,1913), Apidae were sighted at nearby flowers of *Solanum* sp. (Solanaceae). However, the flowers were clearly visited, as evidenced by pollinarium removals and pollen loads on the stigmatic surface of some flowers. We also noticed moth scales, sometimes on the stigmatic surface, and fragments of pollinia on the external surfaces of several flowers. Observations at dusk and by night revealed several flower visitors. Pollinators were small to big moths of the family Noctuidae (two morphoespecies) (Fig. 2 B-C). Several Geometridae and Pyralidae moths were sighted at the flowers, but these tiny moths never removed pollinaria. It seems that only the bulky Noctuidae moths were able to exert the pressure needed to fix the pollinaria. The moths are quite passive and may stay for 2-45 minutes on a single inflorescence, often probing several (>10) flowers. The pollination mechanism is as follows: a moth probes a flower in the male phase inserting its proboscis in order to reach the nectar under the column (Fig. 2 B, Fig. 3 A). When leaving the flower, the proboscis is pressed against the viscidium and the pollinarium gets fixed (Fig. 2 C, Fig. 3 B). A pollinarium-bearing moth that visits a flower in female phase inserts its proboscis into the flower tube, then, the distal parts of the pollinia brush the stigmatic surface and clumps of pollen are left there, thus effecting pollination (Fig. 3 C-D). It seems that when a pollinarium is fixed, the moth is momentaneously retained by the rostellum, until the pollinarium is removed. This apparently disturbs the moth, which leaves the inflorescence. The moths used to visit the inflorescences from the basalmost (in female phase) toward the uppermost (in male phase) flowers. However, moths without pollinaria were often observed to return to the base of the inflorescence. In my opinion, there is no reason to think that *S. elatum* is particularly restrictive concerning the moth pollinators. Additional observations will surely reveal that throughout its distribution this terrestrial orchid may be pollinated by guilds of appropriately sized moths. Occasionally, unidentified Dermaptera bugs were sighted probing the flowers. The flower tube is too long for this insect's short mouth-parts. However, these bugs apparently lick the stigmatic surface. In doing so, pollinaria were occasionally fixed on their forelegs (Fig. 2 D). These bugs were not frequent at flowers. Such behaviour does not seem very appropriate for the orchid and probably represents isolate occurrences with minor relevance for this orchid's reproductive success.

About 33.4 % of the flowers (243/728) set fruits and individual fruiting success ranged from 10 to 49.3 %.

BREEDING SYSTEM

According to the experiments in the greenhouse, plants are self-compatible, but pollinator-dependent. This means that pollinators are needed for the plants to set fruits and seeds. No statistically significant differences were recorded between self and cross-pollination (Table

1). Thus, for now there is no evidence of inbreeding depression. In some cases, the ovaries of some emasculated and untouched flowers enlarged, but seeds were never produced.

Table 1: Percentage of fruiting success per treatment. Numbers in parenthesis represent the number of fruits obtained over the number of flowers used in each treatment. N: number of individuals. Obtained Chi-square value (self x cross-pollination) = 0.054. (Non significant, P= 0.8156)

N	Control	Emasculation	Self-pollination	Cross-pollination
14	0 (0/132)	0.(0/149)	94. 9 (150/158)	98. 14 (159/162)

ANECDOTAL EX-SITU OBSERVATIONS

During "ex-situ" observations, diurnal visitors performed pollination in a strange way. Several workers of *Tetragonisca angustula* (Apidae: Meliponini) visited the flowers. Although these bees are too small to reach the nectar at the base of the column, they were interested in the pollen. They visited both flowers in the male and female phase. Since the pollinarium is granular in texture, the bees broke the pollinaria and actively collected their pollen contents, leaving each clinandrium completely empty. The white-creamy pollen loads were stored on the bee's corbiculae. When the bees manipulated the flowers, small fragments of the pollinaria reached the stigmatic surfaces and consequently, several flowers set fruits. The bees visited each pollen-offering flower in each inflorescence, often returning to the base of the inflorescence or to already visited flowers. Such behaviour clearly promotes self-pollination, either in the strictest sense (pollen of a flower on its own stigmatic surface) or geitonogamy. This interaction was never observed in natural conditions, even though *Tetragonisca* bees are widespread in southern and south-eastern Brazil (pers. obs.). A total of 146 fruits developed, which represents about 37 % of the studied flowers.

DISCUSSION

The flowers of *S. elatum* present the characteristic and well-known groundplan of Spiranthinae orchids (Dressler 1993, Rasmussen 1982). In terms of morphological flower features favouring cross-pollination, what has been said for other Spiranthinae orchids (Singer & Cocucci 1999, Singer & Sazima 1999, 2000) is here applicable: the presence of both granular pollinaria and broad stigmatic surfaces increases the chances of cross-pollination. Each granular pollinarium has a pollen content that is enough to pollinate many flowers (Dressler 1993, Singer & Cocucci 1993, Singer & Sazima 1999, 2000). On the other hand, a broad stigmatic surface increases the chances of multiple pollen loads (i.e. from the very inflorescence or from a cospecific one).

Protandry as here recorded for *S. elatum* follows the same pattern previously registred for *Spiranthes* (Catling 1983a, Catling & Catling 1991). In *Spiranthes*, protandrous taxa (including a few agamospermic ones) are pollinated by Bumblebees. Freshly opened flowers present their column directed toward the lip, the viscidium is clearly fresh and adhesive. The stigmatic surface remains inaccessible, thus, flowers at this stage can only act as pollen donors. After 5-30 days, depending on the species, the column changes its position, exposing the stigmatic surface. Now, the viscidium is dry and the flowers can only act as pollen receivers (Catling 1983a). Flower-opening is sequential from the base of the inflorescence toward the apex, and bee pollinators tend to explore the inflorescences in such a sequence. So, bees carrying pollinaria will first explore the oldest and lowermost (in female phase) flowers, thus effecting pollination. Pollinaria will only be removed from younger (in male phase) flowers, at the apex, when the bee is almost leaving the inflorescence, thus avoiding geitonogamous pollination (Catling 1983a). In the case of *S. elatum*, the recorded moth pollinators are quite passive and do not seem to explore the inflorescences as systematically as the Bumblebees that pollinate *Spiranthes* (Catling 1983a). Remarkably, some moths (although without pollinaria) were observed to return to the base of the inflorescence. Then, the possibility of geitonogamous pollination cannot be denied. Protandry was also observed in the tiny flowers of *Mesadenella apiculata* (Spiranthinae). This orchid shows a very different rostellar structure, with a sheathing viscidium which involves an acicular, needle-like rostellar remnant. Such a rostellar structure is a diagnostic for the so-called "*Stenorrhynchos* alliance" (Balogh 1982). In fact, this genus was previously included in a broadly defined concept of *Stenorrhynchos* (Schlechter 1920, Hoehne 1945). According to field observations, *M. apiculata* is pollinated by Augochlorini Halictid bees (pers. obs.) and not by Bumblebees as the presence of protandry should suggest. Remarkably, the deeply studied reproductive biology of the closely related *Stenorrhynchos lanceolatus* showed no evidence of protandry (Catling 1987, Singer & Sazima 2000). Protandry is apparently absent in the Spiranthinae genera *Cyclopogon*, *Pelexia* and *Sarcoglottis*, of the so-called "*Pelexia* alliance" (Singer & Sazima 1999).

Protandry was also found in orchid species of related subtribes, such as Goodyerinae (Ackerman 1975, Singer & Sazima, in press a) and Prescottinae (Singer & Sazima, in press b). Whereas *Goodyera oblongifolia* (Goodyerinae) is pollinated by Bumblebees (Ackerman 1975); *Erythrodes arietina* (also Goodyerinae) is pollinated by a guild of small Apidae bees, including *Paratetrapedia* and *Osiris* species (Singer & Sazima, in press a). In both cases, the pollinaria fix on the dorsal surface of the bee's tongue. Protandry was recently recorded in *Prescottia stachyodes* (Prescottinae), a taxon which is pollinated by Pyralid moths (Singer & Sazima, in press b). One should not forget that the orchid subtribes Spiranthinae, Prescottinae and Goodyerinae have been considered as closely related and were included with other smaller subtribes into the tribe Cranichideae (Dressler 1993). Protandry is obviously very relevant from an ecological point of view since it clearly promotes cross-pollination (Catling 1983a, Ackerman 1975) and its presence has been previously associated with Bumblebee pollination (Catling 1983a). Remarkably, Catling (1983) noticed that the Halictid-pollinated *Spiranthes lucida* is not (or almost not) protandrous. Then, protandry, as described above, was associated to Bumblebee pollination (Catling 1983a).

In the context of the information provided in this contribution, we would like to suggest that protandry as a phenomenon in the Cranichideae tribe, needs reconsideration. Owing to our evidence, protandry is not necessarily linked to Bumblebee pollination and appears in closely related orchid groups. So, protandry is possibly a feature with both ecological and phylogenetical value. Anatomical studies are needed in order to assess if the protandry recorded in subtribe Spiranthinae is homologous to that observed in subtribes Goodyerinae and Prescottinae. If so, the presence of this flower feature would reinforce the evidence for relatedness in these three orchid subtribes. On the other hand, the absence of protandry in taxa closely related to protandric ones may reflect ecological specialisation, linked to pollination biology phenomena. Other pollination strategies, which are present in the orchid subtribe Spiranthinae (e.g.: the dorsally adhesive viscidia of the genera *Cyclopogon*, *Pelexia* and *Sarcoglottis*), may be, from an ecological point of view, as efficient as protandry to promote cross-pollination.

Other forms of protandry have been reported for orchids of the subfamily Epidendroideae (sensu Dressler 1993) (Catling & Catling 1991, Van der Pijl & Dodson 1966). Epidendroid orchids normally display very concave stigmatic surfaces in agreement with the presence of entire, globose pollinia (Dressler 1993). Protandry in these orchids normally involves the blocking of the stigmatic surface by column or rostellar appendices (e.g. column wings) (Catling & Catling 1991, Van der Pijl & Dodson 1966). In other Epidendroid orchids, such as in the genus *Mormodes* (Catasetinae), the stigmatic surface is only exposed after the pollinarium was removed (Van der Pijl & Dodson 1966).

In general, flower features of *S. elatum* such as greenish-white, tubulose, night-scented flowers agree very well with the moth pollination syndrome (Van Der Pijl & Dodson 1966). Godfery (1933) reported crepuscular fragrance production and moth visitation (but not pollination) in *Spiranthes aestivalis* and *S. spiralis*, respectively. These reports were questioned by Catling (1983a), who demonstrated bee pollination and protandry in several *Spiranthes* species. Catling (1983a) reported several diurnal lepidoptera as non-pollinating flower visitors in several *Spiranthes* species. In these orchid species, the viscidium is relatively large, rigid and flat. These flower features are best suited for the large, hard and flat bee tongues rather than the slender and narrow butterfly proboscises. The viscidium of *S. elatum* is comparatively small and slender, thus allowing the fixation on lepidopteran proboscises.

Moth pollination is here reported for the first time in the subtribe Spiranthinae. The also Brazilian *Sauroglossum* cf. *organense* Szlach. does also show protandry and night scent production (pers. obs.), suggesting that this taxon is also moth-pollinated. Remarkably, this species shows bronze-tan coloured flowers (Hoehne 1945 and pers. obs.) and the flowers may not suggest moth-pollination at first glance. Moth-pollination could be expected in the

Brazilian *Eltroprectris triloba* (Spiranthinae), since it shows long-spurred, greenish-white flowers.

Self-compatibility has been reported for several pollinator-dependent Spiranthinae orchids (Catling 1982, Catling 1987, Catling & Catling 1991, Singer & Sazima 1999) and it may be a rule for this orchid subtribe. Some species or varieties of *Spiranthes* and *Stenorrhynchos* were reported either as autogamous or agamospermic (Catling 1983b, 1987). Autogamy (automatic self-pollination) was associated with different degrees of rostellar narrowing. In this case, the stigmatic secretion passively contacts the pollinia allowing self-pollination (Catling 1983b, 1987). In *Sauroglossum elatum* the rostellum is comparatively short, but wide enough to maintain the anther and the stigmatic surface separated. Agamospermy either in *Spiranthes* or *Stenorrhynchos* was reported as a consequence of the proliferation of the embryo sac cells, thus promoting the production of polyembrionic seeds (Catling 1982, Catling 1987). Owing to the results with cultivated plants, the possibility of either automatic self-pollination or agamospermy (at least, as previously reported) seems unlikely. The interaction between *S. elatum* and Meliponi bees under artificial conditions are interesting since the use of orchid pollen by bees is a very rare occurrence (Dressler 1993, Gregg 1991). Yet, in our opinion, it reflects a local resource (pollen) scarcity rather than a well-established plant-insect interaction. Still, since both taxa (*S. elatum* and *Tetragonisca* bees) are widespread in Brazil and may frequently overlap in their distributions, the possibility that this interaction or similar ones could occur in natural conditions cannot be completely denied.

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REFERENCES

Ackerman J. D. 1975. Reproductive biology of *Goodyera oblongifolia* (Orchidaceae). Madroño 23: 191-198.

Balogh, P. 1982. Generic redefinitions in subtribe Spiranthinae (Orchidaceae). Am. J. Bot. 69: 1119-1132.

Catling P. M. 1982. Breeding systems of Northeastern North American Spiranthes (Orchidaceae). Can. J. Bot. 60: 3017-3039.

Catling, P. M. 1983^{a}.Pollination of Northeastern North American *Spiranthes* (Orchidaceae). Can. J. Bot. 61: 1080-1093.

Catling P. M. 1983b. *Spiranthes ovalis erostellata* (Orchidaceae), a new autogamous variety from the eastern United States. Brittonia 35(2): 120-125

Catling P. M. 1987. Notes on the breeding systems of *Saccoila lanceolata* (Aublet) Garay (Orchidaceae). Ann. Miss. Bot. Gard. 74: 58-68

Catling P. M. & V. R. Catling. 1991. A synopsis of breeding systems and pollination in north american orchids. Lindleyana 6(4): 187-210

Dressler R. L. 1981. The orchids. Natural history and classification. Harvard Univ. Press. Cambridge.

Dressler R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press. Portland.

Godfery M. J. 1933. Native British Orchidaceae. Cambridge University press, Cambridge

Gregg, K. B. 1991. Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata*. Lindleyana 6: 214-220.

Hoehne F. C. 1945. Orchidaceas. In: Hoehne F. C. (ed.) Flora Brasilica. Vol XII. Instituto de Botânica, São Paulo.

Rassmusen F. N. 1982. The gynostemium of the neottioid orchids. Opera Bot. 65: 1-95.

Schlechter R. 1920. Versuch einer systematischen Neuordnung der Spiranthinae. Beih. Bot. Centralb. 37: 317-454.

Singer R. B. & M. Sazima. 1999. The pollination mechanism in the “Pelexia alliance” (Orchidaceae: Spiranthinae). Bot. J. Linn. Soc. 131(3): 249-262.

Singer R. B. & M. Sazima. 2000. The pollination of *Stenorrhynchos lanceolatus* (Aublet) L. C. Rich. (Orchidaceae: Spiranthinae) by hummingbirds in southeastern Brazil. Pl. Syst & Evol. 223: 221-227.

Singer R B & M Sazima (in press a). Flower morphology and pollination mechanism in three sympatric Goodyerinae orchids from Southeastern Brazil. Annals of Botany

Singer R B & M Sazima (in press b).The pollination mechanism of three sympatric *Prescottia* (Orchidaceae: Prescottinae) species in southeastern Brazil. Annals of Botany

Van der Pijl L. & C. H. Dodson 1966. Orchid flowers: their pollination and evolution. Univ. of Miami press. Coral Gables, Florida.

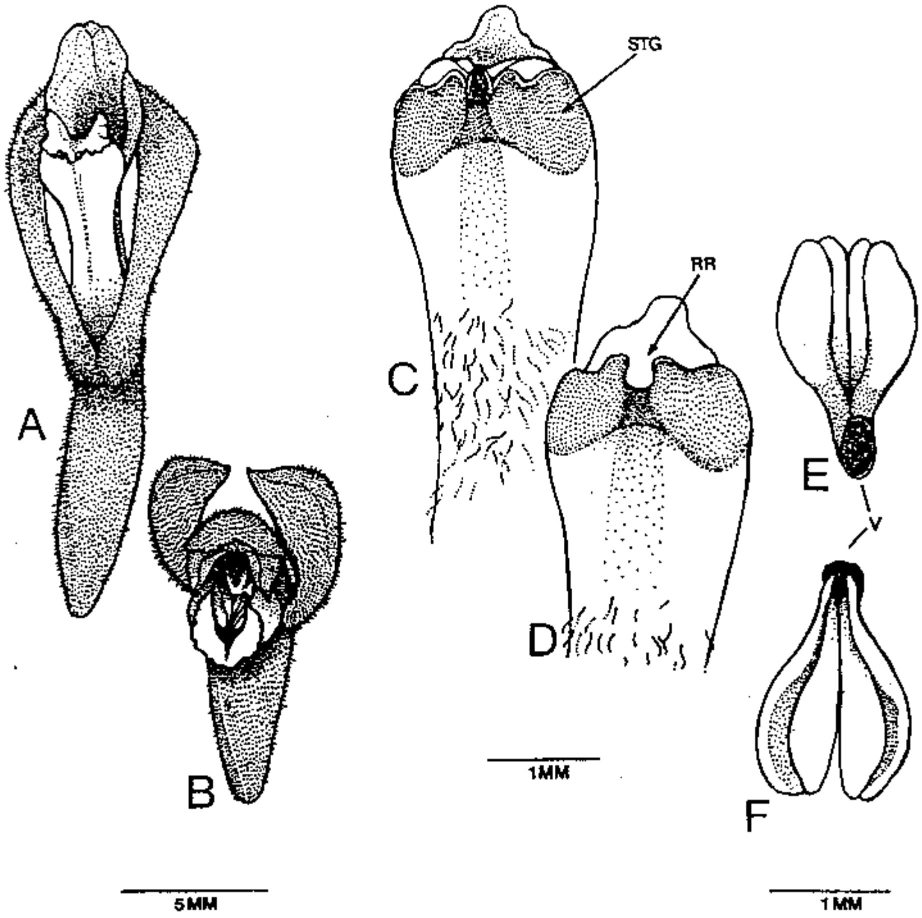

Fig. 1: *Sauroglossum elatum*. Flower features. A-B. Flower. A. Ventral view. B. Frontal view. C-D. Column. C. Intact. D. After pollinarium removal. E-F Pollinarium. E. Ventral view. F. Dorsal view. RR: rostellar remnant. stg; stigmatic surface. v: viscidium.

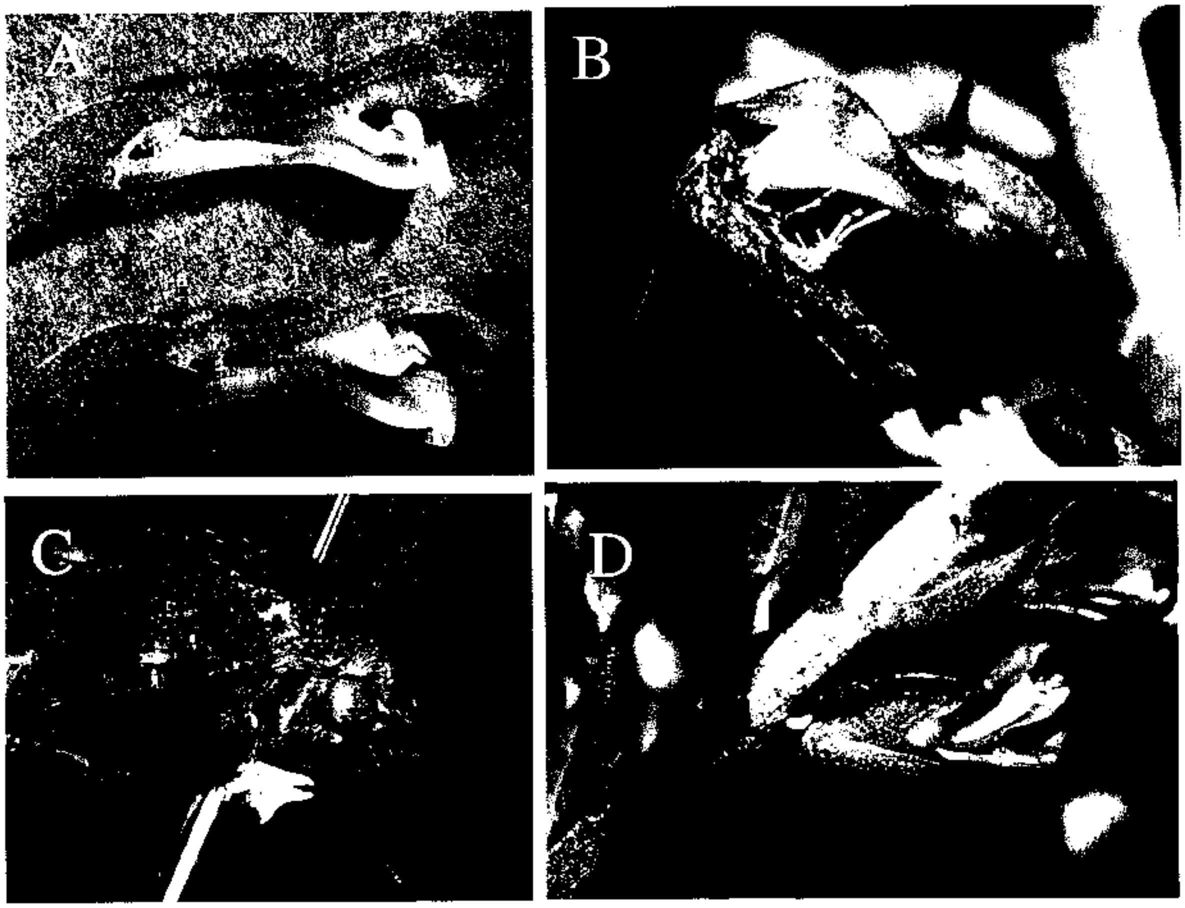

Figure 2. The flowers, their pollinators and visitors. A. Longitudinal cuts of the flower tube showing the male phase (upper flower) and female phase (lower flower). B. Moth probing the flowers. C. Noctuidae moth with pollinarium attached to its proboscis. D. Dermaptera bug with pollinarium fixed on its forelegs

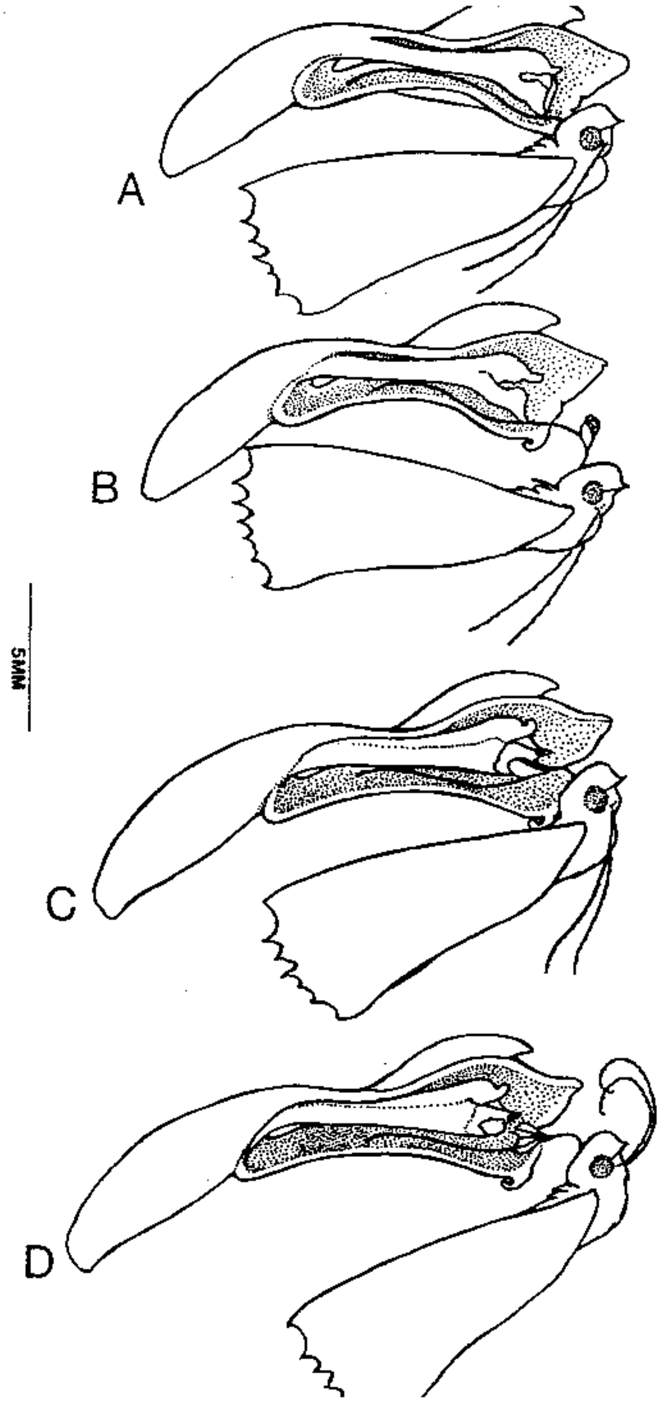

Fig. 3: Pollination mechanism in *Sauroglossum elatum*. A-B. Moth probing a flower in male phase. A. The moth inserts its proboscis to reach the nectar stocked at the column's base. B. The moth dislodges the pollinarium. C-D. A pollinarium-bearing moth visits a flower in female phase. C. The moth inserts its proboscis and brushes the pollinarium against the stigmatic surface. D. Pads of pollen are left on the stigmatic surface.

CAPÍTULO 4:

THE POLLINATION MECHANISM OF THREE SYMPATRIC *PRESCOTTIA* (ORCHIDACEAE: PRESCOTTINAE) SPECIES IN SOUTHEASTERN BRAZIL

Rodrigo B. Singer & Marlies Sazima

Aceito para publicação em Annals of Botany

ABSTRACT

The pollination biology of three *Prescottia* (Orchidaceae: Prescottinae) species was studied in Picinguaba, São Paulo State, southeastern Brazil. Plants are self-compatible but pollinator-dependent and offer nectar as the only reward for the pollinators. *Prescottia plantaginea* Lindl. and *Prescottia stachyodes* Lindl. are pollinated by Pyralid moths (Lepidoptera: Pyralidae). Moth pollination is reported for the first time for genus *Prescottia* and subtribe Prescottinae as well. Pollination by halictid bees (Halictidae) is confirmed for *Prescottia densiflora* Lindl. The pollination mechanism is the same regardless of the kind of pollinator: the pollinaria are fixed on the ventral surface of the insect proboscis through the pad-like viscidium and removed when the insects leave the flowers. A pollinarium carrying insect visiting another flower will brush the stigmatic surface and leave clumps of pollen, thus effecting pollination. *Prescottia stachyodes* is protandrous, a flower feature that increases the chances of cross-pollination. Protandry is, as far as we know, reported for the first time for the genus *Prescottia* and subtribe Prescottinae as well.

Key Words: *Prescottia*, Orchidaceae, Orchids, Prescottinae, moths, Pyralidae, bees, Halictidae, flower morphology, protandry

INTRODUCTION

The neotropical orchid genus *Prescottia* Lindl. contains about 60 species (Dressler 1993), 30 of which have been referred for the Brazilian orchid flora (Hoehne 1945). *Prescottia stachyodes* Lindl. has a noteworthy wide distribution, occuring from northeastern Argentina to Florida (U.S.A) (Ackerman 1995, Correa 1996, Hoehne 1945).

There are only a few pollination reports for subtribe Prescottinae. Wasps and flies have been reported as the pollinators of *Myrosmodes cochleare* (Berry & Calvo 1991) and

Dressler (1993) suggested that the reddish-flowered *Porphyriostachys pilifera* may be pollinated by hummingbirds. Ackermann (1995) suggested the possibility of autogamy in plants of *Prescottia oligantha* (Swartz) Lindl., *P. pellucida* Lindl. and *P. stachyodes* (Swartz) Lindl. occuring in Puerto Rico and the Virgin Islands. More recently, Singer & Cocucci (1999) reported the pollination mechanism in *Prescottia densiflora* Lindl. Halictid bees and (to a lesser degree) syrphid flies were recorded as pollinators (Singer & Cocucci 1999). During our studies on the reproductive biology of southeastern Brazilian orchids, we had the opportunity of studying the pollination biology of three *Prescottia* species, sympatrically occurring in the Brazilian Rain Forest. The aims of the present contribution are: 1) to broaden the knowledge on the pollination biology of South American *Prescottia* species and 2) to highlight the functional flower morphology of this noteworthy orchid genus.

METHODS

Studies were conducted in Picinguaba (Parque Estadual da Serra do Mar, Ubatuba, São Paulo State, ca. 23° 22′ S and 44° 50′ W). Annual rainfall reaches 2600 mm and the annual average temperature is ca. 26° C (Nimer 1977). The climat is tropical-humid (Koeppen 1948) and does not present a well-defined dry-cold season (Nimer 1977). *Prescottia densiflora* Lindl. occurs in natural or man-made grasslands. The studied population occurs in the neighbourhood of the Park base. The study site is surrounded by Atlantic Rain Forest ("Mata Atlântica") vegetation. The pollination biology of this species was monitored through six field visits, from July 23 to July 24, 1999 and October 2 to October 30, 1999. About 15 hs 30 min were spent in observations.

Prescottia plantaginea Lindl. occurs in stands, in grassy or rupicolous, open areas. The studied population occurs near a touristic point locally known as "Casa da Farinha" at an altitude of about 40 m.a.s.l. The pollination biology of this species was studied by means of six field visits, from August 8 to August 14, 2000. About 15 hs 10 min. were spent in observations.

Prescottia stachyodes Lindl. is widespread in the whole Park area, but its pollination biology was studied in a plant stand near "Trilha do Mangue", at about 6 ma.s.l. The surrounding vegetation consists of a short-sized (up to 10 m) forest, locally known as "restinga". The natural pollination of this species was studied by means of five field visits, from June 28 to July 4, 1999 and from June 22 to June 23, 2000. About 22 hs 25 min were spent in observations. The observation period, as a whole, was from 09:00 to 21:00 hs.

In 2000, the fruiting success (as the number of dehiscent fruits divided by the number of produced flowers) was calculated for each species. Twenty inflorescences of *P. densiflora* (totalling 1841 flowers), 8 of *P. plantaginea* (totalling 645 flowers) and 8 inflorescences of *P. stachyodes* (totalling 937 flowers) were then available.

Flower morphology was studied using fresh and pickled (F.A.A 50 %) flowers. Drawings were made using a stereomicroscope with a camera lucida attachement. Plant vouchers are deposited at the Herbarium of the Universidade Estadual de Campinas (UEC). Voucher numbers: *Prescottia densiflora*, R. B. Singer 99/20; *Prescottia plantaginea*, R. B. Singer 99/09; *Prescottia stachyodes*, R. B. Singer 99/08.

The breeding systems were studied through plants cultivated in the greenhouse, at the Universidade Estadual de Campinas. The number of plants per species and flowers per treatment are summarized in Table I. Treatments included untouched flowers (control), emasculation, self-pollination and cross-pollination. Given the peculiar flower morphology (nonresupinate flowers with hidden stigmatic surfaces), the lip (labellum) was removed in order to expose the stigmatic surface. Pollinaria were removed with the help of entomological pins and brushed against the stigmatic surface. Treatments yielding fruits were compared by pairs, trough a $\chi 2$ test (α: 0,05, f. d.: 1). When necessary, Fischer correction was performed. Insect behaviour at flowers was recorded through field notes and photographs. In night-pollinated flowers, insect behaviour was followed with the help of a flashlight, a device which apparently did not disturb insect behaviour. Some insects were captured and sacrified for later identification. Insect vouchers are deposited at ZUEC (Museu de História Natural da Universidade Estadual de Campinas). Throughout this paper, the taxonomical and morphological concepts of Dressler (1993) are followed.

RESULTS AND DISCUSSION

GENERAL PLANT FEATURES: The three studied species share a number of vegetative and flower features: roots are fasciculate and thick. Leaves are rosulate, sessile to petiolate. The inflorescences are terminal, several-flowered spikes (Hoehne 1945). Flower opening is sequential, from the base of the inflorescence toward its top. Flowers are nonresupinate and dome-like (Dressler 1993, Hoehne 1945) (Fig.1A-B, E-F, I-J) . The rostellum is short (Fig. 1C, G and K) and the anther holds a pollinarium made up by four clavate, granulate yellowish pollinia, reduced caudicles and a terminal oblong, pad-like viscidium (Dressler 1993, Rasmussen 1982) (Fig. 1D, H and L). The stigmatic surface is broad, entire to slightly bilobed (Dressler 1993, Rasmussen 1982, Singer & Cocucci 1993) (Fig. 1C, G and K). The flowers offer nectar as the only reward to the pollinators (Singer & Cocucci 1999), which is apparently secreted by the two retrorse lobes at the base of the lip (Dressler 1993). Fruits are capsules bearing a number of minute, dust-like and monoembryonic seeds.

SPECIFIC PLANT FEATURES AND POLLINATION BIOLOGY

Prescottia densiflora: leaves are sessile to atenuate (they were incorrectly referred as "petiolate" in Singer & Cocucci 1999) and broad, but can be absent or somewhat deteriorated during anthesis. The inflorescences are congest and cylindrical and may bear up to 80 flowers, which are whitish (Fig. 2 A-B), sometimes showing delicate pink lines in the sepals. A dense tuft of hairs is visible in the inner surface of the lip, above the column. The flowers emit a musky fragrance during the sunniest hours (Singer & Cocucci 1999) and may keep their fresh appearance for 4-7 days.

Pollination mechanism: our observations confirmed the report of Singer & Cocucci (1999). Halictid bees of genera *Augochlora* and *Pseudoagapostemon* (Fig. 2A-B) were recorded as pollinators. The bees fixed pollinaria on the ventral surface of their proboscis while probing the flowers for nectar. When visiting other flowers, bees carrying pollinaria will brush them against the stigmatic surface, leaving pads of pollen and, thus, effecting pollination (Singer & Cocucci 1999). Bees carrying 1-4 pollinaria were recorded. Syrphid flies (probably of genus *Salpingogaster*) were recorded as flower visitors, but never dislodged pollinaria. Syrphid flies were recorded as co-pollinators of *P. densiflora* in Curitiba (Parana State, Southern Brazil) (Singer & Cocucci 1999). In 2000, about 52.4 % of the flowers (966/1841) set fruits and individual fruiting success ranged from 18 to 90.2 %.

Prescottia plantaginea: leaves are linear to slightly atenuate, light green in colour and often somewhat deteriorated during anthesis. The inflorescences bear up to 120 flowers. Flowers are coriaceous and light green in colour (fig. 2 C). Just after dusk (ca. 18:00 hs.) the flowers emit a sweet, spicy fragrance that can be perceived until 23-24:00 hs. Nocturnal scent emition, 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 Pyralidae moths (two morphoespecies) (Fig. 2 C-D). The moths often displayed a quite passive behaviour, staying for 2-45 minutes on a given inflorescence. They often probed several flowers, but also perched at the inflorescences without exploring the flowers. Pollination mechanism is the following: when the insects probe the flowers searching for nectar, they press the ventral surface of their proboscis against the viscidium, thus fixing the pollinarium. The pollinarium is removed when the moths leave the flower. Pyralid moths were sighted carrying only one pollinarium. A pollinarium-carrying insect that visits another flower will brush the pollinarium (or pollinaria) against the broad stigmatic surface leaving pads of pollen, thus pollinating the flower. In several instances the moths failed in dislodging pollinaria. It seems that in many occasions the slender moth proboscis enters the spur in such an angle that viscidia are not disturbed. Unidentified Geometridae moths were frequently sighted at flowers, but they never removed pollinaria. In 2000, about 30 % of the flowers (193/645) set fruits and individual fruiting success ranged from 0 to 70.5 %.

Prescottia stachyodes: leaves are erect, long-petiolated and lanceolate in shape, being light green in colour, with a whitish median rib. Inflorescences are high, up to 140 cm in length, and may bear up to 180 flowers each. Flowers are protandrous (Fig.3 A-B) and light green in colour (Fig. 2 E). From flower opening and for about three days, the column is presented directed toward the lip (Fig.3 A-B). At this stage, the stigmatic surface is inaccessible and the flower can only act as pollen-donor. After this stage, the column gradually acquires an opposite position, pointed toward the flower entrance (Fig.3 C-D) Now, the stigmatic surface is completely exposed. At this stage, the viscidium is dry and the flower can only act as pollen receiver. The flowers may keep their fresh appereance for about 10-12 days. They emit a strong, sweet and spicy fragrance from dusk (ca. 18 hs) and during the night hours.

Pollination mechanism: Two morphospecies of Pyralidae moths were recorded as pollinators (Fig. 2 E-F). The moths visited the inflorescences for 15-120 seconds, probing several flowers. Insects removed pollinaria when visiting the younger (in male phase) (Fig.3A-B) flowers, toward the inflorescence apex. Except for the protandry, the pollination mechanism is much the same as that of *P. plantaginea*. Moths carrying pollinaria (only insects carrying one pollinarium were sighted) which visited the oldest (in female phase) flowers at the base of the inflorescence, brushed the pollinaria against the broad stigmatic surface, leaving pads of pollen and effecting pollination (Fig.3C-D). In 2000, about 78.65 % (737/937) of the available flowers set fruits and fruiting success per individual ranged from 52.20 to 98.49 %.

BREEDING SYSTEM: results are summarized in Table I. Plants are self-compatible but pollinator-dependent. This means that pollinators are needed by the plants to set fruits. Only *P. densiflora* scored signifficant differences favouring cross over self-pollination (Table 1). This seems to suggest that imbreeding depression could be present in *P. densiflora*. However, larger samplings, with more individuals and repetitions are necesary in order to make conclusions with more confidence. To our knowledge, there are no previous reports on the breeding systems of Prescottinae orchids. Since the plants are self-compatible and the pollinators often visit several flowers of the same inflorescence, part of the fruit set may be due to self-pollination in a broader sense (geitonogamy). Self-pollination in the strictest sense (pollen of the same flower deposited on 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. First, the texture of the pollinarium, which is granular, allows clumps of pollen (instead of whole pollinia) to be left on the stigmatic surfaces. This means that several flowers can be pollinated by the pollen content of one pollinarium (Singer & Cocucci 1999, Dressler 1993). Second, the stigmatic surfaces are broad and slightly convex. This increases the chances of receiving multiple pollen loads (i.e. either from the same inflorescence or not) and, consequently of cross-pollination. In *P. stachyodes* the protandry clearly increases the chances of cross-pollination. Since the younger and uppermost flowers act as pollen donors, insects will fix pollinaria almost when leaving the inflorescence, thus reducing the chances of self-pollination. Insects bearing pollinaria that visit another inflorescence will probably start their visits at the basalmost and older flowers of an inflorescence. These flowers may be in the female phase and will receive pollen pads on their exposed stigmatic surfaces, thus being pollinated. There is still a probability that moths with fixed pollinaria return to the basal (in female phase) flowers, thus promoting self-pollination (geitonogamy). During our observations, this behaviour was only recorded in *P. plantaginea*, which is non-protandrous. Remarkably, *P. densiflora* and *P. stachyodes* displayed surprisingly high fruiting successes (52.4 % and 78.65%, respectively). In a recent review on orchid fruiting success, Neiland & Wilcock (1998) suggested that tropical orchids, either nectarless or nectariferous, display fruiting successes lower than 50 %. In our opinion, a combination of all the above commented factors may account for the high fruit sets.

Table 1: Percentage of fruiting per treatment and species. Numbers in parenthesis represent the number of fuits obtained over the number of flowers used in each treatment. N: number of individuals per species

	N	Control	Emasculation	Self-pollin.	Cross-pollin.
P. densiflora	4	0 (0/ 286)	0 (0/25)	69.2 (18/26)	100 (20/20)
P. plantaginea	4	0 (0/ 126)	0 (0/ 32)	48.04 (49/102)	48.1 (25/52)
P. stachyodes	9	0 (0/ 937)	0 (0/ 211)	93.7 (209/223)	95.9 (209/218)

$\chi 2$ values= *P.densiflora* : 7.47 (significant, P: 0.0065). *P. plantaginea*: 0 (non-significant, P : 0.99). *P. stachyodes*: 1. 06 (non-significant, P> 0.5)

CONCLUSION

Moth pollination is here reported for the first time for the genus *Prescottia* as well as for subtribe *Prescottinae*. Previous reports indicate Calliphorid flies and wasps as co-pollinators of *Myrosmodes cochleare* (Berry & Calvo 1991) and sweat bees (Halictidae) and syrphid flies as co-pollinators of *Prescottia densiflora* (Singer & Cocucci 1999). This later report is here confirmed. This is, as far as we know, the first report of protandry in the genus *Prescottia* and subtribe Prescottinae as well. Protandry does also occur in the rupicolous *P. montana* Barb.Rodr. This species is, according to our preliminar observations, self-compatible but pollinator-dependent. This species does also show flower traits suggesting moth-pollination, such as greenish, night-scented flowers.

Protandry in terrestrial orchids has traditionally been associated with Bumble-bee pollination (Catling 1983 and references therein), since Bumble-bees tend to sistematically visit the inflorescences from the base (with flowers in female phase) toward the apex (with flowers in male phase). Owing to the evidence here presented, this idea needs reconsideration.

Protandry is also known to occur in orchid genera of subtribes Spiranthinae (Catling & Catling 1991, Catling 1983 and references therein) and Goodyerinae (Ackermann 1975, and references therein). According to Dressler (1993), the three orchid subtribes, (Goodyerinae, Prescottinae and Spiranthinae) are phylogenetically related and were included in the tribe Cranichideae, which also includes subtribes Manniellinae, Pachyplectroninae and Cranichidinae (Dressler 1993). The orchid subtribes Spiranthinae and Prescottinae probably are sister groups and share a number of features, such as the granular texture of their pollinaria and velamen type (Dressler 1993). The genus *Prescottia* does also show the two retrorse lobules of the lip (probably nectaries) which are typical in subtribe Spiranthinae (Dressler 1993). Either in subtribe Spiranthinae (Catling 1983) or Goodyerinae (Ackermann 1975) protandry involves column movements. At least in *Spiranthes* (Spiranthinae), column movements are produced through the enlargement of cells at the base of the column (Catling 1983). In *Spiranthes*, cells of the base of the lip may also suffer enlargement (Catling 1983). After checking several pickled flowers (in F.A.A., 50 %) of *P. densiflora* and *P. plantaginea*, we did not find evidence of protandry (in the form of column displacement) for these two species. However, we must remember that protandry does not only involve column displacement, but viscidium drying as well (Catling 1983, Ackerman 1975). It is possible that species lacking the column displacement present viscidium drying at some stage of the flower. This question needs further investigation. Anatomical studies are needed in order to check if the protandry verified in *Prescottia stachyodes* is homologous to these previously recorded in subtribes Spiranthinae (Catling 1983) and Goodyerinae (Ackermann 1975). If so, the evidence for relatedness of the three orchid subtribes will be reinforced.

It could be interesting to perform a better sampling in order to asess how widespread is protandry either in genus *Prescottia* or in subtribe Prescottinae as a whole. Then, the taxonomical and phylogenetic value of this flower feature could be fully understood.

Obviously, further studies are needed, but it is tempting to suggest that protandry could have been present in a common ancestor or evolved early in tribe Cranichideae, thus representing a primitive condition.

Acknowledgements:

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REFERENCES

Ackermann J. D. 1975. Reproductive biology of Goodyer*a oblongifolia* (Orchidaceae). Madroño 23: 191-198.

Ackermann J. D. 1995. An orhid flora of Puerto Rico and The Virgin Islands. Mem. Of NewYork Bot. Gard. 73. Bronx. New York.

Berry, P. E. & Calvo, R. N. 1991. Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleare* (Orchidaceae). Pl. Syst. Evol. 174: 93-101.

Catling, P. M. 1983. Pollination of northeastern North American Spiranthes (Orchidaceae). Canad. J. Bot.61: 1080-1093.

Catling P. M. & V. R. Catling. 1991. A synopsis of breeding systems and pollination in North American orchids. Lindleyana 6(4): 187-210.

Correa M. N. 1996. Orchidaceae. In: F. Zuloaga & O. Morrone (edits). Catálogo de las plantas vasculares de la República Argentina Vol. I. Monographs in Systematic Botany from the Missouri Botanical Garden v. 60. 323 pp.

Dressler 1981. The Orchids.Natural History and Classification. Cambridge, Massachussets. Harvard University Press.

Dressler R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press.

Portland, Or.

Hoehne F. C. 1945. Orchidaceae: In. F. C. Hoehne (edit.) Flora Brasilica. Fasc. 8. Vol XII (2).

Koeppen W. 1948. Climatologia: com un estudio de los climas de la tierra. Fondo de Cultura Económica, México.

Neiland M. R. M. & C. C. Wilcock. 1998. Fruit set, nectar reward and rarity in the Orchidaceae. Am. J. Bot 85 (12): 1657-1671.

Nimer E. 1977. Climatologia da região sudeste do Brasil. Rev. Bras. Geogr. 34:3-48.

Rasmussen F. 1982. The gynostemium of the neottioid orchids. Opera Botanica 65. 96 pp.

Singer R. B. & A. A. Cocucci. 1999. Pollination mechanism in southern Brazilian orchids which are exclusively or mainly pollinated by halictid bees. Pl. Syst. Evol.217: 101-117.

Van Der Pijl & C. H. Dodson 1966. Orchid flowers: their pollination and evolution. University of Miami Press. Coral Gables, Fla.

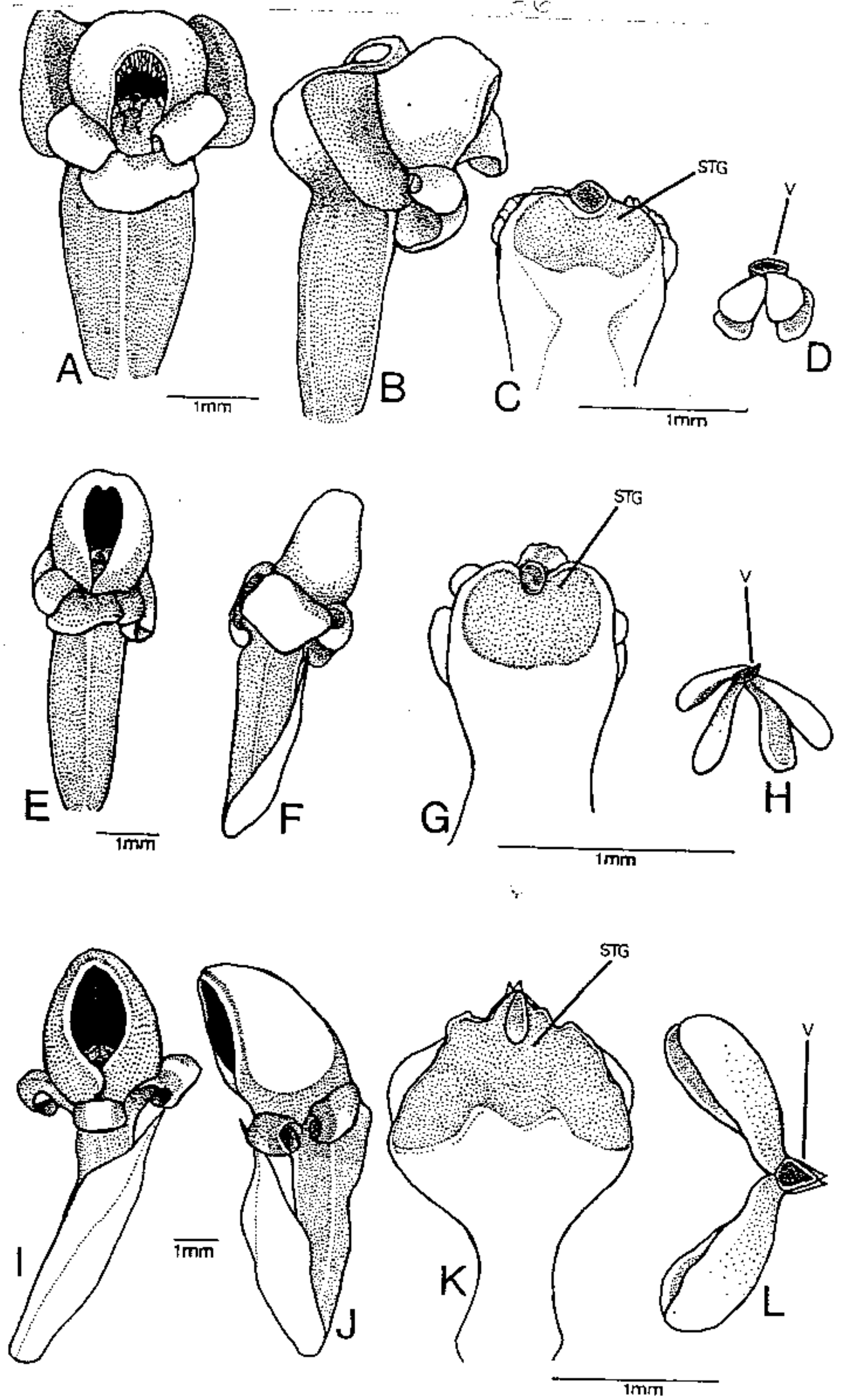

Figure 1. Flower features. A-C: *Prescottia densiflora*. A-B: flower. A: frontal view. B: lateral view. C: column, ventral view. D: pollinarium. E-H: *Prescottia plantaginea*. E-F: flower. E: frontal view. F: lateral view. G: column, ventral view. H: pollinarium. I-L: *Prescottia stachyodes*. I-J: flower. I: frontal view. J: lateral view. K: column, ventral view. L: pollinarium. **stg**: stigmatic surface. **v**: viscidium.

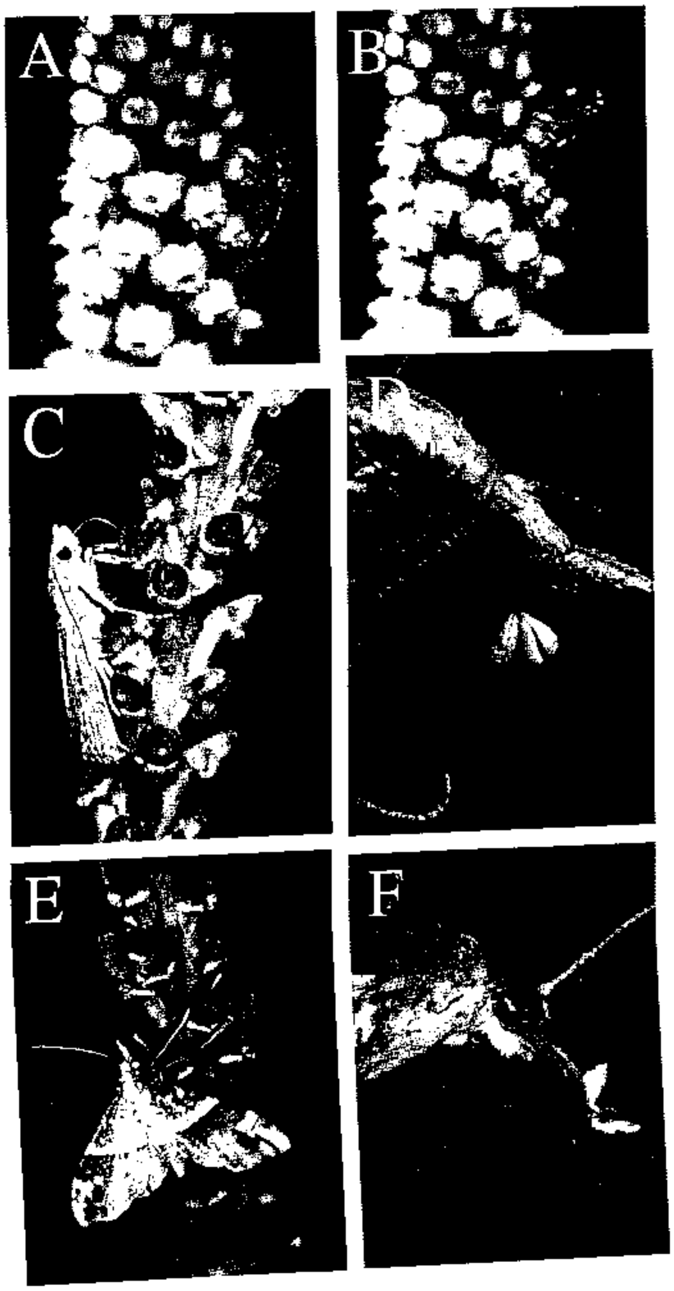

Figure 2. The flowers and their pollinators. A-B. Halictid bee visiting *P. densiflora*. A. Bee probing a flower. B. Bee dislodging a pollinarium. C. Pyralid moth probing the flowers of *P. plantaginea*. D. Moth with a pollinarium of *P. plantaginea* fixed on its proboscis. E. Pyralid moth visiting *P. stachyodes*. F. Pyralid moth with a pollinarium of *P. stachyodes* fixed on its proboscis

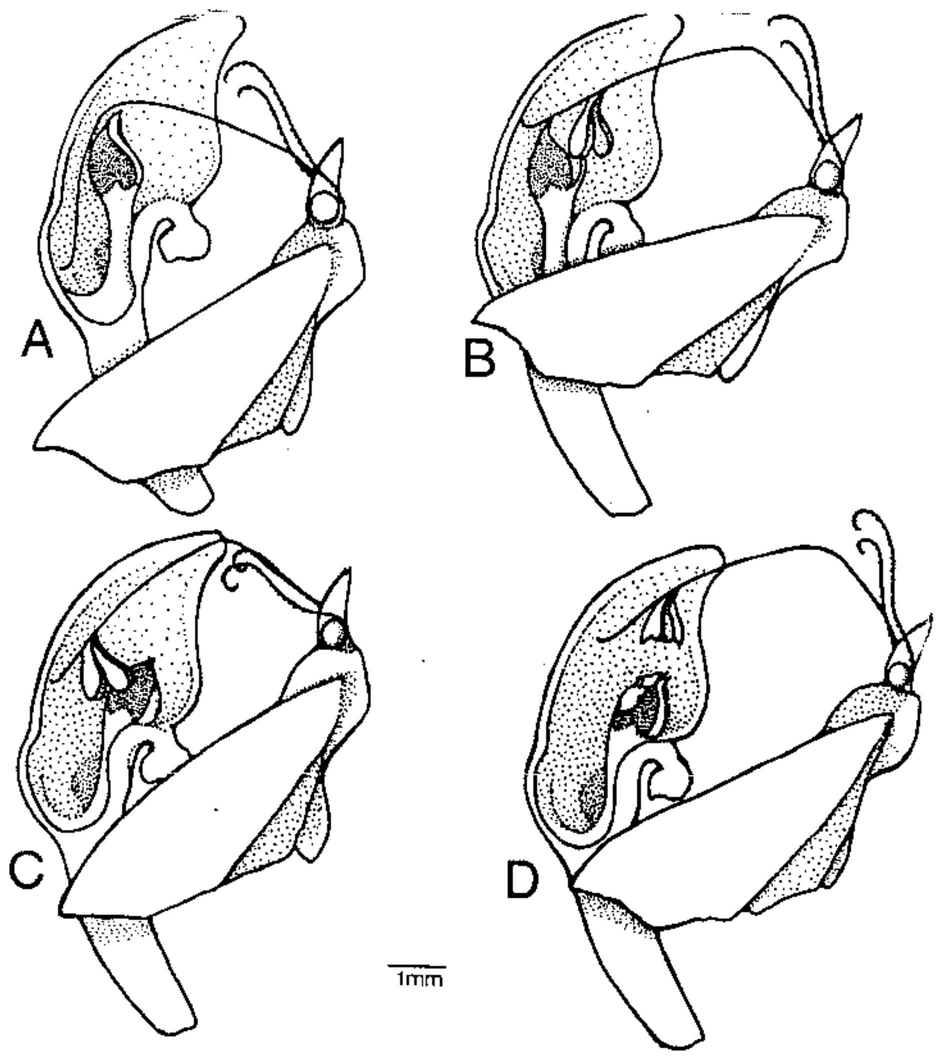

Figure 3. Pollination mechanism in *P. stachyodes*. A: Pyralid moth probes a flower in male phase. B: The insect removes the pollinarium. C: A pollinarium-bearing moth probes a flower in the female phase. D: The stigmatic surface is brushed by the pollinarium and pads of pollen are deposited.

CAPÍTULO 5:

FLOWER MORPHOLOGY, POLLINATORS AND FLOWER VISITORS OF THREE SYMPATRIC GOODYERINAE ORCHIDS FROM SOUTHEASTERN BRAZIL

Rodrigo B. Singer & Marlies Sazima

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ABSTRACT:

The pollination biology of *Aspidogyne argentea*, *Aspidogyne longicornu* and *Erythrodes arietina* was studied in southeastern Brazil. The three species are self-compatible, but pollinator-dependent. These three orchid species offer nectar as the only reward to the pollinators and flower visitors. The two species of *Aspidogyne* have a dorsally-adhesive viscidium, a feature which precludes pollinators other than bees. *Erythrodes arietina* flowers are protandrous and show a ventrally-adhesive viscidium. *Aspidogyne argentea* is visited by halictid bees (here ranked as probable pollinators) and Hesperiid butterflies. *Aspidogyne longicornu* is pollinated by females of *Euglossa* (Euglossini) and visited by the hummingbird *Phaethornis ruber* and by the Euglossini bees *Eulaema seabrai* (females) and *Eulaema cingulata* (males). The pollinarium adheres to the ventral surface of the bee labrum, a very difficult place for the bees to clean. The dorsally-adhesive viscidium in *Aspidogyne* parallels those of the Spiranthinae genera of the so-called "Pelexia alliance". This condition seems to be particularly adaptative under conditions of low-frequence pollinator visits. *Erythrodes arietina* is pollinated by bees of the genera *Paratetrapedia* and *Osiris* which fix the pollinarium on the dorsal surface of their proboscis. Occasionally, these bees removed the pollinaria from their mouthparts by pushing them with the forelegs. In general, in the three studied species, a combination of both, pollinator behaviour and flower morphological peculiarities promote cross-pollination.

Key Words: Orchids, Goodyerinae, *Aspidogyne*, *Erythrodes*, pollination, bees, hummingbirds, butterflies, morphology, viscidium, protandry

INTRODUCTION

Orchids of subtribe Goodyerinae are widespread and particularly diverse in the tropics, specially in tropical Asia (Dressler 1993). All brazilian Goodyerinae orchids were formerly classified under the genus *Physurus* L. C. Rich. (Hoehne 1945) or under the genus *Erythrodes* Blume (Pabst & Dungs 1975). However, Garay (1977) noticed significant morphological differences among the neotropical Goodyerinae and proposed a number of generic segregates, mostly based on rostellar structure. Only a few species of *Goodyera* (Ackerman 1982, Kallunki 1981, Wong & Sun 1999) and *Ludisia* (Van der Pijl & Dodson 1966) have been studied in their reproductive systems or pollination biologies and, as far as we know, there are no studies concerning neotropical species. Studies on *Goodyera* species, showed that these plants are pollinated by Bumble-bees (Apidae) (Ackerman 1975, Kallunki 1981) and *Ludisia* is butterfly-pollinated (Van der Pijl & Dodson 1966). More recently, Ackerman (1995) suggested that a few *Erythrodes* species from Puerto Rico and the Virgin Islands could be autogamous, owing to their relatively high fruit set.

During our studies on the pollination biology of some Brazilian species of the genera *Aspidogyne* Garay and *Erythrodes* Rchb. f., we noticed significant differences in the way the orchid rostellum works during the pollination process. The purpose of the present contribution is thus twofold: 1) to present the first data concerning the pollination biology of neotropical Goodyerinae and 2) to highlight the rostellar differences which promote different pollination mechanisms.

METHODS

The study was conducted in the Brazilian Atlantic Rain Forest at the Reserve of Picinguaba, Municipality of Ubatuba (ca. 23°22´S, 44°50´W), São Paulo State, southeastern Brazil. Annual rainfall reaches 2600 mm and the annual average temperature is ca. 26° C (Nimer 1977). The climat is tropical-humid (Koeppen 1948) and without a well-defined dry-cold season (Nimer 1977). *Aspidogyne argentea* (Vell.) Garay and *A. longicornu* (Cogn.) Garay, are syntopic in the understorey of the shrubby formation known as "restingas ", at ca. 5 m.a.s.l. *Erythrodes arietina* (Rchb. f. & Warm.) Ames, occurs in the understorey of well-defined montane forest formations at about 60 m.a.s.l.

The pollination biology of these three species was studied during several field visits. Phases of anthesis and other flower characteristics were observed *in situ*. Flowers were watched for visitors at different times of the day, from early morning (6:00 hs) until late afternoon (ca. 18:00 hs).The pollination of *A. argentea* was studied by means of eight field visits, from September 26 to September 29, 1999; and from October 1 to October 30, 1999. A total of 36 hs 40 min were spent in observations. The pollination biology of *A. longicornu* was studied by means of 28 field visits, from 22 April-10 June 1998, 30 March-26 April 1999 and 8-10 May 2000. A total of 120 observation hours were spent with this orchid species. The pollination of *E. arietina* was studied by means of seven field visits, from 20-25 October 1998 and 30-31 October 2000. A total of 33 hs 30 min of observation hours were spent with this orchid species. The behaviour of both pollinators and flower visitors was recorded in the field through notes and photographs. Some insects were captured for identification and vouchers are deposited at the Laboratório de Abelhas da Universidade de São Paulo (USP) and at the Moure collection (Universidade Federal do Paraná). Plant vouchers are deposited at the Herbarium of the Universidade Estadual de Campinas (UEC). Voucher numbers: *Aspidogyne argentea*, R. B. Singer 99/06; *Aspidogyne longicornu*, R. B. Singer 98/42; *Erythrodes arietina*, R. B. Singer 98/104.

In 1999, the fruting success (dehiscent fruits divided by the number of flowers produced) was calculated for the three orchid species. Seven inflorescences of *A. argentea* (totalling 135 flowers), 40 of *A. longicornu* (totalling 1794 flowers) and seven inflorescences of *E. arietina* (totalling 571 flowers) were then available.

Breeding systems were studied on plants cultivated in the greenhouse, at the Universidade Estadual de Campinas (Unicamp). The number of plants per species and flowers per treatment are summarized in Table 1. Treatments included untouched flowers (control), self-pollination, cross-pollination and emasculation. Cross-pollination could not be performed in *E. arietina* since the few inflorescences obtained flowered at considerably separated times. Treatments yielding fruits were compared by pairs, trough a $\chi 2$ test (α: 0,05, f. d.: 1). When necessary, Fischer correction was performed.

Flower features were drawn using fresh flowers, with the help of a stereomicroscope with a camera lucida attachment. Flower morphology terminology and general orchid taxonomy follow Dressler (1993) and generic Goodyerinae concepts follow Garay (1977).

RESULTS AND DISCUSSION

General flower features

The present contribution deals mainly with rostellar structure and function. Readers interested in perianth parts shape and measurements are referred to Hoehne (1945) and Pabst & Dungs (1975). The plants show the terrestrial, creeping habit usually found in Goodyerinae (Dressler 1993) and the inflorescences are terminal spikes. The flowers are resupinate and more or less tubulose in shape, bearing noticeable spurs (Fig. 1 A, E and I), where nectar is accumulated and offered to the flower visitors. The flowers of the three species show an indument of hairs (Fig. 1 A, E and I). In the studied species, the column is erect and the rostellum is acute (Fig. 1 B, F and J). The pollinarium is composed of two bipartite pollinia, hard caudicles (Rasmussen 1982, 1986) and a well-developed viscidium (Fig. 1 D, H and L). In the two *Aspidogyne* species, the apex of the lanceolate rostellum detaches as a viscidium, leaving a blunt rostellar remnant (Fig. 1 C and G). In *Erythrodes arietina*, the long viscidium detaches leaving a bifid rostellar remnant (Fig. 1 K). The pollinia consists of a myriad of laminar subunits (Fig. 1 D, H and L) known as "massulae"; condition which is the rule among the Goodyerinae orchids (although not exclusive of this orchid group). Such pollinaria are known as "sectile" (Dressler 1993, Freudenstein & Rasmussen 1997). The stigmatic surface is flat to slightly convex. In the two *Aspidogyne* species the stigmatic surface is broad and almost entire in shape (Fig. 1 B and F). In *E. arietina*, the stigmatic surface is deeply bilobed (Fig. 1 J).

SPECIFIC FLOWER FEATURES AND POLLINATION MECHANISMS

Aspidogyne argentea: The flowers are whitish with noticeable purple dots in the lateral petals and sepals, measuring about 9 mm in length. During the sunniest hours, the flowers emit a sweet, musky fragrance. The viscidium in these flowers is adhesive only through its dorsal surface. Flowers may keep their fresh appearance for 5-10 days.

Pollination mechanism

A number of flower visitors were recorded at the flowers, but further observations are needed to confirm which one is the legitimate pollinator. Insect visits to the flowers occurred during the sunniest hours (10-14 hs), coinciding with the emission of stronger flower fragrances. Hesperiidae butterflies were recorded several times at the flowers, probing many flowers in a single inflorescence and visiting 1-3 inflorescences in the population, spending up to 60-70 seconds as a whole. Anyhow, in no instance the butterflies removed any pollinaria. The small meliponid *Plebeia* cf. *droryana* was occasionally sighted at flowers, but this bee is too small to pollinate them. Medium to relatively big-sized sweat bees (Halictidae) of the genera *Pseudoaugochlora* and *Augochloropsis* were also sighted at flowers. These bees probed the flowers for nectar and it seems that they may be the legitimate pollinators. The few (2) bees we could capture lacked pollinaria, which were possibly lost in the net. Halictid visits to the flowers were very infrequent (1-3 per day) and occurred between 10-14 hs. These bees visited a few (1-3) flowers per inflorescence and 1-2 inflorescences per visit to the population, spending, as a whole, around 5-10 seconds. Small sweat bees of the genus *Agapostemon* were very frequent at flowers, but are also too small to pollinate them. As the viscidium is adhesive through its dorsal surface, it can only be fixed when dorsally pressed. This feature precludes pollinators other than bees (Singer & Sazima 1999). When the bees probe this kind of flowers, they extend their mouth-parts and press the dorsal surface of the viscidium with the labrum. The viscidium then adheres to the ventral surface of the labrum and the pollinarium is removed when the insect leaves the flower (Singer & Sazima 1999). Other flower-visiting animals such as the Hesperidae butterflies, which lack a labrum, only exert pressure against the ventral surface of the column, without fixing or removing pollinaria. Since the only appropriately sized bees we noticed at the flowers were halictids, we support that further fieldwork will confirm these bees as the legitimate pollinators of this orchid species.

In 1999, about 11.11 % of the available flowers (15/135; n: 7 inflorescences) set fruits and the individual fruting success ranged from 0 to 33.33%.

Aspidogyne longicornu: The flowers are whitish and remarkably long-spurred, about 20 mm in length. The spur itself measures around 25-26 mm in length. During the hottest hours the flowers emit a musky, somewhat unpleasant fragrance, which is better perceived by enclosing the flowers in a vial. As for A. *argentea*, the viscidium is adhesive through its dorsal surface. Flowers may keep their fresh appereance for 4-5 days.

Pollination mechanism: Diverse visitors were recorded at the flowers, always during the day-hours. Pollinators are females of *Euglossa chalybeata iopoecila* Dressler and of *Euglossa* cf. *ignita* F. Smith (both, Apidae; Euglossini) (Fig. 2 A-B), which were sighted at flowers from 6:00 to 14:00 hs. These bees are noticeably infrequent at the flowers, consecutive bee visits being separated by 40-120 min intervals. This pollinator infrequence may be related to both 1) pollinators scarcity and 2) trapliner habit (Janzen 1971). The bees normally visited a few flowers (1-3) per inflorescence and 1-5 inflorescences per visit to the population, spending 15-60 seconds as a whole. Those *Euglossas* hover facing the inflorescences, land and extend their mouth-parts in order to probe a flower. As they insert their mouth parts and press the dorsal surface of the viscidium with the ventral surface of their labrum, thus fixing the pollinarium (Fig. 2 B). The pollinarium is removed when the bees leave the flowers. Since the labrum is bent when the bees retract their mouth-parts, the pollinarium remains protected under the head, much in the same way as the Spiranthinae orchids of the "Pelexia alliance" (Singer & Sazima 1999). When a pollinarium-bearing *Euglossa* visits other flowers, the distal parts of the pollinarium touch the stigmatic surface and clumps of massulae are then deposited. The size and shape of the fixed pollinarium, thus avoiding the waste of pollinaria and enhancing the chances of cross-pollination. Only bees carrying a single pollinarium were watched. Since the pollinarium is friable and made up by numerous massulae, many flowers may be pollinated by the pollen content of a single pollinarium (Dressler 1993, Freudenstein & Rasmussen 1997).

The flowers of *A. longicornu* are frequently visited by the hermit hummingbird *Phaethornis ruber* (Phaethorninae) (Fig. 2 C). Consecutive hummingbird visits occured at 20-60 min. intervals. In all cases, the hummingbirds sistematically visited all the fresh-looking flowers, spending up to 120 seconds per visit to the population. The hummingbirds hover in front of the inflorescences, inserting the tip of their bill to lick the nectar (Fig. 2 C). The flowers visited showed that the birds almost emptied most of their spurs. But the birds never removed pollinaria. Although visiting the flowers in a legitimate way, *P. ruber* acts as a nectar thief (Buzato et al., in press) of this orchid species. This can be explained by the nature of the viscidium, which is adhesive only through its dorsal surface. Lacking labrum-like structures suited to exert a dorsal pressure against the viscidium, hummingbirds do not fix pollinaria. The hummingbirds clearly defended the plant population as a foraging patch and showed a territorialist behaviour against the Euglossinis, when both kinds of flower visitors met at the patch. Phaethorninae hummingbirds are said to show a trapliner habit (Sazima et al. 1996, and references therein), but the territorial behaviour of *P. ruber* at these flowers may be explained by the fact that the other flower visitors were bees. Territorialism in *P. ruber* was recently reported by San Martin-Gajardo & Freitas (1999). Another explanation may be related to the flowering period of *A. longicornu*, which flowers during a period of resource scarcity for this hummingbird species. It seems plausible that both the aggressive behaviour of the hummingbirds toward the Euglossinis and the nectar remotion performed by this bird may have a deleterious effect for the reproductive success of this orchid species.

Other flower visitors were females of *Eulaema seabrai* and, more sporadically, males of *Eulaema cingulata* (Apidae: Euglossini) (Fig. 2 D). These bees do not remove the pollinaria since their extended mouthparts are longer than those of the *Euglossa*s and they do not press their labrum against the viscidium. *Phaethornis ruber* did also defend the flowers against these big bees, when both kinds of animals met at flowers. Hesperidae butterflies were occasionally sighted at flowers and for the same reasons cited in *A. argentea*, these insects did not remove pollinaria.

In 1999, about 18 % of the available flowers (323/1794; n: 40 inflorescences) set fruits and the fruiting success per inflorescence ranged from 4.76 to 43.24%.

Erythrodes arietina. The flowers are brownish-white, about 11 mm in length and notably tubulose in shape. They emitt a sweet, though slight fragrance during the day's hottest hours. The flowers are protandrous, with the male phase extending for 1-2 days. In this phase, the column is in a lower position and the flower entrance is very narrow. At this stage the viscidium is adhesive and light gray in colour. After that, the column gradually acquires an uppermost position, exposing its stigmatic surface. The viscidium then becomes dry and shows a dark colouration. Flowers may keep their fresh appearance for about 5 days.

Pollination mechanism: Only legitimate pollinators were sighted at flowers, and these were two bee species of the genus *Paratetrapedia* (Apidae: Tapinotaspidini) and one species of *Osiris*. (Apidae: Osirini) (Fig. 2 E-F). All visits were recorded between 10:00 and 15:00 hs. Consecutive bee visits to the population were separated by 5-120 min intervals. The pollination mechanism is in essence similar to that previously recorded for *Goodyera* species (Ackerman 1982, Kallunki 1981). Pollinaria are removed when a bee visits the uppermost (and younger) flowers, in the male phase. When the insect probes the flowers by introducing their mouth-parts, the pollinarium adheres on the dorsal surface (galeae) of the proboscis (Fig. 2 F, 3 A). The pollinarium is removed when the insect leaves the flower (Fig. 3 B). When a pollinarium-bearing bee visits the lowermost (and older) fresh flowers of an inflorescence, the pollinarium touches the exposed stigmatic surface of the flowers in the female phase (Fig. 3 C), where clumps of massulae are left (Fig. 3 D). Insects visited 1-3 flowers per inflorescence and 1-3 inflorescences per visit to the population, spending up to 20 seconds as a whole. As for the *Goodyera* species (Ackerman 1982, Kallunki 1981), protandry in *E. arietina* promotes cross-pollination. Most recorded insects explored the inflorescences of *E. arietina* from the lowermost flowers toward the apex, without returning to the base, but not as sistematically as the Bumble-bees recorded at *Goodyera* species (Ackerman 1982, Kallunki 1981). This means that the bees occasionally "missed" fresh-looking flowers in their way upward. Only the uppermost and younger flowers in an inflorescence act as pollen donors; pollinaria are then removed when the insect is almost leaving the inflorescence. Thus, when this pollinarium-bearing insect visits another inflorescence, chances are that the first and lowermost flowers it explores will be in the female phase and may be pollinated by the pollen of the preceding inflorescence. Only bees carrying a single pollinarium were observed.

Remarkably, an interesting behaviour was occasionally recorded: both *Osiris* and *Paratetrapedia* bees carrying pollinaria landed at a point of the surrounding vegetation (e.g.: a leaf) and actively removed the pollinaria by extending their mouth-parts and pressing them with their forelegs.

Osiris bees have been recorded as pollinators of some *Palmorchis* (Palmorchideae) species from Panama (Dressler 1984). Significantly, *Osiris* bees are parasitic and *Paratetrapedia* bees are suspected to be one of their hosts (Shanks 1986).

In 1999, about 22.6 % of the available flowers (129/571; n: 7 inflorescences) set fruits.

Fruting success per inflorescence ranged from 9.72 to 40 %.

BREEDING SYSTEMS

The three studied species are self-compatible but pollinator-dependent (Table 1). No untouched or emasculated flowers ever set fruits. These results comply with previous published reports involving species of the genus *Goodyera* (Ackerman 1975, Kallunki 1981, Wong & Sun 1999). In other words, in the three studied species, pollinators are required for the plants to set fruits and seeds. At least *A. argentea* showed a noticeably higher fruit set through cross-pollination (Table 1), suggesting that imbreeding depression may express in this orchis species. However, further studies are desirable in order to get stronger evidence. Anyhow, in the three studied species the behaviour of the flower visitors which tend to visit only a few flowers per inflorescence plus the morphological peculiarities of the flowers (namely, the protandry in *E. arietina* and the dorsally adhesive viscidium of the two *Aspidogyne* species), clearly promote cross-pollination.

Ackerman (1995) suggested that some *Erythrodes* species from Puerto Rico and the Virgin Islands could be autogamous owing to its apparently high fruiting success. Among the Brazilian Goodyerinae orchids we studied, there is at least, one case suggesting the possibility of autogamy (automatic self-pollination). While studying fresh flowers of *Erythrodes austrobrasiliensis* (Porsch) Pabst, we noticed significant rostellar modifications: in this orchid species the rostellum is remarkably short and the viscidium appears already completely dry and separated from the rest of the rostellum. The viscidium is thus non-adhesive and hard, surrounded by hard caudiculae. When we removed pollinaria from the clinandrium, we noticed that many massulae remained at the clinandrium apparently wetted by the abundant stigmatic surface. It seems that owing to the rostellar reduction and narrowing, the sides of the pollinarium are passively wetted by the stigmatic secretion. A similar condition was found by Catling (1987) for some autogamous forms of *Stenorrhynchos lanceolatus* (as *Saccoila lanceolata*).

Table 1: Percentage of fruiting per treatment and species. Numbers in parenthesis represent the number of fuits obtained over the number of flowers used in each treatment. N: number of individuals per species

	N	Control	Emasculation	Self-pollin.	Cross-pollin.
A. argentea	12	0 (0/ 43)	0 (0/34)	47.06 (24/51)	86.11 (31/36)
A. longicornu	6	0 (0/ 101)	0 (0/ 104)	97.3 (36/37)	100 (30/30)
E. arietina	4	0 (0/ 74)	0 (0/ 21)	48.8 (21/43)	————

$\chi2$ values = *A. argentea*: 12.57 (significant, P> 0,01). *A. longicornu*: 0.82 (non- significant, P= 0,55).

IDEAS ON THE ECOLOGICAL VALUE OF SOME FLOWER FEATURES

The biological relevance of sectile pollinaria in Orchidaceae has already been emphasized by Freudenstein & Rasmussen (1997). Since the pollinaria of the Goodyerinae orchids are sectile (composed of smaller subunits, called "massulae"), many flowers can be pollinated by the pollen content of a single pollinarium. On the other hand, each massula derives from a single pollen mother cell (Freudenstein & Rasmussen 1996 and references therein). Thus, the pollen content of each massula represents a different genotype. During the pollination process, clumps of massulae are left on the stigmatic surface and the chances of multiple-genotype contributions to each capsule (fruit) are then increased (Freudenstein & Rasmussen 1997). We would like to add that, since the stigmatic surfaces of Goodyerinae are broad and slightly convex, the possibility of mixed pollen loads (i.e.: either from the same inflorescence or not), and consequently of cross-pollination, is increased.

The three studied species displayed relatively low fruit sets, which fits the patterns suggested for Neiland & Wilcock (1998) for tropical orchids.

The presence of protandry in *E. arietina* is significant in terms of reproductive biology since it promotes cross-pollination (Ackerman 1975, Catling & Catling 1991). Among the Goodyerinae orchids, protandry has already been reported in species of the genus *Goodyera* (Ackerman 1975). Remarkably, the two studied *Aspidogyne* species are non-protandrous, but show dorsally adhesive viscidia instead. From an ecological point of view, it seems that protandry has been "substituted" through a sum of rostellar refinements (e.g.: dorsally adhesive viscidia) and "adequate" pollinator behaviour (e.g.: trapliner habit).

Dorsally adhesive viscidia occur in the Spiranthinae orchids of the so-called "Pelexia alliance" (Singer & Sazima 1999, and references therein), though these viscidia are not morphologically equivalent to those observed in the *Aspidogyne* species. The viscidia of the "Pelexia alliance" are wedge-like in shape, with a turgid membrane holding the rostellar cement. When the viscidium is dorsally pressed (a condition that in nature is only achieved through a bee labrum), the membrane ruptures and liberates the rostellar cement which fixes the pollinarium to the pollinator (Singer & Sazima 1999). The viscidium in *Aspidogyne* seems to be much simpler, consisting of the whole detachable apex of the rostellum, with its dorsal surface being adhesive. Cultivated plants of the genus *Ligeophila* (*L. juruenensis*) also show a dorsally adhesive viscidium. This orchid also shows other remarkable column features, such as an extremely flexible (but not "triggered" as mentionned by Garay 1977) rostellum, perpendicularly disposed to a slightly bilobed stigmatic surface. At least the asiatic Goodyerinae genus *Aenhenrya* Gopalan also shows a dorsally adhesive viscidium (Kumar & Rasmussen 1997). Although dorsally adhesive viscidia apparently arose only once among the Spiranthinae orchids (Singer & Sazima 1999 and references therein), this doesn't seem to be the case for the genera of subtribe Goodyerinae, where dorsally adhesive viscidia may have evolved many times. As for the case of the Spiranthinae, the available evidence does not allow us to ascertain how dorsally adhesive viscidia evolved among the Goodyerinae orchids. However, it seems parsimonious to suppose that this kind of viscidium evolved from an ancestral ventrally-adhesive condition. This question could only be addressed after phylogenetic studies of the whole Goodyerinae orchids were published.

As for the Spiranthinae orchids the advantages of dorsally adhesive viscidia in the Goodyerinae are remarkable: 1) the ventral surface of the labrum (where the pollinarium is fixed) is a difficult place for the bees to clean, 2) the labrum is flexible and bent when the insect is flying, thus the fixed pollinarium remains protected under the insect head and 3) this kind of viscidium seems to be particularly adaptative in a context of pollinator scarcity or pollinators with trapliner habit.

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REFERENCES

Ackerman J. D. 1975. Reproductive biology of Goodyer*a oblongifolia* (Orchidaceae). Madroño 23: 191-198.

Ackerman J. D. 1995. An orhid flora of Puerto Rico and The Virgin Islands. Mem. of New York Bot. Gard. 73. Bronx. New York.

Buzato S., M. Sazima & I. Sazima. In press. Hummingbird-pollinated floras at three Atlantic Forest sites. Biotropica 32. Supplement (3/B).

Catling P. M. 1987. Notes on the breeding system of *Saccoila lanceolata* (Aublet) Garay (Orchidaceae). Ann. Miss. Bot. Gard. 74: 58-68.

Catling P. M. & V. R. Catling. 1991. A synopsis of breeding systems and pollination in North American orchids. Lindleyana 6: 187-210.

Dressler R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press. Portland.

Dressler R. L. 1984. *Palmorchis* in Panama, with a new species where least expected. Orquídea (Mex.) 9: 213-230.

Freudenstein J. V. & F. N. Rassmusen, 1997. Sectile pollinia and relationships in the Orchidaceae. Pl. Syst. Evol. 205: 125-146.

Garay L. 1977. Systematics of the Physurinae (Orchidaceae) in the New World. Bradea 2 (28): 191-208.

Hoehne F. C. 1945. Orchidaceas. In: Hoehne F. C. (ed.) Flora Brasilica. Vol XII. Instituto de Botânica, São Paulo.

Janzen D. H. 1971. Euglossini bees as long-distance pollinators of tropical plants. Science 171: 203-205.

Kallunki J. A. 1981. Reproductive biology of mixed-species populations of *Goodyera* (Orchidaceae) in Northern Michigan. Brittonia 33: 137-155.

Koeppen W. 1948. Climatologia: com un estudio de los climas de la tierra. Fondo de Cultura Económica, México.

Kumar S. C. & F. N. Rassmusen. 1997. The reappearance of *Odontochilus rotundifolius* Blatter and its transfer to *Aenhenrya* Gopalan (Orchidaceae). Novon 7: 81-84.

Neiland M. R. & C. C. Wilcock. 1998. Fruit set, nectar reward and rarity in the Orchidaceae. Am. J. Bot. 85 (12): 1657-1671.

Nimer E. 1977. Climatologia da região sudeste do Brasil. Rev. Bras. Geogr. 34:3-48.

Pabst G. & F. Dungs. 1975. Orchidaceae Brasilienses. Band I. Hildesheim: Brucke

Pijl L. Van der & C. H. Dodson 1966. Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables.

Rassmusen F. N. 1982. The gynostemium of the neottioid orchids. Opera Bot. 65: 1-95.

Rassmusen F. N. 1986. On the various contrivances by which pollinia are attached to viscidia. Lindleyana 1(1): 21-32.

San Martin-Gajardo I & L. Freitas. 1999. Hummingbird pollination in *Besleria mucronata* Hoehne (Gesneriaceae) in southeastern Brazil. Biociências. 7. (2): 13-24.

Sazima I, S. Buzato & M. Sazima. 1996. An assemblage of hummingbird-pollinated flowers in a montane forest in southastern Brazil. Bot. Acta 109: 149-160.

Shanks S. J. 1986. A revision of the neotropical bee genus *Osiris* (Hymenoptera: Anthophoridae). The Wasmann Journal of Biology 44(1-2): 1-56.

Singer R. B. & M. Sazima. 1999. The pollination mechanism in the "Pelexia alliance" (Orchidaceae: Spiranthinae). Bot. J. Linn. Soc. 131(3): 249-262.

Wong K. C. & M. Sun. 1999. Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). Am. J. Bot. 86(10): 1406-1413.

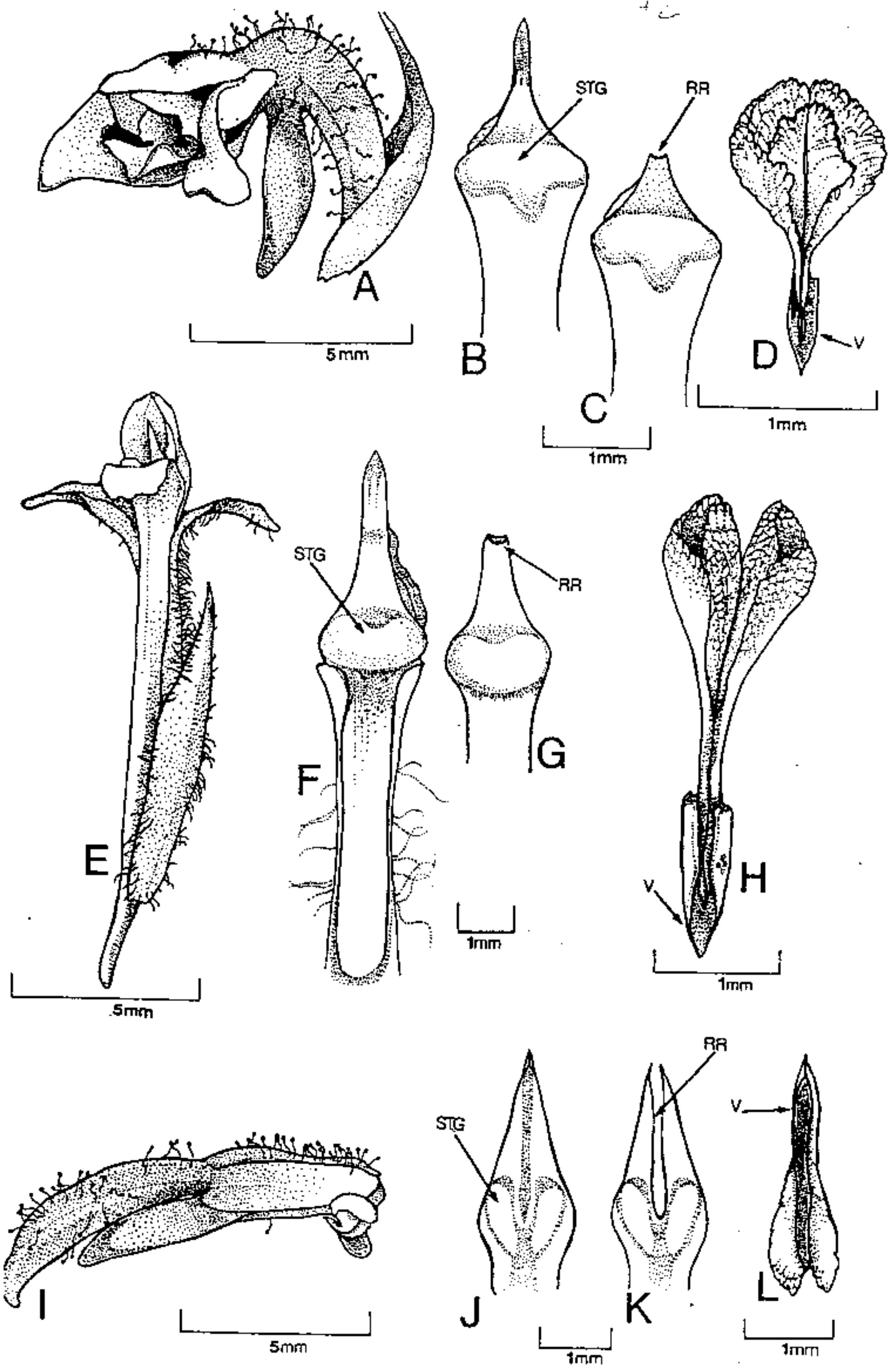

Figure 1. Flower features: A-D. *Aspidogyne argentea*. A. Flower, lateral view. B-C: Column. B. Intact. C. with the pollinarium removed. D. Pollinarium. E-H: *Aspidogyne longicornu*. E. Flower, in frontal view. F-G: Column. F. intact. G. With the pollinarium removed. H. Pollinarium. I-L: *Erythrodes arietina*. I. Flower in lateral view. J-K. Column. J. Intact. K. With the pollinarium removed. L. Pollinarium (ventral view). RR: rostellar remnant. STG: stigmatic surface. V: viscidium.

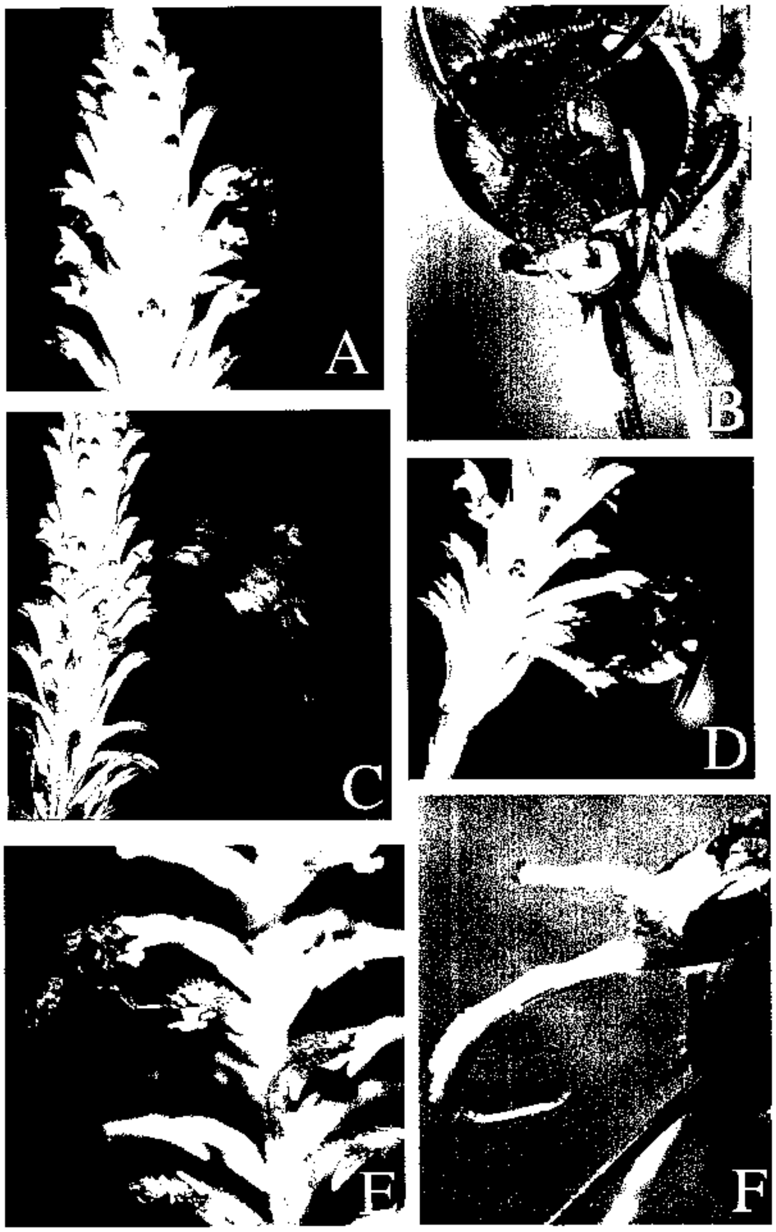

Figure 2. The flowers, their visitors and pollinators. A-D. Visitors and pollinators of *A. longicornu*. A. *Euglossa* cf. *ignita* (pollinator). B. Female of *Euglossa chalybeata iopoecila* with pollinarium attached to the ventral surface of its labrum. C. *Phaethornis ruber* (visitor). D. *Eulaema cingulata* (visitor). E-F. Pollinators of *E. arietina*. E. *Osiris sp.* visiting the flowers. F. Female of *Osiris sp.* with a pollinarium fixed on the dorsal surface of its proboscis.

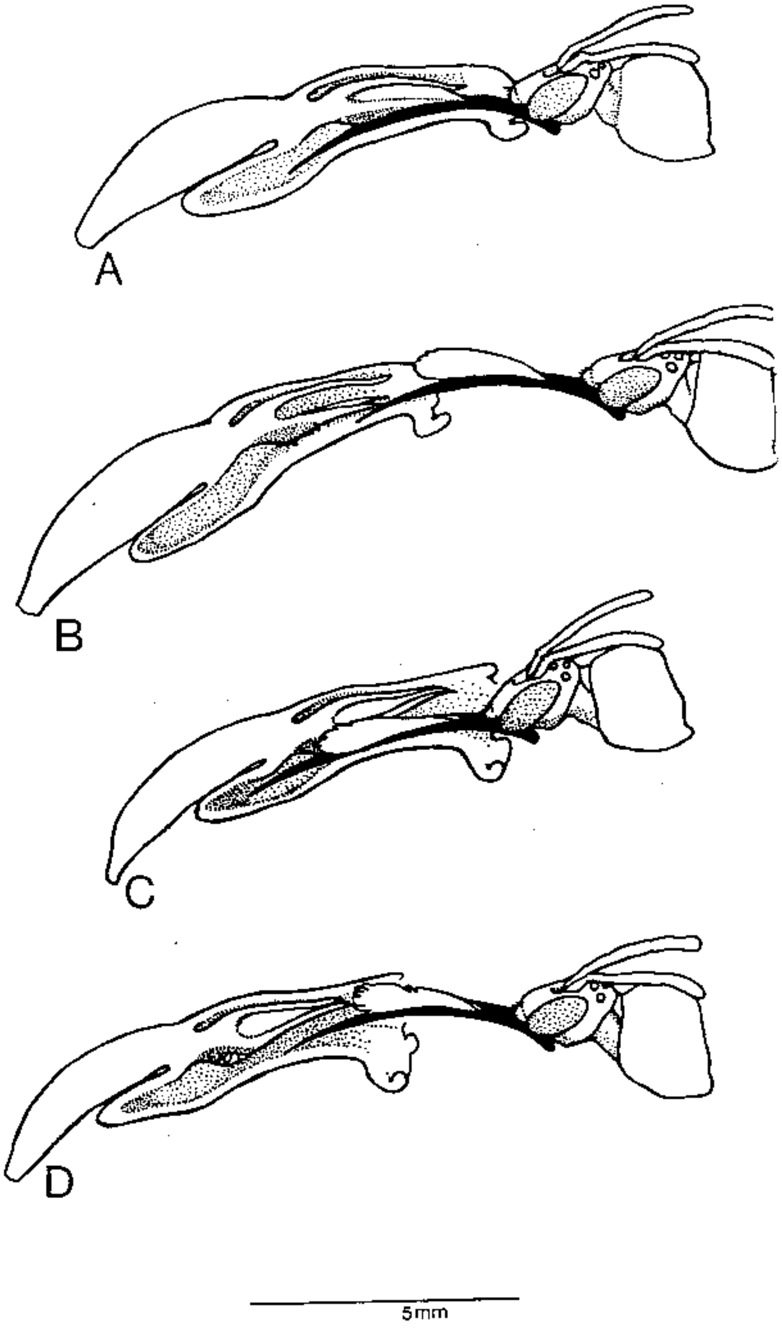

Figure 3 : Pollination proccess in the protandrous *E. arietina*. A: a bee probes a flower in male phase. B: the pollinarium is removed. C: a pollinarium-bearing bee visits a flower in female phase and the distal parts of the pollinarium brush the stigmatic surface. D: clumps of massulae are left on the stigmatic surface.

CAPÍTULO 6:

THE POLLINATION BIOLOGY OF *HABENARIA PARVIFLORA* (ORCHIDACEAE: HABENARIINAE) IN SOUTHEASTERN BRAZIL

RODRIGO B. SINGER

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ABSTRACT

R. B. Singer 2001. The pollination mechanism in Habenaria parviflora (Orchidaceae: Habenariinae) in southeastern Brazil. The pollination biology of *Habenaria parviflora* (Orchidaceae: Habenariinae) Lindl. was studied in Picinguaba, São Paulo State, southeastern Brazil in order to broaden our knowledge on the pollination biology and functional morphology of this terrestrial orchid genus. Plants are self-compatible but pollinator-dependent. The recorded pollinators were crane-flies (Diptera: Tipulidae) females and pyralid moths (Lepidoptera: Pyralidae). The pollination mechanism is the same regardless of the kind of pollinator: the pollinaria are fixed on the insect proboscis through the glove-like viscidium and removed when the insects leave the flowers. A pollinarium-carrying insect visiting another flower will brush the stigmatic surfaces and leave clumps of massulae, thus effecting pollination. This mode of pollinarium fixation is, as far as we know, reported for the first time for the genus *Habenaria* and is a clear consequence of viscidium's morphology. The involute, glove-like viscidium is a unique flower feature among neotropical *Habenaria* species. Flower features promoting cross-pollination are briefly discussed. This is, also, the first orchid pollination report by crane-flies (Tipulidae) in the neotropics.

Key Words: *Habenaria*, Orchidaceae, Orchids, flower morphology, moths, Pyralidae, Tipulidae, crane-flies

INTRODUCTION

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

Most pollination reports for genus *Habenaria* involve different kinds of moths or butterflies (Lepidoptera) (Dressler 1993, Singer & Cocucci 1997), thought *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* Lindl. (as *H. montevidensis*) 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 of *Habenaria*. During our studies on the reproductive biology of southern Brazilian orchids, we had the opportunity of confirming both ideas. The aims of the present contribution are: 1) to broaden our knowledge about the pollination biology of South American *Habenaria* species and 2) to highlight the functional morphology of this orchid species.

METHODS

Studies were conducted in Picinguaba (Parque Estadual da Serra do Mar, Ubatuba, São Paulo, ca. 23° 15′ to 23° 55′S and 44° 45′ to 44° 55′W; altitude at 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 min were spent in observations. The observation period ranged from 19 to 24 hs, 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 some 520 flowers) in 2000. Flower morphology was studied through ethanol 70 % preserved flowers. 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. The breeding system was studied through six bagged plants. Treatments included untouched flowers (control), emasculation, self-pollination and cross-pollination. 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 lantern; 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 taxonomical and morphological concepts of Dressler (1993) are followed.

RESULTS AND DISCUSSION

PLANT FEATURES: owing to a combination of vegetative and reproductive features (well-developed leaves along the stem, plant size and the 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. 1 A) about 6.6 mm in length (Singer & Cocucci 1997). Readers interested in perianth parts size and shape are referred to Hoehne (1940) and Cocucci (1954). Sepals are sturdier and fleshier than the petals. 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, Galetto et al. (1997) provided fine details on the 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. 1 D), one at each side of the column are hidden by the lateral petals (Singer & Cocucci 1997). The anther is erect and holds two pollinaria (until Dressler 1993 such structures were referred as "hemipollinaria"). 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* (Hoehne 1940). Most *Habenaria* flowers show pad-like subcircular, slightly convex viscidia (Cocucci 1954, Hoehne 1940, Singer & Cocucci 1997) which usually fix on 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 hs.) the flowers emit a sweet, spicy fragrance which can be perceived up to 23-24 hs. 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 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 classes of pollinators: when the insect probes the flowers searching for nectar, the proboscis is involved by the glove-like viscidium and the pollinarium is therefore removed. To fix the pollinarium, pressure has to be laterally exerted against the viscidium. This, together with the viscidium shape hinders that both pollinaria be fixed at the same time. In order to remove both pollinaria, insects have to probe the flowers more than once. However, as said above, pyralid moths were sighted carrying only one pollinarium. A pollinarium-carrying insect, which visits another flower, will brush the pollinarium (or pollinaria) against the bulky stigmatic surfaces and clumps of massulae are left there, thus pollinating the flower.

In several instances the moths failed in dislodging pollinaria. It seems that in many occasions the slender moth 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 and, in fact, crane-flies were often seen carrying many pollinaria. Crane-flies are rarely recorded as plant pollinators, but many genera show long proboscises 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* (Listerinae) 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 plantaginea* Lindl. (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* species in Central Argentina (Singer & Cocucci, 1997). *Habenaria hexaptera* (as *H. hyeronimii*) is pollinated by the noctuid moth *Rachyplusia nu* (Noctuidae) and *Habenaria gourlieana* is pollinated by moths of *Agrius cingulatus* and *Manduca sexta* (both, Sphingidae) (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* (fam. Culicidae) and Geometridae moths of genus *Xanthorroe* as well (Thien 1968, Stoutamire 1969). Noteworthy, Voss & Riefner (1983) lately reported that moths of genus *Anageshna* (Pyralidae) also pollinate *H. obtusata*. In all the aforementioned reports, the pollinaria were reported as fixed on the insect eyes. *Habenaria decaryana* from Madagascar does also fix its pollinaria in this way (Nilsson & Jonsson 1985). However, some African *Habenaria* species have been reported to fix their pollinaria on the venter (sternotriby) or in the forelegs (Knuth & al. 1905, Vogel 1954) of their moth pollinators. Pollinarium fixation in the proboscis is, as far as we know, reported for the first time for the genus *Habenaria*.

Reproductive success (number of fruits over produced flowers) could not be calculated, since not bagged inflorescences were cut by the Park inhabitants (the study area is locally used as a parking area). In Central Argentina, a population of *H. parviflora* (reported as *H. montevidensis*) 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 donors) of 0,60 (Singer & Cocucci 1997). This means that 100 pollinaria were removed to pollinate 60 flowers, or, in other words, that more pollinaria were removed tan flowers were pollinated. The fixed pollinaria may in some way disturb the insects. In the Brazilian population, Tipulidae females were often seen trying to clean the pollinaria from their proboscises 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 & Wilcock (1998) affirmation.

BREEDING SYSTEM: results are summarised in Table I. Plants are self-compatible but pollinator-dependent. This means that pollinators are needed for the plants to set fruits. 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 self-pollination in a broader sense (geitonogamy). 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. First, the texture of the pollinarium, which is made up by several subunits ("massulae"). Then, clumps of massulae (instead of whole pollinia) are left on the stigmatic surfaces. This means that several flowers can be pollinated with the pollen content of one pollinarium (Singer & Cocucci 1997). Second, the stigmatic surfaces are broad and convex. This 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 pollinaria *per se* guarantees a considerable degree of genetic diversity (Freudenstein & Rassmusen 1997). According to Freudenstein & Rassmusen (1997) each massula derives from a single pollen mother cell. In other words, 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 & Rassmusen 1997).

TABLE I: Breeding system of *Habenaria parviflora*. Numbers in parenthesis represent the number of fruits produced over the number of flowers used per treatment

Treatments	Control	Self-pollination	Cross-pollin.	Emasculation
Fruiting success %	0 (0/187)	93.3 (28/30)	96.7 (29/30)	0 (0/30)

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REFERENCES

Cocucci, A. E. 1954. Sinopsis de las especies cordobesas de Orchidaceae. Revista Fac. Ci. Ex. Fis. Nat. 2: 1-46.

Correa M. N. 1996. Orchidaceae. In: F. Zuloaga & O. Morrone (edits). Catálogo de las plantas vasculares de la República Argentina Vol. I. Monographs in Systematic Botany from the Missouri Botanical Garden v. 60. 323 pp.

Dressler R. L. 1981. The orchids: natural history and classification. Harvard University Press. Cambridge, Massachussets.

Dressler R. L. 1993. Phylogeny and classification of the orchid family. Dioscorides Press. Portland, Oregon.

Freudenstein J. V. & F. N. Rasmussen. 1997. Sectile pollinia and relationships in the

Orchidaceae. Pl. Syst. Evol. 205: 125-146.

Galetto L., G. Bernardello & G. Rivera. 1997. Nectar, nectaries, flower visitors and breeding systems in five terrestrial Orchidaceae from Central Argentina. J. Plant. Res. 110: 393-403.

Grimaldi D. 1999. The co-radiations of pollinating insects and angiosperms in the cretaceous. Ann. Missouri Bot. Gardn. 86(2): 373-406.

Hoehne F. C. 1940. Orchidaceae: In. F. C. Hoehne (edit.) Flora Brasilica. Fasc. 1. Vol. XII (1).

Knuth P, O. Appel & E. Loew. 1905. Handbuch der Blütenbiologie II, 2. Engelmann. Leipzig.

Neiland M. R. M. & C. C. Wilcock. 1998. Fruit set, nectar reward and rarity in the Orchidaceae. Am. J. Bot 85 (12): 1657-1671.

Nilsson L. A. & L. Jonsson 1985. The pollination of *Habenaria decaryana* H. Perr. (Orchidaceae) in Madagascar. Bull. Mus. Hist. Nat. Paris. sect. B. Adansonia 2: 161-166

Singer R. B. & A. A. Cocucci. 1997. Eye attached hemipollinaria in the hawkmoth and settling moth. Pollination of *Habenaria* (Orchidaceae): a study on functional morphology in 5 species from subtropical South America. Bot. Acta 110: 328-337.

Stoutamire W. P. 1968. Mosquito pollination of *Habenaria obtusata* (Orchidaceae). The Michigan Botanist 7: 203-212.

Thien L. B. 1969. Mosquito pollination of *Habenaria obtusata* (Orchidaceae). Am. J. Bot. 56 : 232-237.

Van Der Pijl L. & C. H. Dodson 1966. Orchid flowers: their pollination and evolution. University of Miami Press. Coral Gables, Florida

Vogel S. 1954. Blütenbiologische Typen als elemente der sippengliederung. Bot. Studien 1, I-X. 338 pp.

Voss E. G. & R. E. Riefner Jr. 1983. A pyralid moth (Lepidoptera) as pollinator of thre blunt-leaf orchid. The Great lakes Entomologist 16(2): 57-60.

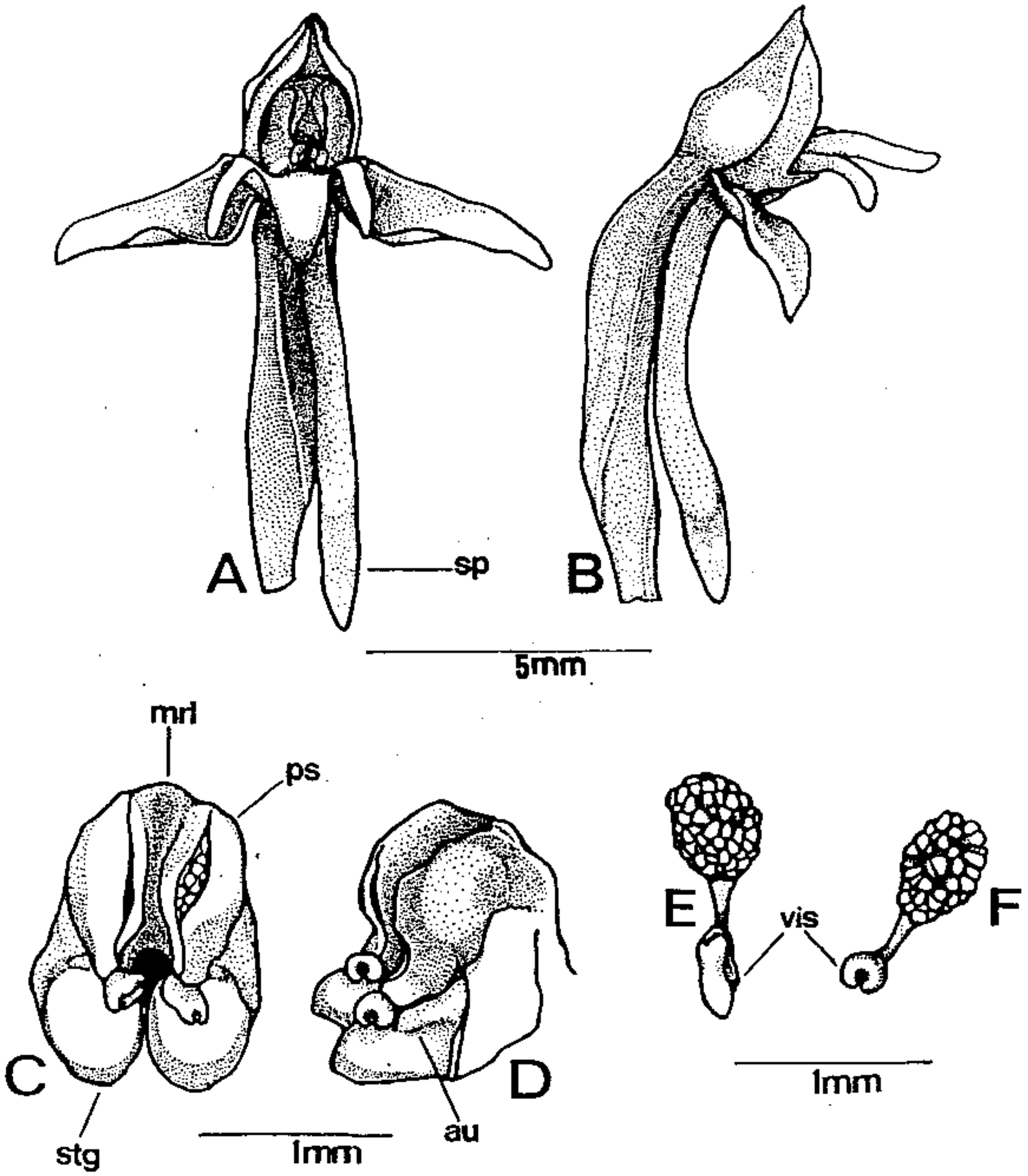

Figure 1: *Habenaria parviflora*. Flower features: **A-B**: flower. **A**: front view. **B**: lateral view. **C-D**: column. **C**: frontal view. **D**: lateral view. **E-F**: pollinaria. **E**: frontal view. **F**: lateral view. **au**: auricle. **mrl**: median rostellar lobe. **ps**: pollen sac. **sp**: spur. **stg**: stigmatic surface. **vis**: viscidium.

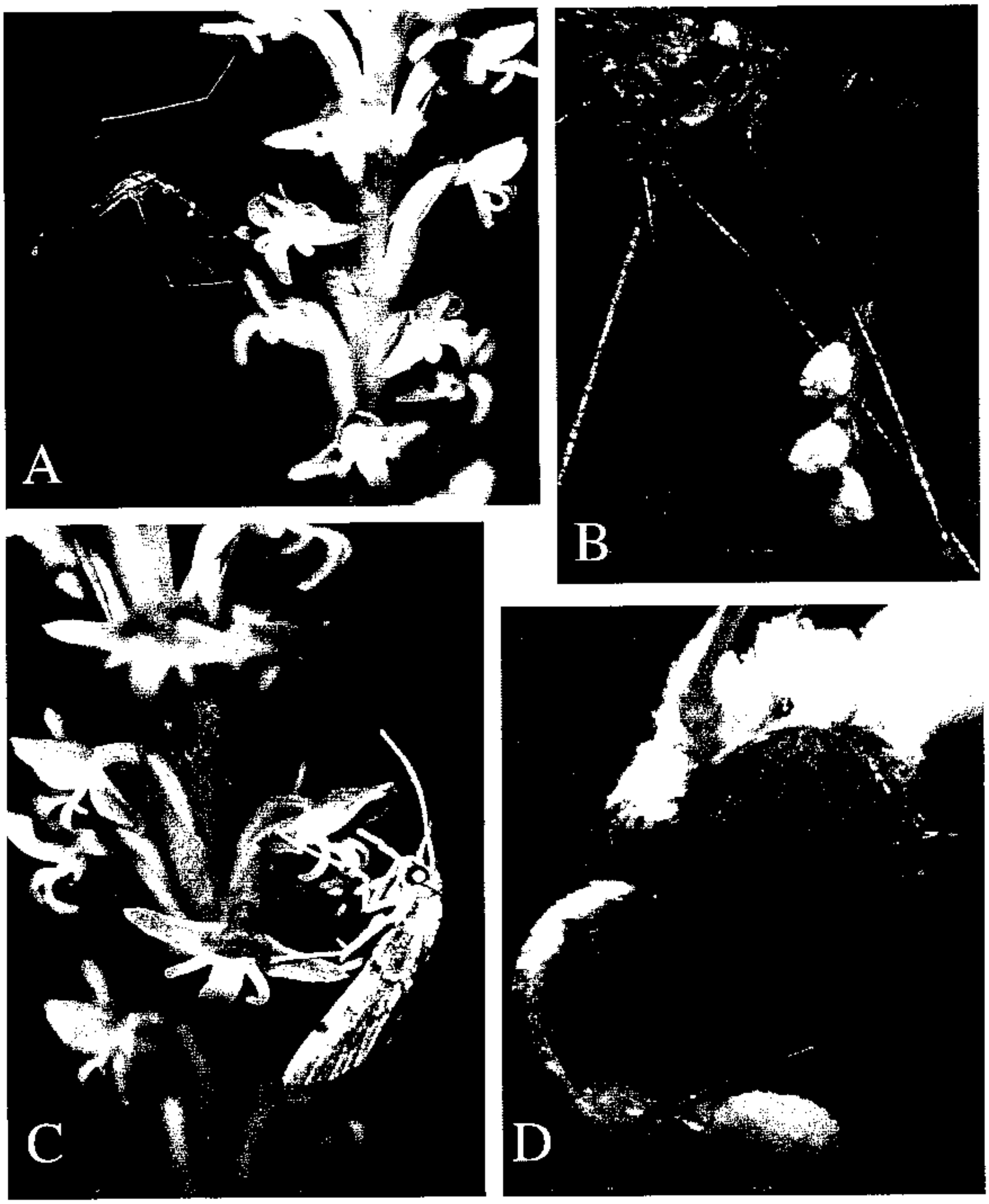

Figure 2. The flowers and their pollinators. A-B. Tipulidae (Diptera) females . A. Insect visiting an inflorescence with a pollinarium attached to its proboscis. B. Specimen with three pollinaria attached to its proboscis. C-D. Pyralid moths. C. Specimen visiting an inflorescence. D. Individual with a pollinarium attached to its proboscis.

CAPÍTULO 7:

ABELHAS EUGLOSSINI COMO POLINIZADORAS DE ORQUÍDEAS NA REGIÃO DE PICINGUABA, SÃO PAULO, BRASIL

Rodrigo B. Singer[1] & Marlies Sazima[1]

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RESUMO - (Abelhas Euglossini como polinizadoras de orquídeas na região de Picinguaba, São Paulo, Brasil) - São estudadas as relações entre as abelhas Euglossini e as orquídeas da região de Picinguaba, Ubatuba, São Paulo, ao longo de um ano. Os machos destas abelhas foram atraídos por meio de iscas químicas (cineol, eugenol, salicilato de metila e vanilina) e examinados à procura de polinários. Em alguns casos foi possível observar as abelhas durante suas visitas às flores. Foram obtidas evidências de polinização por abelhas Euglossini em orquídeas das subtribos Catasetinae (*Catasetum hookeri*, *C. trulla*), Laeliinae (*Cattleya intermedia*), Oncidiinae (*Notylia* sp. e Oncidiinae indet.), Stanhopeinae (*Cirrhaea dependens*, *C. saccata*, *Gongora bufonia*, *Houlletia brocklehurstiana* e *Stanhopea insignis*), e Zygopetalinae (*Promenaea stapelioides*). Apenas *G. bufonia* e *S. insiginis* são localmente polinizadas por uma única espécie de abelha. Dentre as espécies de abelhas Euglossini registradas, 73,74% (11/15) polinizam orquídeas. *Euglossa*, com 88,8 % (8/9) das espécies registradas envolvidas em polinização de orquídeas, é o gênero mais importante. Quatro espécies de *Euglossa*, pertencentes ao subgênero *Glossura* polinizam 63,63 % (7/11) das orquídeas registradas. Em particular, *Euglossa* (*Glossura*) *chalybeata iopoecila* atua como vetor de pólen em 36,36 % (4/11) do total de orquídeas polinizadas por Euglossini na região.

ABSTRACT - (Euglossini bees as orchid polinators in the Picinguaba region, São Paulo, Brazil) - The interactions between the orchids of the Picinguaba region (Municipality of Ubatuba, São Paulo State, Brazil) and their male Euglossini-bee pollinators were studied

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during one year. Male Euglossini bees were attracted by means of chemical baits (cineol, eugenol, methyl salicilate and vanillin) and checked for pollinaria. In some cases, bee behaviour in the flowers could be recorded. Evidence for Euglossini pollination was obtained for orchids belonging to subtribes Catasetinae (*Catasetum hookeri*, *C. trulla*), Laeliinae (*Cattleya intermedia*), Oncidiinae (*Notylia* sp. and Oncidiinae indet.), Stanhopeinae (*Cirrhaea dependens*, *C. saccata*, *Gongora bufonia*, *Houlletia brocklerhurstiana* and *Stanhopea insignis*), and Zygopetalinae (*Promenaea stapelioides*). Only *G. bufonia* and *S. insignis* are pollinated by a single bee species in the studied region. Among the recorded Euglossini, 73,74 % (11/15) of them are involved in orchid pollination. The genus *Euglossa*, with 88,8% of its species carrying pollinaria, was the most important as pollinator. Four *Euglossa* species, belonging to subgenus *Glossura*, pollinate 63,63 % of the recorded orchids. Remarkably, *Euglossa* (*Glossura*) *chalybeata iopoecila* pollinates 36,36% (4/11) of the native Euglossini-pollinated orchids.

1. INTRODUÇÃO

As abelhas Euglossini são exclusivamente neotropicais e de conhecida importância na polinização de numerosas famílias de Angiospermas (Dressler, 1982, Williams, 1982). Recentemente foram descobertas e descritas algumas Euglossini fossilizadas em âmbar, provenientes de Dominica, com uma idade aproximada de 15-20 milhões de anos (Engel, 1999). Este fato sugere que este grupo taxonômico de abelhas vem interagindo com a flora neotropical de longa data.

Machos e fêmeas destas abelhas visitam flores de espécies de Apocynaceae, Bignoniaceae, Convolvulaceae, Gesneriaceae, Marantaceae, Rubiaceae, Costaceae e Verbenaceae à procura de néctar (Dressler, 1982). As abelhas fêmeas utilizam as flores de várias espécies de Bixaceae, Clusiaceae, Cochlospermaceae, Flacourtiaceae, Haemodoraceae, Leguminosae, Ochnaceae e Solanaceae como fontes de pólen (Dressler, 1982). As fêmeas visitam também os caules de numerosas espécies de Anacardiaceae, Burseraceae e Leguminosae, bem como as flores de numerosas Clusiaceae, Euphorbiaceae à procura de resinas para construção do ninho (Dressler, 1982). Os machos destas abelhas visitam flores de diversas espécies de Araceae, Orchidaceae, Euphorbiaceae (*Dalechampia*) e Solanaceae

(*Cyphomandra*) para obter compostos aromáticos (Dressler, 1982; Sazima *et al.*, 1993). A interação dos machos deste grupo de abelhas com numerosas espécies de orquídeas (em particular as da subfamília Epidendroideae, sensu Dressler, 1993), tem chamado a atenção dos pesquisadores desde a época de Darwin (1862). Durante as visitas, os machos coletam ativamente gotinhas de compostos aromáticos que são secretadas em porções especializadas do labelo destas orquídeas. Estas substâncias são acumuladas em uma estrutura esponjosa na tíbia do terceiro par de patas. Ainda não há consenso em relação à utilização destas substâncias pelas abelhas. Acredita-se que, parcialmente ou muito modificadas, possam servir de feromônios para atrair as fêmeas (Dressler, 1982; Williams, 1982). Também foi sugerido que estas abelhas usam combinações destes compostos para atrair vários machos co-específicos na forma de agregados ("leks") e as fêmeas escolheriam algum dos machos para acasalar (Dressler, 1982; Williams, 1982). No entanto, nos estudos sobre o acasalamento destas abelhas, observou-se que não há formação de "leks" (Dressler, 1982; Williams, 1982). Recentemente, Lunau (1992) e Eltz *et al.* (1999) sugeriram idéias alternativas, com base em dados teóricos e experimentais, bem como observações de campo. Os machos acumulam os compostos aromáticos durante muito tempo e sem modificação (Eltz *et al.*, 1999). Por ocasião do acasalamento, os machos liberariam os compostos acumulados e as fêmeas apenas acasalariam com os machos com "melhores" combinações. A combinação de compostos induziria a uma espécie de seleção sexual, atuando como indicadora de "genes bons". Em outras palavras, a combinação de compostos liberados atuaria como uma espécies de "histórico" do macho, indicando a sua longevidade e capacidade de coletar compostos mais diversos e atrativos (Eltz *et al.*, 1999; Lunau, 1992). É interessante salientar que as substâncias aromáticas que as abelhas machos procuram são muito comuns na natureza, ocorrendo nas flores, mas também em madeiras, seivas e resinas (Lunau, 1992), ou seja, os materiais que as fêmeas utilizam para construção do ninho (Lunau, 1992). É possível, ainda, que os materiais utilizados na construção do ninho venham a determinar a preferência dos machos Euglossini por estes compostos aromáticos (Lunau, 1992). Lunau (1992 e com. pess.) acredita que aqueles machos cujos "bouquets" de fragrâncias mais se assemelham ao "bouquet" de fragrâncias do ninho terão maior probabilidade de atrair as fêmeas e acasalar. Durante o processo de coleta destas substâncias, os machos de Euglossini deslocam os polinários e/ou polinizam as orquídeas.

A descoberta de que os machos de Euglossini utilizam compostos aromáticos de orquídeas data dos anos 60 (Dressler, 1967; Van der Pijl & Dodson, 1966). Grande número destes compostos é utilizado na produção de doces, perfumarias, indústria química, bem como em estudos sobre polinização de orquídeas (Dressler, 1976). Em estudos deste tipo são utilizadas iscas (algodão, papel de filtro) embebidas com algum destes compostos, para atrair os machos de Euglossini (Dressler, 1976). Os polinários de orquídeas aderidos aos machos assim atraídos podem ser identificados, com base na morfologia, em nível de gênero ou até de espécie (Dressler, 1976). Esta técnica tem permitido obter dados sobre a polinização de comunidades de orquídeas na América Central e do Sul (Ackermann, 1982; Roubik & Ackermann, 1986; Pearson & Dressler, 1985). No Brasil, existem alguns estudos deste tipo, entretanto, são ainda fragmentários (Braga, 1976; Peruquetti *et al.*, 1999). Portanto, faltam estudos mais consistentes sobre a polinização de orquídeas por este grupo de abelhas. Apresentamos, aqui, um estudo inédito sobre a polinização de orquídeas por abelhas Euglossini em uma área de mata atlântica do litoral norte do Estado de São Paulo, cuja flora de orquídeas foi recentemente estudada (Ribeiro, 1994a, 1994b). Neste estudo abordamos os seguintes aspectos: 1) quais orquídeas desta flora são polinizadas por abelhas Euglossini?; 2) ocorrem padrões taxonômicos? (p. ex.: determinados grupos taxonômicos de Euglossini polinizam determinados grupos taxonômicos de orquídeas?); e 3) há interações espécie-específicas? (p. ex.: interações nas quais uma espécie de orquídea é polinizada apenas por uma espécie de abelha).

2. ÁREA DE ESTUDO E METODOLOGIA

O estudo foi desenvolvido no Núcleo Picinguaba, Parque Estadual Serra do Mar, ca. 23° 22'S, 44° 50'W, litoral norte do Estado de São Paulo. Esta região apresenta cobertura vegetal caracterizada como floresta tropical subúmida (Eiten, 1970). O clima é tropical úmido (Koeppen, 1948). A precipitação média anual atinge 2600 mm, a temperatura média anual é de aproximadamente 21°C e não há uma estação fria-seca definida (Nimer, 1977). Foram feitos censos mensais em quatro pontos de amostragem previamente estabelecidos que foram escolhidos arbitrariamente como representativos da diversidade topográfica / fisionômica dos ambientes do Núcleo Picinguaba. Estes pontos de amostragem são caracterizados brevemente a seguir:

1) Praia da Fazenda, a aproximadamente 20m do alojamento do parque e ca. 4 m.s.m. A vegetação é do tipo restinga, havendo algumas árvores com até 15m de altura. O solo é seco e arenoso.
2) Trilha lateral à "trilha do Mangue" ou "trilha das três lagoas" a ca. 4-6 m.s.m. Na vegetação predominam as árvores altas (15-20m alt.) e o solo é freqüentemente alagado, não arenoso.
3) Trilha secundária nas proximidades da sede administrativa do parque, a ca. 50 m.s.m. A vegetação é variada, ocorrendo árvores muito altas (ca. 20m alt.), mas também clareiras antrópicas (pomares abandonados). O solo apresenta-se com muita freqüência encharcado e tem aparência argilosa.
4) Trilha do Jatobá, ocorre ao lado do rio da Fazenda (seguindo pela trilha da casa da Farinha) a ca. 60 m.s.m. A vegetação aparenta ser a menos alterada pela ação antrópica. As árvores são grandes (> 20 m alt.), sendo o jequitibá (*Cariniana* sp.) e a figueira (*Ficus* sp.) elementos conspícuos. O epifitismo é muito marcante.

No período de fevereiro de 1999 a fevereiro de 2000, foram colocadas, mensalmente em cada ponto de amostragem, quatro iscas químicas diferentes: cineol, eugenol, vanilina e salicilato de metila. As iscas foram colocadas de acordo com as técnicas de Dressler (1971), Williams (1982) e Roubik & Ackermann (1987). Após a colocação das iscas, permanecemos à espera das abelhas por um período de 2-5 horas. Durante o período de observação em cada ponto, as espécies de Euglossini ocorrentes em cada isca foram registradas. Abelhas carregando polinários de orquídeas foram capturadas para uma posterior identificação tanto do inseto, quanto do gênero e/ou da espécie de orquídea. Entretanto, à medida em que as espécies se repetiam, as abelhas eram apenas examinadas (Roubik & Ackermann, 1987) e a seguir, liberadas. Exemplares testemunhas foram depositados no Laboratório de Abelhas da Universidade de São Paulo, bem como na Coleção Moure da Universidade Federal do Paraná, em Curitiba. Como base para identificação dos polinários encontrados nas abelhas foram utilizados os trabalhos de Ribeiro (1994a, 1994b). Em várias ocasiões, encontramos as orquídeas floridas e/ou documentamos a sua polinização pelas abelhas Euglossini. Exemplares testemunho dessas orquídeas estão depositados no herbário da Universidade Estadual de Campinas (UEC).

Quanto à taxonomia e terminologia morfológica dos grupos de orquídeas, seguimos Dressler (1993), e quanto à taxonomia das abelhas Euglossini, seguimos os trabalhos mais recentes de Dressler (1978, 1979, 1982b, 1982c, 1982d) e Kimsey (1982, 1987).

Neste trabalho adotamos a premissa de que as abelhas Euglossini são as polinizadoras das orquídeas cujos polinários carregam. Esta premissa está implícita nos trabalhos deste tipo realizados por outros autores (Ackermann, 1982; Roubik & Ackermann, 1987; Pearson & Dressler, 1982). Adams & Lawson (1993) sugerem que o termo "polinizador" deveria ser aplicado apenas nas circunstâncias em que é possível observar o deslocamento e a deposição de polinários. No entanto, com base em observações de campo, as abelhas que conseguem deslocar os polinários, via de regra, são também capazes de depositá-los, efetuando a polinização. Grande parte do conhecimento sobre polinização de orquídeas em diferentes regiões florísticas dos neotrópicos foi obtida por meio de evidências indiretas, utilizando a técnica das iscas (Ackermann, 1983; Roubik & Ackermann, 1987; Pearson & Dressler, 1985). Embora esta técnica não permita conhecer detalhes do processo de polinização, apresenta a vantagem de produzir resultados bastante confiáveis em um período de tempo relativamente curto.

3. RESULTADOS E DISCUSSÃO

3.1. A COMUNIDADE DAS ORQUÍDEAS

Durante a realização deste trabalho, obtivemos evidências sobre a polinização de 11 espécies de orquídeas, todas da subfamília Epidendroideae (Dressler, 1993): *Cattleya intermedia* Grah. (Laeliinae), *Promenaea stapelioides* (Link & Otto) Lindl. (Zygopetalinae), *Catasetum hookeri* Lindl. (Catasetinae), *Catasetum trulla* Lindl. (Catasetinae), *Cirrhaea dependens* Rchb. f. (Stanhopeinae), *Cirrhaea saccata* Lindl. (Stanhopeinae), *Gongora bufonia* Lindl. (Stanhopeinae), *Houlletia brocklehurstiana* Lindl. (Stanhopeinae), *Stanhopea insignis* Frost ex Hook. (Stanhopeinae), *Notylia* sp. (Oncidiinae), bem como uma espécie da subtribo Oncidiinae não identificada (possivelmente, uma espécie de *Rodrigueziopsis* Schltr.). As orquídeas aqui referidas pertencem às subtribos nas quais já foi relatada a polinização por machos de abelhas Euglossini (Ackermann, 1983; Dressler, 1981, 1993; Roubik & Ackermann, 1987; Van der

Pijl & Dodson, 1966; Williams, 1982). Das espécies relacionadas, vale destacar que este é o primeiro registro sobre a polinização no gênero *Promenaea*. A ocorrência de *Houlletia brocklehurstiana* e *Notylia* sp., antes não mencionadas para a região de Picinguaba (Ribeiro, 1994a, 1994b), está sendo referida com base na ocorrência de numerosas Euglossini com polinários dessas espécies. *Houlletia brocklehurstiana* provavelmente ocorre nas regiões altas de Picinguaba, acima dos 800 m.s.m. Esta espécie de orquídea foi encontrada em Paranapiacaba (litoral Sul de São Paulo) (Obs. pess.; Eduardo Catharino, com. pess.) bem como no Estado do Rio de Janeiro (Dressler, 1982; Miller & Warren, 1994), via de regra em ambientes montanhosos. Machos de *Euglossa chalybeata iopoecila* carregando polinários desta orquídea foram registrados em Tagaçava (litoral norte do Paraná) (R. B. Singer, inédito). As espécies de *Cirrhaea* foram encontradas apenas em estado vegetativo (Ribeiro, 1994a, 1994b), o que torna praticamente impossível a identificação ao nível específico. No entanto, os polinários apresentam caracteres morfológicos distintos que permitem separar as duas espécies encontradas (E. R. Pansarin, com. pess.). A dificuldade de encontrar flores desta planta está associada ao fato da florada das Stanhopeinae ser muito efêmera (dois ou três dias) (Van der Pijl & Dodson, 1966). Estudos aprofundados sobre as espécies de *Cirrhaea* do Estado de São Paulo estão em andamento (E. R. Pansarin, com. pess.). Outra espécie que merece ser comentada é *Cattleya intermedia*. Esta espécie não apresenta os atributos típicos de "flores com fragrância", definidos por Van der Pijl & Dodson (1966). Na realidade, estas flores apresentam uma cavidade nectarífera ou "cunículo" (Dressler, 1981, 1993) adnada ao ovário. Acreditamos que estas flores sejam polinizadas por abelhas de grande porte, em geral, que provavelmente visitam as flores à procura de néctar. Assim, por exemplo na Ilha do Mel (Paraná), onde as abelhas Euglossini são raras, *Cattleya forbesii* Lindl. é polinizada por *Bombus* sp. (Apidae: Bombini) (R. B. Singer, inédito). No entanto, flores de indivíduos desta espécie cultivados em Campinas (SP) atraem machos de *Eulaema nigrita* (Apidae: Euglossini). Aqui vale lembrar que além de coletar fragrâncias em orquídeas e outras fontes florais e não florais, os machos de Euglossini alimentam-se de néctar como qualquer outra abelha. Não registramos a ocorrência de abelhas com polinários de *Huntleya meleagris* Lindl. e *Vanilla chamissonis* Kl., embora estas orquídeas sejam freqüentes na região de Picinguaba. Van der Pijl & Dodson (1966), reportaram machos de *Eulaema meriana* (espécie ausente na área de estudo) como polinizadores de *H. meleagris* na Costa Rica. Em Picinguaba, esta espécie de orquídea é possivelmente polinizada por *Eulaema seabrai* que ocorre na região, mas não é atraída pelas iscas químicas. Não temos evidências sobre a polinização de *Vanilla chamissonis* em Picinguaba. Entretanto, Dressler (1993) refere machos de *Eulaema cingulata,* bem como outras Euglossini não especificadas como polinizadores de *Vanilla* nos neotrópicos. Outro registro, refere-se a alguns machos de *Eulaema nigrita*, com pólen provavelmente de *V. chamissonis*, coletados nos arredores de Uberlândia, Minas Gerais (R. B. Singer, inédito). Por outro lado, é possível que as flores de *V. chamissonis* ofereçam néctar como recompensa, junto com, ou ao invés de fragrâncias, e sejam polinizadas por abelhas de grande porte e não apenas por Euglossini.

Em outubro de 1998, observamos e capturamos nas iscas um macho de *Euglossa chalybeata iopoecila* carregando, na superfície dorsal do labrum, um polinário semelhante ao de *Dichaea pendula* (Aubl.) Cogn. (Zygopetalinae), orquídea abundante na área de estudo e em plena floração nesta ocasião. No entanto, o polinário se perdeu durante o manuseio, não tendo sido possível confirmar sua identidade. Existem vários registros de espécies de *Dichaea* polinizadas por machos de euglosssini (Ackermann, 1983; Dressler, 1981; Roubik & Ackermann, 1987) e em um deles Dressler (1981) comenta que os polinários de *Dichaea* se fixam no clípeo dos polinizadores e se desprendem facilmente durante a captura e o manuseio das abelhas.

3.2. A COMUNIDADE DAS ABELHAS EUGLOSSINI

Ao longo deste estudo, encontramos um total de 15 espécies de abelhas Euglossini (Tabela 1), distribuídas em três gêneros: *Euglossa* (9 spp.), *Eufriesea* (3 spp.) e *Eulaema* (3 spp.). As espécies de abelha, bem como a sua fenologia e ocorrência com polinários de orquídeas ao longo do ano, estão resumidos na tabela 1.

O número de espécies registrado parece proporcionalmente pequeno, se comparado com as 57 espécies de Euglossini conhecidas para o Panamá (Roubik & Ackermann, 1987) e com as 44 espécies referidas por Peruquetti *et al.* (1999) para a mata atlântica (*sensu lato*). No entanto, este último trabalho foi feito com base em dados bibliográficos, bem como em coleções de abelhas de várias instituições (Peruquetti *et al.*, 1999). Estes autores adotaram um conceito muito amplo de mata atlântica, incluindo, por exemplo, as matas de araucária e as matas mesófilas e ciliares do interior dos Estados de São Paulo e Minas Gerais. Neste estudo utilizamos um conceito mais restrito de mata atlântica, referindo-nos apenas à vegetação que acompanha o litoral brasileiro. Das espécies aqui mencionadas, apenas *Euglossa chalybeata iopoecila* havia sido referida para a região de Picinguaba por Peruquetti *et al.* (1999). Acreditamos que o número de espécies de Euglossini obtido esteja muito próximo do real. Mas é provável que, usando uma quantidade maior de iscas, sejam descobertas outras espécies na área de estudo. Algumas espécies foram documentadas apenas por fêmeas forrageando ou coletando materiais de construção para o ninho. Assim, fêmeas de *Euglossa* sp.1 (provavelmente uma espécie do "grupo Intersecta", sensu Dressler, 1978), foram coletadas durante visitas a flores de *Calathea* (Marantaceae) e *Besleria mucronata* (Gesneriaceae) (San Martin-Gajardo & Freitas, 1999, identificada provisoriamente por R.B. Singer como *Euglossa* aff. *sapphirina*). Alguns machos de *Eulaema seabrai* foram coletados durante o comportamento de atração às fêmeas. Por sua vez, uma fêmea de *E. seabrai* foi capturada enquanto coletava lama, possivelmente para utilização na construção do ninho. As demais espécies registradas, foram obtidas por meio de iscas químicas. O eugenol atraiu o maior número de espécies (8 spp.), seguido do cineol e vanilina (7 e 5 spp,, respectivamente), o salicilato de metila atraiu apenas duas espécies de abelha (*E. iophyrra* e, muito raramente, *E. cordata*). Várias espécies de Euglossini foram atraídas por mais de uma isca.

Tabela 1: Fenologia das espécies de abelhas Euglossini em Picinguaba, Ubatuba, SP.
x =ocorrência (sem polinários) X̲= Meses nos quais as abelhas foram encontradas com polinários de orquídeas.

	F	M	A	M	J	J	A	S	O	N	D	J
Eufriesea purpurata (Mócsary)	X̲										x	X
Eufriesea violacea (Blanchard)										X̲	X̲	
Eufriesea violascens (Mócsary)	x											X
Euglossa c. iopoecila Dressler	X̲	x	X̲	x	x	x	x	x	x	X̲	x	X̲
Euglossa cordata L.	x	x	x	X̲		x		x	x	X	x	X̲
Euglossa cf. *ignita* F. Smith	X̲	x	x	x	x				x	x	x	X̲

Euglossa iopyrrha Dressler	X	x	x	x	x	x		x	x	x	x	X
Euglossa pleosticta Dressler		X	x								x	X
E. sapphirina Moure	X	x	x	x	x	x	x	x	x	x	x	X
Euglossa cf. *viridis* (Perty)	X	X	x	x	x				x	x	X	X
Euglossa sp.1												X
Euglossa sp.2	X	X	x		x	x	x	x	x	x	x	X
Eulaema cingulata (Fabricius)	x	x	x	x	x	x		x	x			
Eulaema nigrita Lepeletier	x	x	x						X	x	X	X
Eulaema seabrai Moure	x			x						x	x	

3.3. INTERAÇÃO ENTRE ORQUÍDEAS E ABELHAS EUGLOSSINI

Durante a realização deste trabalho, encontramos abelhas carregando polinários apenas nos meses de outubro a maio (Tabela 1), isto é, nos meses mais quentes e de maior pluviosidade (Nimer 1977).

Dentre estas 15 espécies de abelhas, apenas quatro (26,26% do total) não apresentaram polinários de orquídeas no período de estudo (Tabela 1). Portanto, 73,74% (11/15) das espécies de Euglossini registradas estão envolvidas na polinização das orquídeas. Destas onze espécies, três (27,27%) apresentaram polinários de apenas uma espécie de orquídea. A figura 1 ilustra o local de fixação dos polinários das orquídeas nas abelhas Euglossini. *Euglossa chalybeata iopoecila* foi a espécie que apresentou polinários do maior número de espécies de orquídeas (4 spp.). Oito das nove espécies de *Euglossa* estiveram envolvidas na polinização de orquídeas. Duas (de três) espécies de *Eufriesea* e uma (de três) espécie de *Eulaema* foram documentadas como polinizadoras de orquídeas. Estes resultados indicam maior importância relativa do gênero *Euglossa* em relação aos outros gêneros de Euglossini como polinizadores de orquídeas de mata atlântica. Estudos em outras regiões neotropicais sugerem padrões semelhantes, embora envolvendo faunas e floras mais diversas; além disso foi verificado que cerca de 20-33% das Euglossini nativas não são polinizadoras de orquídeas (Ackermann, 1983; Roubik & Ackermann, 1987). Esta é uma forte evidência de que as abelhas Euglossini não dependem exclusivamente das orquídeas para obter fragrâncias, podendo obtê-las em outras fontes florais ou não florais (Ackermann, 1983).

Com base nos dados obtidos com iscas, bem como nas observações em flores, algumas orquídeas da região de Picinguaba dependem apenas de uma única espécie de abelha como polinizador. Este é o caso de *Gongora bufonia* e *Stanhopea insignis* que dependem das abelhas *Eufriesea violacea* e *Eufriesea purpurata*, respectivamente. No caso de *Promenaea stapelioides*, *Cattleya intermedia* e a Oncidiinae indeterminada, foi registrada também apenas uma espécie de polinizador para cada espécie. Entretanto, *Promenaea stapelioides* é uma planta rara na área de estudo, além de produzir número reduzido de flores. Os polinários da Oncidiinae indeterminada foram observados apenas em machos de *E. iophyrra*, mas como a identidade desta orquídea não está definida, preferimos não concluir sobre sua especificidade em relação à polinização. Provavelmente, como mencionado anteriormente, *Cattleya intermedia* não é polinizada apenas por abelhas Euglossini. Com mais amostragens possivelmente poderemos esclarecer adequadamente se estas orquídeas estabelecem interações espécie-específicas ou não.

3.4. CONSIDERAÇÕES SOBRE A FIXAÇÃO DOS POLINÁRIOS NAS ABELHAS EUGLOSSINI

Os polinários das orquídeas polinizadas pelas abelhas Euglossini habitualmente aderem a áreas do corpo bem delimitadas (Fig. 1) (Dressler, 1981, 1982a; Ackermann, 1983; Williams, 1982). Esta precisão na fixação dos polinários decorre, principalmente, da interação de dois fatores: 1) morfologia floral da orquídea visitada e 2) o comportamento das abelhas nas flores. Ao visitarem as flores, as abelhas pairam defronte delas por alguns segundos, pousam, e coletam as fragrâncias com o primeiro par de patas. Esta coleta de compostos aromáticos é feita repetidas vezes, por meio de uma complexa seqüência de movimentos (Williams, 1982). A seguir, abandonam momentaneamente as flores para transferir as substâncias coletadas ao terceiro par de patas. Os polinários habitualmente aderem às abelhas quando elas abandonam as flores. De modo geral, o local de fixação dos polinários nas abelhas tende a ser constante para cada espécie de orquídea e de abelha (Fig. 1). As abelhas tendem a retornar várias vezes a cada flor, o que poderia resultar em autopolinização. Entretanto, as orquídeas apresentam vários mecanismos para evitar este tipo de polinização. Assim, as flores das orquídeas Stanhopeinae são dicógamas (Van der Pijl & Dodson, 1966). A cavidade estigmática é muito estreita e o polinário recém deslocado precisa desidratar-se, perdendo volume, para poder ser retido na cavidade estigmática. No periodo necessário para isto acontecer, as abelhas têm tempo para visitar outras plantas da mesma espécie, favorecendo a polinização cruzada (Van der Pijl & Dodson, 1966). As flores das espécies de *Gongora* e *Stanhopea* apresentam um mecanismo de polinização semelhante: após a coleta de compostos aromáticos, as abelhas escorregam ou caem das flores (Van der Pijl & Dodson, 1966), ocasião em que os polinários são fixados no escutelo das abelhas (Fig. 1, A-B). Nas grandes flores de *Houlletia* o processo de polinização parece ser simples: as abelhas deslocam os polinários, que são fixados no occipício, quando elas abandonam as flores. Os polinários das flores do gênero *Cirrhaea*, por serem as flores não ressupinadas, são fixados em diferentes partes das patas das abelhas (Van der Pijl & Dodson, 1966) (Fig. 1, D, F-H, K). As orquídeas do gênero *Catasetum* (subtribo Catasetinae) ocorrentes na área de estudo apresentam plantas com sexos separados, tornando impossível a autopolinização (Van der Pijl & Dodson, 1966). Nas flores masculinas, o estipe encontra-se sob tensão e quando as abelhas tocam os filamentos da coluna, o polinário é rapidamente ejetado no dorso das abelhas, no qual se fixa firmemente (Fig. 1, D-F, H-J). Geralmente, o polinário se fixa no escuto e/ou escutelo das abelhas, no entanto, em *E.* cf. *viridis* também encontramos polinários aderidos entre o mesoscuto e a área subalar (Fig. 1, F). Estas fixações 'anômalas' de polinários provavelmente decorrem de abordagens inadequadas da abelha à flor masculina. Várias espécies de *Notylia* (subtribo Oncidiinae) apresentam protandria funcional (Warford, 1982), de modo que nas flores jovens o polinário apenas pode ser deslocado, mas não depositado. Apenas flores de três ou mais dias de duração podem receber pólen. Nesta ocasião, o estigma das flores, antes estreito, apresenta uma fenda suficientemente ampla para receber uma polínia. Aparentemente, além de protândricas, muitas espécies de *Notylia* são autoincompatíveis (Warford, 1992). Em outros gêneros de orquídeas, tais como *Promenaea* e *Dichaea* (subtribo Zygopetalinae) aparentemente é necessário que o polinário diminua de volume, através de desidratação, para que possa aderir ao estigma. Nestes gêneros o polinário é removido junto com o capuz da antera ("anther cap", sensu Dressler, 1993). O capuz da antera cai, liberando o polinário, apenas quando as polínias diminuíram de volume. Este mecanismo é similar ao observado nas orquídeas Stanhopeinae (Van der Pijl & Dodson, 1966) e favorece a polinização cruzada, uma vez que enquanto as polínias desidratam, as abelhas visitam outros indivíduos desta orquídea (q.v. Singer & Cocucci, 1999, para exemplo semelhante em *Pleurothallis luteola*).

3.5. PADRÕES TAXONÔMICOS

A figura 2 resume as interações entre as abelhas Euglossini e as orquídeas nativas da região de Picinguaba. As orquídeas localmente polinizadas por abelhas Euglossini pertencem a grupos taxonômicos nos quais, de um modo geral, a polinização por este grupo de abelhas já foi amplamente documentada (Ackermann, 1983; Dressler, 1981, 1982; Roubik & Ackermann, 1987; Williams, 1982). É interessante salientar que os grupos de orquídeas desta região registrados como polinizados por Euglossini são exclusivamente neotropicais (Dressler, 1993). Uma possível exceção seria *Vanilla chamissonis* (o gênero *Vanilla* é pantropical), se a polinização por abelhas Euglossini na área de estudo for comprovada.

O gênero *Euglossa*, com 88,8% (8/9) das espécies registradas envolvidas em polinização de orquídeas é, de longe, o gênero de abelhas Euglossini com maior importância para esta comunidade de orquídeas. As quatro espécies de *Euglossa* pertencentes ao subgênero *Glossura* polinizam, como um todo, 63,63 % (7/11) das orquídeas registradas. Dentre elas, *Euglossa chalybeata iopoecila* (subgênero *Glossura*: grupo Pilliventris), poliniza a maior diversidade (quatro espécies pertencentes a três subtribos) de orquídeas. Esta subespécie de *E. chalybeata* parece ser endêmica do sul-sudeste do Brasil, ocorrendo de São Paulo até o Paraná (Dressler, 1982b) e diferencia-se da forma típica, de cor verde da região Amazônica, por sua coloração azul-violeta.

O termo co-evolução é usado freqüentemente quando se abordam as inter-relações entre os machos de abelhas Euglossini e as orquídeas que polinizam (Ackermann, 1983). O fato é que em vários dos ecossistemas neotropicais estudados, uma parte significativa das espécies de abelhas Euglossini não poliniza orquídeas (Ackermann, 1983; Roubik & Ackermann, 1987). Além disso, as chamadas interações espécie-específicas (uma orquídea-um polinizador) são pouco freqüentes (Dressler, 1981). É possível que em um contexto ecológico-evolutivo, as orquídeas “aproveitem” condutas preexistentes nas abelhas Euglossini (coletar fragrâncias em diversas fontes florais e não florais) e acabem capitalizando estas abelhas como polinizadoras (Ackermann, 1983; Roubik & Ackermann, 1987). No entanto, é provável que as abelhas Euglossini, como um todo, tenham atuado como um fator catalisador, favorecendo, com sua conduta de coletar compostos aromáticos, a evolução e a diversidade dos grupos taxonômicos de orquídeas que oferecem fragrâncias como recompensa, induzindo a um processo de co-evolução difusa (Roubik & Ackermann, 1987). A Figura 2 resume a interação entre os diferentes grupos taxonômicos de orquídeas e abelhas Euglossini observadas durante a realização deste trabalho.

4. CONCLUSÕES

Os resultados deste estudo confirmam padrões antecipados em pesquisas realizadas em outras regiões neotropicais. As orquídeas nativas de Picinguaba, polinizadas por abelhas Euglossini, de um modo geral, pertencem a grupos taxonômicos onde este fenômeno foi descrito (Ackermann, 1983; Dressler, 1981, 1993; Roubik & Ackermann, 1987). No entanto, este é o primeiro registro sobre polinização para o gênero *Promenaea* (Zygopetalinae). Dentre os gêneros de Euglossini registrados, *Euglossa* possui maior importância na polinização de orquídeas. Apenas dois casos de interação abelha-polinizador representam exemplos devidamente comprovados de interação espécie-especifica (18,18% do total). As abelhas *Euglossa,* do subgênero *Glossura* destacam-se em importância como polinizadores. Uma única espécie de abelha deste subgênero, *E. chalybeata iopoecila,* poliniza quatro espécie de orquídeas, representando 36,36% do total de orquídeas polinizadas por Euglossini. Finalmente, como resultado deste estudo, houve um incremento significativo no conhecimento sobre as espécies de orquídeas bem como das abelhas Euglossini da região de Picinguaba.

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5. BIBLIOGRAFIA

ACKERMANN J.D. 1982. Specificity and mutual dependency of the orchid-Euglossini bee interaction. Biol. J. Linn. Soc. 20:301-314.

ADAMS, P.B. & LAWSON, S.D. 1993. Pollination in Australian orchids: a critical assessment of the literature 1882-1992. Austr. J. Bot. 41:553-575.

BRAGA, P.I.S. 1976. Atração de abelhas polinizadoras de Orchidaceae com auxílio de iscas-odores na Campina, Campinara e Floresta Tropical úmida da região de Manaus. Ciênc. e Cult. 28(7):767-773

DARWIN, C.R. 1862. The various contrivances by which orchids are fertilized. Murray, London.

DRESSLER, R.L. 1967. Why do Euglossini bees visit orchids? Atas do Simpósio sobre a Biota Amazônica 5:171-180.

DRESSLER, R.L. 1976. How to study orchid pollination without any orchids. Proceedings of the Eighth World Orchid Conference. p.534-537.

DRESSLER, R.L. 1978. An infrageneric classification of *Euglossa* with notes on some features of special taxonomic importance (Hymenoptera: Apidae). Rev. Biol. Trop. 26(1):187-198.

DRESSLER, R.L. 1979. *Eulaema bombiformis, E. meriana* and Mullerian mimicry in related species (Hymenoptera: Apidae). Biotropica 11(2):144-151.

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

DRESSLER, R.L. 1982a. Biology of the orchid bees (Euglossini). Annual Reviews Ecol. Syst. 13:373-394.

DRESSLER, R.L. 1982b. New species of *Euglossa* II (Hymenoptera: Apidae). Rev. Biol. Trop. 30(2): 121-129.

DRESSLER, R.L. 1982c. New species of *Euglossa* III. The bursigera species group (Hymenoptera: Apidae). Rev. Biol. Trop. 30(2):131-140.

DRESSLER, R.L. 1982d. New species of *Euglossa* IV. The cordata and purpurea species groups (Hymenoptera: Apidae). Rev. Biol. Trop. 30(4):141-150.

DRESSLER, R.L. 1993. Phylogeny and Classification of the orchid family. Dioscorides Press, Portland.

EITEN, G. 1970. A vegetação do Estado de São Paulo. Bol. Inst. Bot. (São Paulo) 7:1-147.

ELTZ, T., WHITTEN, W.M., ROUBIK, D.W. & LINSENMAIR, K.E. 1999. Fragance collection, storage and accumulation by individual male orchid bees. J. Chem. Ecol. 25(1):157-176.

ENGEL, M.S. 1999. The first fossil *Euglossa* and phylogeny of the orchid bees (Hymenoptera: Apidae: Euglossini). Amer. Mus. Novit. 3272, 14pp.

KIMSEY, L.S. 1982. Systematics of the bees of the genus *Eufriesea* (Hymenoptera: Apidae). Univ. Calif. Publ. Entomol. 95:1-195.

KIMSEY, L.S. 1987. Generic relationships within the Euglossini (Hymenoptera: Apidae). System. Entomol. 12:63-72.

KOEPPEN, W. 1948. Climatologia: com un estudio de los climas de la tierra. Fondo de Cultura Económica, México.

LUNAU, K. 1992. Evolutionary aspects of perfume collections in male Euglossini bees (Hymenoptera) and of nest deception in bee-pollineted flowers. Chemoecology 3:65-73.

MILLER D. & WARREN. R. 1996. Orquídeas do Alto da Serra. Salamandra Editorial, Rio de Janeiro.

NIMER E. 1977. Climatologia da região sudeste do Brasil. Rev. Bras. Geogr. 34:3-48.

PEARSON D.L. & DRESSLER, R.L. 1985. Two-year study of male orchid bee (Hymenoptera: Apidae: Euglossini) attraction to chemical baits in lowland south-eastern Peru. J. Trop. Ecol. 1:37-54.

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. Rev. Bras. Zool. 16 (Supl. 2): 101-118.

RIBEIRO, J.E.L.S. 1994a. Diversidade de orquídeas (Orchidaceae) da Planície Litorânea da Praia da Fazenda (Vila de Picinguaba, Município de Ubatuba, SP) e ocorrência no litoral brasileiro. Anais do II Simpósio de Ecossistemas da Costa Brasileira. Publicação ACIESP p.87.

RIBEIRO, J.E.L.S. 1994B. Distribuição de espécies de Orchidaceae na planície litorânea (restinga) da Praia da Fazenda, Município de Ubatuba (SP). Arqu. Biol. Tecnol. 37(3):515-526.

ROUBIK, D.W. & ACKERMANN, J.D. 1987. Long-term ecology of Euglossini orchid-bees (Apidade: Euglossini) in Panamá. Oecologia (Berlin) 73:321-333.

SAN MARTIN-GAJARDO, I. & FREITAS, L. 1999. Hummingbird pollination in *Besleria mucronata* Hoehne (Gesneriaceae) in southeastern Brazil. Biociências 7(2):13-24.

SAZIMA, M.; VOGEL, S.; COCUCCI, A. & HAUSNER, G. 1993. The perfume flowers of *Cyphomandra* (Solanaceae): pollination by Euglossini bees, bellows mechanism, osmophores and volatiles. Plant Syst. Evol. 187:51-88.

SINGER R.B. & COCUCCI, A.A. 1999. Pollination mechanism in four sympatric epidendroideae orchids from southeastern Brazil. Lindleyana 14(1):49-56.

VAN DER PIJL L. & DODSON, C.H. 1966. Orchid flowers: their pollination and evolution. University of Miami Press, Coral Gables.

WARFORD, N. 1992. Pollination biology: the recyprocal agreement between *Notylia* and *Euglossa viridissima*. Am. Orchid Soc. Bull. 61(9): 885-889.

WILLIAMS, N.H. 1982. The biology of orchids and Euglossini bees. Orchid Biology. Reviews and Perspectives (Arditti, J. ed.). Comstock Publishing Associates, Ithaca v.2, pp.120-171.

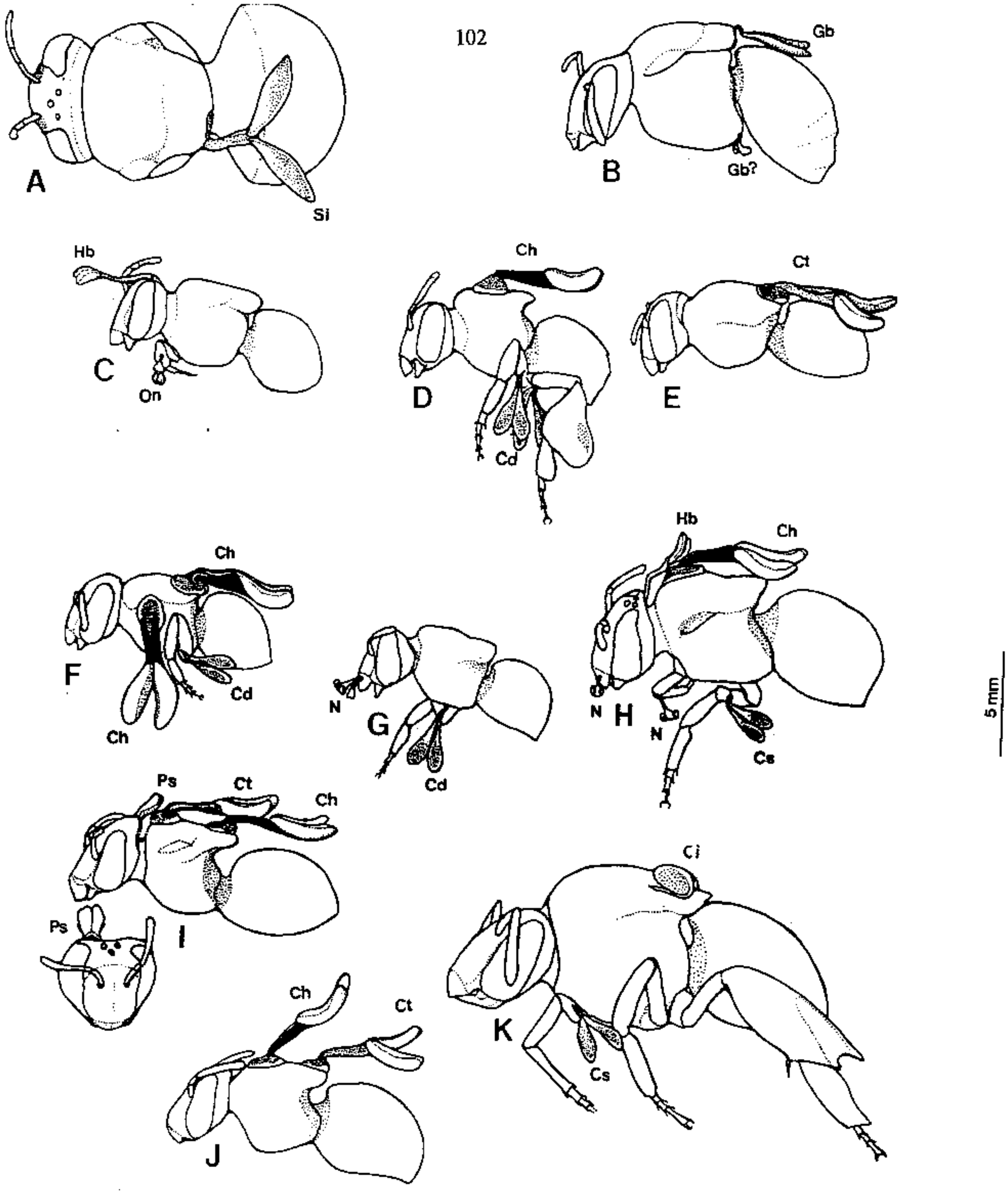

Figura 1: Esquema das abelhas Euglossini polinizadoras de orquídeas na região de Picinguaba, Ubatuba, SP, indicando o local de fixação e formato dos polinários. A: *Eufriesea purpurata*. B: *Eufriesea violacea*. C: *Euglossa iophyrra*. D: *Euglossa cordata*. E: *Euglossa pleosticta*. F: *Euglossa* cf. *viridis* G: *Euglossa sapphirina*. H: *Euglossa chalybeata iopoecila*. I: *Euglossa* cf. *ignita* J: *Euglossa* sp.2. K: *Eulaema nigrita*. Abreviaturas: Cd = *Cirrhaea dependens*. Ci = *Cattleya intermedia*. Ch = *Catasetum hookeri*. Cs = *Cirrhaea saccata*. Ct = *Catasetum trulla*. Gb = *Gongora bufonia*. Hb = *Houlletia brocklehurstiana*. N = *Notylia* sp. On = Oncidiiae indeterminada (*Rodrigueziopsis*?). Ps = *Promenaea stapelioides*; Si = *Stanhopea insignis*.

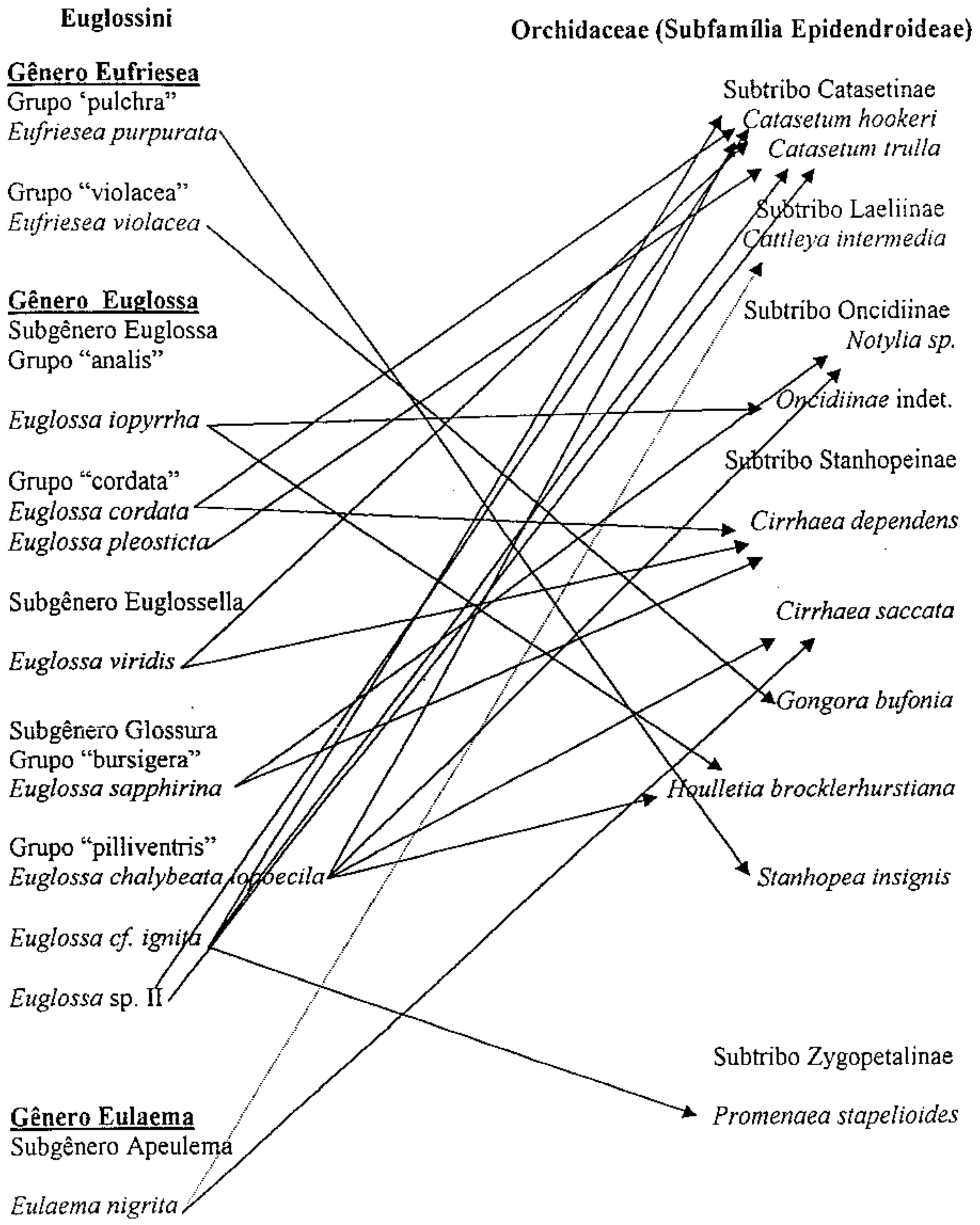

Figura 2: Diagrama resumindo as interações entre os grupos taxonômicos de orquídeas e abelhas Euglossini ocorrentes em Picinguaba, Ubatuba, SP. A linha pontilhada indica uma interação que requer mais evidências (ver texto).

CONCLUSÕES

A) ORQUÍDEAS TERRESTRES (SUBFAMÍLIAS SPIRANTHOIDEAE E ORCHIDOIDEAE)

Verificou-se a existência de vários padrões no que diz respeito à biologia reprodutiva e floral das orquídeas terrestres estudadas. Estas espécies são autocompatíveis, mas polinizador-dependentes, ou seja, é preciso que animais vetores efetuem a polinização para que estas plantas produzam frutos e sementes. A grande maioria das espécies estudadas não apresentou diferenças significativas entre a frutificação decorrente de autopolinização manual e polinização cruzada. Este fato sugere duas coisas:

1) Estas orquídeas, de um modo geral, não manifestam depressão endogâmica;

2) Não ocorrendo mecanismos fisiológicos que evitem ou dificultem a autopolinização, a polinização cruzada e os benefícios que ela traz (na forma de intercâmbio de alelos entre populações e a manutenção da diversidade genética) dependem em grande parte da conduta dos polinizadores.

Idealmente, os polinizadores deveriam: 1) manter certa constância nas flores; 2) visitar as flores favorecendo a polinização cruzada, minimizando a autopolinização (no sentido amplo, incluindo geitonogamia). Em alguns casos, como em *Stenorrhynchos lanceolatus*, a conduta dos polinizadores favorece nitidamente a polinização cruzada, no entanto, a conduta dos polinizadores em algumas orquídeas, tais como *Habenaria parviflora* e *Sauroglossum nitidum* sugere que nelas uma parte dos frutos deve decorrer de autopolinização (no sentido amplo) induzida pelos polinizadores.

Existe uma série de caracteres morfológicos intrínsecos comuns a todas as espécies estudadas, que favorecem a polinização cruzada: a abertura gradativa das flores (da base da inflorescência em direção ao ápice), os polinários friáveis (ora farináceos, ora compostos por mássulas) e as superfícies estigmáticas amplas. Devido à natureza friável dos polinários, a carga de pólen de um único polinário pode ser depositada em numerosos estigmas, o que pode favorecer o fluxo de pólen a longa distância. Nas orquídeas cujos polinários constam de mássulas (subtribos Goodyerinae e Habenariinae), cada uma destas subunidades deriva de uma única célula mãe do pólen. Em outras palavras, cada mássula representa um genótipo. No ato da polinização, várias mássulas são depositadas na superfície estigmática, garantindo que cada ovário receba várias contribuições genotípicas e, portanto, um considerável grau de diversidade genética, mesmo que todas as mássulas derivem de um único polinário.

A superfície estigmática ampla favorece a deposição de grandes quantidades de pólen, mas também incrementa as chances de uma única superfície estigmática receber várias cargas polínicas (por exemplo da mesma inflorescência ou de uma outra coespecífica). Teoricamente, a superfície estigmática poderia atuar como elemento seletivo (por ex: os tubos polínicos decorrentes de polinização cruzada poderiam se desenvolver mais rapidamente do que aqueles de autopolinização). Esta situação poderia, por exemplo, acontecer em *Stenorrhynchos lanceolatus*, na qual os beija-flores polinizadores tendem a visitar (e polinizar) todas as flores disponíveis. Pretendemos abordar estes aspectos em futuras pesquisas.

Algumas adaptações morfológicas de certos grupos de orquídeas terrestres merecem especial destaque não apenas por favorecerem a polinização cruzada, mas também por fornecerem evidências de ordem filogenética e evolutiva. Assim, na chamada "Aliança Pelexia" (gêneros *Cyclopogon*, *Pelexia* e *Sarcoglottis*, da subtribo Spiranthinae), bem como no gênero *Aspidogyne* (subtribo Goodyerinae) ocorrem viscídios dorsalmente adesivos. Na "Aliança *Pelexia*" o viscídio dorsalmente adesivo deve ser homólogo e provavelmente é uma sinapomorfia para este grupo de gêneros. Por outro lado, no gênero *Aspidogyne*, o viscídio dorsalmente adesivo é estruturalmente muito mais simples e não é comparável com o das orquídeas da subtribo Spiranthinae. Portanto, viscídios dorsalmente adesivos devem ter evoluído mais de umas vez na tribo Cranichideae (que contém as subtribos Spiranthinae e Goodyerinae, entre outras). Os motivos ecológicos desta convergência são evidentes. Um viscídio dorsalmente adesivo adere na superfície ventral do labrum das abelhas polinizadoras. Este fato traz algumas consequências: um polinário fixado ao polinizador dificulta mecânicamente a fixação de outros polinários, favorecendo não apenas uma "economia" de polinários, mas também a dispersão de pólen a longa distância. Como o labrum das abelhas é uma peça bucal flexível, que é retraída enquanto a abelha não se encontra forrageando, um polinário fixado em sua face ventral ficará protegido embaixo da cabeça da abelha quando ela estiver voando. Por outra lado, a face ventral do labrum é um lugar que dificilmente as abelhas conseguem limpar. Portanto, a ocorrência de viscídios dorsalmente adesivos e a sua conseqüente fixação na face ventral do labrum das abelhas polinizadoras, resulta ecologicamente vantajosa em contextos nos quais as orquídeas são apenas raramente visitadas pelos polinizadores (como em *Aspidogyne*) e quando as abelhas apresentam condutas de forrageamento do tipo "trap-lining" (percorrendo grandes distâncias). Nestas circunstâncias, polinários com viscídios dorsalmente adesivos promoverão o fluxo de pólen a longa distância. Este fato talvez explique a ocorrência desta estratégia de polinização em subtribos diferentes.

A ocorrência de protandria foi comunicada pela primeira vez para algumas espécies da subtribo Spiranthinae (*Sauroglossum elatum, Mesadenella cuspidata*), Prescottinae (*Prescottia stachyodes*) e Goodyerinae (*Erythrodes arietina*). Este caráter morfológico favorece a polinização cruzada, mas na nossa opinião também apoia a proximidade filogenética destas três subtribos de orquídeas terrestres. Nas três subtribos a protandria envolve movimentos da coluna e, em algum grau, do perianto. No entanto, deverão ainda ser feitos estudos para corroborar se a protandria observada nas três subtribos é, realmente, homóloga. Devemos salientar que a protandria inexiste em orquídeas terrestres que, por exemplo, apresentam outras modificações morfológicas marcantes, tais como viscídios dorsalmente adesivos ou adnação entre os lados do labelo e a coluna (ex.: *Stenorrhynchos lanceolatus*). Este fato nos leva a pensar que a protandria pode ser uma simplesiomorfia para a tribo Cranichideae, que se perdeu várias vezes e de maneira independente em várias espécies da tribo. Outras estratégias de polinização (por exemplo: viscídio dorsalmente adesivo) devem ser tão eficientes quanto a protandria para promover a polinização cruzada e provavelmente a substituíram do ponto de vista ecológico.

Finalmente, como mais um resultado destas pesquisas, a ocorrência de ornitofilia em *Stenorrhynchos lanceolatus* foi confirmada. Verificamos, ainda, que os viscídios dorsalmente adesivos da "Aliança *Pelexia*" (Spiranthinae) e do gênero *Aspidogyne* (Goodyerinae) podem ser manipulados e fixados apenas por abelhas, excluindo qualquer outro tipo de polinizador. Este é também o primeiro relato de falenofilia (polinização por mariposas) nas subtribos Spiranthinae (*Sauroglossum elatum*) e Prescottinae (*Prescottia plantaginea* e *P. stachyodes*).

B) INTERAÇÕES ENTRE ORQUÍDEAS E ABELHAS EUGLOSSINI

De um modo geral, na região de Picinguaba verificamos padrões similares de interação entre orquídeas Epidendroideae e abelhas Euglossini aos já relatados na literatura para regiões da América Central e do Peru. As orquídeas polinizadas por machos de abelhas Euglossini na região de Picinguaba pertencem às subtribos Stanhopeinae, Catasetinae, Zygopetalinae, Oncidiinae e Laeliinae. Estas subtribos haviam sido reportadas na literatura como sendo (ao menos parcialmente) polinizadas por machos de Euglossini. Apenas *Promenaea stapelioides* (localmente polinizada por *Euglossa* cf. *ignita*) parece ser uma adição inédita aos niveis genérico e específico.

De um modo geral, o gênero *Euglossa* (em particular, o subgênero *Glossura*) é o mais importante no que diz respeito à polinização de orquídeas nativas. *Euglossa* (*Glossura*) *chalybeata iopoecila* é, provavelmente, a mais importante polinizadora de orquídeas locais. Esta subespécie de *Euglossa chalybeata* é endêmica da mata Atlântica do sudeste brasileiro. Documentamos a ocorrência de quatro casos de interação espécie-específica (uma orquídea-um polinizador). No entanto, apenas a polinização de *Gongora bufonia* por *Eufriesea violacea* e de *Stanhopea insignis* por *Eufriesea purpurata* (esta interação esta sendo estudada em detalhe por E. Pansarin. E. Pansarin, com. pess.)foram devidamente comprovadas. Os outros dois casos precisam, ainda, de dados adicionais. Como consequência destas pesquisas, vários taxa de Orchidaceae (*Houlletia brocklehurstiana*, *Notylia* sp., Oncidiinae indet.) e de Euglossini estão sendo citados pela primeira vez para esta região.