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*Short Communication*

# Facultative autogamy in *Cyrtopodium polyphyllum* (Orchidaceae) through a rain-assisted pollination mechanism

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**Abstract.** *Cyrtopodium* includes ~42 species, among which is *Cyrtopodium polyphyllum* (Vell.) Pabst ex F. Barros that occurs in a rainforest in south-eastern Brazil. Its non-rewarding flowers, which attract Centridini bees by deceit, are rain-assisted self-pollinated, a phenomenon rarely found in orchids and other plant families. In addition, self-pollination has never been reported in Cyrtopodiinae and data on the pollination of South American orchids are scarce. Flowers were observed at different times of the day, on both sunny and rainy days, to record floral morphology, visitors and the effects of rainfall on flowers. On rainy days, water accumulates on the stigma and dissolves the adhesive substance of the stigmatic surface. A viscous drop thus forms, which contacts the pollinarium. When evaporation makes the viscous drop shrink, the drop moves the pollinarium with the anther onto the stigmatic surface and promotes self-pollination. Fruit set in natural habitat was low, with 2.4% at one study site, where a similar value (2.2%) was recorded in flowers self-pollinated by rain. In *C. polyphyllum*, facultative self-pollination assisted by rain is thus an important strategy that guarantees fruit set when pollinator's visits are scarce, which is common in species pollinated by deceit.

## Introduction

Orchid flowers are mostly pollinated by biotic vectors (van der Pijl and Dodson 1966). Pollination through abiotic factors, such as rain, occurs more rarely in Orchidaceae and has been mainly reported for self-pollinated species (van der Pijl and Dodson 1966; Catling and Catling 1991). Autogamy via a variety of mechanisms has been documented for many different genera of Orchidaceae (van der Pijl and Dodson 1966; Catling and Catling 1991). According to Catling (1990), ~350 orchid species are autogamous. In both self-compatible *Oeceoclades maculata* (Lindl.) Lindl. and *Liparis loeselii* (L.) Rich., autogamy through self-pollination is caused by a physical disturbance of the anther through the action of raindrops (Catling 1980; González-Díaz and Ackerman 1988), although the mechanism of each species differs. Catling (1980) stated that, although rainfall may be important since it improves the chances of autogamy in cloud forest orchids, this phenomenon has never been documented in South American Rainforest species. Furthermore, even though autogamy is well known for North American orchids (Catling 1990; Catling and Catling 1991), conclusive data on South American species have only been presented for *Polystachya estrellensis* Rchb. f. in south-eastern Brazil (Pansarin and Amaral 2006).

The Neotropical genus *Cyrtopodium* (Epidendroideae: Cymbidieae: Cyrtopodiinae) comprises ~42 species distributed from southern Florida to northern Argentina (Batista and Bianchetti 2004). The centre of diversity of the genus is the Brazilian 'cerrado' (Batista and Bianchetti 2004). Nevertheless, *Cyrtopodium polyphyllum* (Vell.) Pabst *ex* F. Barros (i.e. *Cyrtopodium paranaense* Schltr.), the unique species of this genus that occurs along the Brazilian coast mainly from south to south-eastern Brazil (Hoehne 1942), grows in sandy soils or on rocks. Like other *Cyrtopodium* species (Chase and Hills 1992), *C. polyphyllum* offers no reward to its pollinators, and attracts Centridini bees by food deceit (L.M. Pansarin, E.R. Pansarin and M. Sazima, unpubl. data).

Flowers of *Cyrtopodium polyphyllum* were manipulated to test the occurrence of rain-assisted autogamy and observations were made to investigate the mechanism involved in this rain-assisted self-pollination. This paper reports the first case of rain-assisted pollination in Cyrtopodiinae and in a South American species occurring in the Atlantic rainforests. The influence of rain-assisted self-pollination on the increase of natural fruit set and, consequently, on the reproductive success of this Brazilian species is also discussed.

## Material and methods

Populations of *Cyrtopodium polyphyllum* were studied at two sites at Parque Estadual da Serra do Mar: Picinguaba and Praia da

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Fortaleza (50 km apart from each other), both located in municipality of Ubatuba (~23°22′S, 44°50′W; 0–50 m asl), a region characterised mainly by submontane rainforests in the State of São Paulo, south-eastern Brazil. Climate is tropical-humid ('Af.'; see Köppen 1948), with a maximum annual rainfall of 2600 mm, an average annual temperature of 22°C and no well defined dry-cold season even during the so called dry months (from May to September), with a mean precipitation of 118 mm/month. The wet season occurs from October to April, with a mean precipitation of 285 mm/month and air relative humidity more than 80% (Data source: Instituto Agronômico de Campinas, Campinas, Brazil). Both studied populations occur in the restinga scrub, and grow on rocks or in sandy soils. In October, each plant develops a new pseudobulb and sometimes, simultaneously, a lateral, paniculate and erect inflorescence that produces up to ~130 resupinate flowers. The main flowering period occurs in November.

Data on pollination were obtained at both study sites during the 2005 flowering period, from 15–21 November, 28 November to 1 December, 8–9 December and 12–14 December. Flowers were observed at different times of the day, from 0800 h to 1400 h, totaling 90 h, on both sunny and rainy days. The effects of rainfall on flowers were recorded through field notes and photographs of 1359 flowers (30 plants) at Praia da Fortaleza. At the end of each observation day these inflorescences were bagged with tulle to exclude potential biotic pollinators. Details of flower and column morphology were observed under binocular stereomicroscope. Plant voucher (Ubatuba, XI.2005, *L. Mickeliunas and E.R. Pansarin 48*) is deposited at the Herbarium of the Universidade Estadual de Campinas (UEC).

Treatments to investigate the breeding system of *Cyrtopodium polyphyllum* were performed in their natural habitat by using flowers previously bagged with tulle. The experimental treatments included manual self- and cross-pollinations, and emasculations. Thirty flowers (three plants) were used per treatment for each study site. Data on flower production, occurrence of self-pollination through rain and otherwise natural fruit set were recorded. Treatments were randomly applied to each inflorescence, with flowers of different ages. Natural fruit set (open pollination) of *Cyrtopodium polyphyllum* was recorded for both study sites. At Praia da Fortaleza, 2028 flowers (30 plants) were sampled in August 2005, while at Picinguaba 2380 flowers (30 plants) were sampled in July 2006.

Fruit set was recorded when fruits were dehiscent. The number of flowers/fruits sampled varied and depended on the total production of each year. Seed quality (e.g. presence of developed embryos) was checked by placing them in a 1% solution of 2,3,5-triphenyltetrazolium chloride (Lakon 1949).

## Results

Flowers of *Cyrtopodium polyphyllum* last about three weeks, are yellow-greenish and present a central callous on the lip resembling an elaiophore, but anatomical studies revealed that no edible oil is produced (L. M. Pansarin, M. de M. Castro and M. Sazima, unpubl. data). The arched column presents a concave and transversally disposed oval stigma (Fig. 1*A*, *B*). The pollinarium is separated from the stigmatic surface by a thick rostellum. On rainy days, water accumulates in the stigma cavity and dissolves the stigmatic secretion. A viscous drop thus forms, which contacts the pollinarium (Fig. 1*C*, *D*). When evaporation makes it shrink, this drop moves the swollen pollinarium with the anther onto the stigmatic surface, promoting self-pollination (Fig. 1*E*, *F*). Seven to 9 days are necessary for the (very viscous) drop to evaporate after rainfall, which may last several days. This self-pollination process occurred in 89 (6.5%) of the 1359 flowers, of which 30 (2.2%) actually developed fruits. However, when considering actual fruit set (30) of the self-pollinated flowers (89), then the percentage is much higher 33.7%. The population of Picinguaba, albeit smaller, developed a similar percentage of fruit set after self-pollination by rain (L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data). Occasional records about this phenomenon were made on scattered individuals.

No potential pollinators of *C. polyphyllum* were recorded in activity on rainy and cloudy days. However, on sunny days the flowers were pollinated by *Centris labrosa* and *C. tarsata* (Apidae: Anthophorinae), whose visits were scarce since they were attracted to the non-rewarding flowers by deceit (L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data). *Cyrtopodium polyphyllum* is self-compatible and presents high fruit set after manual treatments: 16.66% and 63.33% in self-pollinated flowers and 63.33% and 76.6% in cross-pollinated flowers at Picinguaba and Praia da Fortaleza, respectively. No fruits developed after emasculations. Under natural conditions (open pollination, through rain and/or bees), fruit set was low, with 1.34% (Picinguaba) and 2.42% (Praia da Fortaleza). Fruit set through self-pollination by rain was 2.2% (Praia da Fortaleza). If one considers, for instance, the fruit set under natural conditions of 2.42% and that 2.2% were due to self-pollination by rain, then only 0.2% would have been induced by biotic vectors. Fruits obtained in treatments (cross and self-pollination) and under natural conditions (open pollination, through rain and/or bees) presented a high percentage of viable seeds, over 90%, on average (L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data).

## Discussion

Among Orchidaceae, autogamy can occur in different ways (see reviews by van der Pijl and Dodson 1966; Catling 1980, 1990; Catling and Catling 1991). An uncommon mechanism of self-pollination, autogamy assisted by rainfall as the one reported here for *Cyrtopodium polyphyllum*, differs somewhat from those reported for *Oeceoclades maculata* (González-Díaz and Ackerman 1988) and *Liparis loeselii* (Catling 1980). In *O. maculata*, self-pollination through raindrops occurs after anther disturbance. The stipe then dries and bends down so that pollinia hang near the stigma (González-Díaz and Ackerman 1988). In *L. loeselii*, the pollinaria fall out and remain suspended from the rostellum edge until a raindrop hits the raised and hinged anther causing it in turn to hit the suspended pollen masses, pushing them around the edge of a ridge onto the stigmatic surface (Catling 1980). Whereas in *C. polyphyllum* the process depends on water accumulation in the stigmatic cavity, dissolution of the adhesive stigmatic substance and further slow evaporation of the drop, moving the swollen pollinarium

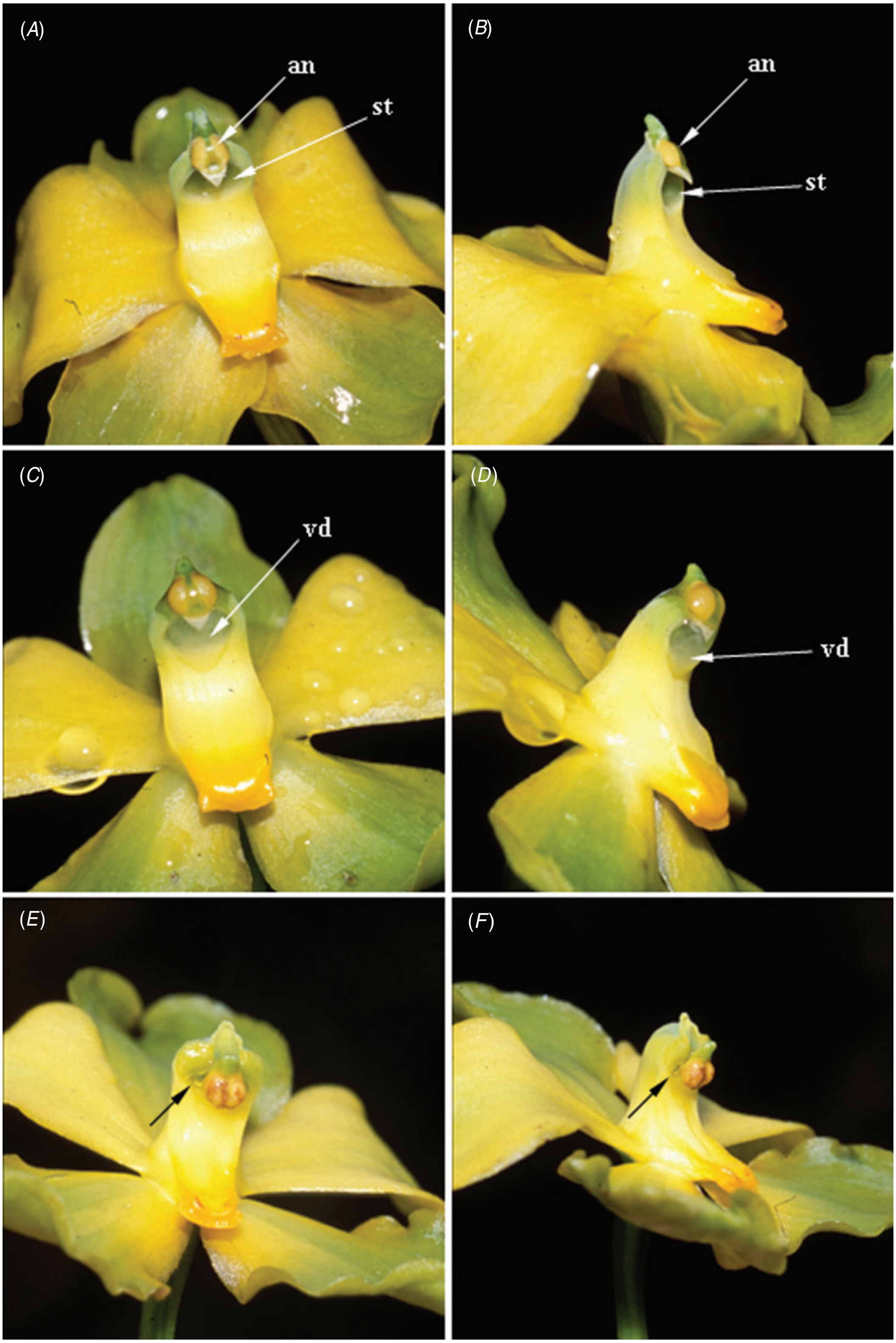


**Fig. 1.** Flowers of *Cyrtopodium polyphyllum* in frontal and lateral views. (*A*, *B*) Flowers showing the concave and oval stigmatic cavity (st) and the pollinarium with the anther cap (an) (arrows). The rostellum prevents the pollinarium from contacting the stigma. No raindrops are retained in the stigmatic cavity of these flowers. (*C*, *D*) Flowers with a great, viscous drop (vd) on their stigma (arrows). Note the swollen pollinarium with the anther cap. (*E*, *F*) Flowers whose pollinarium and anther were disturbed. The drop shrank through evaporation and the pollinarium and anther were moved onto the stigma. Remains of the drop stay in the stigmatic cavity (arrows).

onto the stigmatic cavity. However, it is not clear why rain drops accumulated on the stigmatic surface of only 6.5% of the flowers, but it may be related to flower position in the inflorescence, and/or flower age and variation in column and stigma morphology.

*Liparis loeselii* and *Oeceoclades maculata* are naturally autogamous and rainfall increases the incidence of automatic self-pollination (Catling 1980; González-Díaz and Ackerman 1988). In contrast, *Cyrtopodium polyphyllum* is pollinated by Centridini bees (L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data), and although by deceit, its pollination mechanism is similar to the elaiophor-bearing Cyrtopodiinae *Grobya amherstiae* Lindl. (Mickeliunas *et al*. 2006), and of species of Ornithocephalinae (Vogel 1974) and Oncidiinae (Vogel 1974; Schlindwein 1995; Singer and Cocucci 1999). Furthermore, in spite of being self-compatible, *C. polyphyllum* is not naturally autogamous and depends on a pollen vector, like the self-compatible Cyrtopodiinae *Grobya amherstiae*, which also depends on a biotic vector for pollination (Mickeliunas *et al*. 2006). In *C. polyphyllum*, self-pollination assisted by rain only occurs under high amounts of precipitation and therefore high air humidity, which reaches more than 80% in summer in Ubatuba region. The occurrence of prolonged periods of rainfall that promote facultative self-pollination in *C. polyphyllum*, and considering the actual fruit set of almost 34%, is thus an important means to assure fruit production in periods when pollinator's visits are scarce or absent.

Scarcity of bee visits, as is the case with *Cyrtopodium polyphyllum*, may be a rule in non-rewarding flowers pollinated by deceit (Ackerman 1986, 1989; Montalvo and Ackerman 1987; Zimmerman and Aide 1989). Besides, climatic factors such as cloud cover and precipitation, wind speed, air temperature, solar radiation and humidity exert strong influences on the activity of bees (including Centridini bees in Neotropical regions) and, consequently, on their foraging behaviour and visitation to flowers (see review by Roubik 1992). Since precipitation is a frequent phenomenon in the region of Ubatuba, and rain may persist for one week or more during the wet season, the occurrence of facultative self-pollination through rain in *C. polyphyllum* is favoured and can be an important reproductive strategy. Furthermore, self-pollination [i.e. autogamy that does not require pollen transfer by insects or other animal vectors (Lloyd and Schoen 1992)] may be an extreme adaptation to provide reproductive assurance in pollinator-poor habitats. Self-compatibility and autogamy are hypothesised to be particularly advantageous when the probability of pollen transfer between plants is low (Baker 1955; Stebbins 1957; Holsinger 1986; Cox 1989; Moeller and Geber 2005). According to Dressler (1981), shifts between rewarding and deceptive pollination systems have occurred many times in the evolution of the Orchidaceae and these deceptive systems seem to be derived from rewarding species in the family (Ackerman 1986). Some authors argue that reward production can be energetically expensive and resources used to produce this reward could be better expended in other functions which may ultimately increase the fitness of those individuals lacking pollinator rewards (Ackerman 1986). The natural fruit set of rewardless species, as reported here for *C. polyphyllum*, is generally very low. In the case of *C. polyphyllum*, pollinated by centridini bees which perform mainly cross-pollinations (L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data), an occasional crossing event may be sufficient to infuse enough genetic variability to diminish any effects of inbreeding depression from frequent self-pollinations (see review by Tremblay *et al.* 2005). Conversely, *C. polyphyllum* presented a high rate of fruit abortion in natural conditions, with only 2.2% of 6.5% of pollinations resulting in developed fruits. This rate of fruit abortion may indicate resource limitation, as has been reported for several other orchid species (Montalvo and Ackerman 1987; Ackerman 1989; Zimmerman and Aide 1989; Borba and Semir 1998).

Nevertheless, it is worth stressing that in *Cyrtopodium polyphyllum* self-pollination is facultative, and only occurs on wet days when biotic pollinators (i.e. Centridini bees) are absent from the environment (Roubik 1992; L. M. Pansarin, E. R. Pansarin and M. Sazima, unpubl. data), whereas in *Liparis loeselii* (Catling 1980) and *Oeceoclades maculata* (González-Díaz and Ackerman 1988) rainfall increases the incidence of self-pollination. As pointed out by Catling (1980), it is worth paying more attention to the significance of rain as an auxiliary agent of autogamy, especially in forests with high rainfall and richness of orchid species, as is the case with the study sites (Ribeiro 1994).

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