Short communication

Reproductive biology of *Echinodorus longipetalus* (Alismataceae): Sexual morphs, breeding system and pollinators

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ABSTRACT

The floral biology, pollinators and breeding system of *Echinodorus longipetalus* Micheli were studied in a marshy area of the district of Taquaritinga (State of São Paulo), southeastern Brazil. *E. longipetalus* is gynodioecious and as far as is known, this is the first record of unisexual flowers, besides perfect flowers, in *Echinodorus*. Proportion of female individuals in the studied population is 50% and produces 31% more flowers than hermaphrodites. Perfect and pistillate flowers of *E. longipetalus* are similar in appearance and are pollinated by several species of Hymenoptera (mainly by *Xylocopa* (*Neoxylocopa*) *suspecta* Moure & Camargo). Perfect flowers offer pollen as a reward. Pistillate flowers attract floral visitors by deceit with their staminodes that resemble the stamens of the perfect flowers. Visits to pistillate flowers are quick (1–2 s), while visits to perfect flowers last up to 120 s. The perfect flowers are self-compatible and produce fruits through spontaneous self-pollination (control flowers), whereas the pistillate ones only set fruits through cross-pollinations. Perfect and pistillate flowers set more fruits under natural conditions than in manual treatments, respectively. Although the pistillate and perfect flowers bear a strong similarity, the selective pollinator behavior seems to be responsible for the increase of fruit set in perfect flowers.

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1. Introduction

The family Alismataceae is subcosmopolitan in distribution and comprises 11 genera and about 75 aquatic or semi-aquatic species. Two genera are known in the Neotropics: *Sagittaria* L. and *Echinodorus* Rich. *ex* Engelm. The 25 species of the genus *Sagittaria* are subcosmopolitan. *Echinodorus* is the largest genus within Alismataceae and comprise 26 species distributed among the Western hemisphere, from northern United States of America to Argentina (Haynes and Holm-Nielsen, 1994). In the State of São Paulo (southeastern Brazil), the genus *Echinodorus* is represented by seven species, including *Echinodorus longipetalus* Micheli (Pansarin and Amaral, 2005). The main morphological characteristic that distinguishes between the Neotropical genus *Echinodorus* and *Sagittaria* is the presence of perfect and unisexual flowers, respectively (e.g., Haynes and Holm-Nielsen, 1994; Pansarin and Amaral, 2005). The Brazilian species of *Sagittaria* seem to be monoecious

(Haynes and Holm-Nielsen, 1994; Pansarin and Amaral, 2005). Nevertheless, at least two species are gender dimorphic and present monoecious and dioecious populations: *S. latifolia* L. (Wooten, 1971; Sarkissian et al., 2001) and *S. lancifolia* (Muenchow, 1998).

Data on the reproductive biology of the Neotropical species of Alismataceae are rare in the literature and are only available for *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli in south-eastern Brazil. *E. grandiflorus* presents two subspecies, *E. grandiflorus* ssp. *aureus* and *E. grandiflorus* ssp. *grandiflorus* (Haynes and Holm-Nielsen, 1994). According to Vieira and Lima (1997), *E. grandiflorus* ssp. *aureus* is self-compatible, while *E. grandiflorus* ssp. *grandiflorus* is self-incompatible. Both subspecies are mainly pollinated by bees that take nectar or collect pollen from their flowers. In contrast, data on the reproductive biology of the subcosmopolitan genus *Sagittaria* are more common in the literature (see Wooten, 1971; Muenchow, 1998; Sarkissian et al., 2001).

Based on records of the floral morphs, pollinators, pollination mechanisms, breeding system and fruit set under natural conditions, the present paper reports aspects of the reproductive biology of *E. longipetalus*.

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2. Materials and methods

2.1. Study site

Reproductive biology of *E. longipetalus* was studied in the district of Taquaritinga (approximately 48°29′53″W, 21°44′44″S), in State of São Paulo (southeastern of Brazil) during the 2006 flowering season. The region presents a mean altitude of 512 m. Mean temperature is 24 °C and mean annual rainfall is 1600 mm. Climate is of the "Cwa" type (mesothermic with a dry winter season), according to Köppen classification (1948). This region is characterized by a 71% air relative humidity and a well-defined dry-cold season even during the so called dry months (April to September) and wet months (October to March). It is mainly covered with mesophytic semidecidous forests (Pinto, 1989).

2.2. Study species, sexual morphs and flower features

E. longipetalus is a marshy herb occurring in several localities in the State of São Paulo. This species is easily recognized by its linear-lanceolate leaves with pellucid markings forming a network independent from the veins and by its large and numerous white flowers produced in 2–11 whorls and on a raceme-like cyme (Pansarin and Amaral, 2005).

Data on the anthesis period, flower longevity, pollen availability and odor production were recorded for both the hermaphrodite and female plants. All flowering individuals within the population were counted and sexed and number of flowers produced were recorded on pistillate (30 inflorescences; 30 plants) and hermaphrodites (30 inflorescences; 30 plants).

The morphological features of 120 fresh flowers (60 perfects and 60 pistillates) collected at the study site were recorded under a binocular stereomicroscope. All the flowers were examined in order to detect the possible presence of nectaries, which was detected in populations of *E. grandiflorus* (Vieira and Lima, 1997). Measurements were made directly from floral structures using a caliper rule. In order to test pollen viability 30 perfect flowers collected during the morning hours (at about 04:30 h), before insect visitation, were used. Slides were prepared with macerated anthers over a drop of aceto-carmine glycerol jelly (Radford et al., 1974). For odor verification, flowers at anthesis were placed in Petri dishes. Plant voucher: Taquaritinga, I.2007, *E.R. Pansarin* 1243, was deposited at the Herbarium of the Universidade de São Paulo, FFCLRP (SPFR).

2.3. Floral visitors

Data on the pollinators and visit behavior to the flowers of *E. longipetalus* were observed and pollinators were captured for later identification from 13 to 18 December 2006. The daily observation period was from 05:00 to 13:00 h, totaling 48 h.

2.4. Breeding system

The experimental treatments to investigate the breeding system of *E. longipetalus* were made in the field in December 2006, and included four treatments: manual self- and cross-pollinations, emasculations (agamospermy) and spontaneous self-pollination (control flowers). They involved 30 flowers (3 inflorescences; 3 plants) per treatment for hermaphrodite plants and 30 flowers (3 inflorescences; 3 plants) of female plants. Treatments were randomly applied to each inflorescence and were performed during the morning hours by using previously bagged flowers. Bagged buds were emasculated before flower anthesis, at about 05:00 h. Cross- and manual self-pollinations were per-

formed between 09:00 and 10:00 h. The fruit set under natural conditions (open pollination) of 1606 flowers (30 inflorescences; 30 plants) of hermaphrodite plants and 2324 flowers (30 inflorescences; 30 plants) of female individuals were recorded. The number of flowers setting fruits (pistillate and perfects) under natural conditions (open pollination) was compared using a *t*-test for independent samples using Statistica 6.0 (StatSoft Inc., 2003). The number of achenia produced in each treatment, as well as in 200 receptacles (30 inflorescences; 30 plants) of hermaphrodites and 200 receptacles (30 inflorescences; 30 plants) of females collected under natural conditions (open pollination) was also recorded.

3. Results

3.1. Study species, sexual morphs and flower features

Both female and hermaphrodite plants of *E. longipetalus* flower simultaneously between November and January, with a flowering peak in December. The inflorescence of both female and hermaphrodite plants is a raceme-like cyme with up to 130 flowers produced in 2–11 whorls. The studied population presents a ratio of 164 hermaphrodites to 166 females in the 2006 flowering period, i.e., 1:1 ratio. Both perfect and pistillate flowers are similar in size (4–6 cm diameter), 3-merous, actinomorphic, pedicelate, and have persistent, free and green sepals (9.1–18.9 mm × 6–11.3 mm) surrounding the mature fruiting aggregate. Petals are delicate, white, free and measure 2–3 cm × 1.5–3 cm. Perfect flowers presents 30–42 stamens. Stamens show versatile anthers, opening through lateral and longitudinal slits. Pollen release is gradual and facilitated by vibratory movements (see floral visitors). Pollen viability (perfect flowers) is 99.5% ($N = 6000$). Pistillate flowers present rings of sterile stamens. Staminodes (pistillate flowers) and stamens (perfect flowers) are yellow and similar in appearance (Fig. 1A and B). Sterile stamens (1.4–1.9 mm long) are smaller than fertile stamens (2.4–2.8 mm long), presenting rudimentary and indehiscent anthers. Apocarpic gynoecia present numerous carpels over a convex receptacle. Each carpel shows a single ovule and a terminal and rudimentary stigma. Flower opening occurs between 04:30 and 05:00 h and each flower (pistillate and perfect) lasts 7–8 h. Anther dehiscence (perfect flowers) occurs between 30 and 60 min after flower anthesis. Pistillate and perfect flowers emit a sweet fragrance perceptible in the warmest hours of the day. Fruits (achenia) are dispersed between January and February. The lack of secretion in both flower morphs indicates the absence of the nectaries in *E. longipetalus*. Proportion of females in the studied population was about 50% and produced 31% more flowers than hermaphrodites.

3.2. Floral visitors

Flowers of *E. longipetalus* were visited by several species of beetles, hoverflies and bees. All floral visitors were observed collecting pollen from perfect flowers. Nevertheless, only species of bees acted as pollinators (Table 1) because beetles and hoverflies eat pollen directly from anther of perfect flowers. The pistillate flowers possess yellow staminodes that attracted the bees by deceit. As a consequence, the visits to pistillate flowers were quick (1–2 s), while visits to perfect flowers lasted up to 120 s. Bees sometimes only hovered in front of the pistillate flowers without landing on them. All the bees except *Xylocopa* (Fig. 1C–D) presented a similar behavior. They landed on the petals and moved to the reproductive organs, or directly on the receptacle (Fig. 1E) or on the stamens (Fig. 1F). The bees collected pollen using their anterior and middle legs, making circular movements and
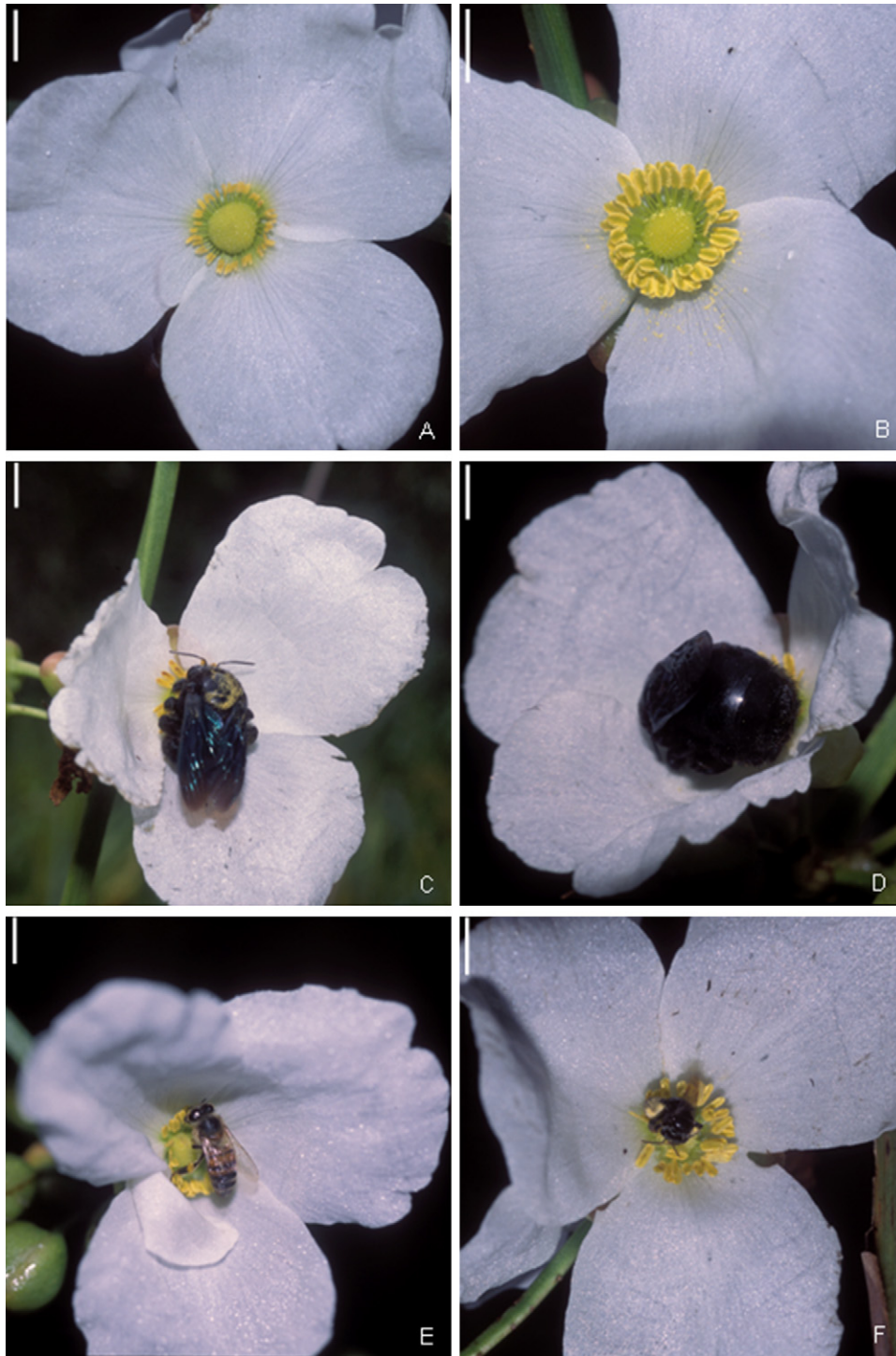
Fig. 1. *Echinodorus longipetalus* Micheli. (A) Pistillate flower. (B) An intact perfect flower. Note the pollen grains on the petals and on the apocarpic ovary, self-pollinating the flower. (C–D) *Xylocopa suspecta* performing buzz-pollination in a perfect flower. Note that this large bee vibrate all stamens simultaneously. (E) *Apis mellifera* collecting pollen. (F) *Melipona* sp. collecting pollen. Note that *Apis* and *Melipona* collect pollen from a single anther. Scale bars: A–F = 2 cm.

generally exploring all anthers. During collection, they contacted the stigmas with their legs and their ventral body parts, where loads of pollen were observed. *Xylocopa* landed on the receptacle, their legs explored all the stamens (Fig. 1C–D) and then performed buzz-pollination. During the vibratory movements, large quantities of pollen grains were observed being deposited on stigmas. All the bee species (except *Apis mellifera* (Fig. 1E) that only visited flowers in the morning hours) were recorded visiting flowers during all the flower life.

3.3. Breeding system

E. longipetalus is self-compatible, producing fruits through spontaneous self-pollination (perfect flowers; Table 2). Pistillate flowers are obligatorily cross-pollinated. No fruits developed after emasculations (agamospermy). Under natural conditions, fruit set (total number of achenia produced) was high. Fruits were mature 1 month after pollination. There was no significant difference between the mean fruit set resulting from pistillate and perfects

Table 1
Bee species collected on the flowers of *Echinodorus longipetalus* in Taquaritinga—SP, and their body length

Bee species	Bee length (mm)
Andrenidae	
Psaenythia bergi Holmberg	12.0
Anthophoridae	
Exomalopsis fulvopilosa Spinola	10.0
Xylocopa (*Neoxylocopa*) *suspecta*	28.0
Moure & Camargo (Fig. 1C–D)	
Apidae	
Apis mellifera L. (Fig. 1E)	15.0
Trigona spinipes Fabricius	8.0
Melipona sp. (Fig. 1F)	8.5
Plebeia droryana Friese	4.0
Plebeia sp.	5.0
Halictidae	
Augochlora sp.	10.5
Dialictus sp.	5.5
Megachilidae	
Megachile sp.	10.5

under natural conditions (open pollination; t-test = -1.430, d.f. = 29, $P = 0.163$). Production of achenia by receptacles was low in all treatments (40–65.6) when compared with fruit formed under natural conditions (143–184.7). Breeding system of *E. longipetalus* is summarized in Table 2.

4. Discussion

Among the Alismataceae that occur in the Neotropics, the production of unisexual flowers, as documented here for *E. longipetalus*, has only been reported for *Sagittaria*, a subcosmopolitan genus with three species occurring in the State of São Paulo (Pansarin and Amaral, 2005). The production of perfect (*Echinodorus*) vs. unisexual (*Sagittaria*) flowers has been the most used parameter for distinguishing between both Neotropical genera within the family (Haynes and Holm-Nielsen, 1994; Pansarin and Amaral, 2005) and as far as is known, this is the first record of unisexual flowers and gynodioecy (populations with both females and hermaphrodite plants) in *Echinodorus*.

Although nectar is the most widespread floral reward within Alismataceae and is recorded in species of *Sagittaria* (Wooten, 1971; Muenchow, 1998; Sarkissian et al., 2001; Pansarin, personal observation), *Damasonium* (Vuille, 1987), *Baldellia* (Vuille, 1988), *Caldesia* (Gituru et al., 2002) and *E. grandiflorus* (Vieira and Lima, 1997), *E. longipetalus*, offer solely pollen as a reward. Data on pollinators of *Echinodorus* are scarce in literature, but as well as reported here for *E. longipetalus*, *E. grandiflorus* also is pollinated by bees, some of which perform buzz-pollination on the flowers (Vieira and Lima, 1997) in a similar way as *Xylocopa suspecta*. Visitation frequencies (data not presented) and time of visit are higher on perfect than on pistillate flowers and can be related to the bee capacity to recognize rewardless pistillate flowers.

Although pistillate and perfect flowers bear a strong similarity, the selective pollinator behavior and consequent increase of visits on perfect flowers (data not presented) seems to be responsible for the increase in the fruit set of perfect flowers. According to Ågren and Schemske (1991) and Schemske and Ågren (1995), in species with unisexual flowers deceit pollination system presents a challenge to pollinators, as selection favors individuals that can distinguish between non-rewarding pistillate flowers and reward-producing male or perfect flowers. As a consequence, open-pollinated perfect flowers of *E. longipetalus* produce more achenia than open-pollinated pistillate. These results contrast with some gynodioecious species in which pistillate flowers produce much more fruits than perfect ones (e.g., Beaumont et al., 2006 and references therein). Although in the studied population of *E. longipetalus* hermaphrodites produce more fruits than females, other ecological and genetic factors, as size and quality of seeds and seed germination (Delph, 1990) and/or pleiotropic effects of sex-determining genes (Charlesworth, 1981) and inbreeding depression (Wolfe, 1995) increase fitness of females within gynodioecious populations (Charlesworth, 1981).

Although the population of *E. longipetalus* presents a similar number of hermaphrodites and female plants, previous studies report that in most gynodioecious species, female plants are less frequent than hermaphrodites (see Delph, 1990, 1996; Beaumont et al., 2006). Thus, the proportion of females (ca. 50%) in the studied population is relatively high. Additionally, female plants *E. longipetalus* produce 31% more flowers than hermaphrodites. Several studies report that female outcrossing advantage is supported by evidence of greater inbreeding in hermaphrodites compared to females and by accompanying inbreeding depression (see Schultz and Ganders, 1996). Assuming that pistillate flowers, which produce no pollen are less costly, some researchers argue that females can allocate more resources to seed production (e.g., Delph, 1990).

As it is reported here for perfect flowers of *E. longipetalus*, some species of *Sagittaria* (Wooten, 1971; Sarkissian et al., 2001), *Damasonium* (Vuille, 1987), *Baldellia* (Vuille, 1988) and *Caldesia* (Gituru et al., 2002) are also self-compatible, but unlikely *E. longipetalus*, they depend on a pollinator for pollen transfer. The inflorescences of monoecious *Sagittaria*, therefore, produce pistillate flowers at basal nodes and male flowers at distal whorls and thus, the occurrence of self-pollination is avoided through protogyny (see Wooten, 1971; Haynes and Holm-Nielsen, 1994; Sarkissian et al., 2001; Pansarin and Amaral, 2005). In the case of *E. longipetalus*, the fruit set of pistillate flowers occurs obligatorily through cross-pollinations. In gynodioeciuos species production of perfect flowers act as pollen donors within the populations and pollen is always outcrossed onto pistillate morphs (Beaumont et al., 2006). This xenogamous seed set in the pistillate morphs results in highly heterozygous offspring (Beaumont et al., 2006). The visits to rewardless pistillate flowers of gynodioecious *E. longipetalus* occur because it resembles the polleniferous perfect flowers. Both perfect and pistillate flowers are similar in size. Furthermore, the yellow staminodes of the pistillate flowers

Table 2
Percentage of flower setting fruits and fruit set (total number of achenia produced) of *Echinodorus longipetalus* per treatment and under natural conditions (open pollination)

Treatments	Perfect flowers		Pistillate flowers	
	Flower setting fruits (%)	Fruit set (No.)	Flower setting fruits (%)	Fruit set (No.)
Spontaneous self-pollination (control)	77 (23/30)	922	–	–
Self-pollination	97 (29/30)	1,711	–	–
Cross-pollination	100 (30/30)	1,829	97 (29/30)	1,902
Open pollination	98 (1579/1606)	36,944	85 (1976/2324)	28,596

Figures in brackets indicate the number of flower setting fruits/flowers.

strongly resemble the anthers of the perfect flowers, and both morphs have a similar number of flowers opening simultaneously. All this evidence leads to the hypothesis that the pollination of *E. longipetalus* is an example of intersexual mimicry where pistillate flowers mimic conspecific males or perfect flowers and pollination occurs through deception mechanisms (Baker, 1976; Bawa, 1980; Ågren and Schemske, 1991; Schemske and Ågren, 1995).

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