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Reproductive biology of *Echinodorus grandiflorus* (Alismataceae): evidence of self-sterility in populations of the state of São Paulo

Biologia reprodutiva de Echinodorus grandiflorus *(Alismataceae): evidência de auto-esterilidade em populações do estado de São Paulo*

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Resumo

A biologia floral e reprodutiva de *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli foram estudadas em populações nativas do interior do estado de São Paulo. A espécie floresce no verão e suas flores oferecem tanto néctar quanto pólen como recurso. O néctar é secretado por nectários localizados na base dos carpelos marginais, opostos às pétalas. Os polinizadores (abelhas sociais e solitárias), no entanto, foram observados coletando apenas pólen. As visitas, que podem durar de um a poucos segundos até mais de um minuto, acontecem desde o momento da abertura das flores (ca. 5:30 h) até o fenecimento. Cada flor dura cerca de oito horas. Todos os indivíduos das populações produzem apenas flores hermafroditas. A porcentagem de grãos de pólen viáveis é de 75%. As populações estudadas são auto-incompatíveis e, como conseqüência, polinizadores são necessários para transferência de pólen. Em condições naturais e a partir das polinizações cruzadas realizadas manualmente, todos os receptáculos apresentaram aquênios maduros. Através das análises dos tubos polínicos das flores autopolinizadas manualmente, e devido ao fato dos aquênios derivados desse tratamento abortarem cerca de 30 dias a partir das autopolinizações, aparentemente, as populações de *E. grandiflorus* estudadas apresentam um mecanismo de auto-esterilidade de ação tardia.
Palavras-chave: Alismataceae, auto-compatibilidade, biologia da polinização.

Abstract

The reproductive biology and the pollination of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli were studied in populations native to interior of the State of São Paulo, Brazil. This species blossoms in summer and its flowers offer both nectar and pollen as rewards to pollinators. Nectar is produced in nectaries located at the base of the marginal carpels, opposite the petals. However, the effective pollinators (social and solitary bees), were recorded collecting pollen only. Visits, which can last from one or a few seconds to more than one minute, occur during the whole flower lifespan. Each flower opens at about 5:30 a.m. and lasts circa eight hours. All plants in the studied populations produce only hermaphrodite flowers. Percentage of pollen viability is 75%. The studied populations are self-incompatible and, as a consequence, pollinators are needed to transfer pollen among individuals. In natural conditions and after hand cross-pollinations, all receptacles presented mature achenes. Based on the analyses of pollen tube growth from hand self-pollinated flowers, and as a consequence of achenes abortion circa 30 days after self-pollinations, the populations of *E. grandiflorus* studied apparently presents a mechanism of late-acting self-sterility.
Key words: Alismataceae, pollination biology, self-compatibility.

Introduction

Alismataceae is subcosmopolitan and it is represented in the temperate, subtropical and tropical regions of both hemispheres (Haynes & Holm-Nielsen 1994). This family includes circa 80 species distributed in approximately 11 genera, three of which occur in Brazil: *Echinodorus* Rich., *Sagittaria* L. and *Helanthium* (Benth. & Hook.f.) Engelm. *ex* J.G. Sm. *Echinodorus* is one of its largest genera, with 26 species distributed exclusively on the American Continent, from Northern United States to Argentina (Rataj 1978). In the State of São Paulo, *Echinodorus* is represented by seven species that grow in aquatic habitats, since they are found in fresh or briny water or on marshy soils (Pansarin & Amaral 2005).

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Data on the floral and reproductive biology involving neotropical species of *Echinodorus* are scarce in the literature and only concern two species: *E. longipetalus* Micheli (Pansarin 2008) and *E. grandiflorus* (Cham. & Schltdl.) Micheli (Vieira & Lima 1997), which are pollinated by native bees (Vieira & Lima 1997; Pansarin 2008).

The main characteristic used to differentiate *Echinodorus* and *Sagittaria*, two genera occurring in Brazil, is the production of hermaphrodite and unisexual flowers, respectively (Haynes & Holm-Nielsen 1994; Pansarin & Amaral 2005). Yet, Pansarin (2008) reported the occurrence of gynodioecy for *E. longipetalus*, in populations of rural areas of the State of São Paulo. According to the classification by Haynes & Holm-Nielsen (1994), *E. grandiflorus* present two subspecies. Both occur in the State of Minas Gerais and present different reproductive systems: *E. grandiflorus* (Cham. & Schltdl.) Micheli ssp. *aureus* (Fasset) Haynes & Holm-Nielsen is self-compatible, while *E. grandiflorus* (Cham. & Schltdl.) Micheli ssp. *grandiflorus* Haynes & Holm-Nielsen is self-incompatible (Vieira & Lima 1997). Nevertheless, phylogenetic analyses based on morphological (Lehtonen 2008) and molecular (Lehtonen & Myllys 2008) data show that *E. grandiflorus* is part of a paraphyletic group. Based on such data, Lehtonen (2008) currently considers *E. grandiflorus* ssp. *aureus* as a synonym for *E. floribundus* (Seub.) Seub. and *E. grandiflorus* ssp. *grandiflorus* as a synonym for *E. grandiflorus*.

Echinodorus grandiflorus (*sensu* Lethonen 2008) occurs in South America and in Florida (U.S.A.). In the state of São Paulo, it is a widespread species, found throughout the state (Pansarin & Amaral 2005). Its flowers are hermaphrodite (Vieira & Lima 1997; Pansarin & Amaral 2005; Pansarin 2009), and offer both pollen and nectar to their visitors (Vieira & Lima 1997).

Campbell (1987) discussed the problems brought on by generalizations made in studies on floral biology carried out in a single study region. When studies involving widely distributed species are carried out in more than one area, differences are observed with regard to their pollination systems (Smith & Snow 1976; Cole & Firmage 1984). Based on these assertions, the present work investigated the reproductive system of *E. grandiflorus* in populations native to the rural area of Ribeirão Preto, State of São Paulo, through studies on floral morphology, pollinators and pollination mechanisms, reproductive systems and fruiting rates in natural environments.

Material and methods

Place of study

This study was carried out in marshy areas of rural areas around Ribeirão Preto, State of São Paulo, namely in the townships of Jaboticabal (circa 21°15'S and 48°19'W), Luiz Antônio (circa 21°33'S and 47°42'W), Matão (circa 21°16'S and 48°22'W) and Sertãozinho (circa 21°08'S and 47°59'W). The region of Ribeirão Preto is located in the northwestern part of the State of São Paulo and has a mean altitude of 555 m, with regular reliefs and some plateau areas. Mean annual temperature varies between 17°C and 28°C. Climate is mesothermic, with humid summers and dry winters, considered as "Cwa" in Köppen's classification (1948). The rainy season and high temperatures occur from October to March, and the dry season from May to August. Dark red latosol, sandy phase, originating from sandstones, covers almost half of the territory of the region. Purple latosol is found in the lower parts of the territory and it originates from the decomposition of basalt rocks (*e.g.*, Centurion *et al.* 1995; Pissarra *et al.* 2004). The native flora is predominantly composed by mesophytic, seasonal semi-deciduous forests. Yet, the advance of monocultures, initially coffee and, more recently, sugar cane, has significantly reduced native wood areas in that region. Nowadays only forest fragments remain, essentially on river and creek banks (Pinto 1989).

Studied species

Echinodorus grandiflorus is found in aquatic habitats and on marshy soils (Pansarin & Amaral 2005). It is easily recognized by it large leaf blades usually oval, with cordate base and translucent marks forming points or points and lines. *Echinodorus grandiflorus* also has very characteristic paniculiform cymose inflorescences that produce many white flowers. Gynoecium is apocarpic and androecium presents many stamens free from each other. Fruits are achenes (Haynes & Holm-Nielsen 1994; Pansarin & Amaral 2005).

Floral phenology and morphology

Visits to field were conducted monthly, from November 2007 to March 2008, in order to determine

the phenological patterns of *Echinodorus grandiflorus* in the studied populations. To do so, we surveyed leaf, inflorescence, flower, and fruit production periods. We also controlled the time and sequence of anthesis, the duration of each flower, in addition to the reward availability and production period checked while observing pollinators and pollination mechanisms. During flowering period, between October and March, field visits were intensified to obtain more data on the floral and reproductive biology of this species.

The fresh material of flowers of *Echinodorus grandiflorus* collected in the townships of Matão (n = 98; 49 plants; 49 inflorescences), Jaboticabal (n = 60; 3 plants; 3 inflorescences), Sertãozinho (n = 60; 4 plants; 4 inflorescences) and Luiz Antonio (n = 30; 1 plant; 1 inflorescence) was analyzed with the help of magnifying glass binoculars. We observed the form, symmetry, disposition and size of floral structures such as sepals, petals, anthers, and related them to both the morphological features of the pollinators and the pollination mechanism (Faegri & van der Pijl 1980).

To investigate the occurrence of gynodioecy, the production of pollen grains by the anthers of fresh flowers collected in the field (n = 98) was verified according to Pansarin (2008). Furthermore, to ascertain pollen grain viability, anthers were macerated in a solution of acetic carmine and observed under an optical microscope (Radford *et al.* 1974). For each flower 200 pollen grains were sampled.

The anatomical analyses of the nectar secreting structures were carried out on newly opened flowers (n = 6) collected in the field. Flowers were previously fixed in FAA 50% (Johansen 1940) and dehydrated in butyl series (tertiary butyl alcohol), embedded in paraffin according to the method described in Sass (1951) and cut with a rotative microtome. The cuts (4 μm) were then stained with safranin and Astra blue 1%, and the slides were mounted in synthetic resin.

Plant material was deposited at the herbarium of the University of São Paulo (SPFR): *E.R. Pansarin & J.F. Sicchieri 1275, 1288, 1289* and *1290.*

Reproductive system

Between November 2008 and February 2009, treatments were carried out to verify the reproductive system of *Echinodorus grandiflorus* in the populations of the township of Sertãozinho (4 plants; 5 inflorescences), Matão (3 plants; 4 inflorescences), Jaboticabal (3 plants; 3 inflorescences) and Luiz Antonio (2 plants; 2 inflorescences). Four types of treatments were performed on inflorescences previously enclosed in tulle bags (Kearns & Inoye 1993): hand self-pollination (n = 240), spontaneous self-pollination (n = 189), cross-pollination (n = 129) and emasculation (n = 60). Treatments were randomly applied to each inflorescence. Floral buds were emasculated before the occurrence of anthesis (circa 5:00 a.m.) and cross- and self-pollinations were performed between 8:30 a.m. and 10:30 a.m.

In addition, both hand self-pollinations (n = 30) and cross-pollinations (n = 30) were carried out on individuals that had been collected in the field (population of Matão) and were cultivated for over a year in the aquatic plant tanks of the Sector of Botany (Department of Biology, FFCLRP, USP), municipality of Ribeirão Preto (circa 21º09'S and 47º51'W). Pollen tube growth of the aborted flowers produced through self-pollinations was analyzed. Flowers were fixed in Carnoy's solution for 24 h, and then transferred to 70% alcohol. To mount the slides, the material was then immersed in NaOH for a mean time of 30 minutes, washed in distilled water, stained with aniline blue, compressed under a coverslip and observed under a fluorescence microscope (Martin 1959).

Fruiting rates in natural environment (open pollination) were verified on 5,965 receptacles (30 infrutescences; 30 plants) collected in the population of the township of Sertãozinho.

The number of flowers producing fruits, as well as the total number of achenes yielded after our treatments and on the infrutescences collected in the field were also assessed. Fruiting rates (in the field and after treatments) were quantified when achenes were mature. The number of fruits was estimated by weighing 200 achenes on a precision analytical balance (0.0001 g).

Flower visitors

Field visits to observe flower visitors and the pollination process of the species were conducted in two of the studied populations, in the townships of Sertãozinho and Matão. The observations of the Sertãozinho population were performed from November 24th to December 03rd, 2008. In the township of Matão, they took place between December 05th and 07th, 2007 and between December 21st and 24th, 2008. In both

places, they occurred between 5:30 a.m. and 1:30 p.m. (between flower opening and withering), totaling 136 hours.

Flower visitors were collected, identified and deposited in the collection of the Zoology Museum of the University of São Paulo (MZUSP).

Results

Floral phenology and morphology – In all the studied regions, *Echinodorus grandiflorus* grows in marshy habitats, on river and creek banks, in waterlogged places or on marshy soil. During the drier periods of the year, in autumn and winter, plants do not have leaves and only present roots, stems and subterranean rhizomes. In spring, with the first rains (October), rhizomes resprout and each plant produces various petioled, rosulate leaves with very evident cordate blades. Each individual then develops one or more, albeit rarely two, lateral inflorescences that produce up to 250 flowers. Flowering period is from November to February (spring to summer), with a peak in December and January. During flowering, each plant may present up to 15–20 open flowers per day, which open at sunrise (approximately 5:30 a.m.) and last circa 8 hours. No morphological differences were registered among the flowers of the different studied populations.

Flowers are trimerous, pedicelled and hermaphrodite (Fig. 1a). Sepals (5.6-7.3 × 4.5-7.3 mm) are green, coriaceous, and persistent, and protect the achenes while they ripen. Petals (2-2.5 × 2.3-2.5 cm) are white, frail and ephemeral. Stamens (ca. 25-30) are arranged in various whorls around the gynoecium (Fig. 1a-b). Anthers measure 1.6 mm, are versatile and all present viable pollen grains. Anther opening coincides with flower anthesis. Pollen grain viability is 75% (n = 19,600). The apocarpic gynoecium presents numerous pistils, each bearing a single ovule and a rudimentary stigma at apex. Mature achenes are scattered between February and March.

The flowers of *Echinodorus grandiflorus* offer both nectar and pollen to their visitors. Nectar is secreted at the base of the peripheral carpels, opposite the petals (Fig. 2a). The secretory tissue is composed of a single layer of oval, juxtaposed epidermal cells (Fig. 2b) that have a strongly stained, dense cytoplasm, and a well-developed nucleus (Fig. 2b), characteristic of metabolically active cells. The subjacent tissue comprises parenchymatous cells that are apparently not involved in the process of nectar secretion.

Reproductive system

The populations of *Echinodorus grandiflorus* here studied are self-sterile and only produce fruits through cross-pollinations

Figure 1 – a-b. *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli – a. Detail of a flower, note the numerous stamens around the apocarpic ovary and the nectar guides at petal base; b. *Trigona spinipes* Fabricius collecting pollen from flower anthers, note their corbiculae full of pollen grains. Scale bars = 1 cm.

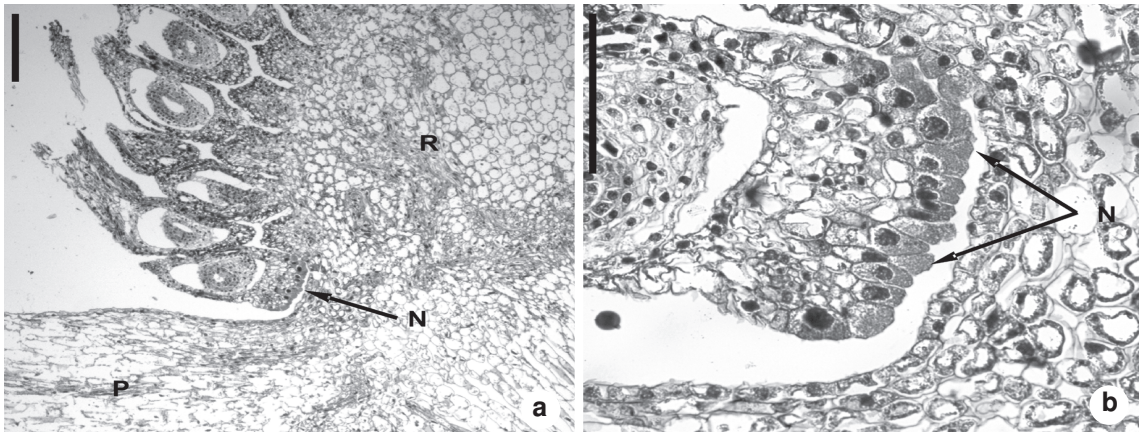

Figure 2 – a-b. Longitudinal sections of a flower of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli – a. detail of the receptacle (R) and the nectary (N), located on the margin of the more distal carpel (arrow), opposite the petal (P); b. detail of the nectary (N) evidencing the nectar secretory epidermal cells (arrow). Scale bars = 100 μm.

(Tab. 1). No fruits were formed in flowers that were emasculated, manually self-pollinated or spontaneously self-pollinated (untouched flowers) (Tab. 1). Thus, a pollinator is necessary to transfer pollen among different individuals of the populations. Fruits are mature circa 45 days after cross-pollinations. In natural conditions, fruiting rates are high (Tab. 1). All the sampled (cross- and open-pollinated) flowers presented mature achenes (predated receptacles were excluded). Achene production in each receptacle is higher after cross-pollinations (mean of 250 fruits) than in natural conditions (mean of 190 fruits). The reproductive system of *E. grandiflorus* is presented in Table 1.

Achenes yielded after hand self-pollination treatment aborted tardily, circa 30 days after manipulations. The analyses of the pollen tube growth of the manually self-pollinated flowers showed that the tubes had reached the ovules, but no evidence of fertilization was found.

Flower visitors

The flowers of *Echinodorus grandiflorus* are visited by various species of beetles, flies and bees. Nevertheless, only species of solitary and social bees really act as pollinators (Tab. 2). All the bee species collect pollen from the flowers. We never observed bees accessing the nectar produced in the nectaries located at the base of the marginal carpels. Beetles were seen feeding on floral pieces (petals, stamens and pistils), damaging flowers and often making them inaccessible to effective pollinators. Flies of the family Bombyllidae were the only visitors to collect nectar from the flowers. Nevertheless, their long proboscides give them access to the reward without touching the stamens and, therefore, they were not seen acting as pollinators of *E. grandiflorus*.

All the bee species except *Xylocopa suspecta* presented similar behaviors when collecting pollen. They usually landed either on the petals before heading to the stamens or directly on the anthers and pistils. They collected pollen grains from each anther with their front and middle legs and then transferred it to their hind legs. When collecting pollen, bees contacted the stigmas with ventral part: legs, thorax and abdomen (Fig. 1b), where pollen grains get attached. *Xylocopa suspecta* landed directly on the receptacle (anthers and pistils) and collected pollen from various stamens simultaneously by buzz-pollination. During these vibratory movements, great quantities of pollen were also deposited on the stigmas. Bees of the family Halictidae sometimes performed buzz-pollination, but on one anther at a time.

Bees begin visiting flowers immediately after opening and continue as long as they were open. Each visit lasts from one or a few seconds to more than a minute. Individuals of *Trigona spinipes* (Fig. 1b) stayed even more than one minute on each single flower. Visits only took place on sunny and cloudy days. Since there is no flower anthesis when it rains, no visits were registered in these conditions. The number of bee visits could not be checked due to the great number of individuals exploiting rewards simultaneously on a one inflorescence. Bee dimension was not a limiting factor to pollination, since different-sized species were registered as pollinators of *Echinodorus grandiflorus* (Tab. 2).

Table 1 – Reproductive system of *Echinodorus grandiflorus*: percentage of flowers that formed fruits and fruiting rate (quantity of achenes produced) after each treatment carried out and in natural conditions (open pollination). Between parentheses is the number of flowers that formed fruits/flowers.

Treatment	Flowers that formed fruits (%)	Quantity of achenes produced (n)
Cross-pollination	100 (129/129)	32,250
Manually self-pollinated	0 (0/240)	-
Spontaneously self-pollinated	0 (0/189)	-
Open pollination	100 (5.965/5.965)	1,133,350
Emasculation	0 (0/60)	-

Discussion

Among the genera of Alismataceae growing in Brazil (*Echinodorus* Rich., *Sagittaria* L. and *Helanthium* (Benth. & Hook. f.) Engelm. *ex* J.G. Sm.), the production of hermaphrodite flowers is the main characteristic used to distinguish *Echinodorus* (including *Helanthium* – a genus segregated from two species classically recognized as *Echinodorus*, Lehtonen & Myllys 2008) from *Sagittaria* (Haynes & Holm-Nielsen 1986, 1994; Pansarin & Amaral 2005). Nevertheless, gynodioecy (*i.e.*, hermaphrodite and female flowers) was registered and documented for *E. longipetalus* in populations of the State of São Paulo (Pansarin 2008). The present study confirms that all the populations of *E. grandiflorus* studied comprise individuals that exclusively produce hermaphrodite flowers, corroborating previously published data (*e.g.*, Rataj 1978; Haynes & Holm-Nielsen 1986, 1994; Pansarin & Amaral 2005), including those on *Helanthium* (Lehtonen & Myllys 2008).

In all the studied populations, we verified that *Echinodorus grandiflorus* offers both nectar and pollen as a reward, which had been previously described in a study carried out in the township of Viçosa, in the State of Minas Gerais (Vieira & Lima 1997). Although nectar is the most common reward among Alismataceae species, since it is described in *Baldellia* (Vuille 1988), *Caldesia* (Gituru *et al.* 2002), *Damasonium* (Vuille 1987) and *Sagittaria* (Wooten 1971, Sarkissian *et al.* 2001), and although bees were watched exploiting this kind of reward in flowers of *E. grandiflorus*, in addition to pollen grains (Vieira & Lima 1997), during our observations, hymenoptera were only seen collecting pollen from the flowers. In the studied populations, nectar was only exploited by flower visitors (Bombyllidae flies) that are not involved in the pollination process of this species. As is the case with *E. grandiflorus*, in the studied populations, the exclusive collection of pollen by bees was also reported for the hermaphrodite flowers of *E. longipetalus* (Pansarin 2008). Female flowers of *E. longipetalus* are pollinated by deceit, since their staminodes, albeit smaller, look like the stamens of hermaphrodite flowers (Pansarin 2008).

The presence of nectary at the base of the marginal carpels opposite the petals, as in the flowers of *E. grandiflorus,* is common in Alismataceae species that offer nectar as a reward. This family comprises other genera that also secret nectar at the base of the filaments and petals (Pansarin 2009).

Studies of floral biology involving species of *Echinodorus* (Vieira & Lima 1997; Pansarin 2008) reveal that the flowers are exclusively pollinated by bees, which is confirmed by the present report. Other insects, as beetles (Vieira & Lima 1997, Pansarin 2008) and flies (Pansarin 2008), only act as flower visitors. The observations of pollinator behavior on the flowers confirm that social and solitary bees of different sizes act as pollinators of *Echinodorus grandiflorus*. Some species should be highlighted because they were reported by other pollination studies on species of this genus. *Exomalopsis fulvopilosa* was also registered as a pollinator of *E. grandiflorus* in the State of Minas Gerais (Vieira & Lima 1997) and of *E. longipetalus* in the interior of the State of São Paulo (Pansarin 2008). Another species registered as a pollinator of *E. grandiflorus* in populations of Minas Gerais is *Protodiscelis echinodori* (Vieira & Lima 1997), while *Xylocopa* (*NeoXylocopa*) *suspecta* was also documented on flowers of *E. longipetalus* (Pansarin 2008).

The family Alismataceae presents a great diversity as for the reproductive system of its species. Genus *Sagittaria* comprises dioecious and monoecious species (Wooten 1971; Sarkissian *et al.* 2001). The flowers of *Caldesia grandis* Samuel.

Table 2 – Bee species (pollinators) collected on flowers of *Echinodorus grandiflorus* and their body length (mm).

Bee species	Body length (mm)
Apis mellifera L.	14.5
Augochlora sp.	10.0
Dialictus sp.	6.0
Exomalopsis fulvopilosa Spindola, 1853	10.5
Pseudoaugochloropsis sp.	8.0
Protodiscelis echinodori Melo, 1996	7.0
Thygater analis Lepeletier, 1841	12.0
Trigona spinipes Fabricius, 1793	9.0
Xylocopa (*Neoxylocopa*) *suspecta* Moure & Camargo, 1988	28.0

(Gituru *et al.* 2002), *Baldellia ranunculoides* (L.) Parl. var. *ranunculoides* and *Baldellia alpestris* (Cosson) Vasc. (Vuille 1988), and those of various species of *Damasonium* (Vuille 1987) are hermaphrodite and self-compatible. Although the species of *Sagittaria* occurring in this region are self-compatible, all are monoecious, and their inflorescences produce female flowers at base and male ones at apex, so that self-pollination is avoided by protogyny (Haynes & Holm-Nielsen 1994; Pansarin & Amaral 2005). *Echinodorus longipetalus* is gynodioecious: the hermaphrodite flowers are self-compatible, while the female ones necessarily need cross-pollination to yield fruits (Pansarin 2008). The occurrence of self-sterility, as seen in populations of *E. grandiflorus* from interior of the State of São Paulo, is rare in that family but had been previously reported for this species in the municipality of Viçosa, Minas Gerais (Vieira & Lima 1997) and for a subspecies of *Baldellia ranunculoides* (Vuille 1988). According to Vieira & Lima (1997), following the classification of Haynes & Holm-Nielsen (1986, 1994), *E. grandiflorus* ssp. *aureus* is self-compatible, while *E. grandiflorus* ssp. *grandiflorus* is self-incompatible. Currently both subspecies have been heightened to the category of species by Lehtonen (2008). *Echinodorus grandiflorus* ssp. *aureus* is now a synonym for *E. floribundus*, while *E. grandiflorus* ssp. *grandiflorus* is a synonym for *E. grandiflorus* (Lehtonen 2008).

The most common mechanisms that tend to avoid self-fertility in Alismataceae are pre-pollination barriers. Within this family, the occurrence of protogyny was reported for species of *Sagittaria* (Pansarin & Amaral 2005), while protandry occurs in *Damasonium* (Vuille 1987). Although the species of *Sagittaria* occurring in the neotropics are all monoecious (*e.g.*, Rataj 1978; Haynes & Holm-Nielsen 1986, 1994; Pansarin & Amaral 2005), at least two North-American species (*i.e.*, *S. latifolia* Willd. and *S. lancifolia* L.) have dioecious populations and, consequently, need cross-pollination (Wooten 1971; Sarkissian *et al.* 2001). Gynodioecy also favors the formation of fruits by cross-pollination in *Echinodorus longipetalus* (Pansarin 2008).

The populations of *Echinodorus grandiflorus* studied clearly show a post-pollination barrier that prevents the production of fruits as a result of self-pollinations. Hand self-pollination treatments indicate the occurrence of a late-acting self-incompatibility system, since the tubes present themselves well formed but the pistils abort circa 30 days after self-pollination. Some authors reveal that in systems of late-acting self-sterility, there are no differences in pollen tube growth between self- and cross-pollination treatments (*e.g.*, Seavey & Bawa, 1986). According to Gibbs (1990), mechanisms of late-acting self-incompatibility have proved relatively common in neotropical species, and were reported for various tree species (see Freitas & Oliveira 2002). Nevertheless, for the populations of *E. grandiflorus* here analyzed, studies will be needed to confirm if ovules fertilize in flowers self-pollinated by hand and verify the kind of late-acting self-sterility mechanism involved.

Studies on tropical species of Alismataceae are extremely important, since many taxa are difficult to identify morphologically. Above all, in Brazil, the understanding of this family taxonomy presents gaps, mainly with regard to *Echinodorus* (Lehtonen 2008). Furthermore, features classically used to delimit genera, as the presence of unisexual (*Sagittaria*) or hermaphrodite (*Echinodorus*) flowers (Haynes & Holm-Nielsen 1986, 1994; Pansarin &

Amaral 2005), can no longer be considered, since female flowers were found in populations of *E. longipetalus* (Pansarin 2008). As a consequence, research on the relations between the neotropical species of *Echinodorus* and *Sagittaria*, their pollinators and pollination mechanisms, their forms of reproduction, as well as the elaboration of phylogenetic hypotheses based on morphological and molecular features are needed to understand the taxonomy and the evolution of this subcosmopolitan family of monocots.

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