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SHORT COMMUNICATION

Evidence of trimonoecy in Phyllanthaceae: *Phyllanthus acidus*

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Abstract Pollen from staminate flowers and pistillate flowers with "staminodes" of *Phyllanthus acidus* Skeels were analyzed under scanning electron microscopy, and tests of pollen viability and in vitro germination were carried out to verify possible similarities between the three types of flowers. The results show that pistillate flowers with "staminodes" are bisexual, indicating the occurrence of trimonoecy in this species.

Keywords Pollen · Scanning electron microscopy · Trimonoecy · Staminode · *Phyllanthus acidus*

Introduction

Polygamy is the condition where staminate, pistillate, and perfect (bisexual) flowers occur on the same plant or on different plants (Cruden and Lloyd 1995; Sakai and Weller 1999; Ainsworth 2000). Darwin (1877) included two subgroups of polygamous plants: those where each of the three floral morphs is usually found on different plants (trioecious) and those where the three floral morphs are on the same plant (trimonoecious or polygamomonoecious).

Phyllanthus L. comprises about 800 species distributed into over 50 sections, with distribution in different environments and vegetation formations in tropical regions of the world (Silva and Sales 2004). The evolution of unisexual flowers is a topic of great interest to plant biologists (Kinney et al. 2007). Studies about types of flowers in Phyllanthaceae are restricted to a description of duodichogamy in *Bridelia tomentosa*, a Chinese tree species (Luo et al. 2007).

Despite the fact that dioecy is particular prevalent in Euphorbiaceae (Renner and Ricklefs 1995), flowers in Phyllanthaceae—sister group of Euphorbiaceae (APG II 2003)—are described as mono- or dioecious (Kathriarachchi et al. 2006; Judd et al. 2009). *Phyllanthus acidus* is native to South America and cultivated as a fruit tree (Kathriarachchi et al. 2006). This species presents pistillate flowers, pistillate flowers with staminodes, and staminate flowers. According to Webster (1957), these staminodes may apparently act as functional stamens. Due to this observation, we tested whether the pollen from staminodes is viable through a study of pollen morphology, viability, and germination tests.

Trimonoecy is relevant in taxonomic classification and studies of floral biology. Besides, pollen grain is an important indicator of infrageneric relationships, mainly between African and tropical American species (Webster and Carpenter 2002; Chen et al. 2009).

Based on Webster's hypothesis (1957), we investigated different types of flowers of *Phyllanthus acidus* Skeels and show a case of trimonoecy in *Phyllanthus* for the first time.

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Materials and methods

Flowers of *Phyllanthus acidus* Skeels were collected from three cultivated plants growing on the campus of Universidade Estadual de Campinas, Campinas, São Paulo State, Brazil. A voucher specimen has been deposited in the Herbarium UEC (143516). After collection of flowers, stamens and staminodes were measured with a digital caliper rule (Mitutoyo) to check allometric similarities.

For scanning electron microscopy (SEM) analysis, adult flowers were fixed in FAA_{50} (Johansen 1940) for 24 h, dehydrated in a graded ethanol series, and critical-point-dried with CO_2 (Horridge and Tamm 1969). Samples were attached to aluminum stubs and coated with gold (30–40 nm). Observations were carried out on a Jeol JSM-5800 LV.

The analyses of pollen viability from stamens and staminodes were performed on slides with macerated

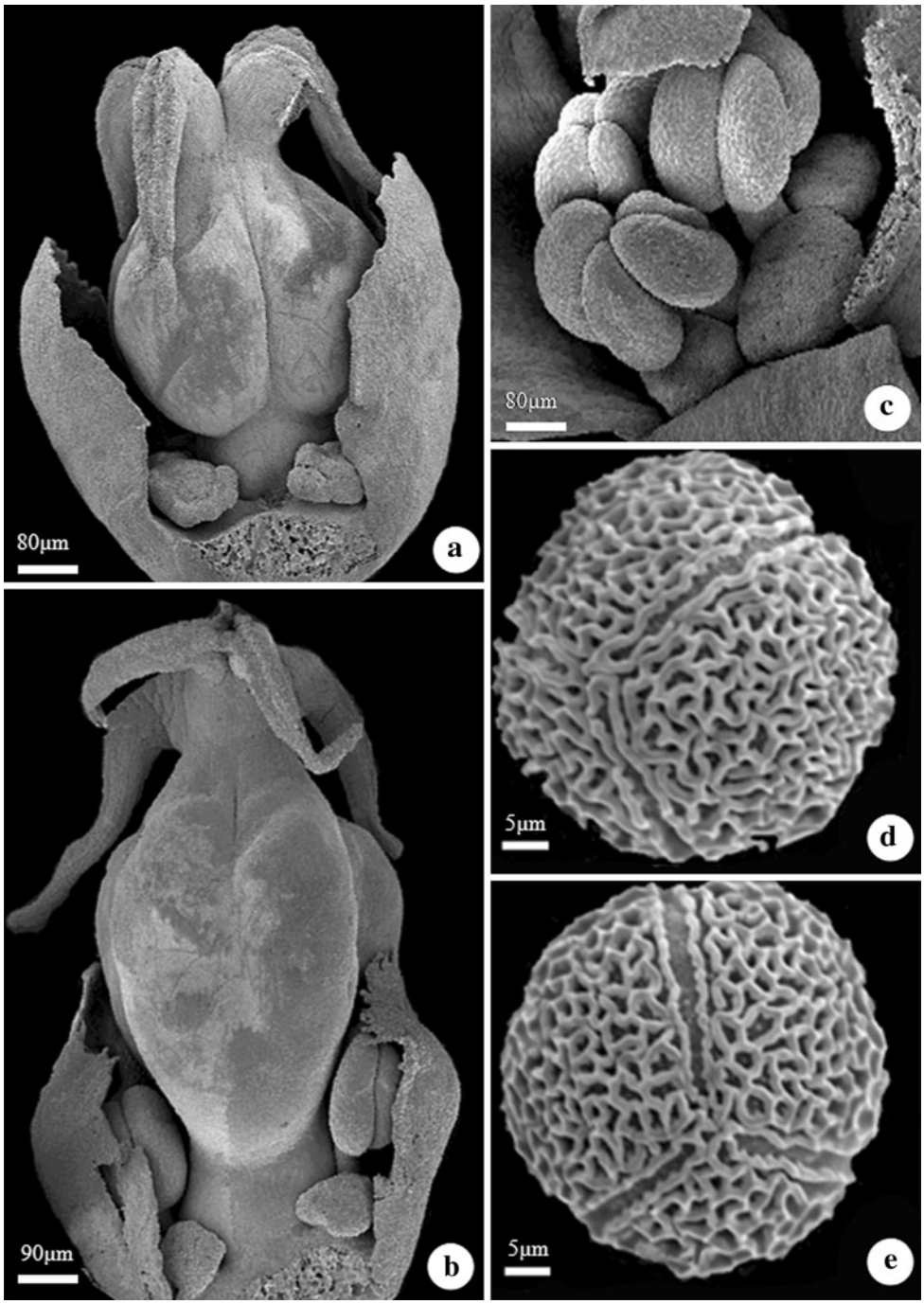

Fig. 1 Scanning electron microscopy of flowers of *Phyllanthus acidus* Skeels. **a** Pistillate flower. **b** Pistillate with staminodes flower. **c** Staminate flower. **d–e** Pollen grains from pistillate with staminodes (**d**) and staminate (**e**) flowers

anthers stained with basic fuchsin/malachite green (Alexander's stain, Alexander 1980) and acetic carmine (Dafni 1992). Pollen grains from stamen and staminodes were collected from flowers in pre-anthesis stage and considered viable when totally colored and spherical.

Some pollen grains isolated from stamen and staminodes were immediately submitted to in vitro germination in a culture medium (Kearns and Inouye 1993).

Results

Three types of flowers are found on the same individual of *P. acidus*: pistillate without staminodes (Fig. 1a), pistillate with staminodes (Fig. 1b), and staminate (Fig. 1c). Fruit development is observed in pistillate flowers both with and without staminodes. Allometric analysis of stamens from pistillate with staminodes and staminate flowers indicated that these structures present the same size (1.02 mm ± 0.02) and shape (Figs. 1b, 1c).

Morphological analysis of pollen grain under SEM showed that both are identical (Fig. 1d, 1e), classified as spheroidal, tricolporate, and with a regulate-reticulate ornamentation. Staining and in vitro germination tests also presented the same positive results for viability and pollen tube growth.

Discussion

The choice of viability tests depends on the species, pollen state, and staining conditions, since results can differ between staining techniques (Slomka et al. 2010). Pollen viability is the capability to germinate and grow, but viable pollen grains may not germinate, in vitro or in vivo, if the conditions are not right (Dafni and Firmage 2000). Several methods have been developed to test viability and other similar or more restrictive parameters such as stainability, pollen maturity, germinability, vigor, and fertility (Dafni and Firmage 2000). There are essentially four different methodologies to test viability: (a) cytoplasmic stains, (b) enzyme reactions, (c) germination, and (d) fruit and seed set (Dafni and Firmage 2000; Nepi and Franchi 2000). General cytoplasmic stains (Alexander's stain, acetic carmine) are easy and fast to use and are in some cases correlated with in vitro germination or fruit set (Dafni and Firmage 2000) to avoid overestimation of pollen viability, because stained grains may be not fully fertile (Nepi and Franchi 2000). The germination methods can be performed in vitro or in vivo. In vitro germination of pollen grains under appropriate conditions (temperature, culture medium) is rapid, simple, and often correlated with fruit set and seed set (Ferrara et al. 2007).

The results showed that pistillate flowers with staminodes are, in fact, bisexual, meaning a trimonoecy case in *P. acidus*. Analysis of pollen grain under SEM agreed with past description for pollen from staminate flowers of *P. acidus* (Punt 1967; Chen et al. 2009).

Trimonoecy, contrary to gynodioecy and gynomonoecy, is rare (Ainsworth 2000). It has been suggested that dioecy is the end point in the pathway of breeding system evolution (Kinney et al. 2007) and can evolve directly from monocliny, or pathways involving diclinous intermediates are implicated (Weiblen et al. 2000). However, monoecy is widespread in Phyllanthaceae (Silva and Sales 2004), whilst dioecy is one of the characters consistent with ancestry in Phyllanthaceae shared with Putranjivaceae—included in Euphorbiaceae *sensu latu* as Phyllanthoideae (Wurdack et al. 2004).

In this study, results of allometric analysis, morphology of stamens, and tests applied to pollen from stamens and "staminodes" were sufficient to suggest trimonoecy. Studies in natural populations have been carried out to corroborate those observations obtained from cultivated plants. Although sections *Cicca* (that contains the single species *P. acidus*) and *Aporosella* form a strongly supported neotropical clade, the genus *Phyllanthus* is not monophyletic (Kathriarachchi et al. 2006). Examination of morphological/anatomical characters may still yield more significant indicators of phylogenetic relationships in Phyllanthaceae (Wurdack et al. 2004). In this context, this finding of trimonoecy may be helpful for future insights into the taxonomy and phylogeny of this group.

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