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Pollinic characterization of *Raulinoa echinata* R. S. Cowan (Rutaceae), *Dyckia brevifolia* Baker and *Dyckia ibiramensis* Reitz (Bromeliaceae), reophyte and saxicolous endemic species of river Itajaí-Açu, Santa Catarina, Brazil

Caracterização polínica de Raulinoa echinata *R.S. Cowan (Rutaceae),* Dyckia brevifolia *Baker e* Dyckia ibiramensis *Reitz (Bromeliaceae), espécies reófitas e endêmicas do rio Itajaí-Açu, Santa Catarina, Brasil*

Denise Monique Dubet da Silva **MOUGA**[1, 4]; Bruna Tereza **POSSAMAI**[2]; Enderlei **DEC**[3] & Sílvio Murilo Cristóvão da **SILVA**[3]

ABSTRACT
Aiming to contribute with elements about the reproduction of the reophytes *Raulinoa echinata*, *Dyckia brevifolia* and *Dyckia ibiramensis* as well as to support their taxonomic definition, their pollinic characterization was carried out. Fresh pollen grains were prepared by acetolysis. Observations occurred on a light microscope and a scanning electron microscope. Measures were done of the polar (P) and equatorial diameters (E) and thickness of the exine and are expressed as means in micrometers. *R. echinata:* monad; radial symmetry; medium size; P=49,07±3,33; E=33,95±3,94; prolate (ratio P / E); *ambitus* subtriangular; 3-aperturated with long *colpi* and lalongate endoapertures; exine striated with thickness 1.54 ± 0,32. *Dyckia brevifolia:* monad; bilateral symmetry; medium size; P=25,16 ± 3,02; E=43,16±3,50 x 28,12±3,26; *ambitus* elliptical; monoaperturated with one long *sulcus;* exine reticulate with thickness 1,66 ± 0,31. *D. ibiramensis:* monad; bilateral symmetry; medium size; P=29,54±3,17; E=42,36±3,60 x 28,43±3,38; *ambitus* elliptical; monoaperturated with one long *sulcus*; exine reticulate with thickness 1,73 ± 0,28. Data obtained can allow to verify hydrophily for the three species as well as suggest *Raulinoa* proximity with *Metrodorea* and *Esenbeckia* (both Pilocarpinae) and confirm *D. brevifolia* e *D. ibiramensis* to Pitcairnioideae.
Keywords: *cutia-de-espinho*; hydrophily; palynology; pollen; *sarandi*.

RESUMO
Visando contribuir com elementos sobre reprodução das reófitas *Raulinoa echinata*, *Dyckia brevifolia* e *Dyckia ibiramensis* e apoiar sua definição taxonômica, foi realizada sua caracterização palinológica. Grãos de pólen frescos foram acetolisados. Observações ocorreram em microscópio de luz e eletrônico de varredura. Fizeram-se medidas do diâmetro polar (P), equatorial (E) e da espessura da exina, expressas como médias, em micrômetros. *R. echinata*: mônade; simetria radial; tamanho médio; P = 49,07 ± 3,33; E = 33,95 ± 3,94; forma prolato (relação P / E.); âmbito subtriangular; 3-aperturado com colpos longos e endoaberturas lalongadas; exina estriada com espessura 1,54 ± 0,32. *Dyckia brevifolia*: mônade; simetria bilateral; tamanho médio; P = 25,16 ± 3,02; E = 43,16 ± 3,50 x 28,12 ± 3,26; âmbito elíptico; monoaperturado com um sulco longo; exina reticulada com espessura 1,66 ± 0,31. *D. ibiramensis*: mônade; simetria bilateral; tamanho médio; P = 29,54 ± 3,17; E = 42,36 ± 3,60 x 28,43 ± 3,38; âmbito elíptico; monoaperturado com um sulco longo; exina reticulada com espessura 1,73 ± 0,28. Os dados podem permitir verificar a hidrofilia das três espécies, sugerir a proximidade de *Raulinoa* com *Metrodorea* e *Esenbeckia* (ambos Pilocarpinae) e confirmar *D. brevifolia* e *D. ibiramensis* em Pitcairnioideae.
Palavras-chave: cutia-de-espinho; hidrofilia; palinologia; pólen; sarandi.

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[1] Departamento de Ciências Biológicas, Universidade da Região de Joinville (Univille), Rua Paulo Malschitzki, n. 10, CEP 89219-710, Joinville, SC, Brasil.

[2] Laboratório de Abelhas (Label), Univille, Joinville, SC, Brasil.

[3] Associação Catarinense de Assistência e Defesa do Meio Ambiente (Academa), Ibirama, SC, Brasil.

[4] Autor para correspondência: dmouga@terra.com.br.

INTRODUCTION

The reophytes constitute a particular group of riparian vegetation, confined to the bed of rivers and streams with rapids, occurring above water level, without distancing much from the banks, but being regularly affected by the floods and characterized by resisting the floods caused by heavy rains in the area of the headwaters of the river (VAN STEENIS, 1981).

Klein (1979) points out that reophytes must cope not only with the strong currents caused by periodic flooding, when they are totally or partially submerged, but also with the intense solar rays incident on the rocks when the waters are shallow. Several botanical families include reophytes species (VAN STEENIS, 1987) and, for the southern region of Brazil, Klein (1979) lists 21 families, of which Euphorbiaceae, Leguminosae and Myrtaceae are the most representative. Although showing several adaptations to their environment, the reophytes do not show a unique feature that could identify them as such. In fact, the rheophyte condition apparently implies a set of hydromorphic and xeromorphic characteristics which, together, make it possible to survive in extreme adverse conditions, submergence with turbulence or high exposure to solar irradiation in the rheophyte environment.

On the river Itajai-Acu, in the state of Santa Catarina (SC), southern Brazil, (figure 1), along about 80 kilometers between the municipalities of Lontras and Blumenau, near the hydroelectric plant of *Salto Pilao*, there are shores, rapids and rocky islands where some endemic riparian reophytes thrive (figure 2), named locally *cutia-de-espinho, gravata* or *sarandi*.

Figure 1 – Emplacement of the river Itajai-Acu, southern Brazil.

Among these, there are *Raulinoa echinata* R. S. Cowan (Rutaceae), in groupings of discontinuous occurrence only on the right side of the river and *Dyckia brevifolia* Baker and *Dyckia ibiramensis* Reitz (Bromeliaceae), also in disjunct populations (ROGALSKI, 2007). *D. brevifolia* occurs on the banks and rocky islands of the river Itajai-Acu and *D. ibiramensis* presents a restricted distribution on a course of 1.1 km along the banks of the river Itajai do Norte (a tributary of the former river) and can be classified as a rare species, as defined by Kruckeberg & Rabinowitz (1985), showing a grouped distribution pattern, with few individuals isolated.

Rutaceae R. S. Cowan comprises of 155 *genera* with 1600 species, mostly tropical and subtropical in distribution and contain many members of economic importance (CHASE *et al.*, 1999). *Raulinoa,* a monospecific genus, is comprehended in Pilocarpinae (Galipeeae < Rutoideae < Rutales) (KAASTRA, 1982). There is a lack of elements, including palynological ones, to ascertain the proximity of *Raulinoa* with the other genera of Pilocarpinae (MORTON & TELMER, 2014).

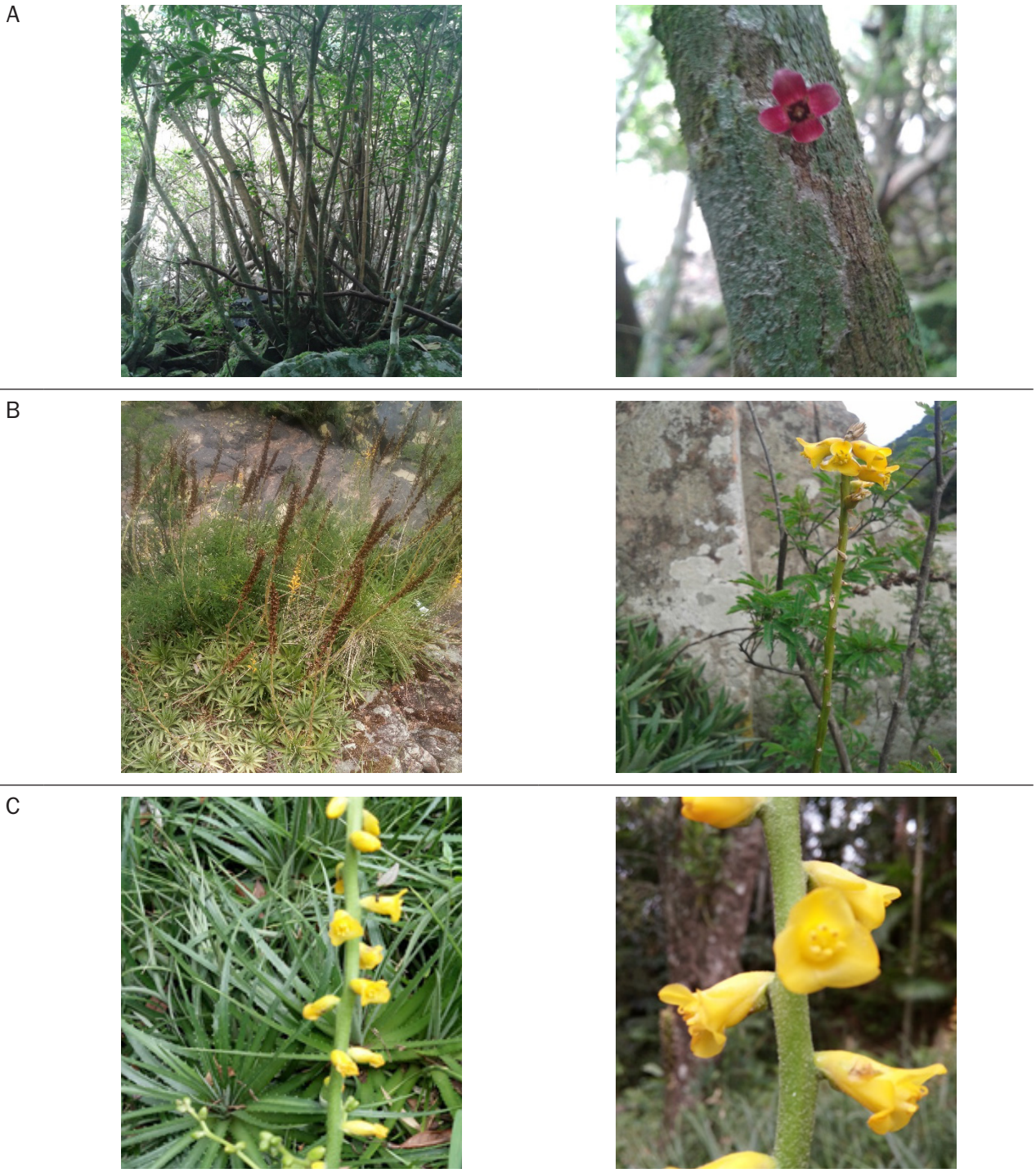

Figure 2 – Reophytes species on the river Itajai-Acu, in the state of Santa Catarina, southern Brazil: A) *Raulinoa echinata*; B) *Dyckia brevifolia*; c) *Dyckia ibiramensis*.

Bromeliaceae Juss. Comprises of 3.172 species and 58 *genera* (LUTHER, 2008) and approximately 40% of these species and 73% of the *genera* can be found in Brazil (LEME & MARIGO, 1993). *Dyckia* Schult. *genus* includes about 130 species (LUTHER, 2008) and is known in the new circumscription of Pitcairnoideae (Bromeliaceae) according to Givnish *et al.* (2007). In SC, *Dyckia* has 10 officially occurring species and, among these, as reophytes, *D. brevifolia, D. ibiramensis* and *D. distachya* (FLORA, 2017). The latter species occurs only on the banks of the rivers Uruguay and Paraná. For the river Itajai-Acu, *D. brevifolia*.has not been yet studied palynologically and *D. ibiramensis* was previously analyzed by Halbritter (2016d) who worked only with herborized material.

The focused *taxons* are endangered as the environment is threatened by the construction of dams and hydroelectric plants on the banks of the river where they thrive (*R. echinata*-VU, *D. ibiramensis*-EN, *D. brevifolia*-DD) (BRASIL, 2014). On the other hand, there is scarcity of studies, for the mentioned species, about the mode of reproduction and the pollinic characterization. These aspects imply in different types of pollination which in turn may involve diverse strategies for the preservation of the menaced taxa. These features are also important in terms of *adaptive radiation, being additive traits in* phylogenetic studies.

In this perspective, this work aimed to contribute to the palynological delineation of three reophytes species on river Itajai-Acu, in view of reproductive aspects, taxonomic proximities and conservation.

MATERIAL AND METHODS

STUDY AREA

The area where these species thrive (27°03'25" S, 49°31'04" W), according to Köeppen's classification, has climate Cfa subtropical, with annual average temperature 18-19°C, annual average relative humidity 75-80% and annual average rainfall 1300-1500 mm, with constant rains in summer (LOBO, 2007). The soil is of the alachloric type, altitude is on average 150 m and the relief of the region is mountainous, with marked slope amplitudes, valleys embedded and bent strands, often exceeding 45° degrees (CESAP, 2007). It is located in the physiographic zone of the southeast basin of the river Itajai-Acu and the vegetation is riparian submontane rain forest, with seasonal fluvial influence (RODRIGUES, 2000).

METHODOLOGY

Samplings were taken from *R. echinata*, *Dyckia brevifolia* and *D. ibiramensis* in the municipality of Ibirama, state of Santa Catarina, on 17 Mar. 2016 and 25 Nov. 2016, for the vouchers' preparation (exsicates), which were stored at Label's Herbarium, at the University of the Region of Joinville. Closed floral buds were conserved in glass bottles, containing glacial acetic acid, pure for analysis (P.A.), the botanic identification and the sampling date. Bottles were sealed with rubber covers until the moment of preparation of the glass slides. For the analysis of the pollinic material, the anthers of the floral buds were taken off and torn for the liberation of the pollen grains that were then submitted to the acetolysis method (ERDTMANN, 1960). Recommendations of Salgado-Laboriau (1973) were followed with the utilization of Kisser's gelatin, closing the slides with paraffin as well as taking photographs. Measures of the pollen grains were taken in a delay of seven days, from the pollen slides, observed on the light microscope. The pollen grains of each species were photographed 25 times in each of the views, polar (P) and equatorial (E), utilizing all slides. The characterization of the form of pollen grains of *R. echinata* was performed by calculating the ratio P/ E. For each analyzed species, in relation to the size of the pollen grain, we verified, in both views, the maximum size, the minimum size, the average size and the standard deviation. The number and type of apertures was observed as well as details of the ornamentation of the exine. All details were registered and described according to the terminologies of Barth & Melhem (1988) and Punt *et al.* (2007). The observations took place under a light microscope Bioval with equipment Dino-Eye Microscope Eye-Piece Camera, associated with the software DinoCapture 2.0 at the University of the Region of Joinville and on a scanning electron

microscope (SEM) of the University of the State of Santa Catarina. Pollen slides were deposited at the pollen reference slides collection of Label-Bee Laboratory, at the University of the Region of Joinville.

RESULTS

Results are showed on figures 3 and 4. Measures are in micrometers (μm). The pollen grains of the three species are in monads.

R. ECHINATA (figure 3, items 1a, 1b, 1c, 1d; figure 4, items 1a, 1b)

Form: radial symmetry, isopolar, outline in polar view subtriangular, medium size, prolate.
Apertures: 3-aperturated with long *colpi* and lalongate endoapertures.
Exine: average exine thickness 1,54 (minimum 1,04, maximum 2,32, standard deviation 0,32), ornamentation of the grains, at SEM, striated.
Measures: average equatorial diameter 33,95 (minimum 26,04, maximum 40,41, standard deviation 3,94); average polar diameter 49,07 (minimum 43,91, maximum 54,84, standard deviation 3,33); P/E 1,46.

DYCKIA BREVIFOLIA (figure 3, items 2a, 2b, 2c, 2d; figure 4, items 2a, 2b)

Form: bilateral symmetry, heteropolar, outline in polar view elliptic, medium size. The shape is flattened on the proximal side and convex on the distal one.
Apertures: 1-sulcate, with one long *sulcus (colpus)* on the distal side. *Sulcus* with wide and light-colored border.
Exine: average exine thickness 1,66 (minimum 1,07, maximum 2,23, standard deviation 0,31), ornamentation of the grains, at SEM, reticulated, except on the borders of the *colpus*.
Measures: average greater equatorial diameter 43,16 (minimum 33,43, maximum 51,08, standard deviation 3,50); average smaller equatorial diameter 28,12 (minimum 23,23, maximum 35,34, standard deviation 3,26); average polar diameter 25,16 (minimum 20,07, maximum 32,28, standard deviation 3,02).

D. IBIRAMENSIS (figure 3, items 3a, 3b, 3c; figure 4, items 2a, 2b)

Form: bilateral symmetry, heteropolar, outline in polar view elliptic, medium size. The shape is flattened on the proximal side and convex on the distal one.
Apertures: 1-sulcate, with one long *sulcus (colpus)* on the distal side. *Sulcus* with wide and light-colored border.
Exine: average exine thickness 1,73 (minimum 1,30, maximum 2,60, standard deviation 0,28), ornamentation of the grains, at SEM, reticulated except on the borders of the *colpus*.
Measures: average greater equatorial diameter 42,36 (minimum 36,58, maximum 53,98, standard deviation 3,60); average smaller equatorial diameter 28,43 (minimum 20,63, maximum 32,49, standard deviation 3,38); average polar diameter 29,54 (minimum 21,54, maximum 33,50, standard deviation 3,17).

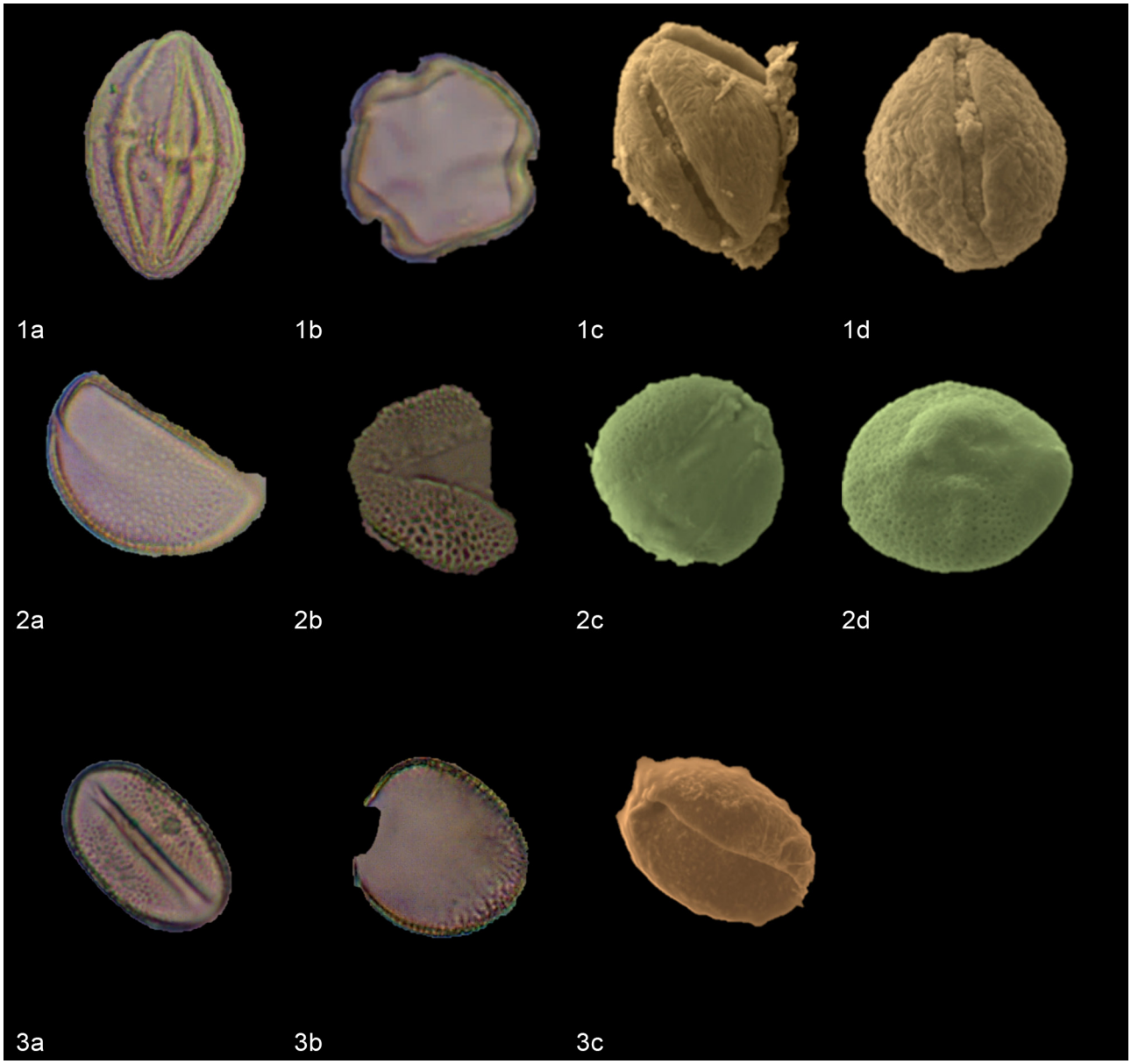

Figure 3 – Photomicrographs of pollen grains of: *R. echinata* in light microscope (1a equatorial view, 1b polar view) and in SEM (1c and 1d, equatorial view); of *D. brevifolia* in light microscope (2a equatorial view, 2b polar distal view with aperture on side, oblique) and in SEM (2c polar view, 2d equatorial view); of *D. ibiramensis* in light microscope (3a polar view, 3b equatorial view) and in SEM (3 c, polar view).

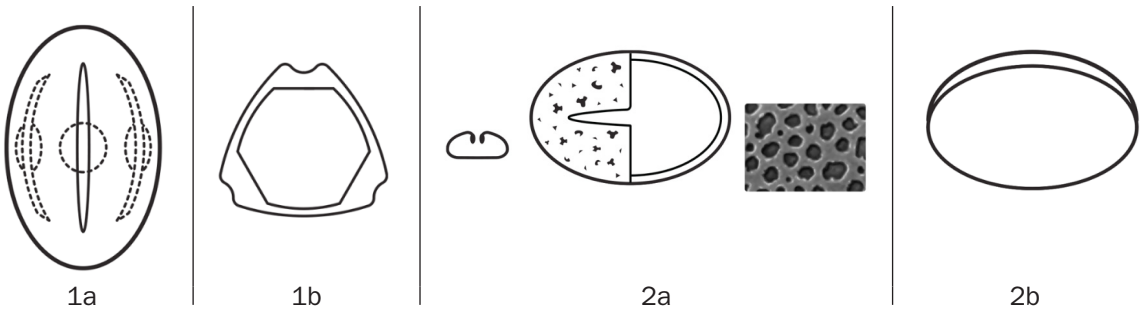

Figure 4 – Palynograms of *R. echinata* (1a-equatorial view; 1b-polar view) and *Dyckia brevifolia* and *D. ibiramensis* (2a-in center, polar distal view; on the left equatorial cross-section view; on the right, exine ornamentation; 2b-equatorial side view).

DISCUSSION

R. ECHINATA

The pollinic characterization obtained in the present study places *R. echinata* in the general pattern of Rutaceae grains, that is, in monads, 3-colporate, prolate, with reticulate exine, as reported by Barth (1980; 1982; 1983; 1985) in the *genera Zanthoxylum* L., *Pilocarpus* Vahl*, Esenbeckia* Kunth and *Helietta* Tul., which considered the family euripalinological. On the other hand, Groppo *et al.* (2010) in a study with the *genus Hortia* Vand., proposed the *taxon* as stenopalinological as it shows a great morphological similarity. According to Dutra *et al.* (2017), pollinic studies about Rutaceae confirm the family as euripalynous due to the diversity of the morphology of the grains, despite the fact that some *genera* show constant pollinic morphology in their species.

The characterization achieved in this study can contribute to sustain the proximity of *Raulinoa* with *Metrodorea* and *Esenbeckia*, which are also inserted in Pilocarpinae as, in general, the evolutionary relationships considering the whole tribe Galipeeae (composed of subtribes Galipeinae and Pilocarpinae) are scarcely studied. According to Oliveira *et al.* (2015), in any tree where the sister-group relationship of *Metrodorea* is clear, *Esenbeckia* is sister to *Raulinoa* or to the clade ((*Raulinoa (Balfourodendron, Helietta*)). *Metrodorea* has eight species, all native to Brazil, with few records in Suriname and Bolivia and *Esenbeckia* has 29, native to the Neotropical region, with greater diversity in South America (OLIVEIRA, 2007). On the other side, according to Darosci (2009), the seed analysis of *R. echinata* showed common morphological characteristics of Rutaceae but also others not described for the family and, in addition, wood analysis showed characteristics which are common among *taxa* with a high degree of specialization as well as characteristics not previously described for Rutaceae.

R. echinata plants have monoclinous flowers attached to the stems (COWAN & SMITH, 1973). Observations about pollination were done on the plants, which grow on the border of the river, aiming to verify the possible pollen transport vectors (chiropterophily, anemophily, entomophily, etc.) and it was noticed that, due to the proximity of the river, the plants are covered, in the time of reproduction, by a mantle of moisture which condenses into micro-droplets of water, that drip between the anthers of the flower, and could take the pollen grains to the stigmas (Silva, *pers.com*.). Thus self-pollination by autogamy (where pollen is transferred to the female part of the same flower) or by geitonogamy (when pollen is transferred to another flower on the same plant) could occur. However, it is possible that a mechanism of hydrophily could also occur as the plants are very close to the river, which regularly overflows from its bed. There are historical registers of floods of the river for all months of the year, but with greater intensity for the period of May to November with a peak in October (CORDERO & MEDEIROS, 2007), the month that is reported by Cowan & Smith (1973) as the blooming period. In this case, reproduction could occur by cross-pollination (allogamy) as pollen would be delivered from the stamen of one flower to the stigma of a flower on another plant of the same species. Anyway, transportation by water is a feature in *R. echinata* as the seeds are able to float on water (hydrocory), which favors their transportation by the river and thus contributes to thicken and expand the vegetal biomass on riparian environments (DAROSCI & PAULILO, 2011).

DYCKIA BREVIFOLIA AND *D. IBIRAMENSIS*

The pollen characteristics found for *Dyckia brevifolia* and *D. ibiramensis* match, in a general way, the pattern described for *Dyckia genus,* that is, pollen grains in monads, with bilateral symmetry, elliptic in polar view outline, heteropolar, medium-sized (26-50 µm), monosulcated, heterobrochate (perforated microreticulated to reticulated) as stated by Ehler & Schill (1973), Pire & Schinip (1992), Antunes & Santos (2013), Dorneles *et al.* (2014), Wanderley *et al.* (2014) and Halbritter (2015, 2016a, b, c, e, f, g, h, i, j, k).

Mez (1891-1894; 1896; 1934-1935) made the first attempt to apply pollen morphology to the systematics of Bromeliaceae. Based on the types of pollen apertures, he classified the subfamilies Pitcairnoideae and Tillandsioideae as stenopalynous, due to the fact that their pollen grains were

predominantly monocolpate, while Bromelioideae was considered euripalynous as there were different types of apertures (including the tribes Poratae and Sulcatae), in addition to inaperturate grains (tribe Integrae). However, the classic Pitcairnioideae subfamily has received the critic of being paraphylic (GIVNISH *et al.*, 2011). In this point of view, the subfamily, considered stenopalynous, is paraphylic. A strong support, in cladistics, was met for *Dyckia-Encholirium* clade (both *genera* of Pitcairnioideae) (CRAYN *et al.*, 2000), a finding that is largely consistent with the results of Varadarajan & Gilmartin (1988). However, this late study conflicts with that of Terry *et al.* (1997) about the other *genera* (*Fosterella* and *Navia*) and thus confirms Pitcairnioideae as polyphyletic (CRAYN *et al.*, 2000).

Studies done of the floral features and ecological observations in Pitcairnioideae lineages indicate various ways of passing the pollen grains on: ornithophily (humming birds) is found in the majority of the species (BERNARDELLO *et al.*, 1991; SAZIMA *et al.,* 1996; VOSGUERITCHIAN & BUZATO, 2006) but melithophily (SIQUEIRA-FILHO, 1998; WENDT *et al.*, 2002; ARAÚJO *et al.*, 2004; HMELJEVSKI, 2007), psycophily (VARASSIN & SAZIMA, 2000; LENZI *et al.*, 2006) and chiropterophily (SAZIMA *et al.*, 1989; 1995) also occur. According to Rogalski (2007), for *D. brevifolia*, there is the probability of the forwarding of diaspores (hydrocory) towards the individuals which thrive downstream. Buneker *et al.* (2013) effectively showed partially emerged inflorescences of flooded plants, in flood period (figure 5).

Figure 5 – Partially emerged inflorescences of flooded plants of *Dyckia brevifolia*, in overflowing period. From Buneker *et al.* (2013).

Plants that live totally or partially submerged undergo four ways of pollination by water (hydrophily) (COX, 1998): the pollen grains go out of the surface of the water and are transported by floating male flowers until falling into the female flowers; some species free filamentous or oval pollen that form pollinic pouches or they wrap their spherical pollen into longiline tubes; in other species, filaments or pollinic grains subject to mucilage are transported under the water by the currents or pollen denser than water is freed and, finally, there is the combined mode where there is a combination of the surface pollination with the underwater one (this happens with species that never reach the surface even on the driest periods). According to Cole (2003), species with restricted distribution tend to be self-compatible although self-compatibility and self-incompatibility may coexist in the same *genus* (WENDT *et al.*, 2002). In Bromeliaceae, most species are self-compatible (MCWILLIAMS, 1974; MARTINELLI, 1994).

As watercourses, such as rivers and streams, rainwater and floods carry with them pollen from plants that are flowering on the banks (SALGADO-LABOURIAU, 2007), the morphological characterization of the pollen grains of the focused species can help the verification of the hypothesis of pollination by hydrophily, by the analysis of samples of the water of the river, during the period of floods. This information may support their conservation, besides contributing to biological knowledge.

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