Morphology and micromorphology of the seed coats of species
of Echinodorus (Alismataceae) from Brazilian Northeastern

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Abstract: The morphology and the micromorphological characteristics of the surface of seeds of ten species of Echinodorus Rich. & Engelm. ex. A. Gray which occurs in northeastern Brazil were examined using scanning electron microscopy. The diversity of the observed ornaments allowed to distinguish the seed testa of the studied species as follows: scalariform in E. pubescens (Martius) Seubert, tenuous ribbed in E. tenellus (Martius) Buchenau, tenuous reticulate in E. reticulatus Haynes & Holm-Nielsen, reticulate channeled in E. grandiflorus (Chamisso & Schlechtendal) Micheli subsp. aureus (Fassett) Haynes & Holm-Nielsen, reticulated in E. glandulosus Rataj, reticulated-foveate in E. paniculatus Micheli and E. lanceolatus Rataj, and reticulated tenuous foveate in E. palaefolius (Nees & Martius) MacBride, E. subalatus (Martius) Grisebach and E. macrophyllus (Kunth) Micheli subsp. scaber (Rataj) Haynes & Holm-Nielsen. The complete description presents observations of the form of the component cells of the testa of these seeds, their anticlinal boundaries with respective cell junctions and cell wall thicknesses. The results suggest the importance of the seed micromorphology as a synthetic character to infra-generic classification of Echinodorus.

Keywords: Monocotyledons. Aquatic plants. SEM. Seed surface.


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INTRODUCTION
The Alismataceae have emergent or floating aquatic species. The family is represented by 12 genera and approximately 80 species with sub-cosmopolitan distribution (Haynes & Holm-Nielsen, 1998). Sagittaria Linnaeus and Echinodorus Rich. & Engelm. ex. A. Gray are genera with relatively low diversity, but are the only neotropical taxa of this family (Lot & Novelo, 1984; Haynes & Holm-Nielsen, 1989). The genus Echinodorus comprises 26 species (Haynes & Holm-Nielsen, 1994) occurring predominantly in the tropical regions of South America.

The genus Echinodorus was divided by Fassett (1955) into the sub-genera Echinodorus Fasset and Helianthium Fasset based on the insertion of the anthers into the filament and on the number of carpels. Leaf form, the types of translucent marks on the leaves, and the number and arrangement of the pericarp glands are other principal taxonomic characteristics used within the family (Micheli, 1881; Lot & Novelo, 1994; Haynes & Holm-Nielsen, 1994). Additionally, Haynes & Holm-Nielsen (1985) indicated that the length of the rostrum of the fruit is taxonomically important. Seed characteristics, however, have not yet been employed for taxonomic purposes. They were not considered in a recent revision of Echinodorus (Rataj, 2004) and in phylogenetics analysis (Lehtonen, 2006; Lethonen & Myllys, 2008).

Rogers (1983) and Micheli (1881) described the presence of slightly punctated testa surfaces in species of Echinodorus. The linear and slightly incurvate outline of the embryo lends an uncinate-curved shape to the seed (Micheli, 1881). In fact, the seed morphology of Echinodorus was described by Haynes & Holm-Nielsen (1985) as U-shaped. The recurvate shape of the seed is an adaptive characteristic, allowing the emergence of the hypocotyls through the micropyyle of the seed, breaking through the less rigid basal region of the achene (Kaul, 1978).

The establishment and renewal of Echinodorus populations in semi-arid regions is dependent on the ability of the seeds to resist long periods of desiccation in temporary aquatic habitats and high soil temperatures. Those temporary habitats are resulted of the climate from the Brazilian semi-arid region (Leprun, 1984-1985). In intermittent aquatic ecosystems, seed viability is related to dormancy (Salisbury, 1942), as well as, longevity, allowing germination to be delayed until environmental conditions are favorable (Yeo, 1965; West & Whigham, 1976).

As there is very little information currently available concerning the biology of aquatic plants from intermittent aquatic environments in the semi-arid region of Brazil, a morphological analysis of the seeds of these plants should contribute to our understanding of adaptive strategies encountered in these environments.

The present work examined the morphological and micromorphological characteristics of the seeds of species of Echinodorus from the semi-arid region of Brazil, in a search for taxonomic characters useful in distinguishing these taxa.

MATERIALS AND METHODS
Seeds were obtained from mature fruits collected in the field, with the criteria that these seeds were easily separated from the achenes present on the floral axis, or that the axis was quite dry. Exceptions were made for the species Echinodorus reticulatus Haynes & Holm-Nielsen, E. grandiflorus (Chamisso & Schlechtendal) Micheli subsp. aureus (Fassett) Haynes & Holm-Nielsen, and E. macrophyllus (Kunth) Micheli subsp. scaber (Rataj) Haynes & Holm-Nielsen, whose seeds were obtained from herbarium collections. The examined species are listed below: E. glandulosus Rataj – Ceará: Aiuaba, estrada para Assaré, 09/05/2002, L. Q. Matias 352 (EAC, ICN); Antonina, 18/05/2003, L. Q. Matias 421 (EAC, ICN).
E. grandiflorus subsp. aureus, Ceará: Crato, Granjeiro, 11/12/1933, G. D. Luetzelburg s.n. (IPA 22490); E. lanceolatus Rataj – Ceará: Granja, 17/07/2003, L. Q. Matias 421 (EAC, ICN).
As no detailed protocols were encountered for the preparation of *Echinodorus* seeds for viewing surface ornamentation under scanning electron microscopy (SEM), a number of different techniques were initially tested. Treating seeds with a 50/50 solution of ether/chloroform (Wilkinson, 1983) followed by ultrasonic washing for 15 minutes was found to remove waxes and oily substances (derived from the achene glands) that adhere to the seed surface as the fruit dries. The seeds were subsequently dried at 25 °C for five days, fixed to aluminum supports (stubs), layered with ten to 15 nm of gold by sputtering Balzers SCD 005, and maintained under a vacuum until observation. Samples were examined using a Joel JSM 6060 Scanning Electron Microscopy at 20 K = kv, and digital electro-photomicrographs obtained at 1,440 dpi. Comparisons between the different species were made on the basis of observations of the cells located near the micropyle. Terminology for seed surface characteristics as seen under SEM were based on Murley (1951 *apud* Stearn, 1973), Barthlott (1981, 1984), and Behnke & Barthlott (1983). Terminology for morphological characters was based on Radford *et al.* (1974) and the Systematics Association Committee for Descriptive Biological Terminology (1960).

**RESULTS**

The majority of the seeds of the species of *Echinodorus* examined were obovate in lateral view (Figures 1, 7, 11, 13, 15, 17, 19). Only *E. paniculatus* (Figure 3), *E. lanceolatus* (Figure 5), and *E. reticulatus* (Figure 9) demonstrated oblong seeds. The slightly recurved outline of the embryo lends a U-shaped form to the seed. The cells composing the testa had a distinct longitudinal alignment as seen in a lateral view, and this orientation is determined by the outline of the embryo. As such, those cells situated near curved regions have distinctly modified isodiametric forms. A well-defined central elliptic depression were observed in the seeds of *E. subalatus* (Figure 13), but was less evident in *E. palaefolius* (Figure 17). The seed surface was reasonably smooth in both *E. tenellus* and *E. reticulates*, but ornamented in the other species.

The patterns of micro-texturing or secondary sculpturing observed on the seed surfaces included: tenuous ribbed (Figures 1-2), reticulate-foveate (Figures 3-4), reticulate channeled (Figures 7-8), tenuous reticulate (Figures 9-10), scalariform (Figures 11-12), reticulated tenuous foveate (Figures 13-14), and reticulated (Figures 19-20).

Diagnostic characteristics related to the primary sculpturing of the seed surfaces included: 1) cell forms, 2) the types of cell wall edges and periclinal wall junctions, 3) the thickness of the anticlinal walls.

*Echinodorus tenellus* showed a tenuous ribbed surface formed by oblong cells with only slightly pre-eminent anticlinal walls (Figures 1-2). The anticlinal walls of this species are thin, not juxtaposed, sub-equal, and rectilinear.

*Echinodorus paniculatus* and *E. lanceolatus* showed a reticulate-foveate pattern, with circular cells and slightly thickened, pre-eminent and juxtaposed anticlinal walls (Figures 3-4, 5-6, respectively).

*Echinodorus grandiflorus* subsp. *aureus* showed polygonal cells, with four pronounced anticlinal walls that are sub-equal, slightly circular, thickened, and with intercellular spaces (principally in the angular regions), lending a reticulated-canaliculated aspect to the seed surface (Figures 7-8).

*Echinodorus reticulatus* showed polygonal, with three to five, slightly pre-eminent, thick anticlinal cell walls, constituting a tenuous-foveate pattern (Figures 9-10). The periclinal walls were rugose and differ in this aspect from all the other species examined.
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Figure 1-6. Longitudinal outline of *Echinodorus* seed-coat. (1-2) *E. tenellus*-seed (1) and tenuous ribbed seed surface (2); (3-4) *E. paniculatus*-seed (3) and reticulated foveate seed surface (4); (5-6) *E. lanceolatus*-seed (5) and reticulated foveate seed surface (6).
Figure 7-12. Longitudinal outline of *Echinodorus* seed-coat. (7-8) *E. grandiflorus* subsp. *aureus*-seed coat (7) and reticulated channeled seed surface (8); (9-10) *E. reticulatus*-seed (9) and tenuous reticulated seed surface (10); (11-12) *E. pubescens*-seed (11) and scalariform seed surface (12).
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Figure 13-20. Longitudinal outline of *Echinodorus* seed-coat. (13-14) *E. subalatus*-seed (13) and reticulated tenuous foveote seed surface (14); (15-16) *E. macrophyllus* subsp. scaber-seed (15) and reticulated tenuous foveote (16); (17-18) *E. palaefolius*-seed and reticulated tenuous foveote seed surface (18); (19-20) *E. glandulosus*-seed (19) and reticulated seed surface (20).
Echinodorus subalatus (Figures 13-14), E. macrophyllus subsp. scaber (Figures 15-16), and E. palaefolius (Figures 17-18) showed a reticular delineation of the seed surface, although their fovea are deeper, constituting a reticulate-foveate pattern. The cells were polygonal, with 4-5 sides, and have thick juxtaposed anticlinal walls of different sizes.

Echinodorus pubescens showed regularly aligned cells, with a predominance of oblong cells with four juxtaposed anticlinal walls that are thick and rectilinear, giving the testa a scalariform pattern (Figures 11-12).

The testa of E. glandulosus showed polygonal cells, with 4-5 anticlinal walls, pronounced, rectilinear, and juxtaposed cell walls, constituting a reticulate ornamentation pattern (Figures 19-20).

DISCUSSION
The seed morphology of the examined species of Echinodorus showed similar patterns to the description presented by Kaul (1978) and Haynes & Holm-Nielsen (1985) for others species of the genus. As explained by Kaul (1978), the recurvate shape of the seed is thus an adaptive character and very important to the establishment of seedlings.

Micromorphological examinations revealed that the testa of all analyzed species were similar in terms of cell distribution in lateral view, all of them forming distinct longitudinal alignments. As these cells vary in form, depending on the outline of the embryo, they do not furnish any taxonomically useful information. But the patterns of seed coat ornamentation, cell form, anticlinal cell wall thickness, anticlinal cell wall junctions, and the form of the anticlinal wall junctions yield important taxonomic criteria for the studied species. Those epidermal characters were applied to others aquatic plants in systematic treatments (Chance & Bacon, 1984; Shaffer-Fehre, 1991; Chuang & Ornduff, 1992; Chuang & Constance, 1992; Suseela et al., 1998), recognizing their importance as diagnostic characters as well (sensu Stuessy, 1990).

The dwarf plants passing as E. tenellus are the basis of the subgenus Helianthium (Fassett, 1955) and that species presented the more distinctive seed coat sculpture: tenuous ribbed surface. The others species are representatives of the subgenus Echinodorus (Fassett, 1955) and they had an evident reticulated pattern, excepted E. pubescens, due to regularly aligned cells, with a predominance of oblong cells with four juxtaposed anticlinal walls. In that way, the section Palaeolii proposed by Rataj (2004) seems not to be an uniform group, because of the differences of the micromorphology of the seed coats of E. pubescens, E. subalatus, and E. palaefolius.

Among the species with a reticulate seed surface pattern, E. glandulosus demonstrated the most defined and compact delineation, with rectilinear anticlinal walls with predominantly right angles that lend a geometric aspect to the seed surface. This pattern contrasts greatly with that of E. grandiflorus subsp. aureus, where the non-juxtaposed sinuous walls of the anticlinal cells generate a reticulate-cannulate pattern.

Echinodorus macrophyllus (Kunth) Micheli and E. scaber Rataj are representative species of the section Macrophyllii Rataj (Rataj, 2004). The recognition at infraspecific level of E. macrophyllus subsp. scaber by Haynes & Holm-Nielsen (1986) was justified by the not distinctive fruit morphology. Then, a future analysis of the seed micromorphology of both species may be important to resolve this taxonomic problem.

Echinodorus subalatus, E. palaefolius, and E. macrophyllus subsp. scaber demonstrated similar micromorphological characteristics, having polygonal cells with juxtaposed and rectilinear anticlinal walls around the foveas that generated a reticulate tenuous-foveate pattern. However, E. subalatus, E. palaefolius (Palaeolii section sensu Rataj, 2004), and E. macrophyllus subsp. scaber (Macrophyllii section) belong to different infraspecific categories. Therefore, those sections seem an artificial taxonomic arrangement. In spite of the similarities among the seeds of these species, it is possible to distinguish E. subalatus by the presence of an elliptical and well-defined central depression.

Seed coat ornamentation allows the taxonomic separation of E. tenellus, E. pubescens, E. reticulates, and
E. grandiflorus subsp. aureus; while E. paniculatus and E. lanceolatus demonstrate great similarity in terms of the micromorphological characteristics of their seeds.

Echinodorus paniculatus and E. lanceolatus differ from others studied species by having circular cells and an ample central fovea. However, the similarities between the seed surfaces of these two species inhibit the use of their micromorphological characteristics for taxonomic purposes. In the same way, the vegetative and floral morphology of those species is quite similar (Matias, 2007, Matias et al., 2007). But, the species could be identified only by the presence of glands in fruit (Rataj, 1968) and the anatomical characters of the scapes (Matias et al., 2008).

Although the micromorphological characteristics of the seed surfaces did not permit the distinction of all the species examined, the observed patterns allow the identification of groups of similar species, which may be important for future infra-generic classification. As such, the ornamentation patterns and the characteristics related to the primary sculpturing seen in E. lanceolatus and E. paniculatus, and in E. subalatus, E. palaefolius, and E. macrophyllus subsp. scaber may represent two similar groups. Additionally, these characteristics should be taken into consideration in the infra-generic classification of Echinodorus as synthetic character.

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