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Anatomy of the root of eight species of emergent aquatic macrophytes from the upper Paraná river, Paraná State, Brazil floodplain

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ABSTRACT. The upper Paraná River floodplain is characterized by the existence of several aquatic and transitional habitats between the aquatic and terrestrial environment, influencing the presence and distribution of aquatic macrophytes. Samples were taken from different places and permanent slides were prepared for analysis and capture of images with the objective of comparing the anatomy of the roots of eight species of emergent aquatic macrophytes. The species feature uniseriate epidermis with narrow and long cells, cortex composed of uniseriate or biseriate exodermis, with or without thickening, aerenchyma with great gaps, uniseriate endodermis, with or without thickening, continuous or interrupted pericycle, and central cylinder with variable number of xylem poles.

Key words: aerenchyma, root, Alismataceae, Cyperaceae, Polygonaceae, Pontederiaceae.

RESUMO. Anatomia da raiz de oito espécies de macrófitas aquáticas emergentes da planície alagável do alto rio Paraná, Estado do Paraná, Brasil. A planície alagável do alto rio Paraná é caracterizada pela existência de vários habitats aquáticos e transicionais entre o ambiente aquático e terrestre, influenciando na presença e distribuição das macrófitas aquáticas. Com o objetivo de se comparar a anatomia das raízes de oito espécies de macrófitas aquáticas emergentes, foram realizadas coletas em diferentes locais e preparadas lâminas permanentes para análise e captura de imagens. As espécies possuem epiderme unisseriada com células estreitas e longas, córtex composto por exoderme unisseriada ou bisseriada, com ou sem espessamento, aerênquima com grandes lacunas, endoderme unisseriada, com ou sem espessamento, periciclo ou interrompido e cilindro central com número variável de polos de xilema.

Palavras-chave: aerênquima, raiz, Alismataceae, Cyperaceae, Polygonaceae contínuo, Pontederiaceae.

Introduction

The studies on the biology and ecology of continental aquatic ecosystems began in environments located in temperate areas. Only a few decades later did they come to be performed in the tropics. In recent decades, studies in Brazilian aquatic continental ecosystems showed great progress. This progress is due to factors such as: the profusion and variety of aquatic ecosystems, some of which still in their natural state; the need to manage aquatic environments already altered by anthropic action; and the great interest for biodiversity in recent decades (THOMAZ; BINI, 2003).

Most medium or large rivers have flooded adjacent areas that, together with the main channel, constitute the systems denominated river-floodplains. In tropical areas, these systems show significant temporal variation in their physical, chemical and biotic factors. Such variations are associated, mainly, with the alterations in

hydrometric levels, which have come to be known as it has been attributed the concept of “flood pulses” (JUNK et al., 1989). This comprises one flooding phase and a drought phase, with oscillations in hydrometric levels, influencing water speed, depth of the aquatic environments and surface area of the plain submitted to flooding (NEIFF, 1990).

The upper Paraná river floodplain is characterized by the existence of several aquatic and transitional habitats between aquatic and terrestrial environments, with distinctions regarding the degree of communication with the main river and tributaries, morphometry and hydrodynamics. As such, each of these habitats possesses individual limnologic characteristics, which influences the presence and distribution of organisms, such as aquatic macrophytes (THOMAZ et al., 1997).

In tropical aquatic environments in general, the climatic conditions tend to favor the growth of aquatic plants. Among these, the high average

temperatures and intense solar radiation can be mentioned. In addition to these factors, there are anthropic pressures that can induce the emergence of appropriate conditions for the development of these plants, such as the construction of artificial reservoirs and eutrophication (BIANCHINI-JÚNIOR, 2003).

According to Pott and Pott (2003), aquatic vegetation is dynamic, adjusting itself to natural alterations resulting from hydrological cycles. Aquatic macrophytes have great adaptation ability, great ecological breadth, and are endowed with an impressive phenotypic plasticity, easily observed in amphibious plants, macrophytes emerged with aerial leaves. According to Esteves (1998), these plants withstand long drought periods, becoming terrestrial forms with deep anatomical, physiologic and morphologic modifications.

According to Crawford (1987), the soils subject to flooding determine alterations in the plants that become tolerant to flooding. There are two interpretations of the strategies of tolerant plants to hypoxia: according to Crawford (1987), some metabolic routes would be activated when the plants are flooded, while, according to Armstrong (1979), the plants would not need alternative metabolic routes inasmuch as their morpho-anatomical adaptations would make possible the diffusion of oxygen from the aerial part to the roots and, as such, they would maintain aerobic breathing. Joly (1994) suggests there can be both metabolic as morpho-anatomical strategies, with an interaction between them.

According to Thomaz et al. (2004), the species of this study, *Echinodorus grandiflorus*, *Sagittaria montevidensis* (Alismataceae), *Eichhornia azurea*, *Pontederia cordata* (Pontederiaceae), *Oxycaryum cubense* (Cyperaceae), *Polygonum ferrugineum*, *P. hydropiperoides* and *P. stelligerum* (Polygonaceae), belong to the ecological group of the emergent aquatic macrophytes and are found on the margins of bodies of water. These species are subject to the natural oscillations in water level and can, frequently, be submerged temporarily. The morphologic modifications induced by the submersion vary considerably among the plants that live in this condition. Some of them differ only in the degree of lignification, and others undergo such phenotypical modifications that they can be (erroneously) recognized taxonomically as different species (SCULTHORPE, 1985).

The roots of emergent aquatic macrophytes face an environment of almost total absence of oxygen, needing artifices to assure appropriate amounts of

that gas. To that end, extensive systems of air spaces in the parenchyma (aerenchyma) are very frequent. This aerenchyma is constituted by a system of aeration channels, leaving from the roots to the stem and even reaching the leaves (SCULTHORPE, 1985). Still according to the same author, this compensatory system of aerenchyma, in addition to assuring provisions of oxygen, offers great mechanical resistance, allowing the maximum possible force with the minimum possible tissue.

Related botanical works regarding the anatomy of emergent aquatic macrophytes of the Paraná River floodplain are scarce; one could mention the one by Silva-Brambilla and Moscheta (2001), which discusses the leaf anatomy of six species of *Polygonum*. Important anatomy works exist with some of the species of this study, accomplished by authors from different regions. Thus, the objective of this work is to compare the anatomy of the roots of eight species of emergent aquatic macrophytes, belonging to different botanical families, which happen in two systems of the Paraná river floodplain, the Baía river and the Paraná river itself.

Material and methods

Echinodorus grandiflorus (Cham. and Schtdl.) Micheli, *Polygonum ferrugineum* Wedd. and *Polygonum stelligerum* Cham. were collected in the Baía river, a tributary of the Paraná river, in the district of Taquarussu, Mato Grosso do Sul, State. *Eichhornia azurea* (Sw.) Kunth and *Pontederia cordata* L. were collected in the ressaco do Leopoldo; *Oxycaryum cubense* (Poepp. and Kunth) Lye and *Polygonum hydropiperoides* Michx. were collected in the ressaco do Pau Véio and *Sagittaria montevidensis* Cham. and Schtdl. were collected at the Lagoa das Pombas, all of them flooded areas of the margins of the Paraná river, area of Porto Rico, State of Paraná. The collection period was between the months of October 2007 and March 2008.

The botanical material was fixed and conserved in FAA 50 (JENSEN, 1962). For the analysis of the anatomy of the roots, the medium portion was used in the making of permanent sheets, with botanical material includes in historesin (GERRITS, 1991). They were split up in rotary microtome, red-faced with Toluidine blue and mounted in Permount (O'BRIEN et al., 1964). The identification of Casparian band was accomplished with Sudan IV in glycerin (JENSEN, 1962).

The pictures of the morphology of the roots were obtained with a digital camera, and the photomicrographs were accomplished with

photographic equipment coupled to the microscopy Olympus BX50, equipped with system of capture digital Zoom Former Browser.

Results and discussion

The roots of all of the studied species are foreign, aquatic, relatively long and thin (Figures 1 and 2). The roots of *Sagittaria montevidensis* (Figure 2A) and *Echinodorus grandiflorus* (Figure 2B and C) differ from the other for the spongy aspect of the first one and for the extensive lateral roots of the second one.

With regard to anatomical structure, there is also apparent similarity in the distribution of the tissues. So, the epidermis, the cortex and the central cylinder of these macrophytes were compared.

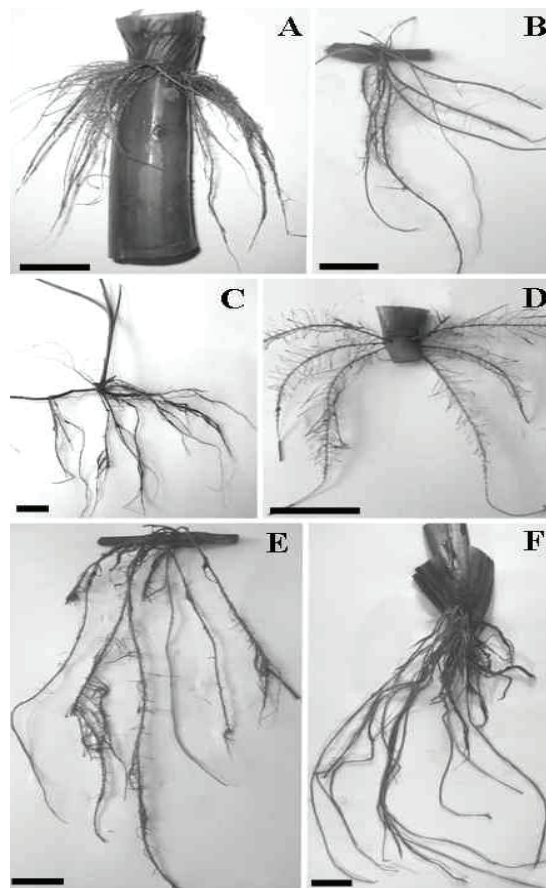


Figure 1. Morphology of the roots. A: *Polygonum ferrugineum*. B: *Polygonum hydropiperoides*. C: *Oxycaryum cubense*. D: *Eichhornia azurea*. E: *Polygonum stelligerum*. F: *Pontederia cordata*. Bar: 2 cm (A, B, E); 3 cm (C, D, F).

The epidermis of the roots of *Echinodorus grandiflorus*, *Eichhornia azurea*, *Oxycaryum cubense*, *Sagittaria montevidensis*, *Polygonum ferrugineum*, *P. hydropiperoides* and *P. stelligerum* is composed by

narrow and long cells, in cross-section, with thin walls. In the three species of *Polygonum* many epidermal cells almost come dehydrated (Figure 3D, A and F), breaking up easily. Bona and Morretes (2003) also observed dehydrated cells in the epidermis of the emerged macrophytes *Bacopa salzmanii* and *B. monnieroides*, although most of the aquatic plants presented root hairs.

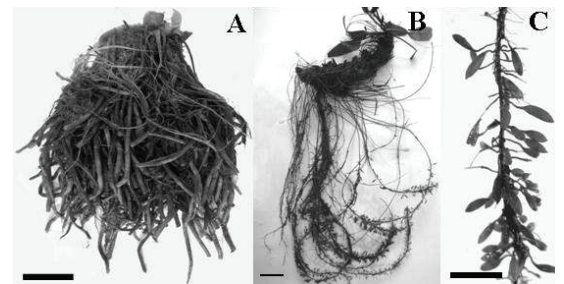


Figure 2. Morphology of the roots. A: *Sagittaria montevidensis*. B and C: *Echinodorus grandiflorus*. C: detail of extensive lateral roots. Bar: 1 cm (C); 3 cm (A, B).

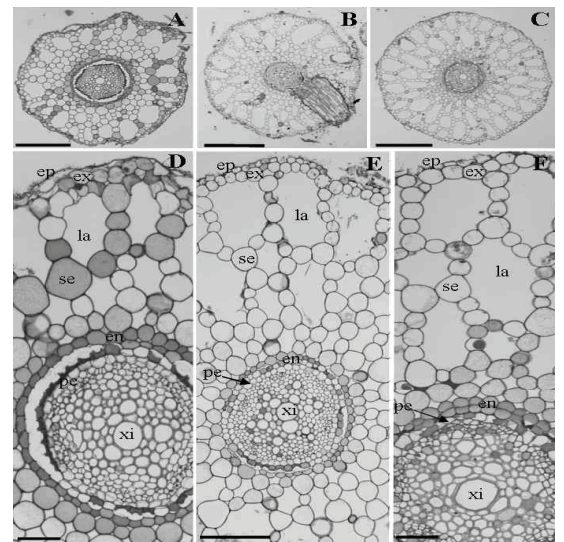


Figure 3. Root of Polygonaceae in cross-section. A and D: *Polygonum ferrugineum*. B and E: *Polygonum hydropiperoides*. C and F: *Polygonum stelligerum*. (en: endodermis, ep: epidermis, ex: exodermis, la: lacunae, pe: pericycle, se: septa, xi: xylem). Bar: 400 μ m (B, C); 200 μ m (A, E); 50 μ m (D, F).

Although according to Sculthorpe (1985) most of the aquatic plants presents root hairs, this was observed only in *Oxycaryum cubense* (Figure 5C). The presence or not of root hairs in aquatic plants seems to be quite variable, regardless of whether they are submerged or not (BONA; MORRETES, 2003).

In *Pontederia cordata*, the epidermis probably disrupts with the ripening of the organ, staying with an

exodermis with subtle thickening of the tangential external walls (Figure 4D and E).

Processes similar to this had already been observed by Seago-Jr. et al. (2000a) for this species, and also by Bona and Morretes (2003), for *Bacopa salzmanii* and *B. monnieroides*. Considering that these authors also found only the exodermis in those species, this aspect deserves embryological studies of the aquatic roots.

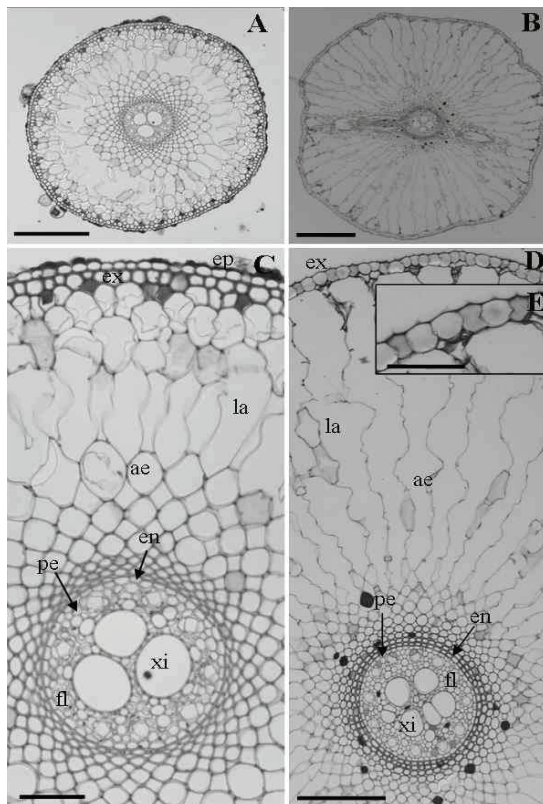


Figure 4. Root of Pontederiaceae in cross-section. A and C: *Eichhornia azurea*. B and D: *Pontederia cordata*. (ae: aerenchyma, en: endodermis, ep: epidermis, ex: exodermis, fl: phloem, la: lacunae, pe: pericycle, xi: xylem). Bar: 400 µm (B); 200 µm (A, D); 100 µm (E); 50 µm (C).

The exoderme of *Oxycaryum cubense* is multiseriate and the first layer possesses thickening in 'O' and the other three layers are, progressively, less thickened (Figure 5B), while the exodermis of *Eichhornia azurea* is slightly thickened (Figure 4C). Fabbri et al. (2005) observed exodermis thickening in *Paspalum modestum* and *Paspalum wrightii*, and Seago-Jr. et al. (2000b), in *Caltha palustris* and *Nymphaea odorata*. Coan et al. (2002) registered exodermis slightly with cells thickened in six aquatic species of Eriocaulaceae.

Perumalla et al. (1990) have suggested that all of the suberized exodermis probably possess Casparian bands. However, Bona and Morretes (2003) have appeared

the need of larger studies that can prove the presence or absence of those grooves in the exodermis.

Most of the cortex is constituted of aerenchyma in all of the analyzed species, with great gaps radially arranged (Figures 3A, B and C, 4A and B, 6A and 7A), or smaller spaces (Figure 5A). The gaps are separate among themselves by septum with eight cellular layers in *Polygonum ferrugineum* (Figure 3A) and with 12 cellular layers in *P. hydropiperoides* and *P. stelligerum* (Figure 3B and C). The cells of the septum are different in size and they are prolonged longitudinally, with few intercellular spaces in *P. ferrugineum* (Figure 3D). This aerenchyma type was observed by Deuner et al. (2007) in another species of the same gender, *Polygonum punctatum*, by Seago-Jr. et al. (2005) in *Rumex crispus* (Polygonaceae) and other aquatic species, and by Seago-Jr. et al. (2000b), for *Nymphaea odorata*.

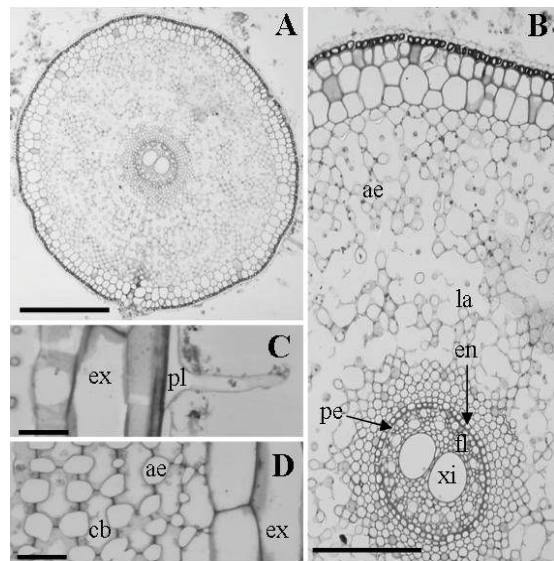


Figure 5. *Oxycaryum cubense* (Cyperaceae). A: cross-section. B: cortex. C: outlying area in longitudinal section. D: cortex in longitudinal section. (ae: aerenchyma, cb: cells with arms, en: endodermis, ex: exodermis, fl: phloem, la: lacunae, pe: pericycle, pl: root hairs, xi: xylem). Bar: 400 µm (A); 200 µm (B); 50 µm (C, D).

The aerenchyma in *Sagittaria montevidensis* (Figure 6B), *Pontederia cordata* (Figure 4D) and *Eichhornia azurea* (Figure 4C) is composed by septum of cells totally dehydrated, what disables the counting of the cellular layers. Dehydrated cells in the aerenchyma were observed in the studies of Fabbri et al. (2005), in *Paspalum modestum*; Seago Jr. et al. (2005), in *Pontederia cordata* and *Glyceria maxim*; Bona and Morretes (2003), in *Bacopa salzmanii* and *B. monnieroides*; Coan et al. (2002), in *Tonina fluviatilis*; Seago-Jr. et al. (2000a), in *Pontederia cordata*; Schussler and Longstreth (2000), in *Sagittaria lancifolia*, and for Longstreth and

Borkhsenius (2000), for the same species and still in *Thalia geniculata* and *Pontederia cordata*.

In the study of Fabbri et al. (2005), the aerenchyma in the roots resulted in the death and subsequent cellular collapse. Schussler and Longstreth (2000) related the lyse of the cortical cells with a type of programmed cellular death, call of PCD - programmed cellular death, however, they did not observe cellular death in *Sagittaria lancifolia*. Longstreth and Borkhsenius (2000) mentioned that, during the formation of the aerenchyma of *S. lancifolia*, the cells prolonged parallel to the ray of the root, reducing in perpendicular to the plan of the ray, forming long and fine rows, without causing, necessarily, the cellular death. The authors verified the cellular ultrastructure and found organelles, as the Golgi apparatus and mitochondria.

In *Oxycaryum cubense*, the aerenchyma is constituted by 12 to 15 layers of short bractiform cells (Figure 5B and D). This cell type was also observed by Menezes et al. (2005), for *Cyperus papyrus*, and by Rodrigues and Estelita (2004), for *Cyperus giganteus*, who consider this characteristic aerenchyma from the family Cyperaceae. Coan et al. (2002) also found bractiform cells in the aerenchyma of aquatic species of Eriocaulaceae, where a variation occurs in the size and number of 'arms', which, according to the authors, can be used in the phylogeny of the family.

The exodermis of *Sagittaria montevidensis* is constituted by two cellular layers (Figure 6C), as described by Andrade et al. (2007) for this species. Its first stratum there are cells almost dehydrated among others of normal respiration. In the second stratum, with cells with larger diameter and sinuous walls, occasional groups of smaller cells are inserted (Figure 6C). These structures were not mentioned by Andrade et al. (2007) by this species, and by Longstreth and Borkhsenius (2000), for *Sagittaria lancifolia*. Hence, the sinuosity in the walls of the exodermis cells was registered by Perumalla et al. (1990), a characteristic usually associated with the presence of the Casparian bands, according to Bona and Morretes (2003). Seago-Jr. et al. (2000a) observed Casparian bands in the thickened exodermis of *Pontederia cordata* with the use of more specific techniques. Casparian bands in the exodermis were also identified by Seago-Jr. et al. (2000b), for *Caltha palustris* and *Nymphaea odorata*, and by Barnabas (1996) and Perumalla et al. (1990), for several species. In the present study, no species presented Casparian bands in the exodermis.

In young roots of *Echinodorus grandiflorus*, great part of the cells of the aerenchyma is dehydrated, breaking up easier than in the previous species,

during the handling (Figure 7A). That process culminates in the total loss of this portion of the cortex in older roots, in which the central cylinder stays protected by a much thickened endoderm, with reinforcement in 'O' (Figure 7B and C). No similar process was found in the researched literature, which evidences the need of the continuation of the studies with this species. However, it is possible deducing that the breaking of the dehydrated cells of the aerenchyma in young roots turns all the portion expresses to this tissue.

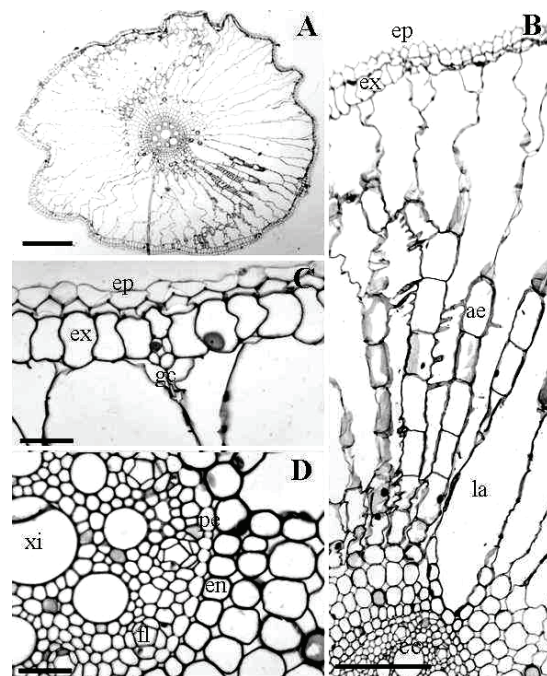


Figure 6. *Sagittaria montevidensis* (Alismataceae) in cross-section. A: root. B and C: cortex and detail. D: central cylinder. (ae: aerenchyma, cc: central cylinder, en: endodermis, ep: epidermis, ex: exodermis, fl: phloem, gc: group cellular, la: lacunae, pe: pericycle, xi: xylem). Bar: 400 μ m (A); 200 μ m (B); 50 μ m (C, D).

This species presents extensive lateral roots in the third apical of the roots of larger caliber (Figure 2B and C), which differ of the others not only morphologic, but anatomically as well. The epidermis is fragmented, with the exoderm of flat cells composing from one to two layers without thickening (Figure 8C). The cortical parenchyma possesses up to layers of more or less isodiametric cells, with thin walls and small intercellular spaces (Figure 8A and C). The endoderm is not thickened and, in the central cylinder, the pericycle is composed of cells of varied size, possessing three xylems poles (Figure 8B). The parenchymatic cortex with tiny spaces and the

reduced and less lignified central cylinder are outstanding differences between the two types of roots.

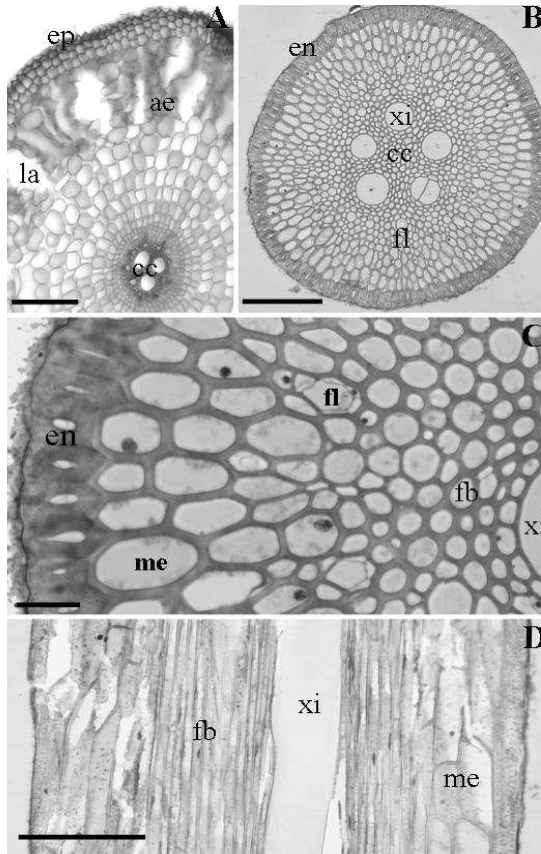


Figure 7. *Echinodorus grandiflorus* (Alismataceae). A: cross-section of young root. B: central cylinder of the root ripens. C: detail of the illustration B. D: longitudinal section. (ae: aerenchyma, cc: central cylinder, en: endodermis, ep: epidermis, fb: fibers, fl: phloem, la: lacunae, me: macrosclereids, xi: xylem). Bar: 200 μ m (A, B, D); 50 μ m (C).

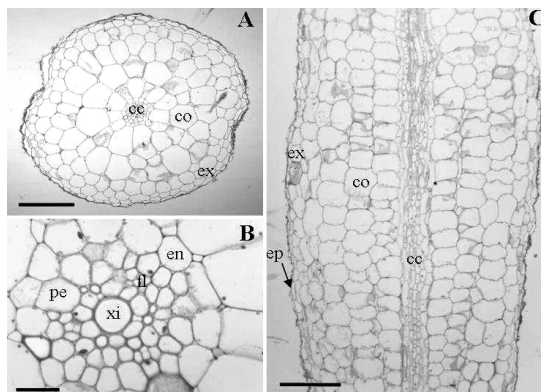


Figure 8. Extensive lateral root of *Echinodorus grandiflorus*. A: cross-section. B: detail of the central cylinder of the illustration A. C: longitudinal section. (cc: central cylinder, co: cortex, en: endodermis, ep: epidermis, ex: exodermis, fl: phloem, pe: pericycle, xi: xylem). Bar: 400 μ m (A); 200 μ m (B); 50 μ m (C).

Seago-Jr. et al. (2005) suggest eight different patterns for the development of the aerenchyma. In the development of the aerenchyma of the expansigeny type, found in the families Polygonaceae and Pontederiaceae, the expansion of intercellular spaces originates gaps without causing collapse or cellular death. In the species *Polygonum ferrugineum*, *P. hydropiperoides*, *P. stelligerum* (Figure 3A, B and C), the resulting pattern is classified as the type expansigeny honeycomb, while, in *Eichhornia azurea* and *Pontederia cordata* (Figure 4C and D), as the type expansigeny radial.

In the development of the aerenchyma of the type lysisigeny, found in the families Cyperaceae and Alismataceae, the gaps appear after the collapse and, a lot of times, there is cellular death. *Oxycaryum cubense* (Figure 5B) has got classified pattern, for the authors, lysisigeny tangential, which the collapse and the death are preceded by the separations of the tangential cellular, maintaining the rows radiates cellular relatively intact. In the *Sagittaria montevidensis* and *Echinodorus grandiflorus* (Figures 6B and 7A) possess pattern of the type lysisigeny packet, in that the willing cells in rows radial collapse, originating wide radial gaps. That new terminology of Seago-Jr. et al. (2005) has been used, recently, for other authors as Mühlenbock et al. (2007), Shimamura et al. (2007) and Liang et al. (2008).

The aerenchyma formation, however, cannot be considered as a rule among the aquatic macrophyte. In the same study of Seago-Jr. et al. (2005), among the 85 studied species, many of them do not develop this tissue, belonging to the orders Poales, Gunnerales, Saxifragales, Malpighiales, Fabales, Ericales, Lamiales and Asterales.

In the endoderm of *Polygonum ferrugineum*, *P. hydropiperoides*, *P. stelligerum*, *Eichhornia azurea*, and *Oxycaryum cubense* were not observed Casparian bands. In *Sagittaria montevidensis* and *Pontederia cordata*, these grooves are less evident and, in the young roots of *Echinodorus grandiflorus*, very evident. The endoderm possesses quick thickening in *Pontederia cordata* (Figure 4D). In *Polygonum ferrugineum* and *P. stelligerum*, some cells of the endoderm suffer collapsing. The endoderm in *Eichhornia azurea* (Figure 4C), *Oxycaryum cubense* (Figure 5B) and in *Echinodorus grandiflorus* (Figure 7B and C) demonstrate a thickening in 'O'. In this last one, the thickening is larger, causing the reduction of the cellular lumen. Coan et al. (2002) did not find Casparian bands in the endoderm of species of Eriocaulaceae, just curing. Barnabas (1996) tells absence of such grooves and curing from the endoderm for *Egeria densa* and *Eichhornia crassipes*. However, Seago-Jr. et al. (2000b), Bona and

Morretes (2003), Rodrigues and Estelita (2004), Menezes et al. (2005) and Andrade et al. (2007) observed Casparian bands and thickening in the endoderm of different aquatic species.

In study of Seago-Jr. et al. (2000a and b), Bona and Morretes (2003), Rodrigues and Estelita (2004), Menezes et al. (2005) and Seago-Jr. et al. (2005), the young endodermis possesses important peridermatic, originating cellular layers of the medium portion of the cortex, which this area, with the ripening of the root, becomes an aerenchyma. According to these authors, in some species the first layers originated from the endoderm come thickened, with cells radially disposed. This thickening of the first layers that start from the endoderm can be observed in *Eichhornia azurea* (Figure 4C), *Pontederia cordata* (Figure 4D), *Oxycaryum cubense* (Figure 5B) and *Sagittaria montevidensis* (Figure 6D). In *Polygonum ferrugineum* (Figure 3D), *P. hydropiperoides* (Figure 3A), *P. stelligerum* (Figure 3F) and *Echinodorus grandiflorus* (Figure 7A), this thickening is not evident, although the radial disposition of the cells suggests the meristematic activity of the endoderm, what it will only be able to be confirmed with ontogenetic studies.

In the central cylinder of all of the species, the uniseriate pericycle with cells with fine walls, come with variations in the size and cellular form, in *Polygonum ferrugineum* (Figure 3D), *P. stelligerum* (Figure 3F), *Echinodorus grandiflorus* (Figure 7B and C), and *Oxycaryum cubense* (Figure 5B). It is interrupted by phloem poles in *Sagittaria montevidensis* (Figure 6B), *Eichhornia azurea* (Figure 4C), *Pontederia cordata* (Figure 4D) and *Echinodorus grandiflorus* (Figure 8B), however it is continuous in *Polygonum hydropiperoides* (Figure 3E). In the study by Coan et al. (2002), the pericycle of some Eriocaulaceae is also interrupted by protoxylem elements, which arrive until the endodermis.

There is difficult to identify the xylem poles and also the location of the phloem, especially in *Polygonum ferrugineum* (Figure 3D), *P. hydropiperoides* (Figure 3E) and *P. stelligerum* (Figure 3F). In the other species, the xylem poles are in variable number, from five to six in *Pontederia cordata* (Figure 4D); eight in *Sagittaria montevidensis* (Figure 6D), from nine to ten in *Eichhornia azurea* (Figure 4C), eleven in *Echinodorus grandiflorus* (Figure 7B and C), and twelve in *Oxycaryum cubense* (Figure 5B). Sculthorpe (1985) also told difficulty to identify the vascular bundles in some species of aquatic plants, since many exhibit several degrees of reduction.

In the central cylinder of *Echinodorus grandiflorus* occurs predominance of fibers around of the

vascular bundles (Figure 7D). In the most external portion of the central cylinder prevail macrosclereids in up to four layers (Figure 7D). According to Sculthorpe (1985), some genders of Alismataceae possess more evident thickening in the central cylinder, information confirmed for *Echinodorus grandiflorus*.

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