

Morphological and anatomical patterns in Pontederiaceae (Commelinales) and their evolutionary implications



Danilo José Lima de Sousa^{a,*}, Vera Lucia Scatena^b, Ana Maria Giulietti^a, Aline Oriani^b

^a Programa de Pós-Graduação em Botânica, Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Feira de Santana, Bahia, Brazil

^b Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP, Brazil

ARTICLE INFO

Article history:

Received 28 February 2015

Received in revised form

19 November 2015

Accepted 24 November 2015

Available online 27 November 2015

Keywords:

Aquatic plants

Brazilian semi-arid

Evolution of vegetative characters

Water availability

ABSTRACT

Pontederiaceae include six genera and approximately 35 species of aquatic plants. The family exhibits great variation in morphology that makes the characterization of species and the understanding of infrafamilial relationships difficult. Twenty species were studied from collections made at the reproductive stage, aiming to establish morphological and anatomical patterns to better understand the taxonomy and evolution of the family. In order to include all species of the family, herbarium specimens were analyzed together with information available in the literature. Four morphological patterns were established for the family: Pattern I—stems with short internodes and alternate, petiolate leaves; Pattern II—stems with long internodes and alternate, petiolate leaves; Pattern III—stems with long internodes and alternate, sessile leaves; Pattern IV—stems with long internodes and verticillate, sessile leaves. The stems have atactosteles and in the species of Pattern I they are rhizomatous. The leaf petiole, the reproductive axis, the inflorescence bract petiole and the peduncle have monosteles and are distinguished from one another by the number of rings of collateral vascular bundles, and by the presence or absence of a fistula. The morphological patterns may represent synapomorphies of infrageneric groups and are related to the life form of the species. Based on current phylogenies, Pattern I is a plesiomorphic condition, including emergent species, and Patterns III and IV are the most derived and include submersed species. This is consistent with variation in water availability in the environment having influenced the diversification of Pontederiaceae during the course of their evolution.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

The family Pontederiaceae consists of six genera and approximately 35 species of aquatic plants (Cook, 1998; Barrett, 2004). The genera *Eichhornia* Kunth (~8 spp.), *Heteranthera* Ruiz et Pav. (~13 spp.) and *Pontederia* L. (~6 spp.) have pantropical distributions; *Monochoria* Presl. (~7 spp.) occurs in Africa, Asia and Australia; *Scholleropsis* H. Pers. (1 sp.) occurs in Africa and Madagascar, and *Hydrothrix* Hook. (1 sp.) is endemic to Brazil, occurring mainly in the northeast region (Hooker, 1887; Cook, 1996). The centre of diversity of the family is in the Neotropical region, where four genera and about 25 species are found (Seubert, 1847; Schultz, 1942;

Castellanos, 1958; Crow, 2003). Brazil has the largest number of species of the family (23 spp.), with the majority of them occurring in dry areas, in temporary pools, on the margins of rivers, and in streams (Amaral et al., 2014; Sousa and Giulietti, 2014).

The aquatic habit and the tubular and zygomorphic flowers are the main characteristics that distinguish Pontederiaceae (Cook, 1998). The species are perennial or annual herbs with varied life forms, including free-floating, floating-leaved, emergent and submersed plants (Sculthorpe, 1967; Barrett and Graham, 1997). Because they can occur in different types of environments, the species exhibit broad diversity in morphology and have different vegetative and reproductive strategies (Sculthorpe, 1967; Barrett and Graham, 1997; Cook, 1998). Such great morphological diversity makes the characterization of the species and the standardization of their descriptive terminology difficult.

A recent taxonomic study of the family, including about 50% of the species, highlighted the need for detailed morphological study of the vegetative and reproductive organs (Sousa and Giulietti,

* Corresponding author.

E-mail addresses: daniolojs@yahoo.com.br (D.J.L.d. Sousa), vscatena@rc.unesp.br (V.L. Scatena), anagiulietti@hotmail.com (A.M. Giulietti), alineoriani@yahoo.com.br (A. Oriani).

2014) and showed for example, that the inflorescences, frequently described as racemose (Kunth, 1815; Lowden, 1973; Horn, 1985; Cook, 1998; Strange et al., 2004), are in fact cymose (Sousa and Giulietti, 2014). This lack of information makes it difficult to establish homologies in attempting to understand infrafamilial phylogenetic relationships.

Previous studies with Pontederiaceae have examined seed anatomy (Coker, 1907), seedling morphology (Tillich, 1994), reproductive biology (Barrett and Anderson, 1985; Barrett, 1988; Cunha and Fischer, 2009; Cunha et al., 2014), floral morphology (Endress, 1995), and floral anatomy (Strange et al., 2004; Simpson and Burton, 2006). Anatomical data for the vegetative organs can be found only in the work of Olive (1894), who studied the leaves and stems of five species of the family; of Schwartz (1926), who applied the study of vegetative anatomy to the systematics of Pontederiaceae, and of Cheadle (1970), who studied the characters of vessel elements in twelve species of the family.

Data on morphological evolution in the family have been presented by Kohn et al. (1996), using reproductive characters. Hypotheses concerning the origin of the aquatic habit in Pontederiaceae were proposed by Barrett and Graham (1997) that considered the aquatic habit as a plesiomorphic condition in the family thus constituting a homology linking Pontederiaceae and Philydraceae within the Commelinales. These authors discussed the evolution of life forms, duration of life cycle, developmental patterns of leaves and floral morphology and they also considered the emergent life form as plesiomorphic and the precursor of the others (floating and submersed).

Phylogenetic studies have been undertaken in Pontederiaceae based either on morphology (Eckenwalder and Barrett, 1986) or on molecular markers, both plastid genes (Graham et al., 2002) and nuclear genes (Ness et al., 2011). In these analyses, only the genus *Monochoria* appears to be monophyletic while the others form paraphyletic or polyphyletic groups, which shows there is a need to accumulate new morphological and anatomical data in order to better define homologies and re-define taxa. Identifying morphological patterns will probably help to recognize ecological groups in Pontederiaceae, and combined with a future phylogenetic reconstruction, should help to clarify the evolution of vegetative characters and the phylogenetic relationships.

In this context, the objective of the present study was to survey morphological and anatomical patterns and life forms in Pontederiaceae, addressing the following questions: is it possible to establish morphological patterns in Pontederiaceae considering its morphological diversity? Are the morphological patterns related to the different life forms found in the family? Do the morphological patterns reflect taxonomic groups or phylogenetic relationships? What are the probable selective pressures that have driven the evolution of the morphological patterns?

2. Material and methods

For the morphological study, individuals of 20 species in reproductive phase were collected in Brazil, in the states of Bahia, Ceará, Goiás, Mato Grosso and São Paulo, in rivers and temporary ponds during the rainy season (Table 1). Part of the material was made into herbarium specimens and deposited at the HUEFS herbarium and part was fixed in FAA₅₀ (Johansen, 1940) and stored in 70% alcohol for anatomical study.

Herbarium specimens were also studied from the following collections: ALCB, EAC, ESA, GH, HRCB, HRB, HST, HUEFS, HUFSCAR, HURB, HVASF, IPA, MAC, MBM, MOSS, NY, PEUFR, RB, SP, SPF, UEC, UFP and UFPB. Taxa included in this study based on herbarium specimens were as follows: *Eichhornia meyeri* A.G.Schulz, *Eichhornia natans* (P. Beauv.) Solms, *Heteranthera callifolia* Rchb. ex

Table 1
Specimens collected for the morphological study.

Species	Voucher
<i>Eichhornia azurea</i> (Sw.) Kunth	D.J.L. Sousa et al. 239
<i>E. crassipes</i> (Mart.) Solms	D.J.L. Sousa et al. 307
<i>E. diversifolia</i> (Vahl) Urb.	D.J.L. Sousa 99
<i>E. heterosperma</i> Alexander	L.Q. Matias et al. 619
<i>E. paniculata</i> (Spreng.) Solms	D. J. L. Sousa et al. 203
<i>E. paradoxa</i> (Mart. ex Schult. & Schult. f.) Solms	D.J.L. Sousa et al. 319
<i>Heteranthera multiflora</i> (Griseb.) C.N.Horn	D.J.L. Sousa et al. 325
<i>H. oblongifolia</i> Mart. ex Schult. & Schult. f.	D.J.L. Sousa et al. 270
<i>H. peduncularis</i> Benth.	D.J.L. Sousa et al. 327
<i>H. reniformis</i> Ruiz & Pav.	D.J.L. Sousa et al. 210
<i>H. rotundifolia</i> (Kunth) Griseb.	D.J.L. Sousa et al. 209
<i>H. seubertiana</i> Solms.	D.J.L. Sousa et al. 308
<i>H. zosterifolia</i> Mart.	D.J.L. Sousa 400
<i>Hydrothrix gardneri</i> Hook. f.	D.J.L. Sousa et al. 211
<i>Pontederia cordata</i> L.	D.J.L. Sousa 401
<i>P. parviflora</i> Alexander	D.J.L. Sousa 503
<i>P. rotundifolia</i> L. f.	D.J.L. Sousa et al. 339
<i>P. sagittata</i> C.Presl	D.J.L. Sousa 403
<i>P. subovata</i> (Seub.) Lowden	D.J.L. Sousa et al. 329
<i>P. triflora</i> Seub.	D.J.L. Sousa et al. 445

Kunth, *Heteranthera dubia* (Jacq.) MacMill., *Heteranthera limosa* (Sw.) Willd., *Heteranthera mexicana* S. Watson, *Heteranthera spicata* C. Presl, *Monochoria cyanea* (F.Muell.) F. Muell., *Monochoria hastata* (L.) Solms, *Monochoria korsakowii* Regel & Maack, and *Monochoria vaginalis* (Burm.f.) C. Presl. Data from *Monochoria africana* (Solms) N.E.Br., *Monochoria australasia* Ridl., *Monochoria brevipetiolata* Verdc., and *Scholleropsis lutea* H.Pierrier were obtained from a survey of the literature (Solms-Laubach, 1883; La Bathie, 1936; Castellanos, 1958; Lowden, 1973; Horn, 1985; Cook, 1989, 1998) (Table 2). The morphological patterns were proposed based on the following criteria: length of stem internodes, phyllotaxy, and presence or absence of petiole in the leaves. These criteria were established by the authors based on the morphological variation found in the family.

For the anatomical study, the species chosen were those most representative of each recognized morphological pattern: *Eichhornia paniculata* (Pattern I), *Heteranthera reniformis* (Pattern II), *Heteranthera zosterifolia* (Pattern III) and *Hydrothrix gardneri* (Pattern IV). Cross sections were made in the mid-region of the stem internodes, the leaf petiole, the reproductive axis, the petiole of the inflorescence bract and the peduncle. The fixed material was dehydrated in an *n*-butyl series and embedded in historesin (Gerrits and Smid, 1983). The sections were made with a rotary microtome (Leica RM 2245) at a thickness of 8 µm, stained in periodic acid, Schiff's reagent (PAS) and toluidine blue (O'Brien et al., 1964; Feder and O'Brien, 1968) and mounted on permanent slides with Entellan (Merck). For the rhizome we also used material embedded in PEG (Richter, 1985), sectioned at a thickness of 12 µm and stained with basic fuchsin and astra blue (Roeser, 1972). The results were documented with images obtained with a capturing device (Leica DFC 450) attached to a microscope (Leica DM4000B), using LAS software for image digitization (Leica Application Suite V 4.0.0).

The definition of life forms followed the classification of Sculthorpe (1967) which includes: emergent—plants rooted into the substrate, with emerged leaves and inflorescences; free-floating—plants with roots below the water surface, not rooted in the substrate, with emerged leaves and inflorescences; floating-leaved—plants rooted into the substrate with leaves floating on the water surface and the inflorescences emerged; and submersed—plants rooted into the substrate with submersed leaves and emerged inflorescences. The emergent species were classified as erect or procumbent, following Barrett and Graham (1997).

Table 2
Morphological characters and growth forms of the species of Pontederiaceae.

Species	Morphological pattern	Leaf	Phyllotaxy	Leaf blade shape	Life form
<i>Eichhornia azurea</i>	II	Petiolate	Distichous	Obovate to elliptic	Procumbent emergent
<i>E. crassipes</i>	I	Petiolate	Spiral	Ovate to orbicular	Free-floating/erect emergent
<i>E. diversifolia</i>	II	Petiolate	Distichous	Cordiform	Floating-leaved
<i>E. heterosperma</i>	II	Petiolate	Distichous	Obovate to elliptic	Procumbent emergent
<i>E. meyeri</i> ^a	I	Petiolate	Distichous	Cordiform	Erect emergent
<i>E. natans</i> ^a	II	Petiolate	Distichous	Cordiform	Floating-leaved
<i>E. paniculata</i>	I	Petiolate	Spiral	Cordiform	Erect emergent
<i>E. paradoxa</i>	I	Petiolate	Spiral	Lanceolate	Erect emergent
<i>Heteranthera callifolia</i> ^a	II	Petiolate	Distichous	Cordiform	Procumbent emergent/floating-leaved
<i>H. dubia</i> ^a	III	Sessile	Distichous	Linear	Submersed/erect emergent
<i>H. limosa</i> ^a	II	Petiolate	Distichous/spiral	Ovate to rounded	Procumbent emergent/floating-leaved
<i>H. mexicana</i> ^a	III	Sessile	Distichous/spiral	Linear	Submersed/erect emergent
<i>H. multiflora</i>	II	Petiolate	Distichous	Reniform	Procumbent emergent/floating-leaved
<i>H. oblongifolia</i>	II	Petiolate	Distichous	Ovate	Procumbent emergent/floating-leaved
<i>H. peduncularis</i>	II	Petiolate	Distichous	Cordiform	Procumbent emergent/floating-leaved
<i>H. reniformis</i>	II	Petiolate	Distichous	Reniform to ovate	Procumbent emergent/floating-leaved
<i>H. rotundifolia</i>	II	Petiolate	Distichous/spiral	Ovate to rounded	Procumbent emergent/floating-leaved
<i>H. seubertiana</i>	III	Sessile	Distichous	Linear	Submersed/erect emergent
<i>H. spicata</i> ^a	II	Petiolate	Distichous	Cordiform	Procumbent emergent/floating-leaved
<i>H. zosterifolia</i>	III	Sessile	Distichous	Linear	Submersed/erect emergent
<i>Hydrothrix gardneri</i>	IV	Sessile	Verticillate	Filiform	Submersed
<i>Monochoria africana</i> ^b	I	Petiolate	Spiral	Cordiform	Erect emergent
<i>M. australasica</i> ^b	II	Petiolate	Distichous	Cordiform	Procumbent emergent/floating-leaved
<i>M. brevipetiolata</i> ^b	I	Petiolate	Spiral	Cordiform	Erect emergent
<i>M. cyanea</i> ^a	II	Petiolate	Distichous	Cordiform	Procumbent emergent
<i>M. hastata</i> ^a	I	Petiolate	Spiral	Sagittate	Erect emergent
<i>M. korsakowii</i> ^a	II	Petiolate	Distichous	Cordiform	Procumbent emergent
<i>M. vaginalis</i> ^a	II	Petiolate	Distichous	Cordiform	Procumbent emergent
<i>Pontederia cordata</i>	I	Petiolate	Distichous	Cordiform to lanceolate	Erect emergent
<i>P. parviflora</i>	I	Petiolate	Distichous	Ovate	Erect emergent
<i>P. rotundifolia</i>	II	Petiolate	Distichous	Triangular to ovate	Procumbent emergent
<i>P. sagittata</i>	I	Petiolate	Distichous	Sagittate	Erect emergent
<i>P. subovata</i>	II	Petiolate	Distichous	Obovate to rounded	Procumbent emergent/floating-leaved
<i>P. triflora</i>	II	Petiolate	Distichous	Elliptic	Floating-leaved
<i>Scholleropsis lutea</i> ^b	II	Petiolate	Distichous	Reniform	Floating-leaved

^aObserved in herbarium specimens.

^bInformation gathered from literature.

3. Results

3.1. Morphology

Based on our observations of adult, fertile individuals of Pontederiaceae, it was possible to propose four morphological patterns for the family: Pattern I—individuals with short stem internodes and alternate, petiolate leaves (Figs. 1 and 2); Pattern II—individuals with long stem internodes and alternate, petiolate leaves (Fig. 3); Pattern III—individuals with long stem internodes and alternate, sessile leaves (Fig. 4); Pattern IV—individuals with long stem internodes and verticillate, sessile leaves (Fig. 5).

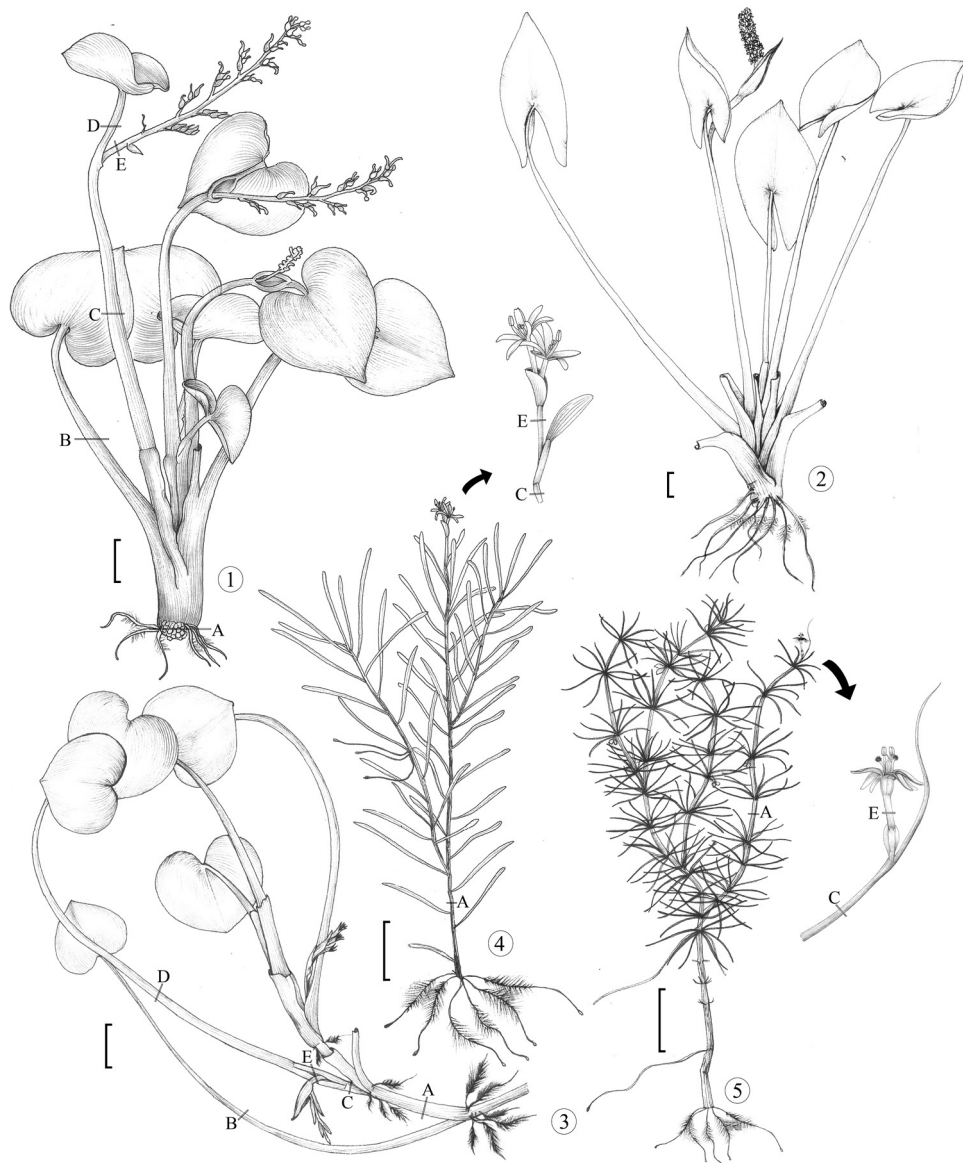
Pattern I includes erect emergent species (e.g. *Eichhornia paradoxa*, *M. hastata* and *Pontederia cordata*) and also the unique free-floating species of the family, *Eichhornia crassipes*; Pattern II includes procumbent emergent species (e.g. *Eichhornia azurea*, *M. cyanea*, and *Pontederia rotundifolia*) and also floating-leaved species (e.g. *Eichhornia diversifolia*, *Pontederia triflora*, and *Scholleropsis lutea*); Pattern III includes submersed and erect emergent species (e.g. *H. dubia*, and *H. zosterifolia*); Pattern IV includes only *H. gardneri*, an obligated submersed species. The morphological pattern of each species of the family, together with information on leaf morphology, phyllotaxy and life forms are provided in Table 2.

The leaves are vaginate, presenting a sheath (sl) with a membranous upward projection, designated in this paper as a ligule (l) (Figs. 6–9). In *Hydrothrix gardneri* (Pattern IV) it can be observed in each node a main sessile leaf with a prominent ligule that encircles the node (Fig. 9). All of the other leaves of the same whorl are borne inside this ligule and have a diminute projection at their base

resembling a reduced ligule. The petiolate leaves of the individuals of Patterns I and II (Figs. 1–3, 6, 7) have blades (bl) of various shapes, cordiform being predominant (Table 2). The sessile leaves are linear in individuals of Pattern III (Figs. 4 and 8) and filiform in those of Pattern IV (Figs. 5 and 9).

In all of the species, when the plant starts flowering, it switches from monopodial to sympodial growth. The main shoot axis becomes determinate and forms a reproductive axis (ra) that bears an inflorescence (Figs. 6–9). The reproductive axis is subtended by a leaf and in its axil the next sympodial unit is originated (Figs. 6–9). In species of Patterns I and II, after the formation of the first reproductive axis, each following reproductive axis is subtended by a modified leaf (ml) that is reduced to the sheath and an apical projection is present (Fig. 7). In most of the species, after the formation of the first reproductive unit, the following units will also be reproductive (e.g. *E. diversifolia*). However, in some species, after the formation of some reproductive units, the individual may return to vegetative growth (e.g. *P. cordata*).

Each reproductive axis bears an inflorescence surrounded by a leaf-like bract that encloses the peduncle (p) (Figs. 6–9). Such leaf-like bracts do not present as prominent a ligule as the vegetative leaves. Between the peduncle and the rachis of the inflorescence there is a bracteole (br) (Figs. 6–9). The exceptions are *E. paradoxa*, in which the peduncle and the bracteole are absent and flowers are enclosed by the bract sheath, and *Heteranthera dubia*, in which the reproductive axis and the peduncle are very reduced and a single sessile flower is born enclosed by a bracteole, a bract and a leaf.



Figs. 1–5. Morphological patterns of Pontederiaceae. Fig. 1. Pattern I: *Eichhornia paniculata* Fig. 2. Pattern I: *Pontederia sagittata*; Fig. 3. Pattern II: *Heteranthera reniformis*; Fig. 4. Pattern III: *H. zosterifolia*; Fig. 5. Pattern IV: *Hydrothrix gardneri*. A = Stem, B = Leaf petiole, C = Reproductive axis, D = Bract petiole, E = Peduncle. Scale bars = 3 cm.

3.2. Anatomy

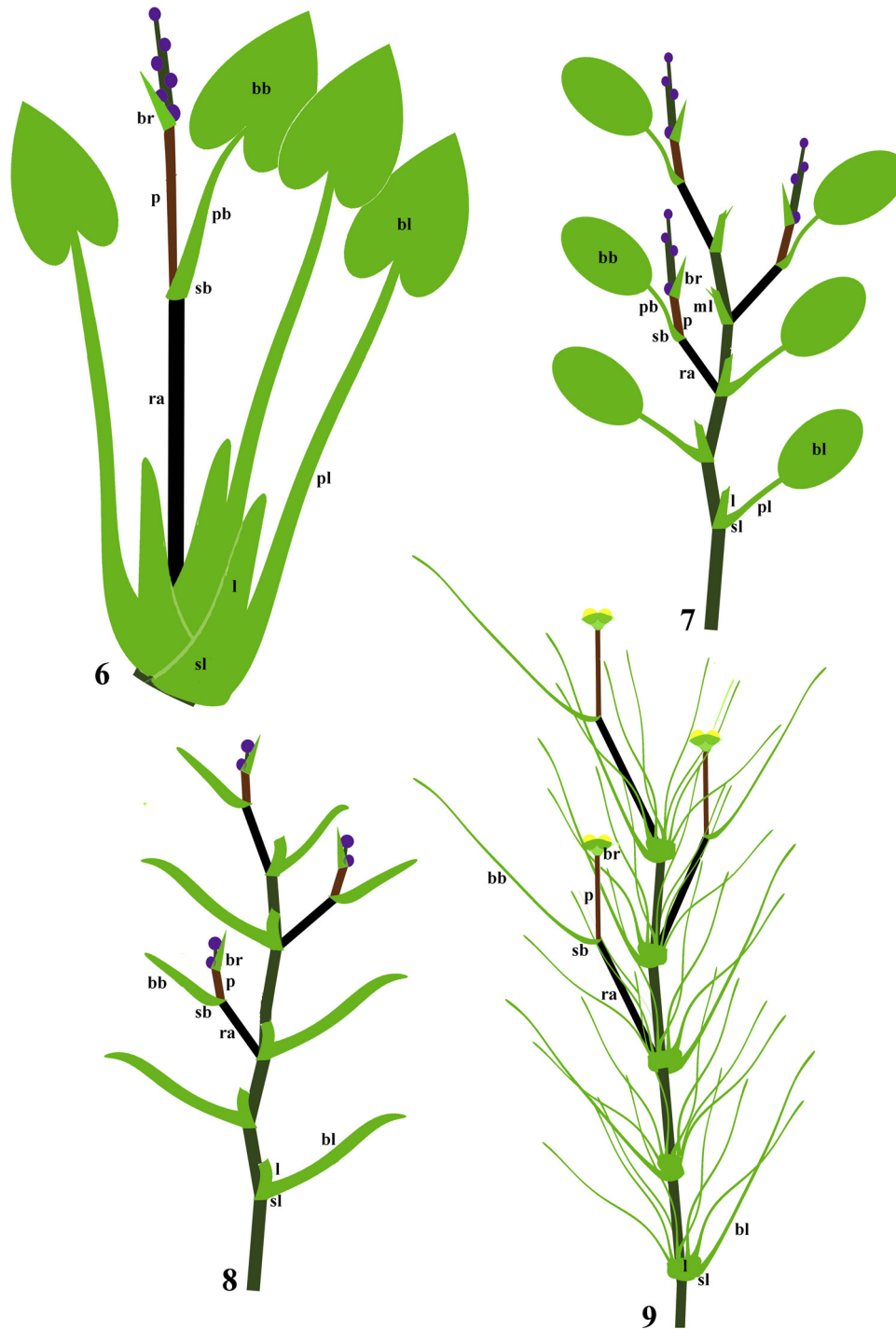
The stem with short internodes, of the species included in Pattern I, has the typical anatomical structure of a rhizome (Figs. 10, 11). It has a single-layered epidermis with thin-walled cells (Fig. 10). The cortex consists of aerenchyma with idioblasts containing phenolic compounds, leaf traces and adventitious roots (Fig. 10). The endodermis exhibits distinct Casparian strips (Fig. 12—arrow). The vascular cylinder is delimited by the pericycle and is composed of vascular plexa and a parenchymatous pith (Fig. 11).

Stems with long internodes, present in the species of Patterns II, III and IV, have a single-layered epidermis with thin-walled cells (Figs. 24, 37, 45), and a cortex of aerenchyma having large air spaces, idioblasts with phenolic compounds and leaf traces (Figs. 23, 35, 43—arrowheads). These air spaces (Figs. 23, 24, 35, 43) are larger than those present in the rhizome (Fig. 10), mainly in the submersed species (Patterns III and IV) (Figs. 35, 43). As in the rhizome, the endodermis exhibits distinct Casparian strips (Figs. 26, 38, 46—arrows). The stele, delimited by the pericycle, is of the atactostele type (Figs. 23, 25, 35, 36, 43, 44).

The leaf petiole (Figs. 13, 27), reproductive axis (Figs. 17, 29, 39, 47), inflorescence bract petiole (Figs. 19, 31) and peduncle (Figs. 21, 33, 41, 49) have the anatomical structure of a monostele. In these organs, the epidermis is single-layered with thin-walled cells and stomata; idioblasts containing phenolic compounds or raphide-shaped crystals may be present (Figs. 14, 15, 18, 20, 22, 28, 30, 32, 34, 40, 42, 48, 50—arrowheads). The cortex is not defined and the vascular bundles surrounded by a double sheath form rings in the aerenchyma (Figs. 13, 17, 19, 21, 27, 29, 31, 33, 39, 41, 47, 49). The air spaces are partitioned by transverse diaphragms (Figs. 14, 18, 20, 28, 32, 40—arrows), which are composed of polygonal cells which form small intercellular spaces at the angles (Fig. 16).

The leaf petiole, present in the species of Patterns I and II, is fistulose (with a central hollow) and the vascular bundles form two concentric rings (Figs. 13, 27). The inflorescence bract petiole has the same anatomical structure as the leaf petiole, but with three rings of vascular bundles (Figs. 19, 31). In both, the fistula is partitioned by transverse diaphragms (Fig. 13—arrow).

The reproductive axis of *Eichhornia paniculata* (Pattern I) is fistulose and partitioned by transverse diaphragms, and has three rings



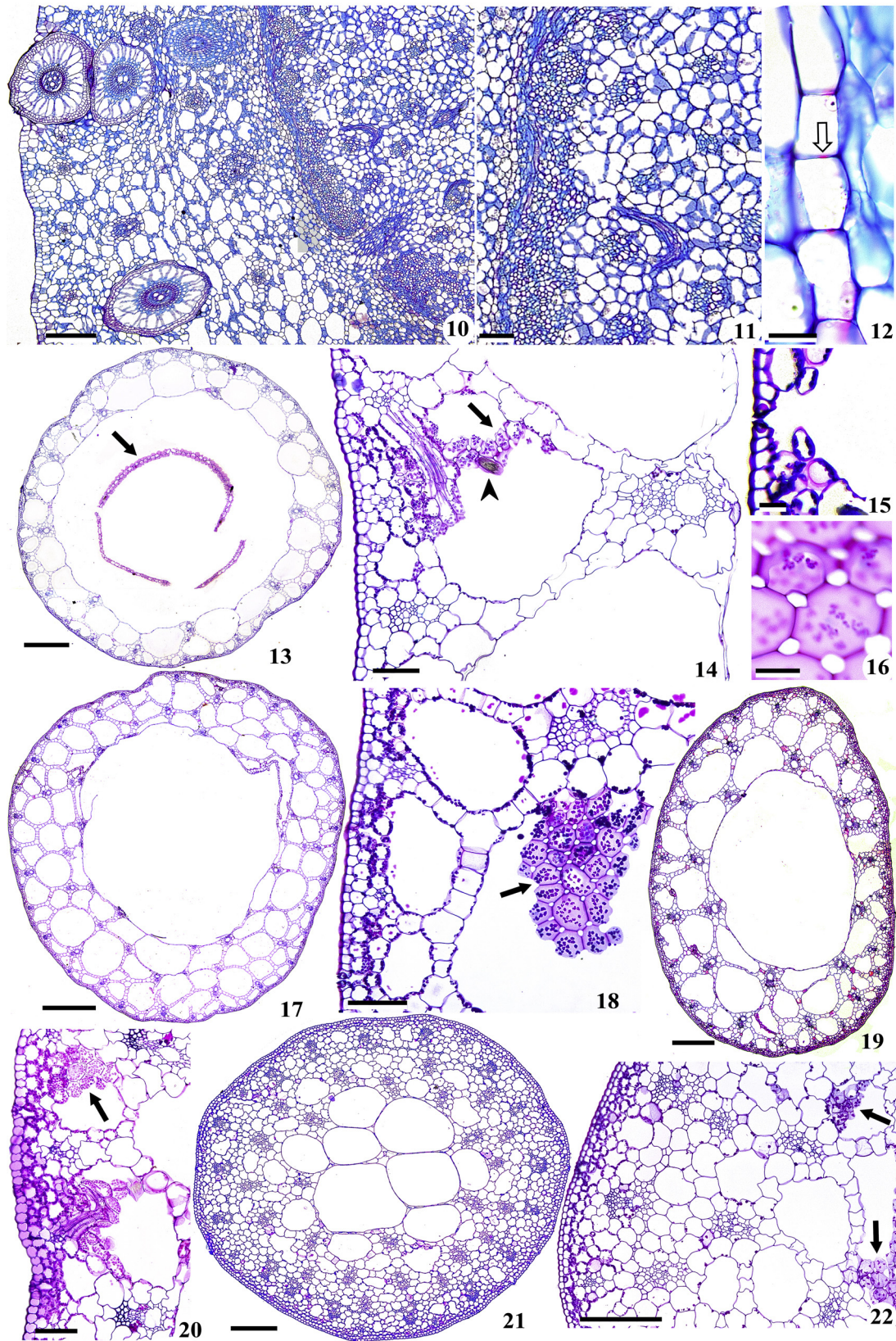
Figs. 6–9. Schematic drawings of the morphological patterns of Pontederiaceae, showing the vegetative and reproductive structures and the sympodial growth. Fig. 6. Pattern I; Fig. 7. Pattern II; Fig. 8. Pattern III; Fig. 9. Pattern IV. sl = Sheath of the leaf, l = Ligule, pl = Petiole of the leaf, bl = Blade of the leaf, ra = Reproductive axis, sb = Sheath of the bract, pb = Petiole of the bract, bb = Blade of the bract, p = Peduncle, br = Bracteole.

of vascular bundles (Fig. 17). In the other species this axis is not fistulose, showing aerenchyma and vascular bundles in the central region (Figs. 29, 39, 47, 48). In *H. reniformis* (Pattern II), the vascular bundles form three concentric rings (Fig. 29), while in *H. zosterifolia* (Pattern III) and *Hydrothrix gardneri* (Pattern IV), there are two rings (Figs. 39, 47). In the latter species the large air spaces also form a ring intercalating between the rings of vascular bundles (Figs. 47, 48).

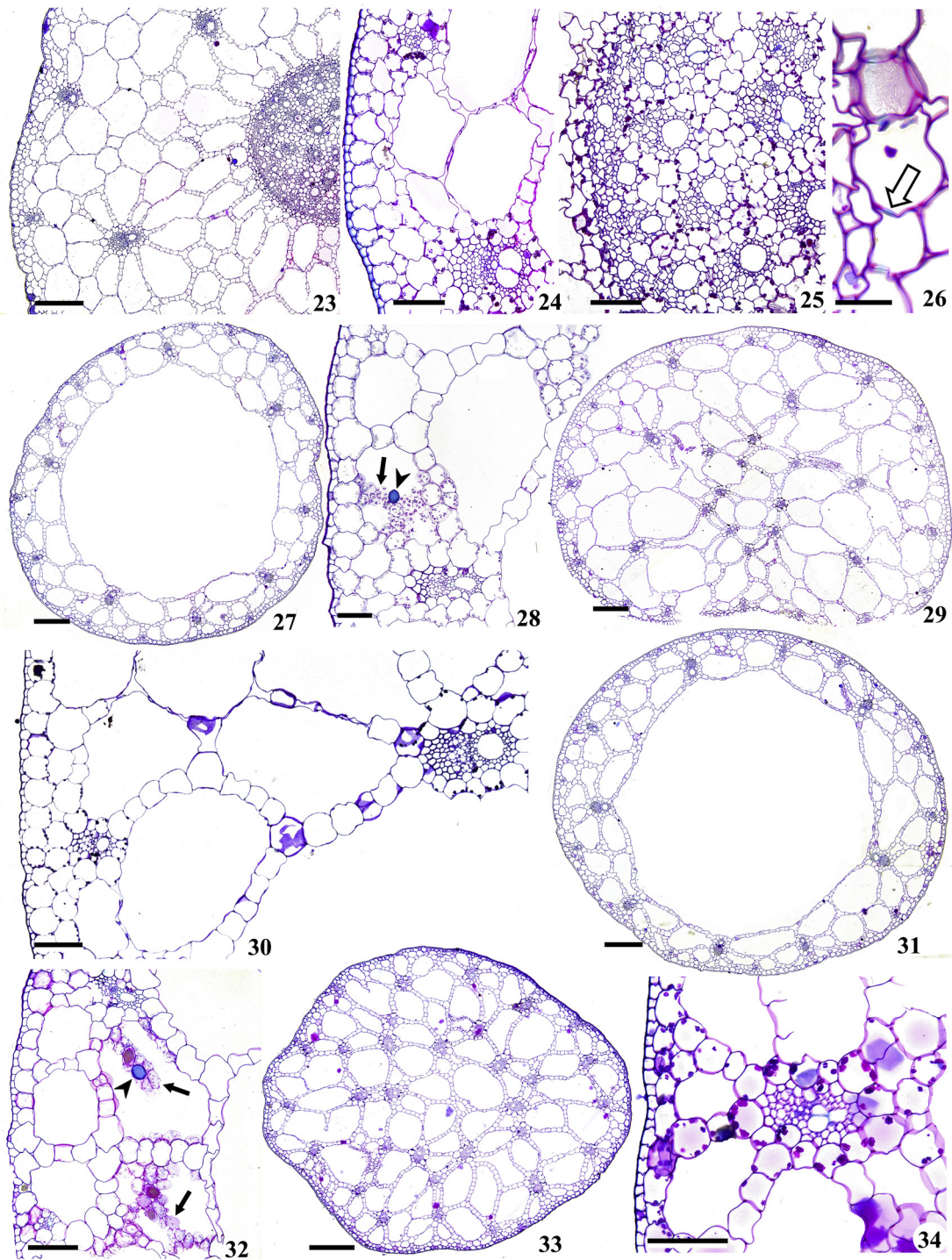
The peduncle shows the same anatomical structure as the reproductive axis (Figs. 33, 41, 49, 50), except for *Eichhornia paniculata*

(Pattern I), in which the reproductive axis is fistulose (Fig. 17) while the peduncle has large air spaces in the central region, delimited by parenchymatous cells (Fig. 21).

In *Hydrothrix gardneri* (Pattern IV) it was observed both in the reproductive axis (Figs. 47, 48) and in the peduncle (Figs. 49, 50) that the vascular bundles are reduced in size, having a smaller number of conducting xylem elements compared to the other species studied.



Figs. 10–22. Anatomical features of *Eichhornia paniculata* (Pattern I), shown in cross sections. Figs. 10–12: Rhizome, general view (10), detail of vascular cylinder (11), detail of endodermis with Casparian strips (12); Figs. 13–16: Leaf petiole, general view (13) and detail of its structure, (14), detail of stoma (15), detail of cells of aerenchyma diaphragm (16); Figs. 17–18: Reproductive axis, general view (17) and detail of its structure (18); Figs. 19–20: Bract petiole, general view (19) and detail of its structure (20); Figs. 21–22: Peduncle, general view (21) and detail of its structure (22). Black arrows = Aerenchyma diaphragm; White arrow = Casparian strips; Arrowhead = Raphide idioblast; Scale bars = 400 μm (Figs. 10, 19, 21, 22), 100 μm (Figs. 11, 14, 18, 20), 20 μm (Figs. 12, 15, 16), 800 μm (Figs. 13, 17).

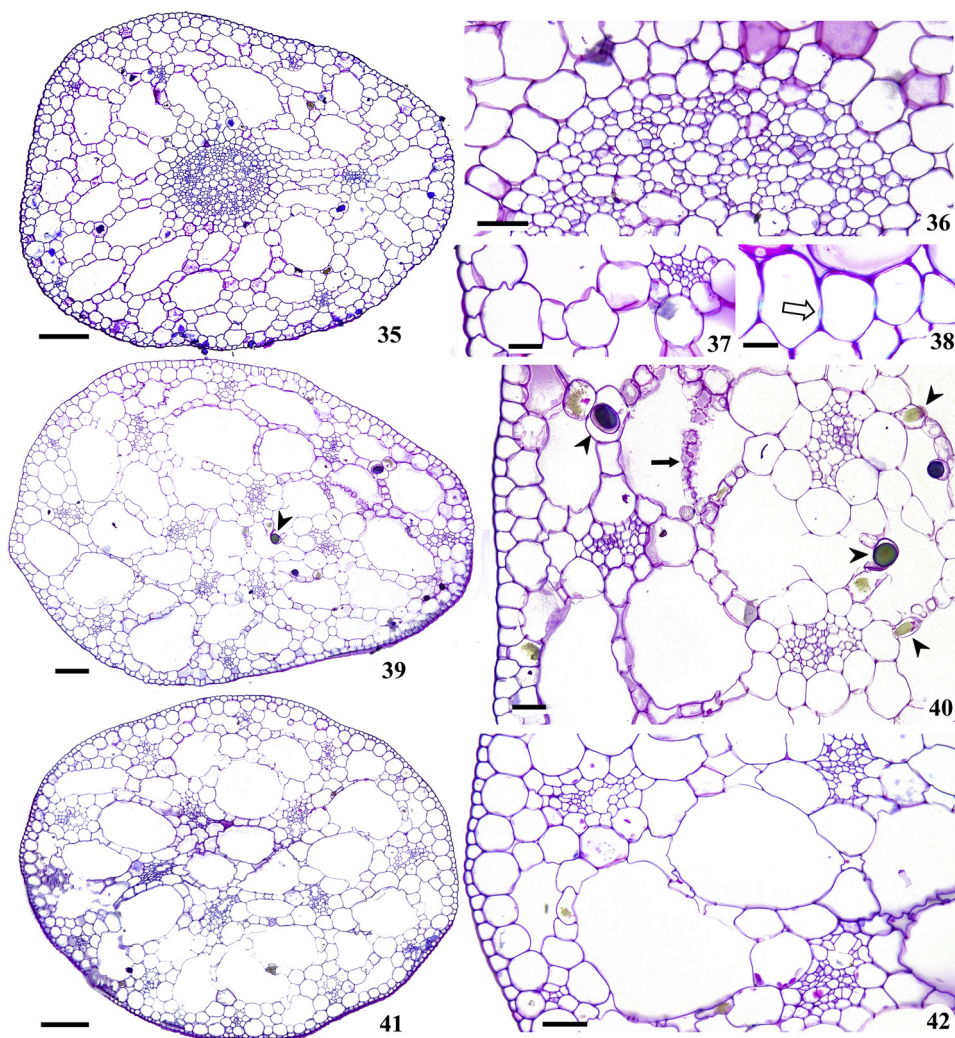


Figs. 23–34. Anatomical features of *Heteranthera reniformis* (Pattern II), shown in cross sections. Figs. 23–26: Stem, general view (23), detail of epidermis and cortex (24), detail of vascular cylinder (25), detail of the endodermis with Casparian strips (26); Figs. 27–28: Leaf petiole, general view (27) and detail of its structure (28); Figs. 29–30: Reproductive axis, general view (29) and detail of its structure (30); Figs. 31–32: Bract petiole, general view (31) and detail of its structure (32); Figs. 33–34: Peduncle, general view (33) and detail of its structure (34). Black arrows = Aerenchyma diaphragm; White arrow = Casparian strip; Arrowheads = Idioblasts with phenolic compounds; Scale bars = 400 μm (Figs. 23, 27, 29, 31, 33), 100 μm (Figs. 24, 25, 28, 30, 32, 34), 20 μm (Fig. 26).

4. Discussion

All of the Pontederiaceae species present a sympodial growth as described to the genus *Monochoria* by Cook (1989). The morphological characters useful for establishing patterns in the family include internode length, phyllotaxy, and presence/absence of leaf petiole. The verticillate phyllotaxy of *Hydrothrix gardneri* comprises

a unique morphological pattern. Rutishauser (1999) analysed the ontogeny of the leaves of this species and showed that the main leaf initiates before the remaining leaves of the same whorl. According to this author, the stem produces an annular bulge around the node of each first-formed leaf and all additional leaves of a whorl arise on this annular bulge.



Figs. 35–42. Anatomical features of *Heteranthera zosterifolia* (Pattern III), shown in cross sections. Figs. 35–38: Stem, general view (35), detail of vascular cylinder (36), detail of epidermis and cortex (37), detail of the endodermis with Casparian strips (38); Figs. 39–40: Reproductive axis, general view (39) and detail of its structure (40); Figs. 41–42: Peduncle, general view (41) and detail of its structure (42). Black arrows = Aerenchyma diaphragm; White arrow = Casparian strip; Arrowheads = Idioblasts with phenolic compounds or raphides; Scale bars = 200 μm (Fig. 35), 40 μm (Figs. 36, 37, 40, 42), 20 μm (Fig. 38), 100 μm (Figs. 39, 41).

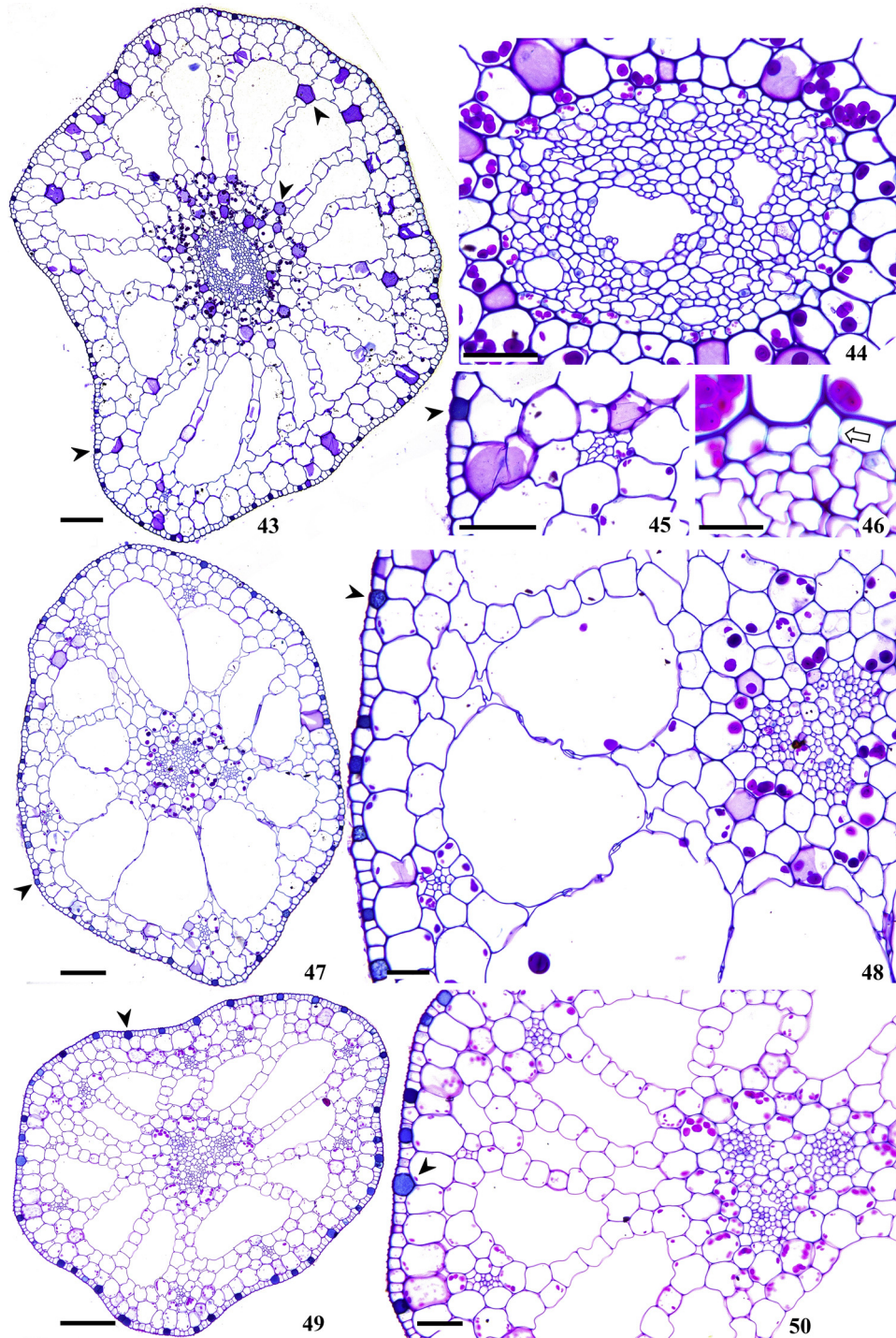
Although *Hydrothrix gardneri* shares some of the characters of other taxa grouped under Pattern III, it is the only obligate submersed species, while the species of Pattern III are submersed but may also become emergent along the margins of ponds and rivers. In addition, *H. gardneri* differs from the other species of the family in having filiform leaves and in details of its floral morphology (Hooker, 1887; Rutishauser, 1999; Sousa and Giulietti, 2014).

Understanding the morphological and anatomical patterns of vegetative and reproductive axes will aid future taxonomic treatments of the family. In this sense, each structure here analyzed (stem, leaf petiole, reproductive axis, inflorescence bract petiole and peduncle) may be distinguished anatomically by its vascular organization. The reproductive axes differ from the petioles by the number of rings of vascular bundles and by the presence or absence of a fistula. These characteristics also distinguish the inflorescence bract petiole from the inflorescence peduncle. The stem is distinguished by having an atactostele, whereas the remaining structures, including the reproductive axis, have monosteles.

Internode length is shown to be an important character for defining morphological patterns and may also suggest water level fluctuation has significantly influenced the evolution of Pontederiaceae. In Pattern I with rhizomatous stems, the short internodes were seen to be a constant character state. In Patterns II and III

however, internode length seems to be related to water level variation. In populations of *Heteranthera rotundifolia* (Pattern II), *H. seubertiana* and *H. zosterifolia* (Pattern III), for example, a series of individuals can be observed growing in a gradual transition of water level from the margins to the centre of a water body. At the margins, where the water level is shallowest, the individuals are emergent and have stems with shorter internodes, although without the typical rhizome structure found in representatives of Pattern I. The individuals growing in the centre of the water body have stems with longer internodes, and those of Pattern III become submersed. In this way, the variation in water level functions as a modulating factor in internode length and often in the life form of these species.

Emergent species predominate in Pattern I, surviving dry periods because of their rhizome. Even *E. crassipes*, the only species of Pontederiaceae that can be free-floating, becomes an emergent when the water level is low or when individuals are located at the margins of a water body. Species of Pattern II are frequently emergent but, depending on the water level, floating-leaved individuals may be found. In Pattern III the species are mostly submersed, but as previously mentioned, may be found as emergent when the water level is low. However, *Hydrothrix gardneri*, the only member of Pattern IV, is always submersed.



Figs. 43–50. Anatomical features of *Hydrothrix gardneri* (Pattern IV), shown in cross sections. Figs. 43–46: Stem, general view (43), detail of vascular cylinder (44), detail of epidermis and cortex (45), detail of the endodermis with Casparian strips (46); Figs. 47–48: Reproductive axis, general view (47) and detail of its structure (48); Figs. 49–50: Peduncle, general view (49) and detail of its structure (50). White arrow = Casparian strip; Arrowheads = Idioblasts with phenolic compounds; Scale bars = 40 μm (Figs. 43, 48, 50), 20 μm (Figs. 44–46), 100 μm (Figs. 47, 49).

The variation in water level, besides influencing the morphology and life form in Pontederiaceae, also seems to have influenced the anatomical alterations of the studied organs. For example, changes in life form from Pattern I to Pattern IV are accompanied by an increase in aerenchyma, principally in the number and dimensions of the air spaces, and by a diminution in the number of xylem conducting elements. The relationship between soil flooding or submersion and the formation of aerenchyma, described by Jackson

and Armstrong (1999), had already demonstrated a tendency for increase in number and size of the air spaces in submersed groups of other angiosperms. The reduction in number of xylem elements has also been described for other families of aquatic plants such as Ceratophyllaceae, Haloragaceae, Hydrocharitaceae, Lentibulariaceae and Potamogetonaceae (Arber, 1920; Metcalfe and Chalk, 1950; Ancibor, 1979; Kaplan, 2001; Lusa et al., 2011).

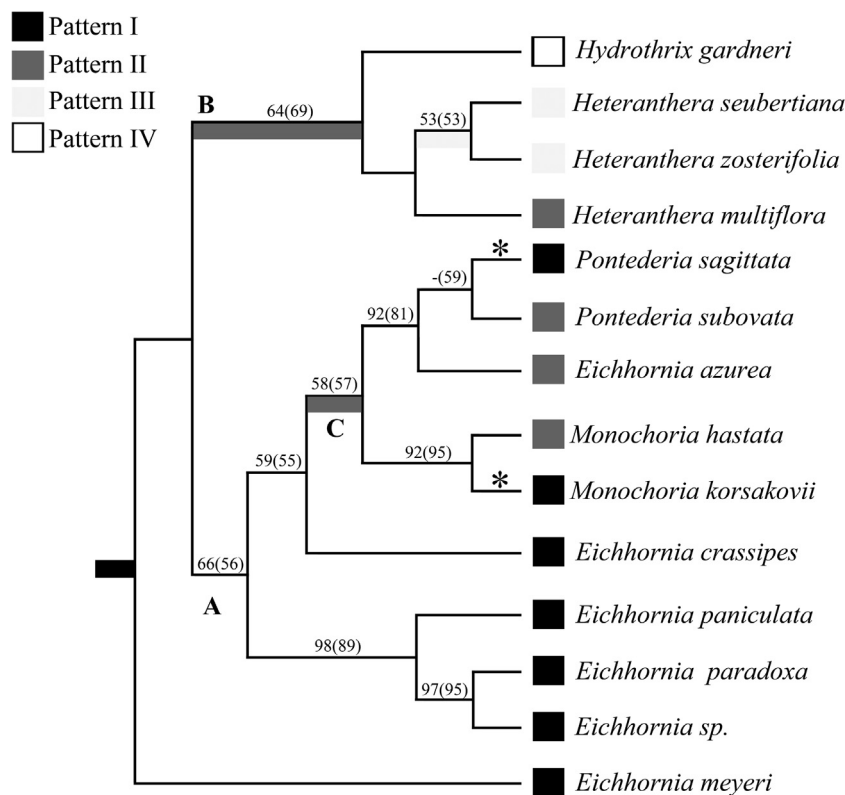


Fig. 51. Distribution of morphological patterns of Pontederiaceae on the maximum parsimony tree obtained from nuclear (Expressed Sequence Tag-EST) and plastid (*ndhF* e *rbcl*) data of [Ness et al. \(2011\)](#). The support values were obtained by bootstrap analysis. Asterisks represent reversions.

It is in the submersed life form that the interaction between the individual plant and aquatic environment is most acute ([Sculthorpe, 1967](#)). However, the adaptations to this direct relationship with the aquatic environment may vary in different plant groups. [Puijalon et al. \(2005\)](#) studied *Berula erecta* (Apiaceae) and *Mentha aquatica* (Lamiaceae) and found contrary responses to water stress. While *M. aquatica* increases in height, in *B. erecta* height and size of the vegetative parts diminish. The same happens in Pontederiaceae, such that species of Patterns III and IV have sessile leaves with linear or filiform blades, while in species of the other patterns the leaves have well-developed petioles which may exhibit adaptations to the floating habit (e.g. *Eichhornia crassipes*) and blades of large size. Reduced size and division of the leaf blade have also been considered to be adaptations to the submersed habit in other groups, e.g. Cabombaceae and Ceratophyllaceae ([Arber, 1920](#); [Sculthorpe, 1967](#)).

It should be pointed out that the morphological patterns established in this study do not define taxonomic groups at generic level, since species of different genera can be found within each pattern. The exception is Pattern IV that includes the monospecific genus *Hydrothrix* and Pattern III that includes only species of *Heteranthera*. Pattern II includes the largest number of genera, with species of *Eichhornia*, *Heteranthera*, *Monochoria* and *Pontederia*, whereas Pattern I includes species of *Eichhornia*, *Monochoria* and *Pontederia*. The fact that species which are often distantly related are included in Patterns I and II shows that these patterns probably had various independent origins and/or reversals during the evolution of the family.

At infrageneric level, the morphological patterns may be useful to support groups. In *Pontederia*, for example, the Patterns I and II differentiate respectively the subgenera *Pontederia* and *Reussia* proposed by [Lowden \(1973\)](#). Within *Eichhornia*, which has been recovered as a non-monophyletic clade formed by four lineages, the

“Azurea group” (comprising *E. azurea*, *E. diversifolia*, *E. heterosperma* and probably *E. natans*) often emerges as monophyletic ([Graham and Barrett, 1995](#); [Graham et al., 2002](#)) and is formed only by species following Pattern II. New phylogenetic analysis (Sousa, D.J.L. et al., unpublished data) are currently in progress to better understand the infrafamilial relationships and to resolve the classification of the family. Morphological patterns identified here appear to be significant and will presumably be important for adding more support to the new taxonomic groups, but alone are not sufficient to justify making taxonomic changes before the final results of on going phylogenetic analyses.

[Barrett and Graham \(1997\)](#), in their phylogenetic and comparative analysis of Pontederiaceae and other families of the Commelinales, found that several characters are homoplasious, having resulted from multiple independent origin or reversal events. The aquatic habit, for example, may have arisen various times within the order or may be homologous for Pontederiaceae and Phylidraceae. However, more recent phylogenetic analyses ([Hopper et al., 1999](#); [Graham et al., 2002](#); [Chase et al., 2006](#); [Saarela et al., 2008](#)) recovered Pontederiaceae as the sister group of Haemodoraceae, reinforcing the idea that the aquatic habit may be a homoplasious state in Commelinales. As regards life forms, the erect emergent state was inferred to be plesiomorphic in the family, and gave rise to the life forms procumbent emergent, floating-leaved and submersed plants, which have arisen independently in some lineages ([Barrett and Graham, 1997](#)).

If we consider the most recent phylogenetic hypothesis for Pontederiaceae ([Fig. 51](#)), based on molecular data ([Ness et al., 2011](#)), Pattern I can be considered as the plesiomorphic state for the family since *Eichhornia meyeri*, the sister group of all other Pontederiaceae species, is included in this pattern ([Fig. 51](#)). This position of Pattern I as the oldest in Pontederiaceae is in agreement with [Barrett and Graham \(1997\)](#), since Pattern I is characterized by erect emergent

plants. Pattern II seems to have arisen independently in lineages B and C, and to have undergone reversals to Pattern I in lineage C (Fig. 51—asterisks). Patterns III and IV can be considered the most derived, having arisen in lineage B (Fig. 51). The close relationship between Patterns III and IV is evidenced by the life form and morphology, both patterns including submersed species with long internodes and sessile leaves. Given that Pattern II is the plesiomorphic state in lineage B, we consider that the submersed life form (Patterns III and IV) must have been derived from the procumbent emergent life form (Pattern II) under the influence of fluctuating water levels.

It is thus evident that environmental factors have acted as modulating influences on the diversification of Pontederiaceae. Morphological characteristics such as the length of the internodes, the presence or absence of a petiole, the dimensions of the leaf blade, proportion of aerenchyma, and reduction of the conducting elements seem to be directly related to fluctuations in water levels in the freshwater environments in which Pontederiaceae evolved.

In the semi-arid region of Brazil, the most important freshwater environments are temporary lakes and ponds (Maltchik et al., 1999; França and Melo, 2006) where Pontederiaceae exhibit a great diversity of species (Sousa and Giulietti, 2014). More than 50% of the species of the family occur in the Brazilian semi-arid region (Amaral et al., 2014), with representatives of all of the four morphological patterns, including *Hydrothrix gardneri*. In the water bodies of this region, individuals of *Eichhornia paniculata*, *E. paradoxa* or *Pontederia cordata* (Pattern I) can be found at the margins, often sympatrically with other species such as *E. diversifolia*, *E. heterosperma*, *Heteranthera oblongifolia*, *H. rotundifolia*, *H. peduncularis* or *P. subovata* (Pattern II) that grow both at the margins and in the centre of the water body.

The water bodies of the Brazilian semi-arid region are predominantly intermittent and show considerable fluctuation in water level. They dry up for a part of the year but fill up during short periods of high rainfall in a flood pulse dynamic (Walker et al., 1995; Capon, 2003; Larned et al., 2010; Alho and Sabino, 2012). The ecological zones evident in these habitats are well-marked by their floristic composition and life form assemblages (Sculthorpe, 1967; Larned et al., 2010). However, these zones vary in area according to fluctuations in water level (Junk et al., 1989; Leyer, 2005; Wantzen et al., 2008; Tabosa et al., 2012; O'Farrell et al., 2011), which may have driven the changes in life form and morphology during the evolution of Pontederiaceae.

This suggests a scenario for how the family diversified: as the water level diminished in more arid periods, individuals of Pattern I, with rhizomes, become dormant, while those of Pattern II possibly continue to develop, accompanying the water surface. An increase in rainfall in wetter periods and the consequent increase in water level could have submersed those individuals of Pattern II located more towards the central part of the water body. Such a repeated submersion over a long time period would probably have been a selective factor driving the establishment of the submersed life form in the family (Patterns III and IV) and related adaptations. This evolutionary scenario is corroborated by the most recent phylogeny of Pontederiaceae (Ness et al., 2011) and by our results and interpretations of the evolution of morphological patterns and life forms in the family.

5. Conclusions

Morphological and anatomical patterns are described for Pontederiaceae and a standardized terminology is proposed for the observed structures, which are important prerequisites for taxonomic and phylogenetic studies of the family currently in progress. The morphological patterns may represent synapomorphies of

infrageneric groups and are related to the life forms of the species and to the evolutionary history of the family. Pattern I is plesiomorphic while Patterns III and IV are more derived and may have evolved from a lineage which would fall within Pattern II. Variation in water level has evidently been important as a modifying factor in the morphological evolution of the family. We conclude therefore that the intermittent water bodies characteristic of the semi-arid region of Brazil would have provided the ideal environment for the evolutionary diversification of Pontederiaceae. Future phylogenetic analyses will make it possible to re-define taxa and to date the origin of infrafamilial groups, linking their appearance with events in geological time and in the family's diversification.

Acknowledgments

We thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for financial support and the anonymous reviewers for their comments that improved the paper. We also thank Charlie Zimmerman for providing language help.

References

- Alho, C., Sabino, J., 2012. Seasonal Pantanal flood pulse: implications for biodiversity. *Oecol. Aust.* 16, 958–978.
- Amaral, M.C.E., Pellegrini, M.O.O., Sousa, D.J.L., 2014. Pontederiaceae. In: Lista de Espécies da Flora do Brasil, 2014. Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB197> (accessed: November 2014).
- Ancibor, E., 1979. Systematic anatomy of vegetative organs of the Hydrocharitaceae. *Bot. J. Linn. Soc.* 78, 237–266.
- Arber, A., 1920. *Water Plants: A Study of Aquatic Angiosperms*. Cambridge University Press, Cambridge.
- Barrett, S.C.H., Anderson, J.M., 1985. Variation in expression of trimorphic incompatibility in *Pontederia cordata* L. (Pontederiaceae). *Theor. Appl. Genet.* 70, 355–362.
- Barrett, S.C.H., Graham, S.W., 1997. Adaptive radiation in the aquatic plant family Pontederiaceae: insights from phylogenetic analysis. In: Givnish, T.J., Sytsma, K. (Eds.), *Molecular Evolution and Adaptive Radiation*. Cambridge University Press, Cambridge, pp. 225–258.
- Barrett, S.C.H., 1988. Evolution of breeding systems in *Eichhornia*, a review. *Ann. Missouri Bot. Gard.* 75, 741–760.
- Barrett, S.C.H., 2004. Pontederiaceae (water hyacinth family). In: Smith, N., Mori, S.A., Henderson, A., Stevenson, D.W., Heald, S.V. (Eds.), *Flowering Plants of the Neotropics*. Princeton University Press, Princeton, pp. 474–476.
- Capon, S.J., 2003. Plant community responses to wetting and drying in a large arid floodplain. *River Res. Appl.* 19, 509–520.
- Castellanos, A., 1958. Las Pontederiaceae del Brasil. *Arq. Jard. Bot. Rio de Janeiro* 16, 147–236.
- Chase, M.W., Fay, M.F., Devey, D.S., Maurin, O., Ronsted, N., Davies, T.J., Pillon, Y., Petersen, G., Seberg, O., Tamura, M.N., Asmussen, C.B., Hilu, K., Borsch, T., Davis, J.I., Stevenson, D.W., Pires, J.C., Givnish, T.J., Sytsma, K.J., McPherson, M.A., Graham, S.W., Rai, H.S., 2006. Multigene analyses of monocot relationships: a summary. *Aliso* 22, 63–75.
- Cheadle, V.I., 1970. Vessels in Pontederiaceae, Ruscaceae, Smilacaceae, and Trilliaceae. In: Robson, N.K.B., Cutler, D.F., Gregory, M. (Eds.), *New Research in Plant Anatomy*. Bot. J. Linn. Soc. 63, 45–50.
- Coker, W.C., 1907. The development of the seed in the Pontederiaceae. *Bot. Gaz.* 44, 293–301.
- Cook, C.D.K., 1989. Taxonomic revision of *Monochoria* (Pontederiaceae). In: Tan, K., Mill, R.R., Elias, S. (Eds.), *The Davis & Hedge Festschrift: Plant Taxonomy, Phytoecology, and Related Subjects*. Edinburgh University Press, Edinburgh, pp. 149–184.
- Cook, C.D.K., 1996. *Aquatic Plant Book*. SPB Academic Publishing, Amsterdam.
- Cook, C.D.K., 1998. Pontederiaceae. In: Kubitzki, K. (Ed.), *The Families and Genera of Vascular Plants*, Vol. 4. Springer, Berlin, pp. 395–403.
- Crow, G.E., 2003. Pontederiaceae. In: Hammel, B.E., Grayum, M.H., Herrera, C., Zamora, N. (Eds.), *Manual de Plantas de Costa Rica*, Vol III: Monocotiledóneas, Missouri Bot. Gard. Press, St. Louis and Inst. Nac. de Biodiversidad; Santo Domingo de Heredia, Costa Rica, 822–828.
- Cunha, N.L., Fischer, E., 2009. Breeding system of tristylous *Eichhornia azurea* (Pontederiaceae) in the southern Pantanal, Brazil. *Pl. Syst. Evol.* 280, 53–58.
- Cunha, N.L., Fischer, E., Lorenz-Lemke, A.P., Barrett, S.C.H., 2014. Floral variation and environmental heterogeneity in a tristylous clonal aquatic of the Pantanal wetlands of Brazil. *Ann. Bot.* 114, 1637–1649.
- Eckenwalder, J.E., Barrett, S.C.H., 1986. Phylogenetic systematics of Pontederiaceae. *Syst. Bot.* 11, 373–391.

- Endress, P.K., 1995. Major evolutionary traits of monocot flowers. In: Rudall, P.J., Cribb, P.J., Cutler, D.F., Humphries, C.J. (Eds.), *Monocotyledons: Systematics and Evolution*, vol. 1. Royal Botanic Gardens, Kew, pp. 43–79.
- Feder, N., O'Brien, T.P., 1968. Plant microtechnique: some principles and new methods. *Am. J. Bot.* 55, 123–142.
- França, F., Melo, E., 2006. Aquatic vascular plants in the semi-arid of Bahia. In: Queiroz, L.P., Rapini, A., Giulietti, A.M. (Eds.), *Towards Greater Knowledge of the Brazilian Semi-Arid Biodiversity*. Ministério de Ciências e Tecnologia, Brasília, pp. 69–72.
- Gerrits, P.O., Smid, L., 1983. A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *J. Microsc.* 132, 81–85.
- Graham, S.W., Olmstead, R.G., Barrett, S.C.H., 2002. Rooting phylogenetic trees with distant outgroups: a case study from the Commelinoid Monocots. *Mol. Biol. Evol.* 19, 1769–1781.
- Hooker, J.D., 1887. On *Hydrothrix*: a new genus of Pontederiaceae. *Ann. Bot.* 1, 89–94.
- Hopper, S.D., Fay, M.F., Rossetto, M., Chase, M.W., 1999. A molecular phylogenetic analysis of the bloodroot and kangaroo paw family, Haemodoraceae: taxonomic, biogeographic and conservation implications. *Bot. J. Linn. Soc.* 131, 285–299.
- Horn, C.N., 1985. A systematic revision of the genus *Heteranthera* (sensu lato; Pontederiaceae) University. In: Ph.D. Dissertation. Univ. of Alabama, Alabama.
- Jackson, M.B., Armstrong, W., 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1, 274–287.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw Hill Book, New York.
- Junk, W.J., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river-floodplain systems. In: Dodge, D.P. (Ed.), *Proceedings of the International Large River Symposium*, Can. Spec. Publ. Fish. Aquat. Sci. 106, 110–127.
- Kaplan, Z., 2001. *Potamogeton* × *fluitans* (*P. natans* × *P. lucens*) in the Czech Republic. I. Morphology and anatomy. *Preslia* 73, 333–340.
- Kohn, J.R., Graham, S.W., Morton, B., Doyle, J.J., Barrett, S.C.H., 1996. Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. *Evolution* 50, 1454–1469.
- Kunth, K., 1815. Pontederiaceae [Pontedereae]. In: Humboldt, F.W.H.A., Bonpland, A.J.A., Kunth, K. (Eds.), *Nova Genera et Species Plantarum*, Ed. 4, Paris.
- La Bathie, H.P., 1936. Notes sur quelques monocotylédones de Madagascar: *Sansevieria*, *Scholleropsis* (Gen. Nov.), *Pontederiacearum* et *Gymnosiphon*. In: Lecomte, H. (Ed.), *Notula Systematicae*, T. 5, Herbarium du Muséum de Paris, Paris, 154–160.
- Larned, S.T., Datry, T., Arscott, D.B., Tockner, K., 2010. Emerging concepts in temporary-river ecology. *Freshw. Biol.* 55, 717–738.
- Leyer, I., 2005. Predicting plant species' responses to river regulation: the role of water level fluctuations. *J. Appl. Ecol.* 42, 239–250.
- Lowden, R.M., 1973. Revision of the genus *Pontederia* L. *Rhodora* 75, 426–483.
- Lusa, M.G., Boeger, M.R.T., Moço, M.C.C., Bona, C., 2011. Morpho-anatomical adaptations of *Potamogeton polygonus* (Potamogetonaceae) to lotic and lentic environments. *Rodriguésia* 62, 927–936.
- Maltchik, L., Costa, M.A.J., Duarte, M.C.D., 1999. Inventory of Brazilian semiarid temporary shallow lakes. *An. Acad. Bras. Cien.* 71, 801–808.
- Metcalfe, C.R., Chalk, L., 1950. *Anatomy of the Dicotyledons*, 2. Clarendon Press, Oxford.
- Ness, R.W., Graham, S.W., Barrett, S.C.H., 2011. Reconciling gene and genome duplication events: using multiple nuclear gene families to infer the phylogeny of the aquatic plant family Pontederiaceae. *Mol. Biol. Evol.* 28, 3009–3018.
- O'Brien, T.P., Feder, N., McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59, 368–373.
- O'Farrell, I., Izaguirre, I., Chaparro, G., Unrein, F., Sinistro, R., Pizarro, H., Rodríguez, P., Pinto, P.T., Lombardo, R., Tell, G., 2011. Water level as the main driver of the alternation between a free-floating plant and a phytoplankton dominated state: a long term study in a floodplain lake. *Aquat. Sci.* 73, 275–287.
- Olive, E.W., 1894. Contributions to the histology of the Pontederiaceae. *Bot. Gaz.* 19, 178–184.
- Puijalón, S., Sagnes, P., Bornette, G., 2005. Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *J. Exp. Bot.* 56, 777–786.
- Richter, H.G., 1985. Wood and bark anatomy of Lauraceae II. *Licaria* Aublet. *IAWA Bull.* 6, 187–199.
- Roeser, K.R., 1972. Die Nadel der Schwarzkiefer—Massenprodukt und Kunstwerk der Natur. *Mikrokosmos* 61, 33–36.
- Rutishauser, R., 1999. Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness of organ identities. *Intern. J. Plant Sci.* 160, 81–103, Suppl. 6.
- Saarela, J.M., Prentis, P.J., Rai, H.S., Graham, S.W., 2008. Phylogenetic relationships in the monocot order Commelinales, with a focus on Philydraceae. *Botany* 86, 719–731.
- Schultz, A.G., 1942. Las Pontederiaceas de la Argentina. *Darwiniana* 6, 4–39.
- Schwartz, O., 1926. Anatomische, morphologische und systematische untersuchungen tiber die Pontederiaceen. *Beih. Bot. Centralbl.* 42, 263–320 (I. Abt.).
- Sculthorpe, C.D., 1967. *The Biology of Aquatic Vascular Plants*. Edward Arnold Ltd., London.
- Seubert, M., 1847. Pontederiaceae. In: Martius, C.F.P., Eichler, A.W., Urban, I. (Eds.), *Flora Brasiliensis*, vol. 3, 85–96.
- Simpson, M.G., Burton, D.H., 2006. Systematic floral anatomy of Pontederiaceae. In: Columbus, J.T., Friar, E.A., Porter, J.M., Prince, L.M., Simpson, M.G. (Eds.), *Monocots: Comparative Biology and Evolution (Excluding Poales)*. Rancho Santa Ana Botanic Garden, Claremont, pp. 499–519.
- Solms-Laubach, H.M., 1883. Pontederiaceae. In: De Candolle, A.E. (Ed.), *Monographiae Phanerogamarum*, vol. 4, pp. 501–535.
- Sousa, D.J.L., Giulietti, A.M., 2014. Flora da Bahia: Pontederiaceae. *Sitientibus Sér. Ci. Biol.* 14, 14–30.
- Strange, A., Rudall, P.J., Prychid, C.J., 2004. Comparative floral anatomy of Pontederiaceae. *Bot. J. Linn. Soc.* 144, 395–408.
- Tabosa, A.B., Matias, L.Q., Martins, F.R., 2012. Live fast and die young: the aquatic macrophyte dynamics in a temporary pool in the Brazilian semiarid region. *Aquat. Bot.* 102, 71–78.
- Tillich, H.J., 1994. Untersuchungen zum Bau der Keimpflanzen der Philydraceae und Pontederiaceae (Monocotyledonae). *Sendtnera* 2, 171–186.
- Walker, K.F., Sheldon, F., Puckridge, J.T., 1995. A perspective on dryland river ecosystems. *Regul. Rivers: Res. Manag.* 11, 85–104.
- Wantzen, K.M., Rothhaupt, K.O., Mörtl, M., Cantonati, M., Tóth, L.G., Fischer, P., 2008. Ecological effects of water-level fluctuations in lakes: an urgent issue. *Hydrobiologia* 613, 1–4.