Chapter 12

Selected Structural Features of the Riverine Plants, *Trapa natans* (Lythraceae) and *Justicia americana* (Acanthaceae)

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http://dx.doi.org/ 10.5772/63709

Abstract

Aquatic, stoloniferous plantlets with floating leaves and nodal, adventitious roots of *Trapa natans* and emergent, stoloniferous, rooted plants of *Justicia americana* from the Oswego River, New York, USA, were investigated to determine root and shoot structures. These riverine eudicots have typical root anatomy with aerenchyma, endodermis, and exodermis. Both species contain stem tissues with endodermis which encircles one stele in *T. natans* and polysteles in *J. americana*. The aquatic stolon of *T. natans* has circumferentially spaced primary xylem with primary phloem. Erect stems of *J. americana* normally lack an endodermis in aerial portions above the water line. Extensive collenchyma is a conspicuous feature under the epidermis in stems of both species. Large stolon cortical cavities are characteristic of *T. natans*, but they are not found in the hypocotyls; in *J. americana* aerenchyma lacunae occur throughout ground tissue. The peduncle of *T. natans* has a stele with a ring of vascular tissue of primary xylem with phloem exterior to the xylem, surrounded by endodermis and air cavities, and pith aerenchyma. Leaves of both species do not contain barrier layers. Petioles of *T. natans* usually contain subepidermal collenchyma and aerenchyma with enlarged lacunae in inflated bladders.

Keywords: anatomy, *Justicia americana*, *Trapa natans*

1. Introduction

The Oswego River is a short (<40 km), north flowing river, whose tributaries connect many of the Finger Lakes of central New York state to Lake Ontario. The river is considered part of a canal system that links Lake Ontario to the famous Erie Canal, which stretches from east to west across New York state; consequently, over the last 188 years, it has been the scene of much river traffic. Its flow has been altered with dredging, dams, and locks to control the ship channel, to produce electricity, and to be part of the canal system. In recent decades, one invasive plant, the infamous water chestnut, *Trapa natans* L., has become a familiar sight in the river and a considerable nuisance to users of the river. It has floating (except with fruit-anchored young stages) stoloniferous ramets with rosettes of rhombic-shaped leaves [1]. The long ignored native water willow, *Justicia americana* (L.) Vahl., is an important shore-line stabilizer and is a stolon- and root-anchored, emergent, linear-leafed [1], sometimes growing in close proximity to the water chestnut.

Figures 1–9. *Trapa natans* and *Justicia american* in habitat settings. 1. Species together at dock in Minetto, New York, June. 2. *Trapa*, view in Oswego River, looking north, early July, 1500 m south of Minetto Dam. 3. *Trapa* at river's edge in June. 4. *Justicia* along river's shore, south of Minetto Dam in early July. 5. *Justicia* along river's shore, south of Minetto Dam, in June. 6. *Trapa*, as in **Figure 2**, in early August. 7. *Trapa* setting, as in **Figure 2**, in early October. 8. *Trapa* setting, as in **Figure 2**, in early November. 9. *Justicia* remnants, as in **Figure 4**, in early November.Abbreviations for all microscopic figures: aer, aerenchyma; cav, cavities; cl, collenchyma; cor, cortex; e, endodermis; f, phloem fiber; st, stele; sx, secondary xylem; v, leaf veins; x, primary xylem; *, periderm; t, trichomes.

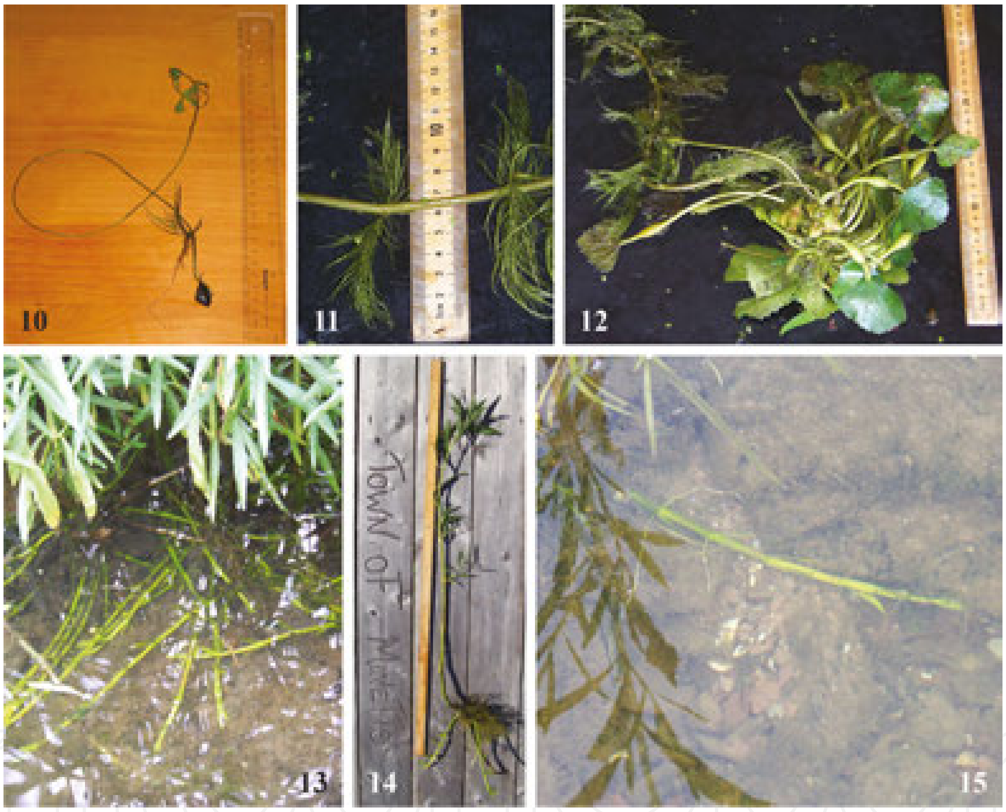
Water chestnut is native to the warm temperate regions of Eurasia and North Africa. It has been of greater interest and study than water willow because of its invasiveness; there is some disagreement as to which decade *Trapa natans* first entered North America and became established. Naylor [2] stated that water chestnut was first recorded in North America near Concord, Massachusetts in 1859, whereas Hummel and Findlay [3] claimed that it was first introduced in 1875, and Pemberton [4] stated that it was in 1884 in Sanders Lake, Schenectady, east central New York, not far from the Oswego River of central New York. Its presence in ponds near Schenectady, New York, was also noted in Britton and Brown [5], and other aspects of its occurrence and control were noted in the 1930s [6, 7]. Arber [8] believed that *Trapa natans* was on its way to extinction, at least in Europe, and in 1974, Ogden [9] mapped its occurrence in New York State only along the Hudson and Mohawk Rivers, east of the Oswego River, but including Schenectady. Its overall growth characteristics have been described by many researchers, especially Groth et al. [10].

Water willow is a native plant [1, 11, 12] that may play roles in shoreline stabilization [13, 14] and in providing breeding habitats [15]. In recent decades, both species have occurred together in the Oswego River, New York, especially in the section south (upriver) of the Minetto Dam that is a small reservoir. In photographs of this section of the Oswego River from the late 1800s, the invasive water chestnut was not observed during summers, and the occurrence of water willow could not be determined [Carl Allen, Minetto, NY, personal communication]. Currently, *Justicia americana* does not appear extensive enough to be important for fish habitat [15, 16]. The senior author's personal observations of the Oswego River before the mid 1980s did not reveal water chestnut or water willow, which might have been disrupted or blocked by boulders installed at various times [Carl Allen, personal communication], including in the 1990s, to reinforce the shore line upriver from the nearby dam after the Minetto Bridge was razed and replaced.

This research was begun as part of the senior author's long-term studies on the development and structure of the root cortex of flowering plants. Prior to this study, roots of these native and invasive riverine plants have been little studied although aspects of the growth and structure of water chestnut had been noted early [8, 17, 18]. In general, relatively few dicotyledons have been examined except, for example, the brief descriptions of Justin and Armstrong [19], Smirnoff and Crawford [20], Perumalla et al. [21], Peterson and Perumalla [22], and Metcalfe and Chalk [23, 24], and the distributions, drawings, and brief descriptions of New York aquatic and wetland plants, including both *Trapa natans* (not then reported in the Oswego River region) and *Justicia americana* (found in the Oswego River), by Ogden [9]; persons (Oswego County Soil and Water Conservation District, personal communication to JLS) involved with river conservation (water levels, shoreline maintenance, etc.) could provide only limited detail about the history of these plants in the Oswego River. Altogether, there is a paucity of reliable information on the shoots and roots of these two species [8, 9, 17, 18, 23–27], especially as structural characteristics may relate to habitat function [13, 27, 28]. It seemed especially troubling that, while Sculthorpe [28] had long ago described some aspects of the adventitious and lateral roots of *T. natans*, recent ecological studies [29, 30] had referred to these as feathery leaves. Sculthorpe [28] had also noted the induced nature of the "floats" or inflated petioles of the rhombic leaves. The presence of characteristic leaf trichomes has also been noted in *Trapa natans* by Metcalf and Chalk [25]. Since we had become aware over the last two decades that there had been an increasing population of both floating and emergent plants along the shores of the reservoir upriver from the lock and dam across the Oswego River in Minetto, Oswego County, New York, we decided to examine the two prominent species, *Trapa natans* (Lythraceae) and *Justicia americana* (Acanthaceae), where they occur together at a small dock (**Figure 1**) and separately (**Figures 1–9**) within 100–1500 m upriver of a hydroelectric dam with lock in Minetto, Oswego County, New York. Here, we present selected developmental and structural observations on root and shoot tissues to illustrate the kinds of anatomical adaptations of native and invasive eudicot species to their riverine habitats.

2. Study of *Trapa natans* and *Justicia americana*

2.1. Plants and study location



Figures 10–15. *Trapa natans* and *Justicia americana*, closeups. 10. *Trapa* seedling with hypocotyl and adventitious roots, nodes with undeveloped leaves. 11. *Trapa* section of stolon with nodes and adventitious roots. 12. *Trapa* rhombic leaves, petioles with bladders, peduncle with young fruits. 13. *Justicia* multiple stolons along shoreline river bottom. 14. *Justicia* an erect shoot with piece of stolon and adventitious roots. 15. *Justicia* single stolon with individual roots.

We studied plants of *Trapa natans* and *Justicia americana*, which grow in the calm, shore-line, or near-shore waters of the Oswego River south (upstream) of the Minetto Dam in Minetto, Oswego County, New York, USA. Here, the Oswego River is a slightly widened reservoir south of a hydroelectric facility, lock/dam, and bridge with a deeper, flowing channel in the middle and eastern sides of the river and the calmer water on the western sides. Hundreds of plants were examined over 15 years.

2.1.1. *Trapa natans* L. (Lythraceae)

Plants were studied from early June through September (2000–2015; see **Figures 1–3**). In some years, ramets grew so prolifically that they pushed up against each other (**Figure 6**). By late October each year, the plants had died and started to disappear (**Figures 7** and **8**). In mid-October 2010, when the plants were already dying, and again in August 2011, after fruits had been produced, the plants were sprayed with Rodeo and large swaths died in the 2011 spraying, but successfully reappeared in 2012 and 2013. Adventitious roots, hypocotyls, stems (the term, stolon, is used instead of rhizome because these stems are not embedded in the rocky substrate of river bed), pedicels, and leaves (petioles and lamina of rhombic leaves) of plantlets were harvested from anchored, floating, rosette plantlets or ramets within 10 m of shore, their branching ramets, clusters of nodal adventitious roots, mature leaves, and peduncles of flowers and fruits (**Figures 3** and **10–12**). The axes of *T. natans* from which specimens were taken varied greatly in dimensions. Roots were mostly 10–65 mm long and less than 1 mm in diameter (**Figure 11**), except for the first adventitious roots arising from the cotyledonary nodes (**Figure 10**), which were much longer and often descended into the substrate river bottom. Stolons harvested were 3–4 mm in diameter, except near the water surface, where they were 5–6 mm. Petioles were proximally and distally 3–4 mm in diameter and 7–10 mm in the bladder (**Figure 12**). Peduncles were expanded from the tip of the stolons to 7–1 0 mm in diameter but were very irregular (**Figure 12**).

2.1.2. *Justicia americana* L. (Acanthaceae)

Plants were studied from June to November within 2 m of the shoreline over the 15 years of study (**Figures 4**, **5** and **9**). *Justicia americana* plants are anchored in the substrate (sand, gravel, rocks, and boulders). Adventitious roots, stolons (sectioned more than 20 cm from their tips), erect stems (submerged portions sectioned within 10 cm of their base and emergent within 20–40 cm of their tips), and some leaf bases were harvested from the sampled stem sections (**Figures 13–15**). Of the plants we sampled, adventitious roots varied greatly in length from a few cm to 30+ cm and in diameter from 0.5 to 1.0 mm. The erect shoots were usually 70–100 cm long, submerged portions 6–8 mm in diameter and aerials varied widely from 2 to 8 mm in diameter. Stolons were 8–12 mm in diameter.

2.1.3. Specimen preparations

For root apical characteristics, root tips from roots of varying lengths were harvested, fixed in FAA or FPA, and processed into paraplast wax by standard techniques; most sectioned material was stained with safranin and fast green FCF [31–33]. For root, stem, pedicel, and leaf structure, hand-cut sections were made and examined either without or with staining; the staining consisted of berberine (BER), berberine–aniline blue (BAB), berberine–toluidine blue O (BTBO), fluorol yellow (FY), sudan red 7B, phloroglucinol HCl, or toluidine blue O (TBO) [34]. Most bright-field observations were done with a Nikon Labophot microscope (SUNY at Oswego), and epifluorescence and differential interference contrast (DIC) observations were done on a Zeiss Axiophot epifluorescence microscope (University of Waterloo, Ontario).

Bright-field, epifluorescence, and laser confocal (LCF) images, including tile scans, were done on a Zeiss LSM700 (SUNY at Oswego).

2.2. Results

Trapa natans and *J. americana* grow in close proximity only at a shore-line, disturbed dock (2000–2015; **Figure 1**). In early June, the plants of *T. natans* are sparse and small (**Figures 1** and **10**) with plants arising from fruits lodged or anchored in the river bottom and with one main axis and one-two branches. In June highly branched plants of *T. natans*, in plantlets or ramets, extensively cover the near-shore, calm waters (**Figures 2** and **3**), and by mid July, they have both horizontal-oriented and vertical-oriented leaves crowded against each other (**Figure 6**); later, all vegetative portions decay and disappear by changes in water levels that disrupt the dying plants (**Figures 7** and **8**). The stands of *J. americana* arise vegetatively from overwintering rhizomes and become extensive along the shoreline by late June and early July (**Figures 4** and **5**). At first, stems elongate from the previous year's stolons with detectable old roots, and each stolon adds new shoots with new roots at nodes; new stems are added in the summer along the muddy river bottom (**Figures 5** and **13–15**), and the erect leafy stems gradually died back in October and early November (**Figure 9**).

2.2.1. *Trapa natans*

The embedded fruits of *T. natans* anchor germinated plants in the river bottom, the single hypocotyls (typically 4–8 cm long from each fruit; **Figure 10**) elongate without primary root emergence; there was no primary root in any of the hundreds of plants examined. The epicotyl elongates into the first internode (**Figure 10**), and a stolon with reduced leaves grows to the water surface. Only when the stolon reaches the surface does a typical rosette of rhombic leaves result (**Figure 3**). Beyond the cotyledonary node (**Figure 10**), adventitious roots soon emerge along the stolon at each node (**Figures 10** and **11**) up to the rosette of rhombic, floating leaves where internode elongation is extremely reduced (**Figures 3** and **12**). The adventitious roots from the cotyledonary node grow horizontally or grow down into the substrate (**Figure 10**). At the other nodes, adventitious roots form asymmetrically and grow outward (**Figure 11**), but there is no observable difference between water roots and other roots, as described by Sculthorpe [28]; roots that emerge horizontally tend to grow horizontally, and roots that emerge vertically tend to grow in that orientation. Of specimens we examined, most adventitious roots measure 10–65 mm long and produce numerous lateral roots to their tips as they age (**Figure 11**), but there are much longer roots from some nodes, especially the cotyledonary node (**Figure 10**). Flowers and later fruits with short peduncles occur among the leaves at the water surface (**Figure 12**).

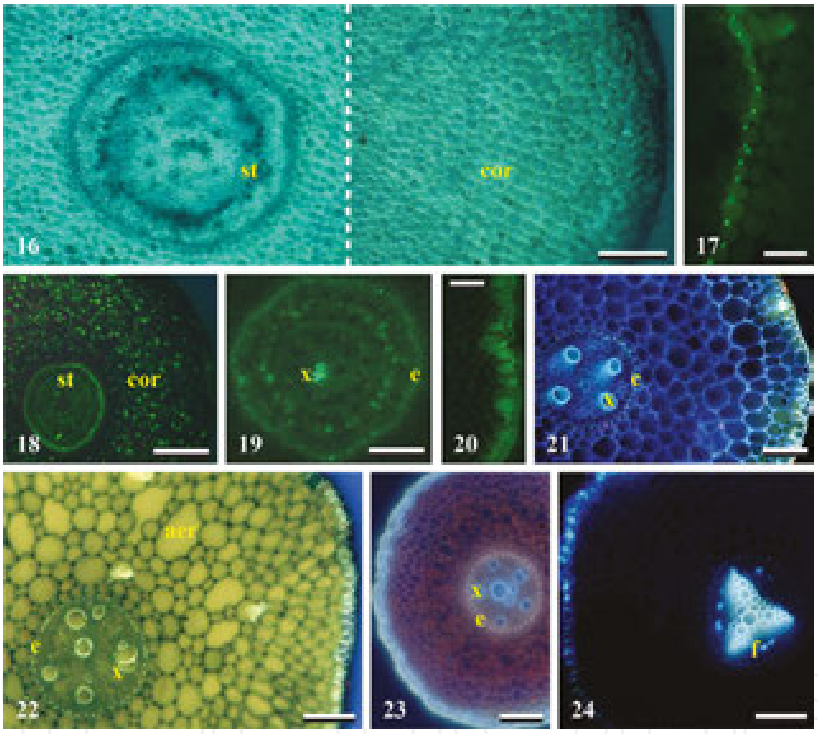
2.2.2. *Hypocotyl*

The hypocotyl has an unusual structure (**Figure 16**); delimited by an endodermis (**Figure 17**), its stele has a little, unevenly distributed, tracheary tissue near its center that takes various forms (**Figures 16**, **18**, and **19**). The tracheary cells are surrounded by rings of extensive immature tissues which never become well differentiated into more xylem or into phloem

(**Figures 16**, **18**, and **19**); a ring of endodermis, which has Casparian bands (**Figure 17**), followed during development by suberin lamellae, prominent on the outer tangential walls (**Figure 19**) surrounds the stele. There is little to no air space in the stele or cortex of the hypocotyl (**Figure 16**). The hypocotyl is often green in the outer cortex, and there is no distinct exodermis, but the epidermis has fluorescent cell walls and the cell layer under it shows some fluorescence (**Figure 20**).

2.2.3. Roots

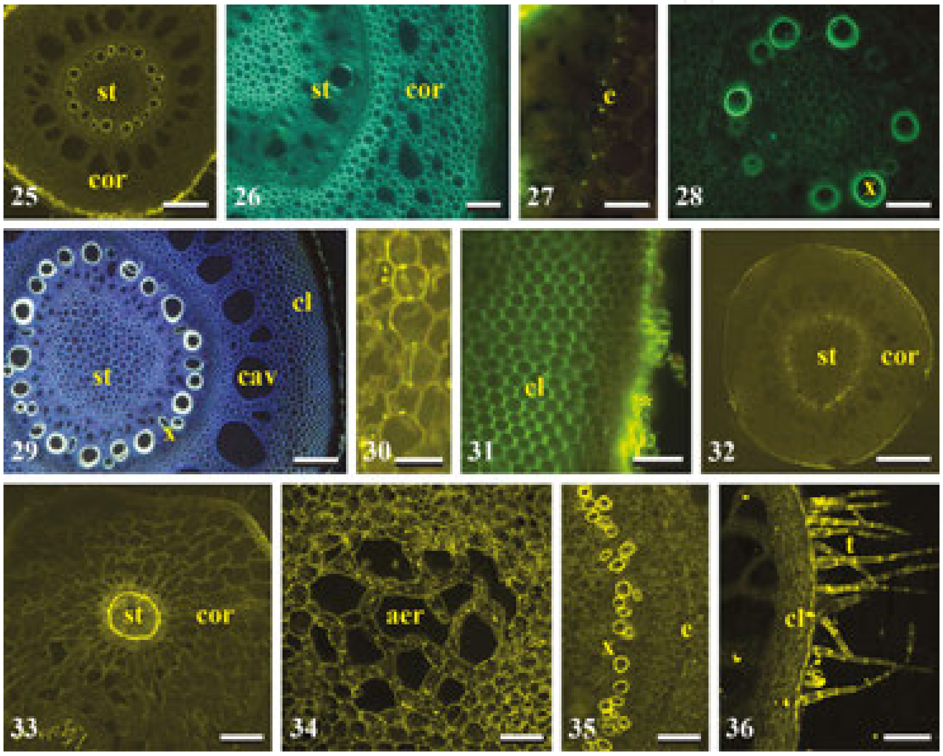
The stele of roots is typically tetrarch (**Figures 21** and **22**), but hexarchy and pentarchy occur in large roots (**Figure 23**) and triarchy in some roots (**Figure 24**). Central metaxylem vessel elements with a discontinuity of mature xylem elements between protoxylem and metaxylem are common (**Figures 22** and **23**).



Figures 16–24. *Trapa natans* hypocotyl and adventitious roots, all transverse sections. 16. Hypocotylar/transition region section in DIC, scale bar = 150 μm. 17. Hypotcotyl endodermis with Casparian bands stained with BTBO, scale bar = 70 μm. 18. Hypocotyl with endodermis delimited stele and cortex berberine stained, scale bar = 200 μm. 19. Hypocotyl stele with few tracheary elements, BTBO, scale bar = 100 μm. 20. Hypocotyl epidermis and hypodermis (exodermis), scale bar = 70 μm. 21. Adventitious root 10 cm long, tetrarch stele, endodermis with Casparian bands, BAB, scale bar = 60 μm. 22. Adventitious root, 7 cm long, protoxylem and metaxylem, phloem, endodermis with Casparian bands, aerenchyma, exodermis, BAB, scale bar = 100 μm. 23. Adventitious root, pentarch stele, metaxylem, protophloem between points of protoxylem, endodermis with Casparian bands and suberin lamellae, exodermis, autofluorescence, scale bar = 150 μm. 24. Adventitious root, triarch stele with limited secondary xylem and phloem fibers, BAB, scale bar = 150 μm.

The root cortex of *Trapa natans* is characterized by aerenchyma in its middle by both schizogeny and expansigeny (**Figures 22** and **23**; for terminology see [32], but it often has asymmetrically larger lacunae in the inner cortex in some anchored, substrate roots. Typically, the endodermis has only Casparian bands (**Figure 21**) in both substrate-bound roots from the cotyledonary node and roots growing only in water from the stolons under floating plantlets. Anchored roots may have faint evidence of extra wall material or suberin lamellae in the endodermis (**Figure 23**). There is usually a biseriate hypodermis with an outer, uniseriate exodermis of Casparian bands (**Figures 21** and **22**) and suberin lamellae (**Figure 23**). The epidermis may be lost. Limited secondary growth with phloem fibers may occur (**Figure 24**).

2.2.4. Stolons

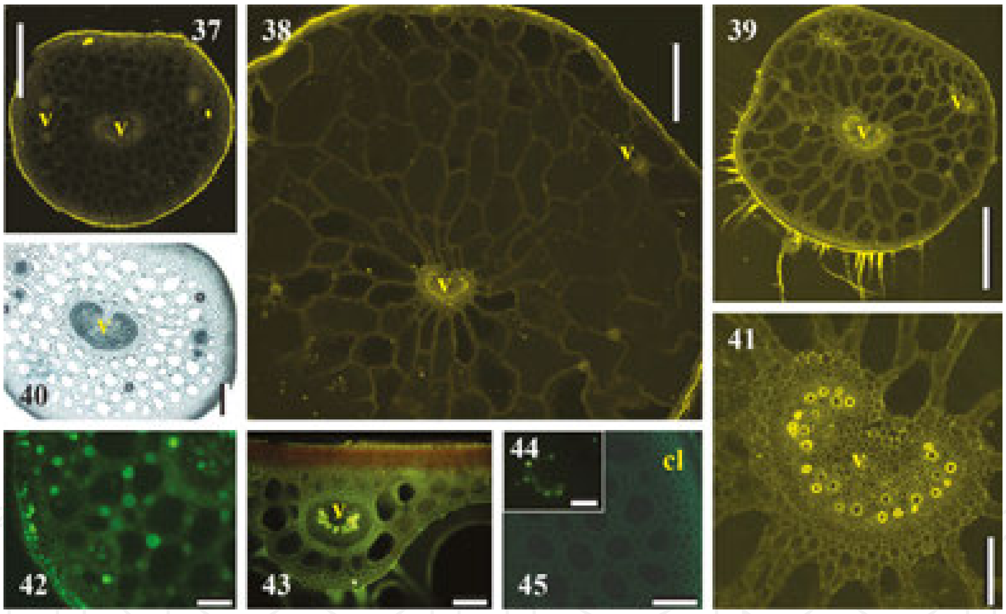


Figures 25–36. *Trapa natans* stolons and peduncles, all transverse sections. 25. Overview of stolon transverse section TS from epidermis to pith, LCF, scale bar = 500 μm. 26. First internode above hypocotyl, DIC, scale bar = 200 μm. 27. First internode, endodermis with Casparian bands, BTBO, scale bar = 70 μm. 28. First internode stele with 6–10 vessel elements and pith, BAB, scale bar = 120 μm. 29. Stolon, typical stele with numerous vessel elements in xylem, cortex with cavities, BAB, scale bar = 250 μm. 30. Endodermis with Casparian bands in stolon, LCF, scale bar = 50 μm. 31. Stolon, collenchyma underlying hypodermis, remnant exodermis, and early periderm, BAB, scale bar = 120 μm. 32. Distal stolon with stele and included aerenchyma, LCF, scale bar = 1200 μm. 33. Peduncle with ring of tightly packed xylem cells, trichomes in lower left, LCF, scale bar = 1000 μm. 34. Peduncle with aerenchyma in pith, LCF, scale bar = 100 μm. 35. Peduncle stele tightly packed xylem, endodermis with Casparian bands, LCF, scale bar = 70 μm. 36. Peduncle multicellular trichomes, collenchyma, LCF, scale bar = 200 μm.

Growth of the epicotyl into a stolon produces stem tissue, which possesses a central core of vascular tissue (overview in **Figure 25**). In the first internode (**Figures 26** and **27**), beyond the hypocotyl, there are usually only 9–11 vessel elements in a ring surrounding a non-aerenchymatous pith (**Figure 28**) with limited phloem; there are lysigenous cavities near the xylem elements that appear to be protoxylem lacunae (**Figure 26**). The endodermis has Casparian bands (**Figure 27**) but never appears to develop suberin lamellae. The cortex of the first internode is characterized by large lacunae, developed mostly by schizogeny with expansigeny, and a multiseriate hypodermis with a uniseriate exodermis, comprising Casparian bands and suberin lamellae. The boundary between hypodermis and mid cortex has collenchyma (**Figure 26**). The epidermis may be often lacking.

The stolon axis beyond the first internode until the distal stolon has a fairly large central cylinder or stele in which there are 15–25 vessels in a ring (**Figure 29**), varying with size of the stolon, outside a large pith; except for protoxylem lacunae in the stele, there is normally no pith aerenchyma throughout most of the length of the stolon. The stele is encircled by an endodermis with Casparian bands (**Figure 30**). There are usually 20 large cavities in the middle of the cortex (**Figure 29**), and there is a wide collenchyma region under a hypodermis and remnant epidermis (**Figure 31**). An initial periderm-like zone develops in many stolons (**Figure 31**). Only the distal stolon, where it leads into peduncle, contains pith aerenchyma, as well as cortical cavities (**Figure 32**).

2.2.5. Peduncles



Figures 37–45. *Trapa natans* leaves—petioles and lamina, transverse sections. 37. Proximal petiole, note aerenchyma lacunae, veins, LCF scale bar = 1000 μm. 38. Petiole bladder, note large aerenchyma lacunae, veins, LCF, scale bar = 1000 μm. 39. Distal petiole, note aerenchyma lacunae and trichomes, veins, LCF scale bar = 1000 μm. 40. Proximal petiole, aerenchyma, vein, TBO, scale bar = 350 μm. 41. Distal petiole vein without endodermis, aerenchyma, LCF, scale bar = 200 μm. 42. Proximal petiole, vein and epidermis, aerenchyma, BAB, scale bar = 80 μm. 43. Leaf lamina, note vein and air spaces, aerenchyma, autofluorescence, scale bar = 400 μm. 44. Petiole vein, BTBO, scale bar = 150 μm. 45. Proximal petiole, epidermis and collenchyma, BAB, scale bar = 120 μm.

Peduncles to flowers and one-seeded drupes are larger than stolons and are characterized by a central core of vascular tissue and pith (**Figure 33**), but the pith has large schizo-expansigenous cavities (**Figure 34**). There is a conspicuous ring of xylem cells with intervening individual parenchyma cells between most tracheids (**Figure 35**); small, inconspicuous phloem elements lie in a ring between the xylem and an endodermis. The endodermis has tangentially elongated cells and long Casparian bands (**Figure 35**). Variously sized lacunae occur in the inner cortex and a collenchyma ring underlies the epidermis, but its cells are not as thick-walled as in other stems. Clusters of multicellular trichomes extend from epidermal cells, usually with a thick cuticle (**Figure 36**).

2.2.6. Leaves

At the nodes in early growth, initially, there are extremely reduced leaves with <1 mm long buds (**Figure 10**; reduced, submersed leaves found above the first few nodes were not studied here; see [28]) and later multiple adventitious roots along the axis under water (**Figure 11**). Leaf petioles are comprised of a longer proximal petiole, inflated mid-petiole bladder, and shorter distal petiole (**Figure 12**); a main vein or vascular bundle and two to eight smaller lateral veins are characteristic (**Figures 37–40**), but branches of the veins occur. The bladder (**Figure 38**) contains more and longer cells and larger lacunae than cells and lacunae in the proximal (**Figures 37** and **40**) and distal (**Figures 39** and **41**), non-inflated portions of the petiole. The aerenchyma arises by schizogeny and expansigeny (**Figures 42** and **43**). There is no endodermis or exodermis (**Figures 41**, **43**, and **44**).

The rhombic leaf lamina has palmate venation with none of the veins containing barrier layers, although a bundle sheath surrounds some veins, especially the mid-vein. Palisade parenchyma is typical and often contains crystals at its base, but spongy mesophyll has a more characteristic lacunar appearance than typical leaf spongy mesophyll (**Figure 43**). Collenchyma under the epidermis is usually present in petioles and even the leaf blade (**Figures 37–40**, **43**, and **45**). The cuticle on the surfaces, especially lower epidermis, is thick (**Figures 37–39**). Multicellular epidermal trichomes are common on the epidermis of petioles and blades beneath major veins (**Figures 39** and **43**).

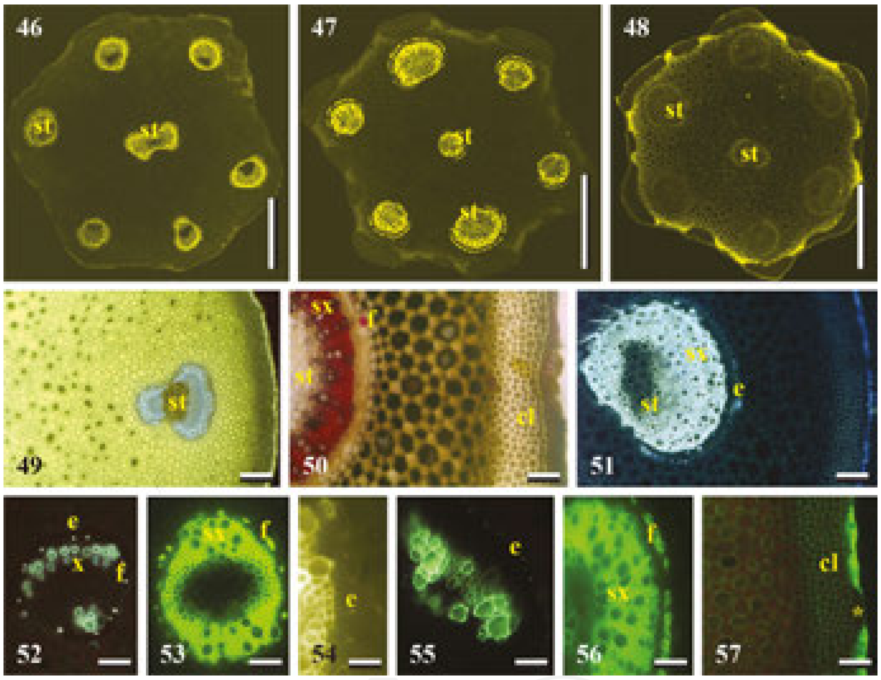
2.3. Justicia americana

In its habitat setting along the shore, *J. americana* overwinters via its underwater stolons, sensu Sculthorpe [28], which run above the shallow river bottom at the shore, and buds which elongate in late spring (**Figures 13–15**) to produce at first a few shoots (**Figures 13** and **15**) and later many stolon branches and upright, erect shoots (**Figures 13** and **14**). These stolons may grow between the boulders (**Figures 13–15**) that had been placed along the shoreline during the 1990s. Stolons initially form a few adventitious roots per node at about the second or third visible node and later produce clusters of roots at the nodes nearest the base of the erect shoots (**Figure 15**). The depth of water varies every year, but 10–50 cm of erect shoots is under the surface of the water at various times during a season.

2.3.1. Stems

The stems of *Justicia americana* include the submerged (**Figure 46**) and aerial portions of the vertical erect stems (**Figures 47**—3rd internode, **48**—5th internode) and stolons (**Figure 49**).

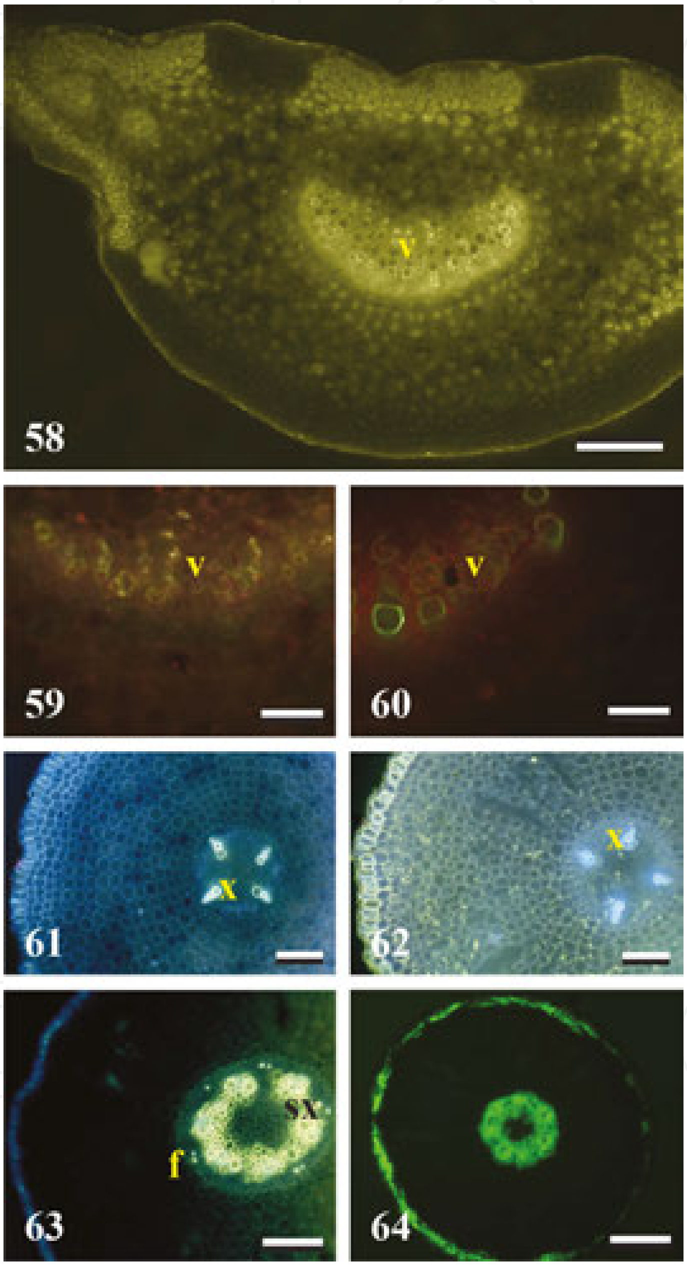
They have six large, peripheral steles (a mushroom shape for each stele in stolons, **Figure 49**) and one or two central bundles with a pith in each stele of their hexangular stems, a polystelic situation. The ground tissue, in which the peripheral steles and central stele(s) of the stems is embedded, is extensive; the peripheral parenchyma and collenchyma can be termed cortex. The steles develop secondary xylem (**Figures 50**, **51**, and **53**) and even show indications that stolons persist for two years with more secondary xylem. Phloem cells and fibers are present as a more or less thin arc around the outer portions on the six peripheral bundles (**Figures 50–53**) and, after typical early primary growth (**Figure 52**), the central bundle has xylem with surrounding small patches of primary phloem and fibers (**Figure 53**). Primary growth shows the position of protoxylem toward the inside of each group of xylem cells, as well as the presence of early phloem fibers and endodermis (**Figures 52** and **53**).



Figures 46–57. *Justicia americana* erect stems and stolons. 46. Submerged erect stem, hexagonal stem, 1 central-doubled, and 6 peripheral steles, LCF, scale bar = 2000 μm. 47. Erect aerial stem third internode, LCF, scale bar = 1000 μm. 48. Erect aerial stem fifth internode, LCF, scale bar = 1000 μm. 49. Stolon, one mushroom-shaped stele, aerenchyma, exodermis near right edge, BAB, scale bar = 150 μm. 50. Stolon, phlorogucinol-stained to show phloem fiber, periderm, scale bar = 100 μm. 51. Stolon, peripheral stele, xylem, few phloem fibers, endodermis, BTBO, scale bar = 120 μm. 52. Stolon, young stele, primary xylem, phloem fibers already present, BTBO, scale bar = 110 μm. 53. Submerged erect stem, central stele, endodermis, BER, scale bar = 100 μm. 54. Aerial stem with endodermis, BTBO, scale bar = 70 μm. 55. Young aerial stem with endodermis, BTBO, scale bar = 100 μm. 56. Aerial stem without endodermis, phloem fibers, BTBO, scale bar = 100 μm. 57. Stolon, collenchyma, BER, scale bar = 200 μm.

Submerged aerial stems usually contain endodermis (**Figure 54**), and aerial portions at the third internode usually do not (**Figure 55**), but early year high waters often have endodermis around each stele (**Figure 56**). Ground tissue from the cortex to the central region contains schizogenous–expansigenous lacunae, and thus, stems have aerenchyma (**Figure 49**). There are an extensive collenchyma band under the epidermis and a hypodermis between the epidermis and the collenchyma that shows exodermal traits (**Figures 50**, **51** and **57**). Accompanying secondary growth in the steles via a vascular cambium is limited cork cambium growth in the outer layer of cortex that produces a few layers of cork cells (**Figures 50** and **57**).

2.3.2. Leaves



Figures 58–64. *Justicia americana* leaves and adventitious roots. 58. Leaf lamina, mid vein, BAB, scale bar = 200 μm. 59. Submerged leaf lamina, vein, no endodermis, BAB, scale bar = 50 μm. 60. Aerial leaf lamina, vein, no endodermis, BAB, scale bar = 50 μm. 61. Short adventitious root, tetrarch, BAB, scale bar = 100 μm. 62. Short adventitious root, tetrarch, schizo-lysigenous aerenchyma, exodermis, FY, scale bar = 100 μm. 63. Long adventitious root, secondary xylem, and phloem fibers, BAB, scale bar = 100 μm. 64. Long adventitious root, secondary growth, remnant exodermis, initial periderm, BAB, scale bar = 200 μm.

Leaves of *J. americana* are structured like mesophytic leaves, except that they have cellulosic thickened cell walls under the epidermis that could be termed collenchyma (**Figure 58**). Whether submerged (**Figure 59**) or aerial (**Figure 60**), veins do not have any barrier layers in their lamina or petioles, and their air spaces are similar.

2.3.3. Roots

The stele typically has three to five protoxylem and protophloem strands or poles (usually tetrarch; **Figures 61** and **62**) and a pith, but metaxylem may rarely occupy the stele center. In older roots, there is limited secondary growth; secondary xylem (**Figures 63** and **64**) is accompanied by phloem fiber formation (**Figure 63**). The cortex is delimited by an endodermis with Casparian bands only (**Figure 61**). The mid-cortex is characterized by radial lacunae which are primarily produced by schizogeny in the mid to out cortex, but some cell deaths occur; therefore, it is termed schizo-lysigenous aerenchyma (**Figure 62**). The biseriate hypodermis has a uniseriate exodermis with Casparian bands and thick suberin lamellae (**Figures 62** and **63**) and in older roots a periderm may start to form (**Figure 64**).

3. Discussion

We have provided anatomical characteristics of two important riverine plants, the infamous, invasive *Trapa natans*, and the native *Justicia americana* that contribute to our understanding for the reasons these species are successful in their respective habitats.

In appearance, the ramets of *T. natans* most closely resemble the drawings in Crow and Hellquist [[1], p. 209] who accurately depicted multiple, anchoring, adventitious roots arising from the cotyledonary node above the extension of the hypocotyl out of the fruit/seed. *Trapa natans* has structural traits which allow it to be well-adapted to its anchored, floating habitat in quiet parts of rivers. *Justicia americana* is equally well adapted to its shoreline habitats by its tough stolons with adventitious roots and upright stems on the shoreline.

Unlike Arber [8] and Menegus et al. [27], we never found evidence of an elongated primary root, seminal roots, or even an aborted primary root outside of the seed/fruit in hundreds of plants, which were connected to the fruits by their stolons, in *T. natans*. Rather, the hypocotyl elongates vertically from the fruit/seed, and the first roots are adventitious roots from the cotyledonary node, arising outside the seed/fruit; there never is a primary root. The variable xylem patterns in the hypocotylar region are a manifestation of a transition from root to stem. Except for the first adventitious roots, adventitious roots are short and have many lateral roots; together these roots are prolific, giving rise to the appearance that they were sometimes interpreted in ecological studies as feathery compound leaves [[30, 35]; published plant guides [36]]; they are roots [1, 28]. Our findings are similar to those reported long ago in illustrations by Schenck [17, 18], although Schenck [17] reported primary root elongation; by our anatomical analysis, this is really hypocotylar elongation in his illustrations as the radicle remains embedded in the fruit/seed [28]. Roots of both species are typical for plants growing in aquatic/wetland conditions with typical endodermis, exodermis, and aerenchyma. Both species also exhibit root apical meristems that Heimsch and Seago [33] classified as tiered dicot with separate initials for the stele, overlying tier of cortical initials, and epidermal-rootcap initials (not presented here).

Lewis [11] found that *J. americana* could reproduce easily by stolon, especially when stolons and young, vertical shoots were not covered by debris. Stolons extend from the base of erect shoots into the river water along the surface of the sand and rocks and among the boulders; some adventitious roots penetrated the sandy-rocky river shore bottom.

In both species, the structures in our photographs are similar to the drawings of Ogden [9], except that we have identified the structures in the organs; clearly, *Trapa* was already well enough known in New York waterways in 1974 for Ogden [9] to include it in his article. Metcalfe and Chalk [23–25, p. 216] noted "conspicuous" endodermis in the stem of three genera (*Andrographis, Barleria, Thunbergia*) of the Acanthaceae and "Casparian thickenings" in some members of the Lythraceae (*Adenaria, Cuphea, Lagerstroemia, Peplis*); their diagram [24, p. 1018] of *Justicia* stem is similar to our photographs.

However, vascular tissue arrangements of *Trapa natans* and *Justicia americana* are unusual for angiosperms, but Scott [37] had earlier reported polystelic stems in aquatic dicots derived from terrestrial plants. In *Trapa*, the hypocotyl superficially resembles a modified root stele in that only a few apparent tracheary cells are centrally located and surrounded by parenchyma, but it does not contain a typical root vasculature with arms of xylem, interspersed with batches of phloem, radiating outward to a pericycle; phloem encircles parenchyma and the limited xylem. While Fahn [38] stated that the primary root had monarch pattern, clearly a radicle never emerges into a primary root in *T. natans*; we interpret the axis as the transition zone of the hypocotyl. Hypocotyls never produce much mature tissue. Thus, after adventitious roots take over the anchorage function, the hypocotyl and fruit effectively may no longer function.

The stems of *Trapa* are siphonostelic with one layer of endodermis surrounding all vascular tissue. In the peduncle, where xylem cells are closely appressed, vascular tissue arrangement is best described as a kind of ectophloic siphonostele [38]; stolons and peduncles differ slightly in their positions of phloem. The early development of the vascular system in the stem of *Justicia americana* was described long ago by Jones [39, named *Dianthera americana* therein]. It has also been deemed to have a monocot-like atactostele [26], but we have shown that each complex vascular bundle or stele is surrounded by an endodermis; therefore, the stem is a polystele with each stele having parenchyma, primary and secondary xylem, and partially surrounding primary and secondary phloem—all within an endodermis. Other species of *Justicia* do not show the same polystelic vascular anatomy in their stems [40, 41], although O'Neill [40] noted what could be endodermis. The interest in the kind of vasculatures which characterize *Trapa* and *Justicia* was addressed long ago by Scott [37], but has not received much attention in recent decades [23, 24]. Our findings of fern-like (not identical to such steles) siphonosteles and polysteles clearly indicate a need for further study of these structural features in other members of their families since they are so prevalent in stems of aquatic and wetland plants [24].

Another distinctive feature of both species is the presence of a wide band of collenchyma under the epidermis in the stems (and even petioles), even when there is considerable secondary vascular tissue, as in *J. americana*; both O'Neill [40] and Zottele and Aoyama [41] noted fairly extensive collenchyma under the epidermis [23, 24]. Collenchyma is clearly a substantive supportive tissue for these plants occupying riverine habitats. The air spaces do not occur within this tissue but internal to it in the next zone of cortex or ground tissue. Aerenchymatous tissue does not occur within individual steles of *Justicia* or in the hypocotyl and most stolons of *Trapa*, although lysigenous cortical cavities and protoxylem lacunae occur in *Trapa*, and schizogenous-expansigenous lacunae occur in the uppermost stolon and its continuation into the peduncle of *Trapa*; small lacunae occur throughout the ground tissue of *Justicia* stems.

While we have reported on the air spaces within these two species, we have not attempted to analyze the presence of an air space continuum between their organs [42]. We note that at the water–air interface, the distal stolons and peduncles show pith aerenchyma, in addition to cortical air spaces [28, 43]. Our descriptions of aerenchyma differ somewhat from Van der Valk [44], but we agree that floating leaves, such as *T. natans*, have enlarged mesophyll spaces which we believe can properly be termed aerenchyma because they have typical aerenchymatous lacunae. Leaves submersed in water during development do not develop typical leaf structures in *T. natans* and lack barrier layers in both *T. natans* and *J. americana*.

The multicellular trichomes which we observed along the petioles and on the abaxial surface of leaves, resembled those in drawings in Metcalfe and Chalk [25] and have been termed hydropoten because they presumably absorb water and minerals [25], but we did not test for this and we note that they are not at all like hydropoten in Nymphaeaceae (Seago, personal observation). The presence of crystals was not presented herein [for *T. bispinosa* 45, 46].

The nature of the stoloniferous growth and structural features of *T. natans*, including siphonostele, endodermis, collenchyma, and cortical air spaces, along with the anchorage of the extensive ramet systems by the fruit-hypocotyl axis and first adventitious roots, must contribute to its success in calmer waterways.

5. Conclusions

This study of the structural traits of both *Trapa natans* and *Justicia americana* reveals the ecological adaptations of each to their respective habitats. The organizations and structures of the tissues in roots, stolons, leaves, and peduncles of *T. natans* contribute to its characteristic sponginess, buoyancy, and flexibility in its floating habit, outpacing native species. The enormous production of ramets with their multiple floating leaves, along with the flowering/fruiting axis, can quickly cover a calm water's surface, probably monopolizing nutrients and space, while limiting light penetration and dissolved oxygen in the water column. The abundant adventitious roots undoubtedly aid in the absorption of nutrients and must contribute to its success in invading riverine habitats. The ability of *J. americana* to provide substrate stabilization and a barrier to shoreline erosion through its network of stolons can be attributed, in part, to its angular stems' structural characteristics, which surely give its stems (stolons and erect stems) strength. Its adventitious roots must also add to its ability to maintain substrate.

The unique stem steles of these two species, their endodermis delimiting these steles, their air space systems, and the peripheral, flexible collenchyma undoubtedly contribute to the respective ecosystem roles, including the respective roles of the invasive [10] versus the shoreline stabilizer [13] species. Unfortunately, we really do not know how extensive these anatomical patterns are in flowering plants [23, 24, 39]. The occurrence of endodermis-enclosed steles in stems, air space tissues and flexible collenchyma, found in these species of eudicots not near the base of the angiosperms, suggests that these structural traits have been conserved over evolution and can appear in more derived families, genera, and species of plants in response to environmental stimuli. The endodermis-enclosed steles of stems, however, are very different from the norm among eudicots.

Acknowledgements

The authors wish to acknowledge the assistance of Dr. Carol A. Peterson, Daryl E. Enstone, and Dr. Simon Chuong, University of Waterloo, Ontario, Dr. Chris Meyer at University of Guelph, Dr. Hilary A. McManus at LeMoyne College, Dr. Julien B. Bachelier at SUNY Oswego, and Marilyn A. Seago for her continuing encouragement and support, especially in collecting specimens.

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