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ABSTRACT Didiplis diandra is a small shoreline aquatic plant scattered throughout eastern North America. In the Atlantic coast states from Virginia to Georgia it has been confused with other species that grow in similar habitats. Leaves of *D. diandra* are heterophyllous, submersed leaves average 15.4 mm long while emersed leaves average 6.3 mm long. A taxonomic key is presented to aid in identification of small shallow water and shoreline aquatic plants including members of the genera *Callitriche, Crassula, Elatine* and *Ludwigia*.

INTRODUCTION During survey work by a North Carolina scientist in 2008 a small aquatic plant of unknown taxonomic identity was discovered in the North Carolina piedmont. Pictures were circulated via the North Carolina rare flora list serve. Even though quick replies allowed readers to recognize the plant as a member of the Lythraceae, observed morphological features did not clearly match key characteristics found in several regional floras, including Radford et al. (1968) and Weakley (2010). The plant was eventually confirmed as Didiplis diandra (Nutt. ex A.DC.) Wood. This is a small aquatic plant of the Lythraceae that is found widely scattered in eastern North America (Weakley 2010). In North Carolina it is on the rare plant list as a significantly rare plant at the periphery of its range, SR-P (Franklin 2010). In the process of identification it became evident that most of the taxonomic literature does not adequately recognize morphological variation in this species and distinction from other similar shallow water and shoreline species.

The taxonomic problem is largely due to heterophylly. Morphological variation in *D. diandra* was recognized by Fassett (1939). In his article on *Elatine* he added a footnote, based on heterophylly, to recognize two forms

of *Didiplis*, forma *terrestris* (Koehne) Fassett and forma *aquatica* (Koehne) Fassett. In a subsequent publication, Fassett (1940, p. 255) further described differences between the two forms, stating that forma *aquatica* had leaves that were "ribbon-like, thin, limp, not narrowed at base," while leaves of forma *terrestris* were "broader, somewhat tapered to each end and of firmer texture."

Most commonly *D. diandra* is seen as a shallow water submersed herb. Radford et al. (1968, p. 738) state that this species is a "submersed aquatic or marsh plant with weak stems." Their accompanying drawing shows leaves that are about 20 times longer than wide. No mention of the emersed form was included in the description. Such omission, though appropriate at that time, resulted in confusion when the rare emersed form develops.

The purpose of this article is to clearly document the morphological variation in leaves and present a taxonomic key to distinguish small shoreline species.

MATERIALS AND METHODS During field work in July 2008, plants of *Didiplis diandra* were discovered and collected at several sites in central North Carolina (Appendix 1). At each site, photographs were taken and voucher specimens collected. It was noted if a population included plants with leaves that

*email address: Charles.horn@newberry.edu Received September 13, 2010; Accepted May 19, 2011. were submersed, floating or emersed. Dried specimens were used for measurement of leaf length and width with a sample size of 50 leaves and using no more than five leaves per stem. Data were analyzed to calculate average, standard deviation and range of leaf size. Voucher specimens were deposited at the Newberry College herbarium (NBYC) and duplicates were distributed to the University of South Carolina (USCH), University of North Carolina – Chapel Hill (NCU) and other herbaria.

To develop a taxonomic key, several regional floras were referenced (Radford et al. 1968; Godfrey and Wooten 1979, 1981) to obtain detailed descriptions. Specimens at NBYC were reviewed to note morphological details and variation in growth patterns to fill gaps in published species descriptions.

RESULTS Populations of *Didiplis diandra* were discovered at three locations in North Carolina, one each in Johnson, Nash and Wake counties. The population along the Little River in Wake County included plants that were either submersed or on saturated mud with floating leaves. Along Little River in Johnston County plants grew submersed near the shoreline in several depressions with standing water. Plants in Nash County were are along the margins of a mill pond where it was evident that the water level had dropped over 0.5 meters, resulting in plants becoming stranded; they grew emersed.

Observation of *D. diandra* at several sites in North Carolina clearly showed that it can produce submersed, floating and emersed leaves. Submersed plants were commonly seen in water up to 10 cm deep and these individuals generated stems up to 20 cm long that commonly branched. Submersed leaves were the longest (Figure 1A, 1B) and had an average leaf length of 15.4 mm (standard deviation 2.3 mm; range 10-22 mm) and width of 1.6 mm (standard deviation 0.5 mm; range 1.0-3.0 mm). In shallow water, typically less than 2 cm, stems grew to the water surface produced shorter floating leaves. Plants also grew stranded along shorelines as a result of receding water levels. These stranded plants (Figure 1C) had adapted to the new microhabitat by producing ascending stems with leaves that were much shorter and firmer than seen on submersed plants. Emersed leaves were 6.3 mm long (standard deviation 1.2 mm; range 3.7–8.4 mm) and 1.2 mm wide (standard deviation 0.3 mm; range 0.6–2.0 mm).

DISCUSSION Heterophylly among aquatic plants is a common phenomenon (Sculthorpe 1967, Kral 1978, Horn 1988). Changes in leaf morphology and anatomy occur as the growing tip of a stem moves from a submersed to emersed habitat. Commonly, juvenile submersed leaves are either highly dissected or linear and developmental changes are seen in leaf size, blade shape and presence of a petiole (Sculthorpe 1967). In the piedmont of the southeast, many submersed plants initially produce long, linear and flaccid leaves on flexible stems. Then, as plants grow to the water surface and as water levels drop, emersed forms develop with stouter stems and shorter, firmer leaves, allowing plants to grow in an emersed habitat as well as produce flowers.

The observed reduction in leaf size between submersed and emersed habitats is a common pattern of heterophyllous aquatic plants that live in shallow water. Undocumented heterophylly apparent between the submersed and emersed forms of *D. diandra* is responsible for some misidentification of this species. Further confusion may occur as, based on observation, only stems with floating and emersed leaves generate flowers which are positioned near, at or above the water surface and have a red coloration. *Didiplis diandra* does not produce petals (Godfrey and Wooten 1981) and no pollinators were seen during field observation.

Other members of the Lythraceae can be confused with D. diandra, mainly due to small flower size. It is the smallest species of the family and commonly grows either submersed with stems elongating to the water surface, or as a decumbent terrestrial herb. All other species of the family in eastern North America (as delineated by Weakley 2010) typically germinate and develop as erect emersed plants, probably being submerged only due to rising water levels. Flowering specimens can be distinguished from other members of the Lythraceae, as D. diandra does not produce petals. The species most commonly confused with D. diandra has been Rotala ramosior (L.) Koehne. Both species are







small shoreline plants; however, the two can be distinguished by several features, as described by Godfrey and Wooten (1981). Didiplis diandra has weakly developing stems which become decumbent, lacks intersepalary appendages, lacks petals, and has indehiscent capsules. Meanwhile, R. ramosior has rigid stems (that allow for vertical growth), triangular intersepalary appendages, four pinkish petals, and dehiscent capsules.

Didiplis diandra can also be confused with small aquatic plants of other families. Morphologically similar species are in the genera Callitriche L. (Plantaginaceae), Crassula L. (Crassulaceae), Elatine L. (Elatinaceae), and Ludwigia L. (Onagraceae). Each of these genera contain species that grow in a shoreline aquatic habitat and that overleap distributionally with D. diandra. The best way to separate these genera is through observation of floral features. Species of Callitriche (six are known in the region of Virginia to Georgia) are also heterophyllous, but can be distinguished from D. diandra either by production of spatulate floating or emersed leaves or flowers that lack sepals and petals (flowers of D. diandra have sepals). Crassula aquatica (L.) Schonland produces a branched stem pattern and leaf bases are connate to the stem; whereas D. diandra stems occasionally branch and leaf bases are attenuate. Four species of Elatine are known from the area and they have sepals and petals, neither of which are fused into a tube, as well as a dehiscent capsule (D. diandra produces a floral tube,

lack petals and has an indehiscent capsule). Five species of opposite-leaved *Ludwigia* were considered because they are less than 10 cm tall and have repent growth, - L. arcuata Walt., L. brevipes (Long ex Britton, Braun & Small) Eames, L. palustris (L.) Ell., L. repens J.R. Forst., and *L. spathulata* Torr. & A. Gray. These species can be distinguished from D. diandra in that they either have a pedicel longer than 4 mm or they have spatulate leaves. Godfrey and Wooten (1981, p. 412) include a note that individuals of L. arcuata in water up to one meter deep may have a growth pattern similar to that of D. diandra with "the leaves flaccid, mostly linear-subulate or narrowly oblanceolate." Shallow water or emersed stems can easily be distinguished in that L. arcuata is pubescent (stems and leaves) and the pedicels are longer than the leaves.

A taxonomic key is provided to assist in identification of small shoreline and shallow water plants that can potentially be confused with *D. diandra* along the Atlantic region from Virginia to Georgia. Species restricted to outer coastal plain and salt marshes were not included. Included are plants that are commonly less than 15 cm tall and have leaves less than 3 cm long (see Appendix 2 for list). An attempt has been made to emphasize vegetative features and to take into account observed heterophylly. Generic and specific description information was obtained from Godfrey and Wooten (1979, 1981) and Radford et al. (1968). Taxonomy follows that of Weakley (2010).

KEY TO GENERA OF ROOTED SHALLOW WATER OR SHORELINE HERBS LESS THAN 15 CM TALL AND WITH LEAVES LESS THAN 3 CM LONG OF THE MID-ATLANTIC REGION, EXCLUDING OBLIGATE OUTER COASTAL PLAIN SPECIES

1.	Leaves alternate or spirally arranged
	2. Stems flattened, leaves clustered at nodes, tufted Juncus (Juncaceae)
	2. Stems terete, leaves alternate
	3. Leaves appearing spirally arranged on elongate stems Mayaca (Mayacaceae)
	3. Leaves distinctly alternate 4
	4. Stipules present, usually fused into a sheath
	Potamogeton (Potamogetonaceae)
	4. Stipules absent
	5. Leaves greater than 1 cm long; ovary inferior Ludwigia (Onagraceae)
	5. Leaves less than 1 cm long; ovary superior Anagallis (Primulaceae)

Figure 1. Variation in growth form of *Didiplis diandra*. A. Submersed plants growing in about 10 cm water picture taken 12 July 2008; B. herbarium specimen of submersed plant, *Horn 18,340*; C. herbarium specimen of emersed plants, *Horn 18,356*.

1.		aves opposite or whorled, at least apically
	6.	Leaves whorled
		7. Most nodes opposite, some nodes with leaves appearing whorled, leaf bases with
		sheaths
	6.	Leaves opposite
		8. Stems mostly emersed and erect
		9. Leaf base auriculate to cordate clasping
		10. Leaves 1–2x longer than wide, punctate glandular on adaxial surface
		<i>Hypericum</i> (Hypericaceae) 10. Leaves 5–10x longer than wide, not punctate glandular
		10. Leaves 3–10x longer than wide, not punctate giandular
		9. Leaf base cuneate to attenuate
		11. Stems pubescent
		11. Stems glabrous
		12. Leaves glandular pubescent Gratiola, Sophranthe (Plantaginaceae)
		12. Leaves glabrous
		13. Leaves generally ovate Lindernia (Linderniaceae)
		13. Leaves linear to oblanceolate
		14. Leaves 10 mm or more long Rotala (Lythraceae)
		14. Leaves less than 7 mm long Crassula (Crassulaceae)
		8. Stems submersed and flaccid or emersed and mostly prostrate
		15. Flowers in axillary glomerules or heads
		16. Inflorescence an axillary head on an elongate peduncle <i>Eryngium</i> (Apiaceae)
		16. Inflorescence a glomerule of several flowers, rarely solitary
		Oldenlandia (Rubiaceae)
		15. Flowers solitary or rarely in pairs, in leaf axils 17
		17. Leaf margins toothed, leaf bases sheathing Najas (Hydrocharitaceae)
		17. Leaf margins entire, bases lacking sheath
		18. Flowers pedicillate
		19. Ovary inferior Ludwigia (Onagraceae)
		19. Ovary superior Bacopa (Plantaginaceae)
		18. Flowers sessile or on pedicles less than 1 mm
		20. Leaves orbicular, as long as wide Micranthemum (Linderniaceae)
		20. Leaves at least 2x longer than wide
		21. Flowers lacking perianth Callitriche (Plantaginaceae)
		21. Flowers with perianth (at least sepals) 22
		22. Most leaves linear to lanceolate, widest near middle
		Didiplis (Lythraceae)
		22. Most leaves oblanceolate to spatulate, widest near apex
		(occasionally ovate)
		23. Most leaves more than 2 cm long
		Ludwigia (Onagraceae)
		23. Most leaves less than 1 cm long Elatine (Elatinaceae)

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APPENDIX 1. Collections of *Didiplis diandra* observed during field work in July 2008.

North Carolina. Johnston County. County Route 1722 (Mudham Road) at Little River, 1.4 mi south of junction with County Route 1723 (Taylor's Mill Road). 35°46.31'N, 78°18.72'W, 12 July 2008. *Charles N. Horn* 18,348 (NBYC).

North Carolina, Nash County. Taylor's Mill Pond of Mocasin Creek at County route 1120 (Taylor's Mills Road); at Johnson County Line. 35°46.77′N, 78°14.88′W. 12 July 2008. *Charles N. Horn 18,356* (NBYC).

North Carolina. Wake County. US 64 Bus at Little River, 1.7 mi west of junction with NC 97, just west of Zebulon. 35°48.06′N, 78°20.55′W. 12 July 2008. *Charles N. Horn 18340* with Gerald Pottern and Linda Holland-Toll (NBYC).

APPENDIX 2. List of species used in development of taxonomic key.

APIACEAE

Eryngium prostratum Nutt. ex A.DC.

CRASSULACEAE

Crassula aquatica (L.) Schonland

ELATINACEAE

Elatine americana (Pursh) Arn. Elatine minima (Nutt.) Fischer & C.A. Mey. Elatine rubella Rydb.

HYDROCHARITACEAE

Egeria densa Planchon Elodea canadensis Michx. Hydrilla verticillata (L.f.) Royle Najas gracillima (A. Braun ex Engelm.) Magnus Najas guadalupensis (Spreng.) Magnus

HYPERICACEAE

Hypericum gymnanthum Engelm. & A. Gray Hypericum mutilum L.

JUNCACEAE

Juncus repens Michx.

LINDERNIACEAE

Lindernia dubia (L.) Pennell Micranthemum umbrosum (J.F. Gmel.) Blake

LYTHRACEAE

Ammannia coccinea Rottb.
Cuphea carthagenensis (Jacq.) J.F. Macbr.
Cuphea viscosissima Jacq.
Didiplis diandra (Nutt. ex A.DC.) Wood
Rotala ramosior (L.) Koehne

MAYACACEAE

Mayaca fluviatilis Aubl.

ONAGRACEAE

Ludwigia arcuata Walt.
Ludwigia brevipes (Long ex Britton, A. Braun & Small)
Eames
Ludwigia linearis Walt.

Ludwigia linifolia Poir. in Lam. Ludwigia microcarpa Michx. Ludwigia palustris (L.) Ell. Ludwigia repens J.R. Forst. Ludwigia spathulata Torr. & A. Gray

PLANTAGINACEAE

Bacopa caroliniana (Walt.) B. L.Rob. Bacopa monnieri (L.) Wettst. Callitriche heterophylla Pursh Callitriche palustris L. Callitriche pedunculosa Nutt. Callitriche peploides Nutt. Callitriche terrestris Raf. emend. Torr. Gratiola neglecta Torr. Gratiola virginiana L. Sophronanthe pilosa (Michx.) Small

POTAMOGETONACEAE

Potamogeton diversifolius Raf.

PRIMULACEAE

Anagallis minima (L.) E.H.L. Krause

RUBIACEAE

Oldenlandia boscii (A.DC.) Chapm. Oldenlandia uniflora L.