RHODORA, Vol. 118, No. 974, pp. 235–242, 2016

doi: 10.3119/15-30; first published on-line May 2, 2016.

NOTE

NEW RECORDS OF *ELATINE AMBIGUA* (ELATINACEAE), A NONINDIGENOUS NORTH AMERICAN SPECIES

Aaron J. Rosman

Department of Natural Resources and the Environment, University of Connecticut, Storrs, CT 06269

Hamid Razifard[1]

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269
[1]Author for correspondence; e-mail: hamid.razifard@gmail.com

Gordon C. Tucker

Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920

Donald H. Les

Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269

Elatine L. (waterwort) is a genus of aquatic flowering plants with about 25 species worldwide (Tucker 1986). Fourteen of the species occur in the New World, including two nonindigenous species (*E. ambigua* Wight and *E. triandra* Schkuhr), which both have a cosmopolitan distribution and are thought to be of Asian origin (Mason 1957; H.R., unpublished data). According to herbarium records, the New World populations of *E. ambigua* have been reported only from California, but *E. triandra* has a wider distribution ranging from southern Canada to the USA, Mexico, and Brazil. In the New England area, *E. triandra* has been reported from Connecticut, Maine, and Massachusetts. This species also has been reported from the USA states of Alabama, Arizona, California, the Carolinas, Colorado, Georgia, Louisiana, Minnesota, Nebraska, Nevada, New Jersey, New York, North Dakota, Oklahoma, Pennsylvania, Texas, Utah, Virginia, and Wisconsin. Considering that both of these species are common weeds of rice fields (DiTomaso and Healy 2007; Moody 1989), rice farming may have been responsible for their initial introduction. Where rice farming does not occur, it is feasible that the popularity of both *E.*

ambigua (H.R., unpublished data) and *E. triandra* (De Wit 1964) as aquarium plants could have contributed to their spread.

Elatine ambigua and *E. triandra* are very similar morphologically, and are distinguished mainly by the presence of a recurved flower stalk in the emersed form of *E. ambigua* (Tucker and Grissom 2012). The submersed forms of the two species are nearly indistinguishable from one another based solely on morphological characters (H.R., pers. obs.), although *E. ambigua* sometimes has slightly longer leaves (Figure 1). The highly reduced morphology of *Elatine* makes it difficult to designate conspicuous taxonomic characters in this group. As a result, *Elatine* species typically exhibit fewer morphological differences than those of other angiosperm genera.

Similar to other *Elatine* species, *E. ambigua* and *E. triandra* are extremely under-represented among herbarium collections that have been made in recent decades. We tested several populations of these species (Table 1) using three genetic markers (ITS, *rbcL*, and *trnK*/*matK*) to evaluate the delimitation of *E. ambigua* and *E. triandra* as separate species. Our sampling included both dry herbarium specimens and freshly collected material for which we made voucher specimens that were deposited at CONN. This comparison included Asian, Australian, European, and North American populations of the two species. We were not able to include the herbarium specimens of *E. triandra* collected in Maine and Massachusetts (housed at, e.g., GH and NEBC) due to their antiquity or lack of sufficient plant material for DNA extraction. One accession of *E. minima* (Nutt.) Fisch. & C.A. Mey. served as the outgroup for our analyses.

We extracted DNA from both fresh and dry material following the method proposed by Doyle and Doyle (1987). We followed the procedures described in Les et al. (2008) to obtain the sequences of ITS, *matK*/*trnK*, and *rbcL*. All the sequences obtained in this study were submitted to Genbank (accession numbers provided in Table 1). We aligned the sequences using MAFFT version 7 (available from http://mafft.cbrc.jp/alignment/server/) with a gap opening penalty of 2.5. Preliminary analyses recovered the same two clades of taxa regardless of the DNA partition used. Consequently, we combined the sequences of the three regions and analyzed the full dataset using PAUP* (Swofford 2002) with the following settings: starting trees were obtained by step-wise addition using tree-bisection reconnection (TBR) as the branch-swapping algorithm; the maximum number of trees was set to 100,000; and polytomies were allowed. We calculated bootstrap support (BS) values using PAUP* by conducting 1000 bootstrap replicates with settings similar to those of the MP analyses,

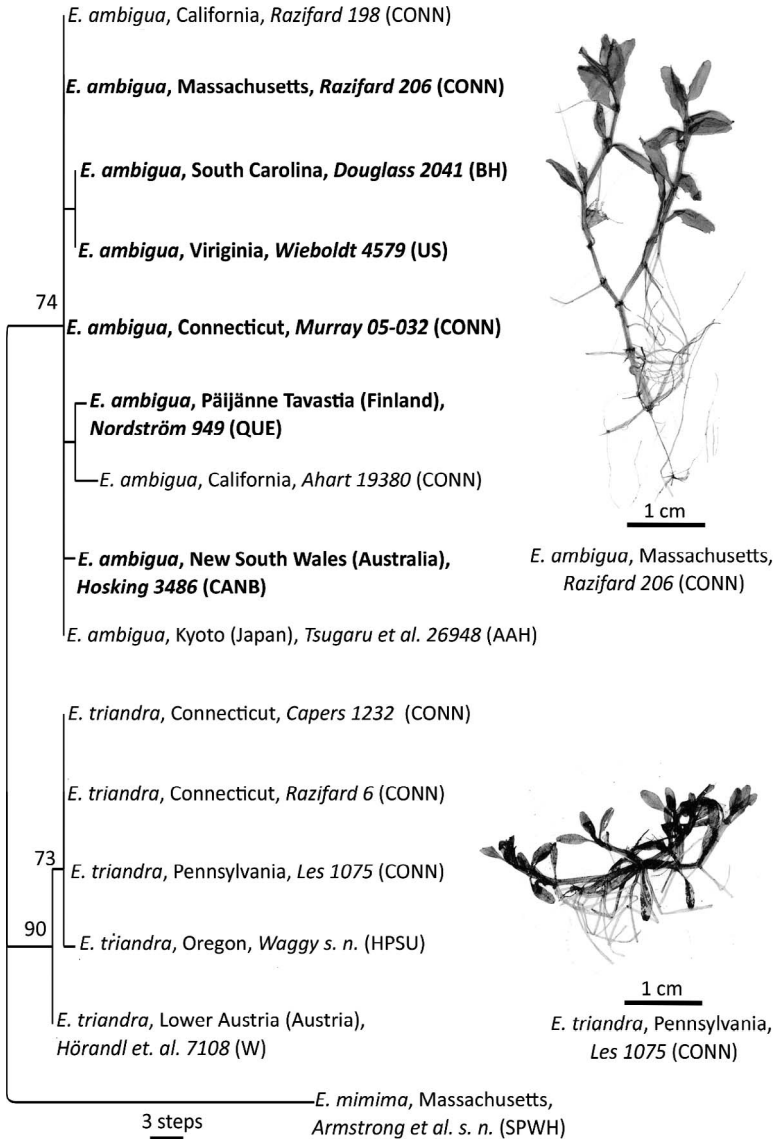

Figure 1. A phylogenetic tree (right) drawn using PAUP* based on the combined ITS, *rbcL*, and *trnK/matK* data. The numbers above nodes are the parsimony bootstrap support values. Only bootstrap values above 50% are presented. The boldface accessions represent records newly reported in this paper. On the right, images of *Elatine ambigua* and *E. triandra* (from dry herbarium specimens) are provided for comparison. The scale bars are provided for both the phylogenetic tree and the plant images.

Table 1. Voucher information for *Elatine*. The material source is distinguished as fresh (F) or dry herbarium specimens (D). Missing data are shown by a long dash (–) in the last column.

Species of *Elatine*	Locality	Herbarium Code	Collector(s) Name and Number	Collection Date	Material Source (F/D)	Genbank Numbers (ITS, *matK*/*trnK*, *rbcL*)
E. ambigua	AUSTRALIA. New South Wales: Narrabri, near south eastern corner of Brigalow Park Nature Reserve, −30.4253°, 149.5981°.	CANB	*Hosking 3486*	12 February 2012	D	KT970416, KT970427, KT970401
E. ambigua	FINLAND. Päijänne Tavastia Region, Lake Vesijärvi, shore of Jussila Manor, in shallow water with *E. hydropiper* L.	QUE	*Nordström 949*	25 August 1982	D	KT970417, KT970429, KT970403
E. ambigua	JAPAN. Kyoto: near Kyudo-Shichiyama-toge Miwa-cho, Amata-gun.	AAH	*Tsugaru et al. 26948*	17 September 1998	D	–, KT970432, KT970406
E. ambigua	USA. California: Butte County, N of Hamilton Road, E of Riceton Highway. Agricultural site. 39.4506111°, −121.7166111°.	CONN	*Ahart 19380*	17 April 2014	F	KT970414, KT970425, KT970399
E. ambigua	USA. California: Butte County, NW of Gray Lodge Wildlife Area, growing in a rice field. 39.36282°, −121.7442°.	CONN	*Razifard 198*	15 May 2013	F	KT970418, KT970430, KT970404
E. ambigua	USA. Connecticut: Middlesex County, Middlefield, Beseck Lake, Area I.	CONN	*Murray 05-032*	11 July 2005	D	–, KT970428, KT970402

Table 1. Continued.

Species of *Elatine*	Locality	Herbarium Code	Collector(s) Name and Number	Collection Date	Material Source (F/D)	Genbank Numbers (ITS, *matK*/*trnK*, *rbcL*)
E. ambigua	USA. Massachusetts: Worcester County, Wachusett Reservoir, Malagasco Brook Cove, submersed, forming a green mat near the shore, 42.3417500°, −071.7490833°	CONN	*Razifard 206*	29 September 2014	F	KT970419, KT970431, KT970405
E. ambigua	USA. South Carolina: Greenville County, Pleasant Ridge State Park.	BH	*Douglass 2041*	24 September 1985	D	KT970415, KT970426, KT970400
E. ambigua	USA. Virginia: King William County, near Scotland Landing, muddy tidal shore and near-shore shallows of the Mattaboni River.	US	*Wieboldt 4579*	12 October 1982	D	–, KT970433, KT970407
E. minima	USA. Massachusetts: Barnstable County: Falmouth, fresh water pond, growing in water near shore.	SPWH	*Armstrong et al. s.n.*	21 July 1989	D	KT970420, KT970434, KT970408
E. triandra 1	AUSTRIA. Lower Austria, on flooded muds, N and W shores of Kufstein pond in Litschau.	W	*Hörandl et al. 7108*	10 September 1995	D	KT970424, KT970436, KT970410

Table 1. Continued.

Species of *Elatine*	Locality	Herbarium Code	Collector(s) Name and Number	Collection Date	Material Source (F/D)	Genbank Numbers (ITS, *matK*/*trnK*, *rbcL*)
E. triandra 2	USA. Connecticut: Litchfield County, West Hill Pond, New Hartford. Shallow water.	CONN	*Capers 1232*	14 September 2005	D	KT970421, KT970435, KT970409
E. triandra 3	USA. Connecticut: Litchfield County, West Hill Pond, New Hartford. Shallow water.	CONN	*Razifard 6*	09 September 2011	F	KT970423, KT970438, KT970412
E. triandra 4	USA. Oregon: Lincoln County, Devils Lake. Grown in the lab from a sediment core retrieved from the lake bed.	HPSU	*Waggy s.n.*	21 September 2000	D	–, KT970439, KT970413
E. triandra 5	USA. Pennsylvania: Berks County, French Creek State Park, Scotts Run Lake, public boat launch. 40.2095556°, −075.7967778°.	CONN	*Les 1075*	04 July 2012	F	KT970422, KT970437, KT970411

except with 10,000 trees for each bootstrap replicate (maxtrees =10,000). The accessions with missing data in ITS region (Table 1) were excluded from the BS calculations to avoid reduction in bootstrap values due to missing data. The combined matrix of the aligned data was deposited in TreeBASE (study number 18438; available at http://purl.org/phylo/treebase/phylows/study/TB2:S18438?x-access-code=ee6bf4447afe7b72691128c7fefe3a95&format=html).

The results are summarized in Figure 1. Our molecular data indicated that *Elatine ambigua* and *E. triandra* are separable by four differences in the ITS alignment (sites 131, 186, 258, and 562), two differences in the *trnK*/*matK* alignment (sites 298 and 457), and one difference in the *rbcL* alignment (site 1078). Also, the molecular survey uncovered six specimens of *E. ambigua*, which previously had been misidentified as other *Elatine* species. These corrected reports represent the first record of *E. ambigua* for Finland, four new state records in the USA (i.e. Connecticut, Massachusetts, South Carolina, and Virginia), and one new state record in Australia (Table 1; Figure 1). All of these records were identified previously as *E. triandra*, except for the Australian record, which was identified as *E. gratioloides* A. Cunn. We annotated all of these collections, as well as other accessions included in our study, using the identification indicated by the molecular results.

Little is known about the introduction history of *Elatine ambigua* or *E. triandra*. The proximity of the Finnish and Australian populations of *E. ambigua* to agricultural sites, especially rice fields, implicates rice farming as a means of introduction for *E. ambigua* in those regions. This also is the case for most North American populations of *E. ambigua*, with the exception of records from the northeastern USA (i.e., Connecticut and Massachusetts), which represent areas where rice farming does not occur. For these localities, it is more plausible that aquarium disposal and/or fish stocking served as their original means of introduction, although supportive evidence currently is lacking and further evaluation of this question is necessary.

A field survey of *Elatine ambigua* conducted in Wachusett Reservoir in Boylston (Worcester County, Massachusetts) found several populations growing within the reservoir. We also observed that *E. ambigua* achieved a much larger population size than many of the other aquatic plant species and dominated most of the shallow areas of the reservoir. Being so abundant, this species clearly limits the growth of native species such as *E. minima*. In Wachusett Reservoir, *E. minima* populations are much smaller both in patch size and in the total number of individuals than the *E. ambigua* populations. At least in this

case, it seems that the introduction of *E. ambigua* has negatively impacted some of the native plant populations within the reservoir. Thus, we recommend more extensive studies of this species to elucidate whether it should be considered as an invasive species in the USA.

ACKNOWLEDGMENTS. We thank Robert S. Capers for assisting with the acquisition of specimens from several herbaria (AAH, BH, CANB, CONN, HPSU, QUE, SPWH, US, and W). We also thank the curators of those herbaria for providing these specimens, and in some cases, for permitting sampling for DNA analysis. We owe special thanks to Jamie Carr for his kind assistance with our field studies at Wachusett Reservoir and to Melissa Culina, Thomas Philbrick, and two anonymous reviewers for useful feedback during the preparation of this paper. We gratefully acknowledge the financial support received for field work and molecular analyses from Office of Undergraduate Research, University of Connecticut (IDEA Grant) and Bamford Foundation (Department of Ecology and Evolutionary Biology, University of Connecticut).

LITERATURE CITED

De Wit, H. C. D. 1964. Aquarium Plants. Blandford Press Ltd., London, UK.

DiTomaso, J. M. and E. A. Healy. 2007. Weeds of California and other Western States. Vol. 1. Aizoaceae–Fabaceae. UCANR Publications, Canada.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.

Les, D. H., S. W. L. Jacobs, N. P. Tippery, L. Chen, M. L. Moody, and M. Wilstermann-Hildebrand. 2008. Systematics of *Vallisneria* (Hydrocharitaceae). Syst. Bot. 33: 49–65.

Mason, H. L. 1957. A Flora of the Marshes of California. University of California Press, Berkeley and Los Angeles, CA.

Moody, K. 1989. Weeds Reported in Rice in South and Southeast Asia. International Rice Research Institute, Los Baños, Laguna, Philippines.

Swofford, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates. Sunderland, MA.

Tucker, G. C. 1986. The genera of Elatinaceae in the southeastern United States. J. Arnold Arbor. 67: 471–483.

——— and E. U. Grissom. 2012. Elatinaceae, pp. 684–685. *In*: B. G. Baldwin, D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, eds., The Jepson manual: Vascular plants of California, 2nd ed. University of California Press, Berkeley, CA.