Systematics of the Cosmopolitan Aquatic Genus *Elatine*

Author(s): Hamid Razifard, Aaron J. Rosman, Gordon C. Tucker, and Donald H. Les
Source: Systematic Botany, 42(1):73-86.
Published By: The American Society of Plant Taxonomists
URL: http://www.bioone.org/doi/full/10.1600/036364417X694584

Systematic Botany (2017), 42(1): pp. 73–86

DOI 10.1600/036364417X694584
Date of publication March 1, 2017

Systematics of the Cosmopolitan Aquatic Genus *Elatine*

Hamid Razifard,[1,4] Aaron J. Rosman,[2] Gordon C. Tucker,[3] and Donald H. Les[1]

[1]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269, U. S. A.
[2]Department of Natural Resources and the Environment, University of Connecticut, Storrs, Connecticut 06269, U. S. A.
[3]Department of Biological Sciences, Eastern Illinois University, Charleston, Illinois 61920, U. S. A.
[4]Author for Correspondence (hamid.razifard@gmail.com)

Communicating Editor: David Morgan

Abstract—The cosmopolitan genus *Elatine* (Elatinaceae) includes about 25 aquatic species of mostly diminutive aquatic plants, whose relationships have not been evaluated using a phylogenetic approach. The taxonomic study of this group has been complicated by the small stature of the plants, their minute reproductive structures, and their cosmopolitan distribution. Consequently, much uncertainty exists with respect to species delimitations, their geographical distributions, and interspecific relationships. To clarify the infrageneric classification of *Elatine* and to provide insights on interspecific relationships within the genus, we conducted a phylogenetic study of nearly all (24) of the currently recognized species using both morphological and molecular data. The tree topology obtained based on morphological data (including vegetative and reproductive characters) was less-resolved than the trees based on molecular data, derived from either nuclear (ITS) or two plastid regions (*matK/trnK* and *rbcL*). However, the tree topology obtained from combined morphological and molecular data was well resolved and placed the morphologically distinctive *E. alsinastrum* as the sister group of the remaining species, which fell within two major clades: a clade of 4-merous-flowered species and a clade of 3-merous species, within which was embedded a subclade of 2-merous species. Although a number of topological differences occurred between the ITS and plastid tree topologies, significant incongruence was observed only for the placements of *E. americana* and *E. hexandra*, possibly resulting from reticulate evolution. *Bergia*, the sister genus of *Elatine*, comprises larger species, which often are mostly helophytic but never truly aquatic. Ancestral state reconstructions based on the ITS tree indicated that a morphological reduction series (in stature and floral merosity) exists among *Elatine* species, which is best explained as a consequence of adaptation to their aquatic life. These phylogenetic analyses also have helped to clarify the infrageneric classification of the genus and to provide a better understanding of the natural and nonindigenous distributions of the species. The new monotypic section ***Elatine*** sect. ***Cymifera***, including *E. brochonii*, is described.

Keywords—Hybridization, ITS, morphology, *rbcL*, *trnK/matK*, waterworts.

As a genus of aquatic angiosperms, *Elatine* L. ("waterworts") includes about 25 species in exclusion of *E. rotundifolia*, which recently has been transferred to *Micranthemum* Michx., Linderniaceae (Razifard et al. 2016a). The name *Elatine* derives from the Greek *elatinos* (i.e. of the fir, of the pine), which was the ancient name for *Kickxia spuria* (L.) Dumort. (Plantaginaceae) (Quattrocchi 1999). Linnaeus (1753) later applied this name to the waterworts in the first volume of his *Species plantarum*.

Elatine and *Bergia* L. ("bergias") together comprise the small family Elatinaceae (Seubert 1845; Britton and Brown 1897; Niedenzu 1925). The family has been variously classified as related to Caryophyllaceae and Hypericaceae (cf. Tucker 1986). Recent molecular studies demonstrated Malpighiaceae as the putative sister family of Elatinaceae (Davis and Chase 2004). *Bergia* and *Elatine*, the two genera of the Elatinaceae, exhibit fundamental morphological differences, which can reasonably be attributed to their specific ecology. All *Elatine* species are aquatic and complete their life cycle either while completely submersed under water (in freshwater lakes, ponds, and vernal pools), or by growing as emergents on mudflats or similarly inundated substrates. Phenotypic plasticity is especially profound among *Elatine* species and enables them to tolerate these different environmental conditions. This plasticity is manifest as variation in shoot height, leaf shape, and flower size within *Elatine* species (Molnár et al. 2015; Fig. 1). Consequently, the mudflat forms often differ from the submersed forms in having larger flowers as well as more rigid stems, shorter internodes, and shorter, broader leaves (Fig. 1A, line drawing). In many cases, this high degree of variability has resulted in questionable new species reports, but further taxonomic studies have rendered numerous species names synonymous within the genus (e.g. Razifard et al. 2016a). In contrast to *Elatine*, submersed forms never have been reported in *Bergia*, a primarily tropical genus whose species persist mainly under more terrestrial conditions or at most as emergent wetland plants.

All but one *Elatine* species (*E. alsinastrum* L.) are opportunistic annual plants (Tucker 1986; Razifard et al. 2016c). Most *Elatine* species grow in the temperate regions of the world and are extremely rare or occur in small patches in their native habitat. Six species have been reported as threatened, endangered, or decreasing in population size: *E. alsinastrum*, *E. americana*, *E. brochonii*, *E. gussonei*, *E. macropoda*, and *E. minima* (IUCN 2016; USDA, NRCS 2016). On the contrary, the Eurasian *E. ambigua* ("Asian waterwort") and *E. triandra* ("three-stamen waterwort") have expanded their distributions to all continents except Antarctica, and also have spread quickly in the U. S. A. (Tucker and Razifard 2014; Rosman et al. 2016).

Seubert (1845) subdivided *Elatine* into two subgenera and three sections. In that classification, subgenus *Potamopitys* (Adanson) Seub. contained only *E. alsinastrum* L., which was distinguished from the other species by its whorled leaves. This species grows in Europe and North Africa and is differentiated further from all other *Elatine* species (subgenus *Elatine*) by the heterophylly of its submersed and emersed shoots, which exhibit morphologically distinct leaves (Popiela et al. 2013). All members of subgenus *Elatine* have opposite leaves, complete their life cycle as aquatic forms (submersed or emersed), and lack heterophylly (Tucker 1986). Subgenus *Elatine* is divided into two sections: section *Elatine* (= sect. *Elatinella* Seub.) and section *Crypta* (Nutt.) Seub. Section *Elatine* includes species that have flowers with six or eight stamens in two whorls; the remaining species, with two or three stamens in one whorl, are assigned to section *Crypta*. Mason (1956) noted that a variable number of stamens (between 3 and 6) could occur on single individuals of *E. heterandra* and expressed some doubt as to the applicability

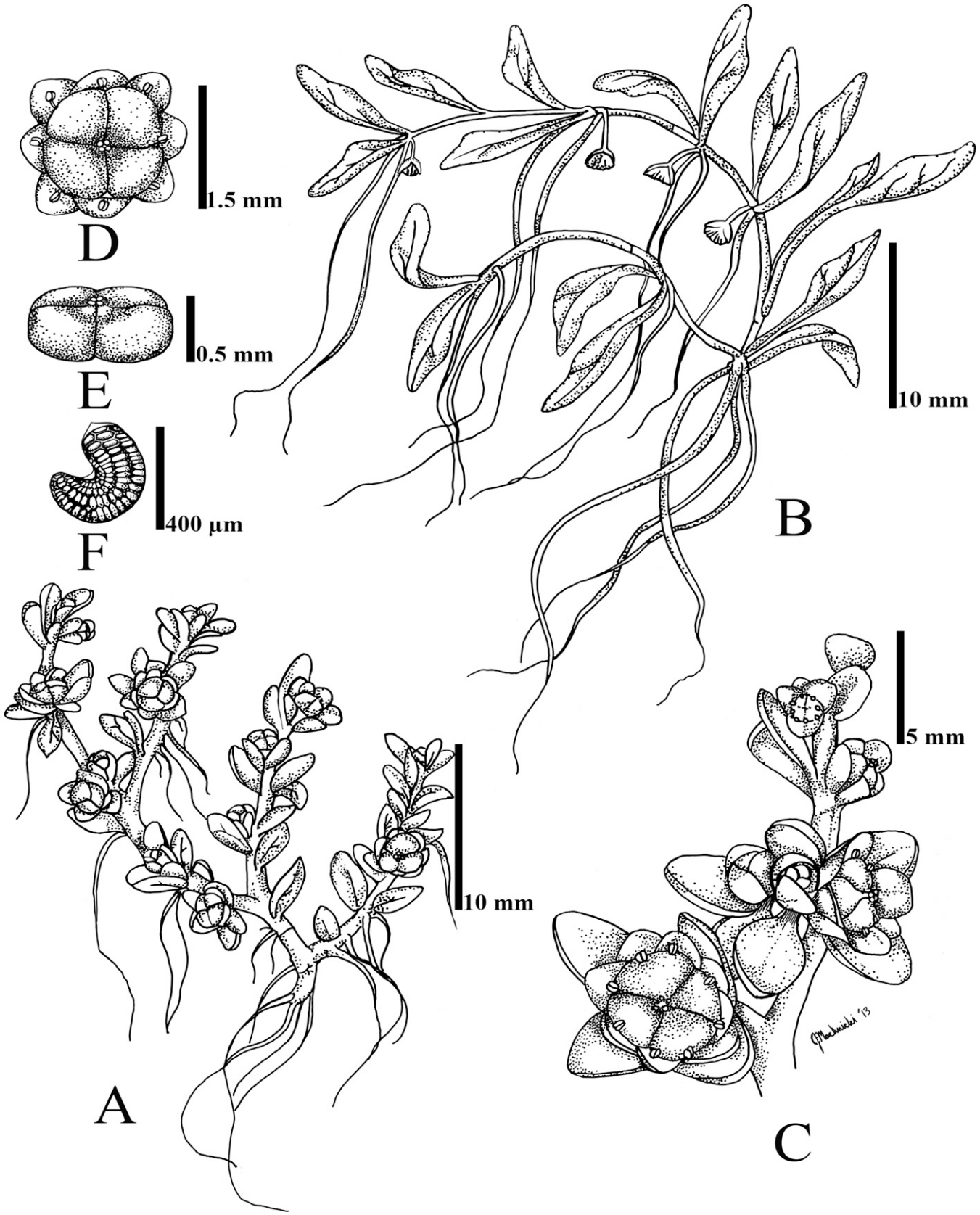

Fig. 1. The general morphology of *E. californica*. A. Emersed form. B. Submersed form. C. Magnified inflorescence. D. Flower with fully developed capsule (polar view). E. Fully developed capsule (equatorial view). F. Seed. The drawing was created by Jessica Machnicki (http://www.jessicamachnicki.com).

of stamen number for infra-generic classification of the genus (Tucker 1986).

Polyploidy is common in *Elatine* although the mechanism of polyploidization (auto- vs. allopolyploidy) remains unknown. The base chromosome number for the genus is $x = 9$ with the sporophytic chromosome number varying between 18 and 108 (Kalinka et al. 2015). *Elatine americana* ($2n$ = 70–72) and *E. hexandra* DC. ($2n$ = 72, 108) have the highest chromosome numbers reported for the genus (Probatova and Sokolovskaya 1986; Pogan and et al. 1990, Kalinka et al. 2015).

Most of the current taxonomic information for the genus is scattered among regional floras. The only monograph of *Elatine* was published by Seubert (1845), which treated only 10 of the presently recognized species. That monograph also was written well before the application of phylogenetic approaches to systematics. As a step toward a modern systematic treatment for *Elatine*, we have undertaken a phylogenetic approach, which for the first time incorporates both molecular data (derived from the nuclear internal transcribed spacer region [ITS], and plastid regions [*matK/trnK* and *rbcL*]), as well as morphological data (including both vegetative and reproductive characters). Our main objectives were to 1) test the previous morphologically-based subgeneric classification of *Elatine* using molecular analyses of a worldwide sample of taxa; 2) gain insights on the geographical origin of two cosmopolitan species (*E. ambigua* and *E. triandra*) in North America; and 3) evaluate the potential for hybridization within the waterworts, and any associated implications for the taxonomy of the group.

Materials and Methods

***Plant Material*—** Accessions of 24 of the previously recognized *Elatine* species (Tucker 1986) and two *Bergia* species (outgroup) were examined in this study (Appendix 1). *Elatine paramoana* Schmidt-M. & Bernal and *E. orthosperma* Dueb. were not included in our analyses due to lack of sufficient material. The *Elatine* accession included in our analyses represent all the major centers of biodiversity of *Elatine*, i.e. Africa, Asia, Australia, Europe, North America, and South America. Samples for DNA extraction were obtained from both dry herbarium specimens and freshly collected material. Voucher specimens were made for the fresh material and deposited at CONN.

***Morphological Data*—** Preliminary species identifications were made using the keys and descriptions provided by Britton and Brown (1897), Tucker (1986), Fernald (1941), and Cook (1968). Through direct observation, the accessions were scored initially for 35 morphological characters. However, seven of those characters were parsimony uninformative and were excluded from the analyses. Also, sepal and petal number were correlated, thus petal number was excluded from the dataset. The resulting dataset included a combination of 26 vegetative and reproductive characters (Table 1). Up to ten individuals per accession, up to 5 leaves, flowers, and fruits per individual, and up to 10 seeds per fruit were scored morphologically. Continuous characters (all maxima) were divided using break points according to the preceding literature. For plants with both submersed and emersed forms, an equal number of accessions from both forms were included. For *E. alsinastrum*, which has both emersed and submersed leaves, only the values for the emersed leaves were recorded due to shortage of submersed leaves among the material available for this study. Total leaf length (blade and petiole) and petiole length were measured separately because the two characters did not have a strong positive correlation (Spearman correlation = 0.38).

***Molecular Data*—** Genomic DNA was extracted from the same accessions used for obtaining the morphological data using the method of Doyle and Doyle (1987) . Both nuclear (ITS) and plastid regions (*rbcL* and *trnK/matK*) were amplified using the polymerase chain reaction (PCR). The PCR protocols and reagent concentrations were as described in Les et al. (2008). The ITS region was amplified using the forward and reverse primers (ITS4, ITS5) described by Baldwin (1992). The external primers described by Tippery et al. (2008) were used to amplify the *rbcL* and *matK/trnK* regions. Internal *rbcL* and *matK/trnK* primers were newly designed for accessions that did not yield a PCR product for *rbcL* or *matK/trnK* regions using the external primers. The internal primers designed for *rbcL* were: rbcLIntF (5′-ATGGGCTTACCAGTCTTGATCG-3′) and rbcLIntR (5′-AACAAAGCCCAGAGTGATTTCT-3′). The internal primers designed for *matK/trnK* were: trnkIntF (5′-GCCCTATGGTTCCAATTAT-3′) and trnkIntR (5′-AGACGATAATAATCGCAGAG-3′). All PCR products were visualized using agarose gel electrophoresis and SYBR-Green dye. Successful PCR reactions were sequenced as described by Tippery and Les

Table 1. Coding of the morphological characters analyzed in this study.

Character	Character state designation
1. Average plant height	tall (> 70 mm) = 0; short (< 70 mm) = 1
2. Stem form	unbranched (< 2 branches) = 0; branched (≥ 2 branches) = 1
3. Stem color	green = 0; red or reddish green = 1
4. Stem thickness	thin (< 3 mm) = 0; thick (> 3mm) = 1
5. Average internode length	long (> 8.5 mm) = 0; medium long (7.25–8.5 mm) = 1; medium (4.9–7.25 mm) = 2; short (< 4.9 mm) = 3
6. Leaf arrangement	opposite = 0; whorled = 1
7. Average leaf length	short (≤ 10 mm) = 0; long (> 10 mm) = 1
8. Average length to width ratio of leaves	≤ 3.61 = 0; > 3.61 = 1
9. Petiole length	short (< 1.06 mm) = 0; long (≥ 1.06) = 1
10. Petiole length to leaf length ratio	< 0.2 = 0; > 0.2 = 1
11. Leaf base	acuminate = 0; cordate = 1
12. Length to width ratio of stipules	> 2.06 = 0; ≤ 2.06 = 1
13. # of flowers per node	> 2 = 0; ≤ 2 = 1
14. Pedicel length	short (< 2 mm) = 0; long (> 2 mm) = 1
15. # of sepals	5 = 0; 4 = 1; 3 = 2; 2 = 3
16. Sepal tip shape	acute = 0; obtuse = 1
17. Sepal length to petal length ratio	< 1 = 0; >1 = 1
18. Stamen #	10 = 0; 8 = 1; 6 = 2; variable 1–6 = 3; 3 = 4; 2 = 5
19. # of stamen whorls	2 = 0; 1 = 1; variable = 2
20. Height to width ratio of capsules	≥ 0.67 = 0; < 0.67 = 1
21. Carpel #	5 = 0; 4 = 1; 3 = 2; variable 2–3 = 3; variable 2–4 = 4
22. Average # of seeds/capsule	> 50 = 0; 13–50 = 1; < 13 = 2
23. Seed shape	near straight (> 90°) = 0; near circular (< 90) = 1
24. Average # of pits in the longest row of the seeds	11–25 = 0; ≥ 25 = 1; <10 = 2
25. Average # of pit rows	> 3.61 = 0; < 3.61 = 1
26. Length to width ratio of seed pits	0.84–1 = 0; 0.36–0.84 = 1; ≤ 0.36 = 2

(2011) using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, California). Contig sequences were assembled using the program CodonCode Aligner 3.7.1 (CodonCode Corporation, Centerville, Massachusetts, available at http://www.codoncode.com/aligner/) and then aligned using the ClustalW algorithm as implemented in the phylogenetic software Mesquite ver. 3.04 (Maddison and Maddison 2015). A few sequences from previous work (Rosman et al. 2016) also were included in our datasets (Appendix 1). Insertions and deletions ('indels') in the ITS and *mat/trnK* datasets were scored as multistate characters using the modified complex indel coding method (MCIC) as proposed by Müller (2006); these data were included along with their corresponding dataset as a matrix of multi-state categorical data.

Phylogenetic Analyses — Aligned morphological and molecular datasets (Table 2) are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.69f22 (Razifard et al. 2017b). The phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) approaches. All MP analyses were conducted using PAUP* (Swofford 2002) with the following settings: starting trees were obtained by 100 random step-wise addition replicates, tree bisection and reconnection (TBR) was the branch-swapping algorithm, the maximum number of trees was set to 100,000, and polytomies were allowed. For datasets that returned the maximum number of trees before the end of each run, a new analysis was conducted by saving 1,000 most-parsimonious trees at each addition sequence (nchuck = 1,000). Bootstrap support (PBS) values for the parsimony analyses also were obtained using PAUP* by conducting 1,000 bootstrap replicates using same settings as those of the MP analyses, except for using one stepwise addition replicate and saving 1,000 trees during each bootstrap replicate (maxtrees = 1,000).

For ML and BI analyses on the 'morphology' and 'indels' datasets, the Mk model of evolution (Lewis 2001) was used, which allows equal probability of transitions between all character states. The molecular datasets (ITS, *matK/trnK*, and *rbcL*) were partitioned with each partition fitted to a specific evolutionary model. The ITS dataset was divided into 18S, ITS1, 5.8S, ITS2, and 28S partitions. The *matK/trnK* dataset was partitioned into coding and non-coding regions. The coding region of *matK/trnK* was further partitioned according to the first, second, and third codon positions. The *rbcL* dataset also was partitioned according to codon position. Models were selected using the program PartitionFinder (Lanfear et al. 2012), with the following chosen under the BIC criterion (Schwarz 1978) for the three data partitions: K80 + I for 18S, 5.8S, and 28S; TrNef + G for ITS1 and ITS2; K81uf + G for all *matK/trnK* partitions and *rbcL* third codon positions; and JC + I + G for *rbcL* first and second codon positions.

Maximum likelihood (ML) analyses were conducted using Garli 2.01 (Zwickl 2006) with two search replicates (searchreps = 2) for 10 million generations (stopgen = 10,000,000). For ML bootstrap analyses, one search replicate was used for 1,000 bootstrap replicates, with each run continued for one million generations. The remainder of settings were as default in Garli.

Bayesian inference (BI) was conducted using MrBayes 3.3.2 (Huelsenbeck et al. 2013). The number of Markov Chain Monte Carlo (MCMC) generations was set to 30 million with a sampling frequency of every 1,000 generations. Two independent runs, each with two simultaneous searches (four independent searches in total), were made. The convergence of results from the two runs was checked by comparing the final average standard deviation of split frequencies (which was < 0.005); Tracer 1.6 (Rambaut et al. 2013) was used to compare the final likelihood and estimated parameters.

The congruence of the different datasets was evaluated by visual inspection of the resulting tree topologies obtained from each separate phylogenetic analysis. In cases of perceived incongruence, several constraint analyses were conducted for all nodes independently using Garli. Each incongruent node was constrained to the position observed in the topology based on the alternative datasets, e.g. ITS versus chloroplast tree topologies and vice versa. The resulting site-specific likelihoods were analyzed using the approximately unbiased (AU) test (Shimodaira 2008) provided in the Scaleboot software package ver. 0.3–3 in R ver. 3.1.3 (R Core Team 2013). Incongruences with p values < 0.05 were considered significant. The '*matK/trnK*+indels' and '*rbcL*' datasets produced congruent topologies, thus the two datasets were combined and analyzed together as 'cpDNA'. Because the accessions of *E. americana* and *E. hexandra* were the source of significant incongruence between ITS and cpDNA datasets (see Results), they were excluded from the combined molecular data analyses ('combined DNA') as well as the combined analyses of morphological and molecular data ('combined morphology + DNA').

Morphological Evolution — All the morphological characters used for phylogenetic analyses (Table 1) were mapped on one of the most parsimonious trees obtained from the ITS dataset. Both character mapping and ancestral state reconstructions (ASRs) were made under the parsimony criterion using Mesquite.

Results

Attributes of the morphological and molecular datasets evaluated in this study are summarized in Table 2.

Morphological Data — The final morphological dataset ('morphology'), including only the informative characters, is provided in Appendix 2. Character 18 (stamen number) had the highest percentage of missing data (16.78%). Two accessions (*E. californica* [3] and *E. triandra* [4]; Appendix 1) had the largest percentage of missing data (55.56%). Among the 26 morphological characters examined, several character states were unique to one or two species. For example, two-merous flowers (characters 15, 18, and 21) were unique to *E. minima* and *E. lorentziana*. A variable number of stamens on the same individual was observed only in accessions of *E. heterandra*. Also, a variable number of carpels was observed in some accessions of *E. minima* (2–3), *E. brachysperma* (2–4), and *E. heterandra* (2–4). Also, two cases of additivity were evident in the morphological dataset. First, *E. hexandra* was intermediate morphologically between *E. brochonii* and *E. macropoda*. By its average petiole length (character 9; ≥ 1.06 mm) and petiole length to leaf length ratio (character 10; > 0.2), *E. hexandra*

Table 2. A Summary of the dataset attributes. Asterisks indicate cases where the maximum number of trees was obtained. Values in the last two columns ('combined molecular + indels' and 'all combined') reflect the exclusion of three *E. brochonii* accessions and six *E. americana* accessions (see Methods). MD = missing data; VC = variable characters; PIC = parsimony-informative characters; PP (BI) = maximum posterior probability from the Bayesian analysis. *Represents the number of accessions after removing the accessions of the potentially hybrid taxa and accessions with a large proportion (> 35%) of missing data.

	ITS	*matK/trnK*	*rbcL*	cpDNA (*matK/trnK* + *rbcL* + indels)	morphology	combined DNA	combined morphology + DNA
# accessions	128	140	140	137	147	121*	121*
# sites/characters	705 (694 nucleotides + 11 indels)	766 (760 nucleotides + 6 indels)	1,303 (0 indels)	1,819	26	2,524	2,550
% MD	2.98	1.94	11.08	10.37	4.60	9.14	9.32
# VC	207	114	54	158	26	388	414
# PIC	176	74	39	113	26	285	311
% PIC	24.96	9.41	3.78	6.21	100	11.29	12.19
# trees (MP)	92000	16003	98,000	100,000	1014	100,000	10,000
tree length (MP)	347	128	68	184	67	583	666
CI/RI (MP)	0.83/0.97	0.94/0.99	0.82/0.97	0.90/0.98	0.61/0.82	0.86/0.97	0.78/0.96
lnL (ML)	−2323.38	−1741.68	−1813.39	−3604.58	−235.78	−5900.34	−6215.86
PP (BI)	−2349.26	−1769.53	1837.51	446.87	−267.18	−5913.83	−6233.21

was more similar to the 8-stamened species of section *Elatine*. However, by its 3 sepals, 3 petals, 6 stamens, and 3 carpels, *E. hexandra* more closely resembled the 6-stamened species of section *Elatine*, i.e. *E. brochonii* and *E. madagascariensis*. *Elatine americana* was intermediate morphologically between *E. ambigua* and *E. chilensis*. Its green stems (character 3) and average stipule length to width ratio (character 12; ≤ 2.06) are most similar to *E. ambigua*; whereas, its seed pit length to width ratio (character 26; ≤ 0.36) are most similar to *E. chilensis*.

***Molecular Data*—**Among the three molecular datasets obtained in this study, the ITS dataset had the highest percentage of parsimony informative sites (24.96%). The *trnk/matk* and *rbcL* datasets had an intermediate (9.41%) and low (3.78%) percentage of parsimony-informative sites, respectively. After excluding accessions with a proportion of missing data > 30% (*E. brachysperma* [5, 9, and 11], *E. chilensis* [1 and 15], *E. fassettiana* [1], *E. hydropiper* [7], *E. macropoda* [4 and 5], *E. madagascariensis*, and *E. rubella* [8, 9, and 11], Appendix 1) and accessions exhibiting significant incongruence between ITS and cpDNA trees, the resulting combined DNA dataset (ITS+*trnK/matK+rbcL*) included 2523 nucleotide positions scored for 121 accessions.

***Phylogenetic Analyses (Morphological Data)*—**The phylogeny reconstructed using the morphological dataset (Fig. 2) was less resolved than those obtained from the molecular datasets (Figs. 3, 4, and 5). However, a few major clades were resolved, which essentially corresponded to the traditional subgeneric classification of the genus *Elatine*. All accessions of *E. alsinastrum* (the sole member of subgenus *Potamopitys*) resolved as a clade that was sister to the remaining *Elatine* species (subgenus *Elatine*). The members of subgenus *Elatine* with 6 or 8 stamens (traditionally categorized within section *Elatine*) did not form a distinct clade on the morphological tree. However, all members of this subgenus with four-merous flowers (*E. californica*, *E. gussonei*, *E. hungarica*, *E. hydropiper*, *E. macropoda*, and *E. ojibwayensis*) resolved as a clade with moderate to low internal support (MP BS = 74%, ML BS = 67%, and PP < 50%).

***Phylogenetic Analyses (Molecular Data)*—**Based on AU test results, instances of statistically significant incongruence ($p < 0.05$) in the placement of *E. americana* and *E. hexandra* were observed between the ITS and cpDNA trees (Figs. 3, 4). All significant incongruence between the ITS and cpDNA datasets was eliminated once the six accessions of *E. americana* and two accessions of *E. hexandra* were excluded. A few instances of statistically non-significant incongruence between the ITS and cpDNA tree topologies also were observed (dashed lines and letters in Fig. 4) as follows. First, contrary to the ITS topology, *E. alsinastrum* did not resolved separately from the rest of *Elatine* species in the cpDNA trees (branch A, Fig. 4). Second, the position of *E. macrocalyx* (branch B) differed by being placed within (by ITS) or separate from (by cpDNA) a clade including *E. triandra* and *E. ambigua* (Fig. 4). Third, the South American species (i.e. *E. ecuadoriensis*, *E. fassettiana*, *E. lorentziana*, and *E. peruviana*) resolved as a clade (MP BS = 78%, ML BS = 86%, and PP = 100%), which included the North American *E. minima* in the ITS tree; however, this was not the case in the cpDNA tree (Fig. 4).

Similar to the cpDNA trees (Figs. 3, 4), *E. alsinastrum* was placed in a clade including *E. brochonii* on the topologies obtained from the 'combined molecular' dataset (Fig. 5). Otherwise, the topology of the combined molecular data tree mostly supported the traditional infra-generic classification

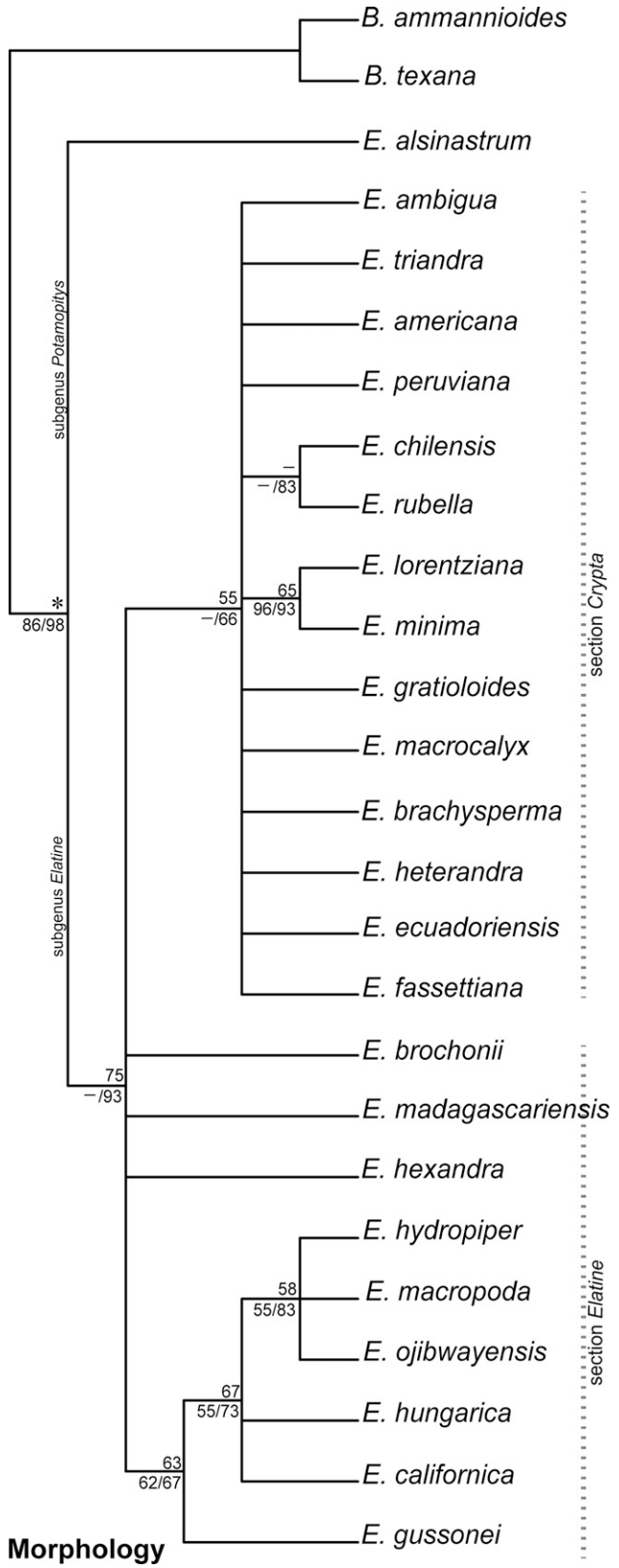

FIG. 2. MP topology (strict consensus) obtained using PAUP* based on the morphological data. Numbers above the branches represent MP BP; the first and the second numbers below the branches represent ML BP and Bayesian PP (converted to percentages), respectively. The asterisks (*) represent values equal to 100. Values < 50 are shown by –; support values are provided for only the nodes that received support > 50 in at least one of the three methods. Infrageneric classification of *Elatine* is shown by branch labels and dotted lines.

FIG. 3. The most parsimonious trees (ITS and cpDNA data) constructed using PAUP*. Tip labels include the species name and its associated geographical area. Multiple accessions of the same species are distinguished with a number that matches the accession number in Appendix 1. Dashed lines represent branches that were shortened to fit the illustration. A scale is provided for each tree.

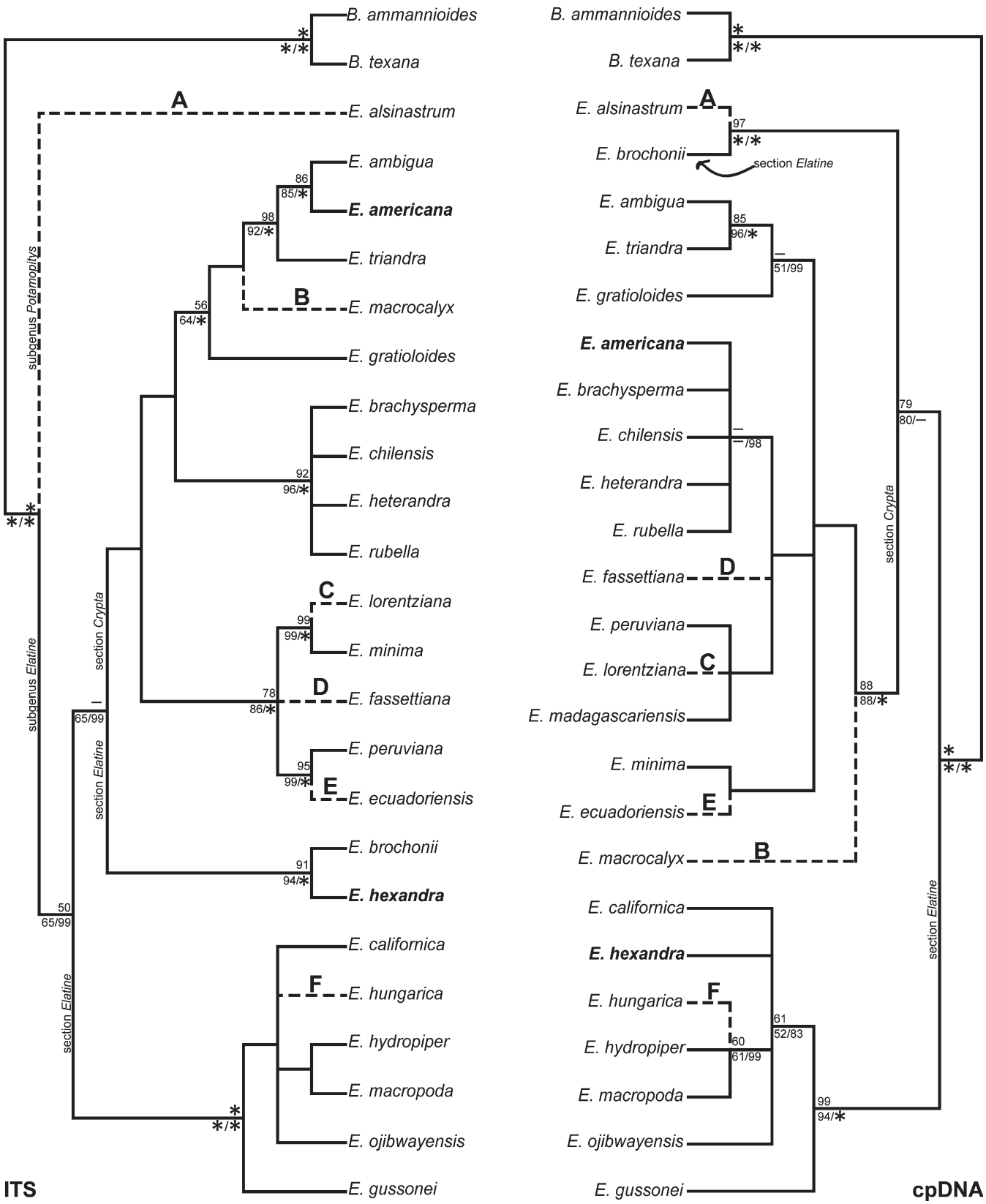

FIG. 4. Condensed ITS and cpDNA trees based on Fig. 3. Species with significant incongruence in their placement between the two trees are shown in bold. Dashed lines and their respective letters distinguish the nodes with non-significant incongruence. Support values are provided as in Fig. 2.

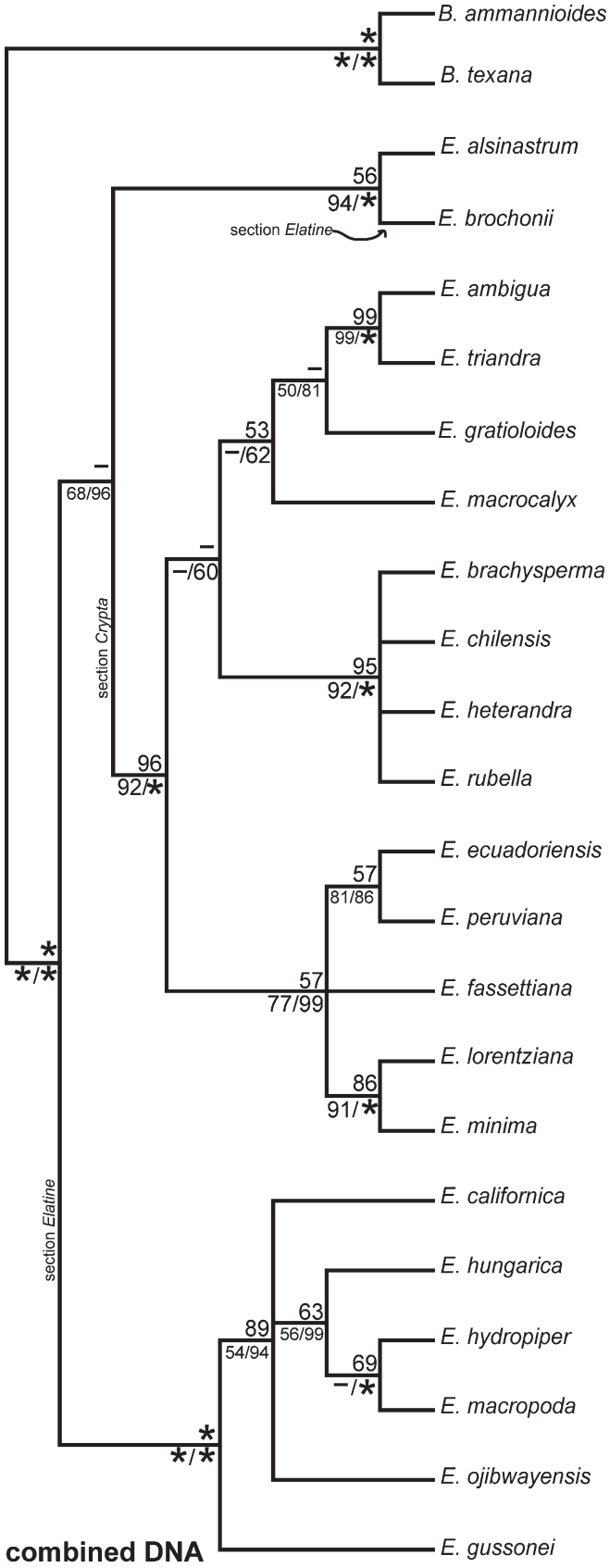

Fig. 5. The 50% majority-rule consensus tree topology built using MrBayes based on the combined molecular data ('combined DNA'). Species with multiple accessions (see Figs. 3, 4) are presented as one terminal branch. Support values are provided as in Fig. 2.

of the genus. With the exception of *E. heterandra*, all species belonging to section *Crypta* were resolved as a separate clade with high support (MP BS = 96%, ML BS = 92%, and PP = 100%). The members of section *Elatine* with 4-merous flowers also resolved as a clade with high support (all three support values = 100%). This result agreed with the topology of the morphology tree (Fig. 2), in which the four-merous species of section *Elatine* also resolved as a clade. Within section *Crypta*, a clade with mixed support (MP BS = 57%, ML BS = 77%, and PP = 99%) was observed for all of its New World members. Within this clade, the North American *E. minima* and South American *E. lorentziana* (the only *Elatine* species having 2-merous flowers), resolved in a clade having moderate to high internal support (MP BS = 86%, ML BS = 91%, and PP = 100%). The Australasian *E. gratioloides* and *E. macrocalyx* were placed together with the Eurasian *E. ambigua* and *E. triandra* within a clade of low statistical support (MP BS = 53%, ML BS < 50%, and PP = 62%). In all of the molecular tree topologies, the accessions of *E. brachysperma*, *E. chilensis*, *E. heterandra*, and *E. rubella* resolved only as a polytomy. This result was due to the fact that the ITS and cpDNA sequences of these taxa were nearly identical.

Phylogenetic Analyses of Morphological plus Molecular Data—After removing the accessions of *E. americana* and *E. hexandra* (sources of significant incongruence), the tree topologies derived from separate analyses of morphological and combined molecular data were in agreement. Therefore, the two datasets were combined and analyzed as one ('combined morphology + DNA'). The ML and BI topologies obtained from the combined data were identical to the topology derived from the combined molecular dataset (Fig. 5). However, the MP tree (Fig. 6) differed from the ML and BI topologies in the placement of *E. alsinastrum*. Similar to the 'morphology' and 'ITS' trees, *E. alsinastrum* (subgenus *Potamopitys*) resolved apart from the remaining *Elatine* species on the MP tree (Fig. 6), a result consistent with the traditional classification of the genus. All trees based on cpDNA and combined molecular datasets, as well as the ML and BI trees obtained from combined morphological and molecular data, similarly resolved *E. alsinastrum* in a clade with *E. brochonii*.

Morphological Evolution—The ASRs based on the ITS tree were depicted for only the morphological characters exhibiting notable evolutionary patterns between the infrageneric groups in *Elatine* (Fig. 6B–C). The node delimiting all members of subgenus *Elatine* showed a transition toward smaller average plant height (character 1), branched stems (character 2), and shorter average leaf length (character 7). The ancestral flower form reconstructed for the genus *Elatine* had 4 sepals, 4 petals, 8 stamens, and 4 carpels. The results of ASRs based on the cpDNA tree are not shown because of uncertainty in the ASRs; i.e. there were several equally parsimonious ancestral states for many of the nodes.

Discussion

The results of this study have provided new insights into the phylogeny, biogeography, extent of hybridization, and patterns of morphological evolution in *Elatine*. In the following sections, we discuss our findings with respect to their applicability for clarifying inter-specific relationships in *Elatine* as well as consequent improvements in the infrageneric classification of the genus.

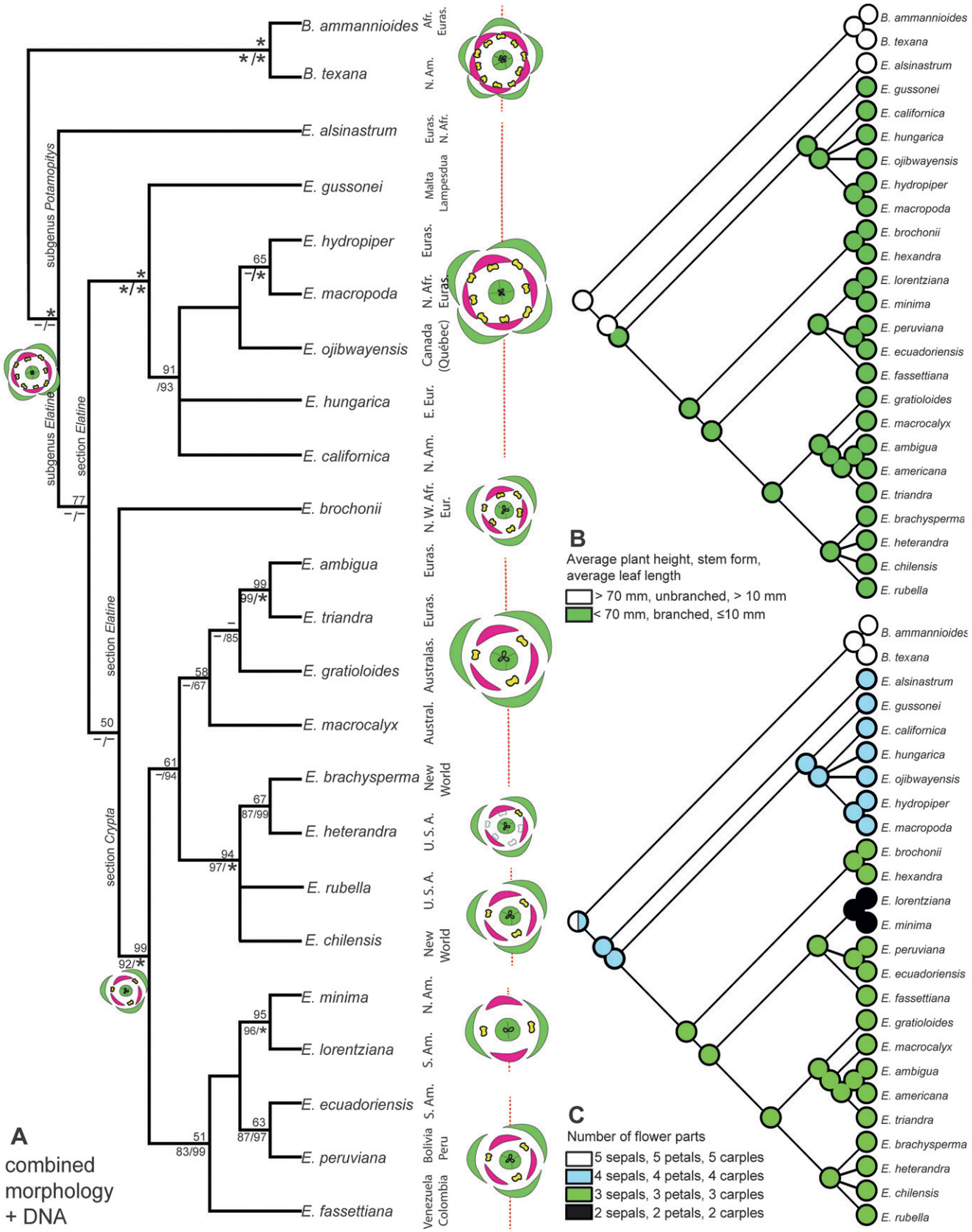

FIG. 6. A. Majority-rule (50%) consensus tree built using PAUP* based on the 'combined morphology + DNA' dataset. The floral structures are provided as diagrams between dashed red lines to the right of the tree. The floral diagram of *E. heterandra* demonstrates its variable number of stamens (1–6). The hypothetical forms of the ancestral flowers based on ASRs on the ITS tree are provided for two clades. The geographical range for each species is provided. The support values are provided as in Fig. 2. B, C. ASRs on characters with similar evolutionary patterns, using the ITS tree topology (Fig. 4).

Phylogeny of Waterworts—Several clades were consistent in all of the phylogenetic analyses conducted herein. First, all members of section *Crypta* resolved as a clade, which also included *E. heterandra* (assigned previously to section *Elatine* because of its variable number of stamens). Second, all members of section *Elatine* that have 4-merous flower parts grouped as a clade. Third, the 6-stamened species within section *Elatine*, except *E. hexandra* in the 'cpDNA' tree, resolved separately from the clade including the remaining members of that section (Figs. 3 and 4). Thus, the traditional taxonomy of subgenus *Elatine* requires some modification (discussed in *Taxonomic Evaluation*) in order to be compatible with the phylogenetic results.

The position of *E. alsinastrum* (the only member of subgenus *Potamopitys*) was not consistent in the phylogenetic analyses conducted here. In all the phylogenetic analyses based on morphological and ITS data, as well as the MP analyses of combined morphological and molecular data, *E. alsinastrum* consistently resolved apart from all other *Elatine* species. However, all analyses based on the 'cpDNA' dataset and the ML and BI analyses based on the 'combined DNA' dataset, supported a close relationship between *E. alsinastrum* and *E. brochonii*. However, results of our AU tests on ITS and cpDNA tree topologies indicated this to be a case of non-significant incongruence. Such incongruence may be attributable to long-branch attraction (reviewed by Bergsten 2005) considering the long branch that separates the clade of *E. alsinastrum* and *E. brochonii* from other species on the cpDNA tree topology (Fig. 3).

Biogeography of Waterworts—Disjunct Distributions—Our phylogenetic analyses revealed four cases of disjunct distributions within waterworts (Fig. 6): a) a Mediterranean-American disjunction within section *Elatine*, between *E. californica* and *E. ojibwayensis* (both endemic to North America) and the other species in section *Elatine* (all Old World species); b) a New World–Australasian disjunction within section *Crypta* between a clade of Eurasian/Australasian species (*E. ambigua*, *E. triandra*, *E. gratioloides*, and *E. macrocalyx*) and the New World members of section *Crypta*; c) a bipolar disjunction within section *Crypta*, between the North American *E. minima* and the southern South American *E. lorentziana*.

Various natural events have been proposed as mechanisms to explain the disjunctions observed in many groups of plants based on the age estimates derived from phylogenetic studies. Examples include long-distance dispersal, fragmentation of a Beringian ancestral range, migratory events between Old World and New World, and continental drift (e.g. Thorne 1972; Les et al. 2003; Wen and Ickert-Bond 2009). Without a chronogram, it is difficult to suggest the most plausible scenarios for the cases of disjunct distribution that occur within *Elatine*. Thus, for future studies, it would be useful to derive age estimates for *Elatine* based on those provided previously for Malpighiaceae (Davis et al. 2002) and the molecular data provided here.

Cosmopolitan Species—*Elatine ambigua* and *E. triandra* are the only waterworts whose biogeographic distributions extend beyond one or two continents (Tucker and Razifard 2014). Although genetically distinct (Figs. 3–6), these two species are highly similar morphologically (Fig. 2). We found only the average length of internodes (character 5) to be a useful character for separating accessions of the two species in this study. Due to their high degree of morphological similarity, many cases of misidentification exist among the herbarium records for these species. Thus, it is difficult to draw any firm conclusions on the biogeographic distribution of either species based solely on the basis of herbarium records. Also, both species grow in very similar habitats (e.g. in shallow areas of lakes, ponds, and rice fields) throughout their distributional range. In the New World, *E. ambigua* has been reported mostly from rice fields (DiTomaso and Healy 2007) and occasionally from lakes that are subjected to fish stocking (Rosman et al. 2016). However, *E. triandra* was reported often in ponds containing cultivated aquatic plants such as water lilies (Fernald 1917), and occasionally in undisturbed habitats (Fassett 1939). Both *E. ambigua* and *E. triandra* are popular aquarium plants (De Wit 1964, H. Razifard, pers. obs.). In fact, one accession of *E. ambigua* used in this study (accession 4, Appendix 1) was obtained through an internet forum specialized in aquarium plants. Therefore, human introductions as a result of rice farming, fish stocking, and aquarium disposal all could have contributed to the spread of these two morphologically and genetically similar species.

Both *E. ambigua* and *E. triandra* seem to be closely related to the Australasian waterworts *E. gratioloides* and *E. macrocalyx* (Figs. 3–6). However, the clades including these species did not receive high statistical support. Thus, it is difficult to determine the continent of origin for *E. ambigua* and *E. triandra* although the molecular analyses provided in this study would implicate an Asian origin for both species. In Europe, *E. triandra* was reported among macrofossils belonging to about 100,000 yr ago (Väliranta et al. 2009), although sufficient evidence (e.g. images) of the macrofossils were missing in that report. However, subfossil seeds of *E. triandra* have been found within samples from up to 5,400 yr of age from the Netherlands (Brinkkemper et al. 2008). These reports suggest that *E. triandra* already had been long-established in Europe through a long-distance dispersal event. However, considering that the seed morphology of *E. ambigua* is nearly identical to that of *E. triandra* (characters 24–27, Appendix 2), the reports of subfossil seeds of *E. triandra* from Europe could, in fact, apply to populations of both species. A previous study on these species revealed one state record of *E. ambigua* in Australia, one record new to Finland, and several state records in the U. S. A. (Rosman et al. 2016).

Implications of Reticulate Evolution—Two *Elatine* species, *E. americana* and *E. hexandra*, resolved with significantly incongruent placements in the ITS and cpDNA tree topologies (Figs. 3, 4). One possible explanation for such incongruence is reticulate evolution, i.e. hybridization. Based on the chromosome counts reported so far, *Elatine americana* ($2n$ = 70–72) and *E. hexandra* ($2n$ = 72, 108) clearly are polyploid, having the highest chromosome numbers known for the genus (Probatova and Sokolovskaya 1986; Pogan et al. 1990; Kalinka et al. 2015). Compared to the lower counts reported in all other *Elatine* species ($2n$ = 18, 54) the higher chromosome numbers as well as their differing placements between ITS and cpDNA tree topologies (Figs. 3, 4), support the possibility that *E. americana* and *E. hexandra* are of hybrid origin. By considering the pattern of morphological additivity with respect to other *Elatine* species (see "Morphological Data" in Results above), as well as the specific placements on ITS and cpDNA trees, one may be able to identify putative parental lineages of *E. americana* and *E. hexandra*. Accordingly, the paternal lineage of *E. americana* seem to be related

to *E. ambigua* and the maternal lineage to some unspecified lineage within the *E. chilensis* clade. It also seems plausible that *E. hexandra* is derived from a hybridization event involving *E. brochonii* and some unspecified lineage within the four-merous clade within section *Elatine*. Furthermore, the distribution of *E. americana* overlaps with its potential parental lineages within the western U. S. A. (Razifard et al. 2016b). Similarly, the distribution of *E. hexandra* overlaps with that of *E. brochonii* and other members of section *Elatine* in the Mediterranean Basin (Popiela et al. 2013). Thus, the biogeography of these waterworts is also consistent with the possibility of their hybrid origin.

Molecular data have proven to be useful for discovering the parental lineages of hybrid species. Several authors (e.g. Les et al. 2009; Hodač et al. 2014) have exploited ITS sequence polymorphisms as indicators of hybrid parental lineages, by identifying the specific alleles and then associating each with a different species. Unfortunately, the lack of divergent ITS sequences among a number of closely-related *Elatine* species precluded a similar approach here. Such results could arise due to concerted evolution of the ITS region, which occurs commonly in sexually-reproducing plants (Hodač et al. 2014) such as waterworts. To overcome this problem, we have conducted a subsequent study (Razifard et al. 2017a) utilizing sequences of low-copy-number nuclear region (e.g. *phytochrome C* or *phyC*), which are not subject to concerted evolution.

Morphological Evolution—*Elatine* species exhibit a clear phylogenetic trend towards an increasingly reduced morphology based on ASRs and the ITS tree topology (Figs. 6B–C). Reduced average plant height (character 1) and lower numbers of flower parts (characters 15, 18, and 21), along with a tendency toward more highly branched stems (character 2), potentially reflect some of the adaptations necessary for the maintenance of hydrophytic forms within subgenus *Elatine*. Morphological reduction is a common feature of aquatic plants and is believed to represent their adaptation to aquatic habitats (Sculthorpe, 1967; Les et al. 1997). By this interpretation, the amphibious, *E. alsinastrum* probably represents an early state in the transition from a terrestrial ancestor toward the truly aquatic species.

Taxonomic Implications—The results of our morphological and molecular analyses have provided a number of insights that can be used to improve the infrageneric classification of *Elatine*. This work has identified two additional characters (average plant height and stem form), which can be used for distinguishing subgenus *Potamopitys* (*E. alsinastrum*) from subgenus *Elatine*. We also observed that *E. hexandra*, along with the four-merous members of section *Elatine* are distinguishable from other *Elatine* species by their longer average petiole length, and greater petiole length to leaf length ratio. Our molecular analyses indicated the placement of *E. brochonii* in a position separate from the remaining species of section *Elatine*. The 2–5-flowered cymes (vs. solitary flowers) also distinguish *E. brochonii* from all other *Elatine* species (Cook 1968). We use these results as justification for recognizing *E. brochonii* within the monotypic section *Cymifera*, which is newly described here (see Taxonomic Treatment).

After excluding *E. brochonii* from section *Elatine*, and taking into account the hybrid origin of *E. hexandra*, section *Elatine* is redefined to include those members of subgenus *Elatine* with four-merous flowers (four sepals, four petals, eight stamens, and four carpels), an average petiole length $\geq$ 1.06 mm, and a petiole length to leaf length ratio > 0.2. In this revised classification, *E. hexandra* stands in a position intermediate between sections *Cymifera* and *Elatine*, and is separate from both sections.

Elatine heterandra, the only *Elatine* species with a variable number of stamens (1–6), formerly was placed within section *Elatine* (Tucker 1986). However, this species resolved within section *Crypta* in both the morphological and molecular analyses conducted in this study. Having mostly three-merous flower parts, *E. heterandra* is morphologically more similar to the species of section *Crypta* (Fig. 2) and being endemic to the U. S. A., has a geographical distribution more similar to New World species within sec. *Crypta* (e.g. *E. brachysperma* and *E. rubella*), than to the mostly Old World species within section *Elatine*. Thus, both morphological and geographical evidence supports the placement of *E. heterandra* within section *Crypta*. With this modification, section *Crypta* is redefined as those members of subgenus *Elatine* having solitary inflorescences, two to three sepals, two to three petals, and two to three carpels.

Considering the results of our molecular analyses, the inclusion of *E. heterandra* (with 1–6 stamens) in section *Crypta* clearly illustrates the inapplicability of stamen number as a sole criterion for distinguishing the sections within subgenus *Elatine*. Although we found no molecular divergence to exist among the accessions of *E. brachysperma*, *E. chilensis*, *E. heterandra*, and *E. rubella* for any of the loci we incorporated, we have preserved their status as separate species considering the consistent morphological differences among them. In this respect, all four species are interpreted to be of fairly recent origin.

Taxonomic Treatment

Elatine sect. **Cymifera** sect. nov. H. Razifard & D. Les—TYPE: *Elatine brochonii* Clav.

Elatine sect. *Elatine* Tucker (1986) (sect. *Elatinella* Seubert [1845]), pro minima parte.

Opportunistic herbs, submersed or growing on exposed but wet substrates. Stems decumbent to erect, branched, 1.5–5 cm long. Stipules lanceolate, margins dentate, apex acute. Leaves ovate, 2.5–4 mm long × 2.1–3.2 mm wide, light green to green, sometimes reddish in emergent plants; apex obtuse; base cuneate; margin entire, hydathodes present; petiole 0.1–0.5 mm. Inflorescences cyme with 2–5 flowers. Flowers sessile. Sepals broadly triangular, 3(4), green, usually equal, sometimes 1 reduced, connate until half the length; tip obtuse. Petals broadly triangular 3(4), white to pink, shorter in length than sepals, sometimes half as long. Stamens 6(8), usually shorter in length than petals. Carpels 3(4); styles 3 (4). Capsules globose, 3(4)-locular. Seeds 5–14 per locule, oblong, straight to slightly curved, length 2–3 times as width; surface pits hexagonal, length 1–2 times width, in up to 8 rows, 13–15 per row.

Elatine brochonii Clav. in Actes Soc. Linn. Bordeaux 37: 63. 1883.—TYPE: FRANCE. Gironde: Saucats, 08 Nov 1883, *Clavaud and Brochon 496* (holotype: BORD, isotype: KFTA, MPU (photo!), TOU (photo!)).

The description of this species is identical to the section. *Elatine brochonii* is a near threatened Mediterranean species (IUCN 2016), reported from Algeria, Morocco, Corsica,

France, and Spain. This species grows inside or on the edges of shallow lakes and vernal pools (Porto et al. 2012) and can be distinguished from other *Elatine* species by its axillary cymes with 2–5 flowers.

***Representative Specimens Examined*—**FRANCE. Saucats, *Neyraut s. n.* (W); *Neyraut s. n.* (W); MOROCCO. Kenitra, Mamora-Wald, ca. 15 km SW Sidi-Yahia-Rharb, *Podlech 53918* (W), PORTUGAL. Fernão Ferro, N 38°33′57″, W09 07′00″, *Porto s. n.* (CONN).

Acknowledgments. We thank Lowell Ahart, Attila Mesterházy, Hossein Akhani, Ursula King, Yu Ito, Stephan Mifsud, and Miguel Porto for providing samples of waterworts from California, Hungary, Iran, Ireland, Japan, Malta, and Portugal, respectively. Also, we greatly appreciate the kind assistance of directors and curators of the following herbaria for providing herbarium specimens, sampling permission, and/or samples for DNA analyses: AAU, AK, B, BH, CANB, CHSC, CONN, DNA, E, GZU, HUH, JEPS, MO, PERTH, QUE, SJNM, TNS, UC, US, and W. We benefitted from useful feedback from David Morgan (Associate Editor) and three anonymous reviewers during the preparation of this paper. Research was funded in part by Bamford Foundation (Department of Ecology and Evolutionary Biology, University of Connecticut). This study is a portion of the PhD dissertation of H. R.

Literature Cited

Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of ribosomal DNA in plants: An example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

Bergsten, J. 2005. A review of long-branch attraction. *Cladistics* 21: 163–193.

Brinkkemper, O., E. J. Weeda, S. J. P. Bohncke, and W. J. Kuijper. 2008. The past and present occurrence of *Elatine* and implications for palaeoenvironmental reconstructions. *Vegetation History and Archaeobotany* 17: 15–24.

Britton, N. L. and H. A. Brown. 1897. *An Illustrated Flora of the Northern United States and Canada*. vol. 2. New York: Dover Publications.

Cook, C. D. K. 1968. *Elatine* L. Pp. 295–296 in *Flora Europaea* vol. 2, eds. T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb. Cambridge: Cambridge University Press.

Davis, C. C., C. D. Bell, S. Mathews, and M. J. Donoghue. 2002. Laurasian migration explains Gondwanan disjunctions: evidence from Malpighiaceae. *Proceedings of the National Academy of Sciences USA* 99: 6833–6837.

Davis, C. C. and M. W. Chase. 2004. Elatinaceae are sister to Malpighiaceae; Peridiscaceae belong to Saxifragales. *American Journal of Botany* 91: 262–273.

De Wit, H. C. D. 1964. *Aquarium plants*. London: Blandford Press Ltd.

DiTomaso, J. M. and E. A. Healy. 2007. *Weeds of California and other western states* vol. 1. Oakland (California): UCANR Publications.

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

Fassett, N. C. 1939. Notes from the herbarium of the University of Wisconsin – XVII. *Elatine* and other aquatics. *Rhodora* 41: 367–377.

Fernald, M. L. 1917. The genus *Elatine* in Eastern North America. *Rhodora* 19: 10–15.

Fernald, M. L. 1941. *Elatine americana* and *E. triandra*. *Rhodora* 43: 208–211.

Hodač, L., A. P. Scheben, D. Hojsgaard, O. Paun, and E. Hörandl. 2014. ITS polymorphisms shed light on hybrid evolution in apomictic plants: A case study on the *Ranunculus auricomus* complex. *PLoS One* 9: e103003, doi: 10.1371/journal.pone.0103003.

Huelsenbeck, J., B. Larget, P. Mark, F. Ronquist, D. Simon, and M. Teslenko. 2013. MrBayes: Bayesian Inference of Phylogeny. http://mrbayes.sourceforge.net/download.php.

IUCN. 2016. The IUCN red list of threatened species. Version 2015–4. http://www.iucnredlist.org. Accessed 02 September 2016.

Kalinka, A., G. Sramkó, O. Horváth, V. A. Molnár, and A. Popiela. 2015. Chromosome numbers of selected species of *Elatine* L. (Elatinaceae). *Acta Societatis Botanicorum Poloniae* 84: 413–417.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Les, D. H., D. J. Crawford, R. T. Kimball, M. L. Moody, and E. Landolt. 2003. Biogeography of discontinuously distributed hydrophytes: a molecular appraisal of intercontinental disjunctions. *International Journal of Plant Sciences* 164: 917–932.

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

Les, D. H., E. Landolt, and D. J. Crawford. 1997. Systematics of Lemnaceae: inferences from micromolecular and morphological data. *Plant Systematics and Evolution* 204: 161–177.

Les, D. H., N. M. Murray, and N. P. Tippery. 2009. Systematics of two imperiled pondweeds (*Potamogeton vaseyi, P. genmiparus*) and taxonomic ramifications for subsection *Pusilli* (Potamogetonaceae). *Systematic Botany* 34: 643–651.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50: 913–925.

Linnaeus, C. 1753. *Species plantarum* vol. 1. Stockholm: Laurentii Salvii.

Maddison, W. P. and D. R. Maddison. 2015. Mesquite: A modular system for evolutionary analysis. Version 3.04. http://mesquiteproject.org.

Mason, H. L. 1956. New species of *Elatine* in California. *Madroño* 13: 239–240.

Molnár, A. V., J. P. Tóth, G. Sramkó, O. Horváth, A. Popiela, A. Mesterházy, and B. A. Lukács. 2015. Flood induced phenotypic plasticity in amphibious genus *Elatine* (Elatinaceae). *PeerJ* 3: e1473, doi: 10.7717/peerj.1473.

Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular Phylogenetics and Evolution* 38: 667–676.

Niedenzu, F. 1925. Elatinaceae. Pp. 270–276 in *Die Natürlichen Pflanzenfamilien* vol. 21, eds. A. Engler and K. Prantl. Leipzig: Verlag von Wilhelm Engelmann.

Pogan, E., A. Jankun, and Z. Sawicka. 1990. Further studies in chromosome numbers of Polish angiosperms, part 22. *Acta Biologica Cracoviensia. Series; Botanica* 31: 1–17.

Popiela, A., A. Łysko, and A. Molnár. 2013. Recent distribution of the Euro-Siberian sub-Mediterranean species *Elatine alsinastrum* L. (Elatinaceae). *Acta Botanica Croatica* 72: 375–386.

Porto, M., A. J. Pereira, R. Rego, J. Forminhão, A. Clemente, J. L. Vitorino, and C. Tauleigne-Gomes. 2012. *Elatine brochonii* Clavaud (Elatinaceae), a new species to the Portuguese flora. *Acta Botanica Malacitana* 37: 173–178.

Probatova, N. S. and A. P. Sokolovskaya. 1986. Chromosome numbers of the vascular plants from the far east of the USSR. *Botanicheskii Zhurnal SSSR* 71: 1572–1575.

Quattrocchi, U. 1999. *CRC world dictionary of plant names: Common names, scientific names, eponyms, synonyms, and etymology* vol. 3. Boca Raton: CRC Press.

R Core Team. 2013. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rambaut, A., M. Suchard, and A. Drummond. 2013. Tracer 1.6. A program for analyzing the results from Bayesian MCMC programs such as BEAST and MrBayes. http://tree.bio.ed.ac.uk/software/.

Razifard, H., D. H. Les, and G. C. Tucker. 2016a. Evidence for the Transfer of *Elatine rotundifolia* to Linderniaceae. *Systematic Botany* 41: 665–671.

Razifard, H., G. C. Tucker, L. Ahart, and D. H. Les. 2016b. Noteworthy collection: *Elatine americana* (Pursh.) Arn. (Elatinaceae). *Madroño* 63: 3–4.

Razifard, H., G. C. Tucker, and D. H. Les. 2016c. *Elatine* L. Pp. 349–353 in *Flora of North America vol. 12*, eds. Flora of North America Editorial Committee. New York: Oxford University Press.

Razifard, H., D. H. Les, and G. C. Tucker. 2017a. Reticulate evolution in *Elatine L.* (Elatinaceae), a predominantly autogamous genus of aquatic plants. *Systematic Botany* 42: 87–95.

Razifard, H., A. Rosman, G. C. Tucker, and D. H. Les. 2017b. Data from: Systematics of the cosmopolitan aquatic genus *Elatine*. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.69f22.

Rosman, A. J., H. Razifard, G. C. Tucker, and D. H. Les. 2016. New records of *Elatine ambigua* (Elatinaceae), a nonindigenous North American species. *Rhodora* 118: 235–242.

Schwarz, G. 1978. Estimating the dimension of a model. *Annals of Statistics* 6: 461–464.

Sculthorpe, C. D. 1967. *Biology of aquatic vascular plants*. New York: St. Martin's Press.

Seubert, M. 1845. Elatinarum monographia. *Academia Caesarea Leopoldino-Carolina Nova Acta* 21: 34–60.

Shimodaira, H. 2008. Testing regions with nonsmooth boundaries via multiscale bootstrap. *Journal of Statistical Planning and Inference* 138: 1227–1241.

Swofford, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.0. Sunderland: Sinauer Associates.

Thorne, R. F. 1972. Major disjunctions in the geographic ranges of seed plants. *The Quaternary Review of Biology* 47: 365–411.

Tippery, N. P., D. H. Les, D. J. Padgett, and S. W. L. Jacobs. 2008. Generic circumscription in Menyanthaceae: A phylogenetic evaluation. *Systematic Botany* 33: 598–612.

Tippery, N. P. and D. H. Les. 2011. Phylogenetic relationships and morphological evolution in *Nymphoides* (Menyanthaceae). *Systematic Botany* 36: 1101–1113.

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

Tucker, G. C. and H. Razifard. 2014. Elatinaceae. Pp. 542–546 in *Flora of Thailand* vol. 11, eds. T. Santisuk and H. Balslev. Bangkok: Department of National Parks, Wildlife, and Plant Conservation.

USDA, NRCS. 2016. The PLANTS Database. Greensboro, North Carolina: National Plant Data Team. http://plants.usda.gov. Accessed 02 February 2016.

Väliranta, M., H. H. Birks, K. Helmens, S. Engels, and M. Piirainen. 2009. Early Weichselian interstadial (MIS 5c) summer temperatures were higher than today in northern Fennoscandia. *Quaternary Science Reviews* 28: 777–782.

Wen, J. and S. M. Ickert-Bond. 2009. Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* 47: 331–348.

Zwickl, D. J. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. Dissertation. Austin: University of Texas.

Appendix 1. Voucher information and GenBank accession numbers for accessions examined. Following the herbarium acronym are the GenBank numbers (ITS, *matK/trnK*, *rbcL* respectively). Asterisks (*) represent previously published sequences. Missing sequences are represented by a dash sign (–). Cultivated accessions are designated as [cult.].

Bergia L. ***B. ammannioides*** **B. Heyne ex Roth**, NAMIBIA, Okavango. *Kolberg & Genspec 2283* (US), KU230363*, –, KU604811. ***B. texana*** **Seub. ex Walp.**, U. S. A., California: Modoc Co., (1) *Taylor 10487* (UC), KU604583, KU604693, KU604812; Butte Co., (2) *Ahart 19799* (CONN), KU230364*, KU604694, –, KU604813.

Elatine L. ***E. alsinastrum*** **L.**, AUSTRIA, Burgenland, (1) *Melzer 8465/4* (GZU), KU604584, KU604695, KU604814; (2) *Barta s. n.* (W), KU604585, KU604696, KU604815; HUNGARY, unspecified location, (3) *Ito & Mesterházy s. n.* (TNS), KU604586, KU604697, KU604816; GERMANY, Brandenburg, (4) *Dürbye 4310* (B), KU230362*, KU604698, KU604817; RUSSIA, Ryazan Oblast, (5) *Ctjabreva s. n.* (US), KU604587, –, –; ***E. ambigua*** **Wight**, AUSTRALIA, New South Wales, (1) *Hosking 3486* (CANB), KT970416*, KT970427*, KT970401*; FINLAND, Päijänne Tavastia Region, (2) *Nordström 949* (QUE), KT970417*, KT970429*, KT970403*; JAPAN, Kyoto, (3) *Tsugaru & al. 26948* (AAH), –, KT970432*, KT970406*; U. S. A., Arizona: (4), *Razifard 213* (CONN), KU604588, KU604699, KU604818, [cult.]; Connecticut: Middlesex Co., (5) *Murray 05-032* (CONN), –, KT970428*, KT970402*; California: Butte Co., (6) *Ahart 19061* (CONN), KU604589, KU604700, KU604819; (7) *Ahart 18723* (CONN), KU604590, KU604701, KU604820; (8) *Ahart 19380* (CONN), KT970414*, KT970425*, KT970399*; (9) *Ahart 19697* (CONN), –, KU604702, KU604821; (10) *Oswald 9974* (CHSC), KU604591, KU604703, KU604822; (11) *Razifard 198* (CONN), KT970418*, KT970430*, KT970404*; Sutter Co., (12) *McCaskill 735* (OSC), KU604592, KU604704, KU604823; Massachusetts: Worcester Co., (13) *Carr s. n.* (CONN), KU604593, KU604705, KU604824; (14) *Razifard 206* (CONN), KT970419*, KT970431*, KT970405*; South Carolina: Greenville Co., (15) *Douglass 2041* (BH), KT970415*, KT970426*, KT970400*; Virginia: King William Co., (16) *Wieboldt 4579* (US), –, KT970433*, KT970407*. ***E. americana*** **(Pursh) Arn.**, CANADA; Québec (1) *Deshaye 91-1422* (QUE), KU604594, KU604706, KU604825; (2) *Marie-Victorin & Germain s. n.* (GH), –, KU604707, –; U. S. A., California: Butte Co., (3) *Ahart 9477* (CONN), KU604595, KU604708, KU604826; (4) *Ahart 19966* (CHSC), –, KU604709, KU604827; Connecticut: New Haven Co., (5) *Brickmeier 26* (CONN), KU604596, KU604710, KU604828; Virginia: New Kent Co., (6) *Strong & Kelloff 1118* (US), KU604597, –, –. ***E. brachysperma*** **A. Gray**, U. S. A., California: Butte Co., (1) *Ahart 19234* (CONN), KU604598, KU604711, KU604829; Butte Co., (2) *Ahart 19411* (CONN), KU604599, KU604712, KU604830; (3) *Razifard 186* (CONN), KU604600, KU604713, KU604831; (4) *Razifard 187* (CONN), KU604601, KU604714, KU604832; Sonoma Co., (5) *Rubtzoff 5400* (GH), –, KU604715, KU604833; Tehama Co., (6) *Razifard 192* (CONN), KU604602, KU604716, KU604834; (7) *Razifard 194* (CONN), KU604603, KU604717, KU604835; (8) *Razifard 195* (CONN), KU604604, KU604718, KU604836; (9) *Oswald & Ahart 7079* (CHSC), –, KU604719, KU604837; Nevada: Washoe Co., (10) *Tiehm 3726A* (GH), KU604605, KU604720, KU604838; Texas: Jeff Davis Co., (11) *Hellquist 16664 & Schneider* (GH), –, KU604721, KU604839. ***E. brochonii*** **Clav.**, MOROCCO, Kenitra, (1) *Podlech 53918* (W), KU604606, KU604722, KU604840; PORTUGAL, Fernão Ferro, (2) *Porto s. n.* (CONN), KU604607, KU604723, KU604841. ***E. californica*** **A. Gray**, U. S. A., California: Butte Co., (1) *Ahart 19964A* (CHSC), KU604608, KU604724, KU604842; Lassen Co., (2) *Ahart 18882* (CONN), KU604609, KU604725, KU604843; (3) *Ahart 20294* (CHSC), KU604610, KU604726, KU604844; (4) *Ahart 20301* (CHSC), KU604611, KU604727, KU604845; (5) *Razifard 196* (CONN), KU604612, KU604728, KU604846; (6) *Razifard 197* (CONN), KU604613, KU604729, KU604847; Merced Co., (7) *Ahart 14674* (CHSC), KU604614, KU604730, KU604848; Modoc Co., (8) *Ahart 14979* (CHSC), –, KU604731, KU604849; (9) *Ahart 18723A* (CONN), KU604616, KU604732, –; (10) *Ahart 20354* (CHSC), KU604617, KU604733, KU604850; Tehama Co., (11) *Razifard 188* (CONN), KU604618, KU604734, KU604851; (12) *Razifard 190* (CONN), KU604619, KU604735, KU604852; (13) *Razifard 193* (CONN), KU604620, KU604736, KU604853; Nevada: Washoe Co., (14) *Tiehm 12615* (OSC), KU604621, KU604737, KU604854. ***E. chilensis*** **Gay**, U. S. A., Arizona: Apache Co., (1) *Heil & Clifford 23176* (SJNM), KU604622, –, KU604855; (2) *Walter & Walter 13458* (SJNM), KU604623, KU604738, KU604856; California: Butte Co., (3) *Ahart 9524* (CHSC), KU604624, KU604739, KU604857; (4) *Ahart 6954* (JEPS), KU604625, KU604740, KU604858; (5) *Ahart 19964* (CHSC), KU604626, KU604741, KU604859; Lassen Co., (6) *Ahart 18752* (CONN), KU604627, KU604742, KU604860; Plumas Co., (7) *Ahart 19023W* (CONN), KU604628, KU604743, KU604861; (8) *Ahart 19023AL*, (CONN), KU604629, KU604744, KU604862; (9) *Ahart 9311* (JEPS), KU604630, KU604745, KU604863; Shasta Co., (10) *Ahart 18779* (CONN), KU604631, KU604746, KU604864; Colorado: La Plata Co., (11) *O'Kane & al. 6608* (SJNM), KU604632, KU604747, KU604865; Nevada: Humboldt Co., (12) *Tiehm 11474* (OSC), KU604633, KU604748, KU604866; Elko Co., (13) *Tiehm 13061* (OSC), KU604634, KU604749, KU604867; Oregon: Harney Co., (14) *Otting 409* (OSC), KU604635, KU604750, KU604868; Linn Co., (15) *Johnston s. n.* (OSC), KU604636, KU604751, KU604869. ***E. ecuadoriensis*** **Molau**, ECUADOR, Loja: Lagunas de Compadre (1) *Terneus & Ramsay 127* (AAU), KU604637, KU604752, KU604870; (2) *Terneus & Ramsay 130* (AAU), –, KU604753, –. ***E. fassettiana*** **Steyerm.**, BOLIVIA, Chapare: (1) *Ritter & Nash 1325* (MO), –, –, KU604871; ECUADOR, Pichincha: Laguna de Yuyos, (2) *Terneus & Terneus 31* (AAU), –, KU604754, KU604872; Azuay, (3) *Ulloa & al. 1285* (MO), KU604638, –, KU604873. ***E. gratioloides*** **A. Cunn.**, AUSTRALIA, New South Wales, (1) *Crawford 7689* (CANB), KU604639, KU604755, KU604874; (2) *Crawford 6239* (CANB), KU604640, KU604756, KU604875; NEW ZEALAND, North Island, (3) *Lange 5332* (AK), KU604641, KU604757, KU604876. ***E. gussonei*** **(Sommier) Brullo, Lanfr., Pavone & Ronsisv.**, MALTA, Insel Gozo, (1) *Karl Rainer* (GZU), KU604642, KU604758, KU604877; Saptan Valley, (2) *Mifsud s. n.* (CONN), KU604643, KU604759, KU604878; (3) *Mifsud s. n.* (CONN), KU604644, KU604760, KU604879. ***E. heterandra*** **Mason**, U. S. A. California: Butte Co., (1) *Ahart 9523* (CHSC), KU604645, KU604761, KU604880; (2) *Ahart 5472* (CHSC), KU604646, KU604762, KU604881; (3) *Ahart 8729* (CHSC), KU604647, KU604763, KU604882. ***E. hexandra*** **DC.**, IRELAND, Galway, (1) *King s. n.* (CONN), KU604648, KU604764, KU604883; AUSTRIA, Steiermark, (2) *Gosch s. n.* (GZU), KU604649, KU604765, KU604884; Lower Austria, **(3)** *Melzer & Helmut s. n.* (GZU), KU604650, KU604766, KU604885. ***E. hungarica*** **Moeszi**, HUNGARY, Southern Hungary, (1) *Ito & Mesterhagy s. n.* (TNS), KU604651, KU604767, KU604886; (2) *Ito & Mesterházy 1626* (TNS), KU604652, KU604768, KU604887. ***E. hydropiper*** **L.**, AUSTRIA, Lower Austria: (1) *Barta s. n.* (W), KU604653, KU604769, KU604888; IRAN, Golestan, (2) *Akhani 17053* (CONN) KU604654, KU604770, KU604889; FINLAND, Vaasa, (3) *Kytövuori 3422* (QUE), KU604655, KU604771, KU604890; U. K., (4) *Razifard 212* (CONN), KU604656, KU604772, KU604891, [cult.]. ***E. lorentziana*** **Hunz.**, Falkland Islands: West Lagoons, *Lewis 1859* (E), KU604657, KU604773, KU604892. ***E. macrocalyx*** **Albr.**, AUSTALIA, Western Australia: Wheatbelt, (1) *Lyons & Lyons 4410* (PERTH), KU604658, KU604774, KU604893; (2) *Latz 17892* (PERTH), KU604659, KU604775, KU604894; (3) *Byrne 2264* (PERTH), KU604660, –, KU604895; South Australia: Epenarra Station, (4) *Risler & Duguid 954* (DNA), KU604661, KU604776, KU604896. ***E. macropoda*** **Guss.**, CANADA, Québec: Montreal Botanical Garden, (1) *Coursel s. n.* (MT), KU604662, –, KU604897, [cult.]; (2) *Morriest 91-045* (MT), KU604663, –, KU604898, [cult.]; (3) *Morriest 95-01* (MT), KU604664, KU604777, KU604899, [cult.]; FRANCE, Pays de la Loire, (4) *Préaubert & Bouvet s. n.* (W), KU604665, KU604778, –; Montrelais, (5) *Chevallier s. n.* (W), KU604666, KU604779, –; Varades (Loire inferieure), (6) *Chevallier s. n.* (GZU), KU604667, –, KU604900; GERMANY, Heidelberg Botanical Garden, (7) *Glück s. n.* (W), KU604668, KU604780, –, [cult.]. ***Elatine madagascariensis*** **H. Perrier**, MADAGASCAR, *Perrier de la Bathie s. n.* (P), –, KU604781, KU604901. ***E. minima*** **(Nutt.) Fisch. & C. A. Mey.**, U. S. A., Alabama: Hale Co., (1) *Haynes 10505* (UNA), –, KU604782,

KU604902; Connecticut: Litchfield Co., (2) *Capers & Selsky 1134/295* (CONN), KU604669, KU604783, KU604903; (3) *Razifard 05* (CONN), KU604670, KU604784, KU604904; (4) *Razifard 09* (CONN), KU604671, KU604785, KU604905; Tolland Co., (5) *Razifard 02* (CONN), KU230361*, KU604786, KU604906; (6) *Razifard 211* (CONN), KU604672, KU604787, KU604907; Massachusetts: Barnstable Co., (7) *Armstrong & al. s. n.* (SPWH), KT970420*, KT970434*, KT970408*; Worcester Co., (8) *Razifard 210* (CONN), KU604673, KU604788, KU604908; New Hampshire: Carroll Co., (9) *Hellquist 247-12* (CONN), KU604674, KU604789, –; Rhode Island: Providence Co., (10) *Les 1062* (CONN), KU604675, KU604790, KU604909. ***E. ojibwayensis*** **Garneau**, CANADA, Québec: TE Jamésie, *Deshaye 91-841* (QUE), KU604676, KU604791, KU604910. ***E. peruviana*** **Baehni & J. F. Macbr.**, BOLIVIA, Chapare, (1) *Ritter & Wood s. n.* (MO), KU604677, KU604792, KU604911; (2) *Ritter s. n.* (MO), KU604678, KU604793, KU604912. ***E. rubella*** **Rydb.**, U. S. A. California: Lassen Co., (1) *Ahart 18883* (CONN), KU604679, KU604794, KU604913; (2) *Ahart 20295* (CHSC), KU604680, KU604795, KU604914; (3) *Ahart 20297* (CHSC), KU604681, KU604796, KU604915; Modoc Co., (4) *Ahart 10292* (CHSC), KU604682, KU604797, KU604916; (5) *Ahart 14980* (CHSC), KU604683, KU604798, KU604917; (6) *Ahart 20351* (CHSC), KU604684, KU604799, KU604918; Riverside Co., (7) *Thorne & al. s. n.* (BH), KU604685, KU604800, KU604919; Tehama Co., (8) *Oswald & Ahart 7153.1* (CHSC), –, KU604801, –; Utah: San Juan Co., (9) *Mietty & al. 22937* (SJNM), –, KU604802, KU604920; Oregon: Harney Co., (10) *Mansfield 93-313* (CIC), KU604686, KU604803, KU604921; Malheur Co., (11) *Brainerd 1406* (CIC), KU604687, KU604804, KU604922; (12) *Mansfield 99-110* (CIC), KU604688, KU604805, KU604923; (13) *Mansfield 06-113* (CIC), KU604689, KU604806, KU604924. ***E. triandra*** **Schkuhr**, AUSTRIA; Steiermark, (1) *Crailsheim & Fuchs s. n.* (GZU), –, KU604807, KU604925; Lower Austria, (2) *Hörandl & al. 7108* (W), KT970424*, KT970436*, KT970410*; Lower Austria, (3) *Barta s. n.* (W), –, KU604808, KU604926; U. S. A., Connecticut: Hartford Co., (4) *Rosman s. n.* (CONN), KU604690, KU604809, KU604927; Litchfield Co., (5) *Razifard 06* (CONN), KT970423*, KT970438*, KT970412*; (6) *Razifard 07* (CONN), KU604691, KU604810, KU604928; (7) *Capers 1232* (CONN), KT970421*, KT970435*, KT970409*; Oregon: Clatsop Co., (8) *Harwood 6903-44* (HPSU), KU604692, –, –; Lincoln Co., (9) *Waggy s. n.* (HPSU), –, KT970439*, KT970413*; Pennsylvania: Berles Co., (10) *Les 1075* (CONN00181024), KT970422*, KT970437*, KT970411*.

Appendix 2. Morphological data scored for *Bergia* and *Elatine* species. Missing data are indicated by ?. The order of morphological characters is the same as in Table 1. Multiple character states shown in parentheses indicate instances in which two or more states of a character are present in the same species.

Bergia ammannioides: 00100020100001000100000000; ***B. texana***: 00100020100001000100000000; ***E. alsinastrum***: 00010120001000111010010001 1; ***E. ambigua***: 0100(0123)0(01)(01)(01)(01)(01)01102120410210011; ***E. americana***: 1100(12)0000001102120410210012; ***E. brachysperma***: 1100000000101021422000411011; ***E. brochonii***: 1100200000000002121200210000; ***E. californica***: 1100200(01)1100111111101111111; ***E. chilensis***: 1110300000000102120410210012; ***E. ecuadoriensis***: 1100?0000000102120 41?2?00?1; ***E. fassettiana***: 1100(12)0000000102120410210001; ***E. gratioloides***: 1100300000011021204102100 11; ***E. gussonei***: 1100100011001111111101111000; ***E. heterandra***: 1110300000011021203204100 11; ***E. hexandra***: 1100200011001121201210011; ***E. hungarica***: 1100200111001111111010111111; ***E. hydropiper***: 1100200111001011101011111111; ***E. lorentziana***: 1100200000011031305103200 01; ***E. macrocalyx***: 1100300000011021204102100 11; ***E. macropoda***: 1100(12)00111001(01)111110111(01)(10)11; ***E. madagascariensis***: 1100300000011221202012 10?1?; ***E. minima***: 1100300000011031305103200 01; ***E. ojibwayensis***: 1100100111001011101011101 11; ***E. peruviana***: 1100100000011021204102200 11; ***E. rubella***: 1110300000001021204102100 11; ***E. triandra***: 1100200000011021204102100 11.