WILEY-BLACKWELL

Cladistics 24 (2008) 218–239

Cladistics

10.1111/j.1096-0031.2007.00177.x

Cladistic analysis of Echinodorus (Alismataceae): simultaneous analysis of molecular and morphological data

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Accepted 8 May 2007

Abstract

Echinodorus is the second largest genus in the aquatic plant family Alismataceae. The genus is naturally distributed in the New World, but many species are known world-wide as popular aquarium plants. The views upon species delimitation and infrageneric classification of the genus have been controversial. Phenotypic plasticity of aquatic plants combined with reduced and presumably convergent morphological structures pose serious problems to classification, emphasizing the need for molecular-level data. A simultaneous cladistic analysis of molecular and morphological data was conducted to resolve the phylogeny of the genus. The results showed *Echinodorus* (as it is currently circumscribed) to be polyphyletic. None of the currently proposed infrageneric classifications of the genus were supported in the light of phylogenetic evidence. Also, many species and subspecies level rankings were found to be unnatural. Monophyly of *Echinodorus* is ascertained by separating *Helanthium* and the monotypic genus *Albidella* from *Echinodorus*. As a result, two new combinations (*Helanthium bolivianum* and *H. zombiense*) are made, and a detailed description of the genus *Helanthium* is provided.

Monocot phylogenetics has received great interest over the last years (Soltis et al., 2005). Within monocots, the Alismatales represent one of the oldest lineages and they therefore have a central role in the study of character evolution of the whole group (Soltis et al., 2005). Aquatic monocots are of special interest, as a longstanding hypothesis favors an aquatic origin for this major group of angiosperms (Les and Schneider, 1995). Within Alismatales Judd et al. (2002) recognized a large "aquatic clade" (Alismataceae, Aponogetonaceae, Butomaceae, Cymodoceaceae, Hydrocharitaceae, Juncaginaceae, Limnocharitaceae, Posidoniaceae, Potamogetonaceae, Ruppiaceae, Scheuchzeriaceae and Zosteraceae). This large clade was further subdivided in two, and the smaller subclade includes Alismataceae ($\approx$ 80 species and 12 genera), Butomaceae (monospecific), Hydrocharitaceae ($>$ 100 species in 18 genera), Limnocharitaceae (seven species in three genera) and Najadaceae ($\approx$ 40 species in one genus) (Les and Haynes, 1995; Les et al., 1997; Chen et al., 2004). Some studies have resolved Limnocharitaceae nested within Alismataceae (Les et al., 1997; Chen et al., 2004), while other studies suggest that they are sister lineages (e.g., Petersen et al., 2006). Butomaceae has been constantly resolved as sister to Hydrocharitaceae–Najadaceae clade, together they seem to be a sister to Alismataceae–Limnocharitaceae clade (Les and Haynes, 1995; Les et al., 1997; Chen et al., 2004).

Alismataceae phylogenetics and taxonomy have remained poorly understood, largely because of morphological reductions and presumed convergent adaptations in aquatic habitats (Les and Haynes, 1995). Most phylogenetic studies of the group (Les et al., 1997; Chen et al., 2004; Petersen et al., 2006) have concentrated on higher level taxonomy, and taxon sampling within Alismataceae have been insufficient to test the monophyly of the genera, or to study genus or species-level relationships. The two largest genera (*Echinodorus* Rich. *ex* Engelm., *Sagittaria* L.) comprises more than half of

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the species in the family, and only few species are included in each of the remaining ≈10 genera. However, especially the boundaries between *Echinodorus*, *Baldellia* Parl., *Caldesia* Parl., *Luronium* Raf. and *Ranalisma* Stapf are considered to be poorly defined (Cook, 1990).

Therefore it is highly important to understand the phylogenetics of genus *Echinodorus*. This genus includes ≈26 species (up to 62 according to Rataj, 2004), and its center of diversity lies in South America (Haynes and Holm-Nielsen, 1994). *Echinodorus* is widely known because of its worldwide importance in the ornamental aquarium plant trade (Haynes and Holm-Nielsen, 1994; Kasselmann, 2003; Lehtonen and Rodríguez Arévalo, 2005).

In recent years several revisions of the genus have been published, with controversial views upon species delimitation and infrageneric classification (Rataj, 1975; Haynes and Holm-Nielsen, 1994; Lot and Novelo, 1994; Rataj, 2004). Rataj (1975, 2004) has used both subgeneric and sectional ranks below genus, while Haynes and Holm-Nielsen (1994) rejected Rataj's sections, but accepted subgenera *Echinodorus* and *Helanthium*, the latter often misspelled as *Helianthium* (see Pichon, 1946).

However, none of the existing revisions have included any phylogenetic analyses, and therefore it is not surprising that several problems in existing classifications were discovered in a recent morphology-based phylogenetic analysis of the genus (Lehtonen, 2006). In this analysis *Echinodorus* was found paraphyletic, as the pseudostoloniferous species of the subgenus *Helanthium* were not placed in monophyletic *Echinodorus sensu stricto* (Lehtonen, 2006). *Albidella nymphaeifolia* was resolved as a basal member of the subgenus *Echinodorus*, although Fassett (1955), Rataj (1975, 2004), and Haynes and Holm-Nielsen (1994) classified it in subgenus *Helanthium*. Furthermore, the analysis rejected practically all of the sections proposed by Rataj (1975, 2004), and also questioned some synonymies made by Haynes and Holm-Nielsen (1986).

In this study our main goal is to investigate the phylogenetic relationships in *Echinodorus* by a simultaneous analysis (Nixon and Carpenter, 1996) of morphological and molecular characters. New DNA sequence data are added to the morphological data set modified from Lehtonen (2006) in order to unambiguously resolve both the phylogeny of the genus and the existing controversy in its taxonomy. Taxon sampling is expanded by adding several new outgroup terminals.

Materials and methods

Taxon sampling and classification adopted

Most of the material used for DNA sequencing in this study was collected from natural populations growing in Argentina, Bolivia, Ecuador, Mexico, Paraguay, Peru, Uruguay and Venezuela by the senior author together with field assistants. Some material was collected and kindly provided for the study by colleagues traveling in other countries. Herbarium material was used when species were not found in the field. Few taxa were collected from cultivated populations, but as hybrids are common in cultivation (Kasselmann, 2003), the purity of these taxa cannot be guaranteed. Ten taxa were coded only for the morphological data matrix, because no molecular data could be obtained (Appendix 1).

Although we believe that classification (including species delimitation) should be the result of an analysis instead of given a priori, we had to limit DNA isolation and sequencing to some individuals due to economical reasons. However, we tried to cover both the morphological and geographic variation in *Echinodorus* as widely as possible by selecting 50 specimens (including *Albidella* and *Helanthium*) of distinct populations for molecular studies (Appendix 1). These populations are used here as terminals instead of a priori delimited species, and therefore also the morphological data are coded for every sampled population. We identified and named the studied plants mainly according to Haynes and Holm-Nielsen (1994). Under this classification our molecular data represent 19 recognized species, but in some cases groups of morphologically united specimens did not match any species diagnosis of Haynes and Holm-Nielsen (1994) or others (Micheli, 1881; Fassett, 1955; Rataj, 1975, 2004). These taxa are treated as unnamed species (sp1, sp2, sp3, sp4). Haynes and Holm-Nielsen (1986, 1994) treated *E. longiscapus* and *E. grandiflorus* as *E. grandiflorus* ssp. *grandiflorus* and *E. floribundus* as *E. grandiflorus* ssp. *aureus*. This classification is in conflict with Rataj's (1969; 1975, 2004) opinion and it was also challenged by the morphological analysis (Lehtonen, 2006). Similarly, Rataj (1967, 1970, 1975) described species *E. gracilis*, *E. osiris* and *E. cylindricus*, which were later synonymized with *E. grisebachii*, *E. uruguayensis* and *E. paniculatus*, respectively, by Haynes and Holm-Nielsen (1994). On the other hand, Holm-Nielsen and Haynes (1985) described the species *E. eglandulosus*, which was not accepted as a separate species by Rataj (2004). *Echinodorus ovalis* was described by Sauvalle (1870), but considered as a synonym of *E. cordifolius* ssp. *cordifolius* by Haynes and Holm-Nielsen (1986, 1994). In these cases we determined our terminals using the narrow concept in order to test the contradicting classifications. In general, however, we tried not to strictly follow any existing classification, but to achieve a reliable basis for one.

We included several representatives of the other genera of Alismataceae (*Alisma*, *Baldellia*, *Caldesia*, *Ranalisma*, *Sagittaria*, *Wiesneria* Micheli) and one species of Limnocharitaceae to test the monophyly of *Echinodorus*. *Butomus umbellatus* (Butomaceae) served

as an outgroup species for the analyses. Although it is not clear whether Alismataceae and Limnocharitaceae should be considered a single family, they clearly form a clade. We consider *Butomus* to be an appropriate outgroup because of its basal position in the sister lineage of Alismataceae–Limnocharitaceae clade (Les and Haynes, 1995; Les et al., 1997; Chen et al., 2004). Our collections concentrated on *Echinodorus*, and most sequences for outgroup terminals were obtained from GenBank. Unfortunately most of the Alismataceae genera are still today very poorly sampled. ITS sequences were available for a variety of Alismataceae species, and *mat*K sequences for a couple of taxa, but otherwise useful sequences were not available. Missing data may cause problems for phylogenetic analyses, but the same is also true regarding insufficient taxon sampling (Zwickl and Hillis, 2002). Wiens (2006) recently revisited the effect of missing data and concluded that even highly incomplete taxa may improve the accuracy of the analysis. Encouraged by these findings we included in our analyses some taxa with ITS sequences and morphological data only. As a result, we coded 70 terminals for the total-evidence analysis.

DNA isolation, amplification and sequencing

Four DNA regions representing both nuclear and chloroplast genomes were selected for sequencing, as they have proved to have high substitution rates in previous phylogenetic studies (Cox et al., 1992; Baldwin et al., 1995; Hilu and Liang, 1997; Grob et al., 2004). The *mat*K region was sequenced from the chloroplast genome, and the second intron of *LEAFY*, 5S non-transcribed region (5S-NTS), and internal transcribed spacer regions (ITS1;2), including 5.8S gene were sequenced from the nuclear genome. The *mat*K region has been commonly used and recommended for phylogenetic studies because of its rapid evolution (Soltis and Soltis, 1998; Hilu et al., 2003). Similarly, ITS has been widely used (Soltis and Soltis, 1998), and studies where the second intron of *LEAFY* gene has been used have revealed great potential especially in lower-level phylogenies due to its high substitution rate and unproblematic amplification (Oh and Potter, 2003; Grob et al., 2004). 5S-NTS is a rapidly evolving locus and therefore useful in studies of closely related species (Sastri et al., 1992; Persson, 2000; Becerra, 2003; Lindqvist et al., 2003).

Total genomic DNA was extracted from silica-dried material by using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and following the instructions of the manufacturer. For herbarium samples, however, we used the modified protocol (30 min incubation, 450 μL of AP1, 50 μL of AE, 10 min elution) by Drábková et al. (2002).

The *mat*K gene was amplified using two external primers, MG1 and MG15 (Table 1). The following polymerase chain reaction (PCR) profile was used: initial incubation at 95 °C for 2 min, followed by 30 cycles of 1 min at 95 °C, 1 min at 60 °C and 1 min at 72 °C. The cycle ended with a 7 min extension at 72 °C. Fragments were purified with the QIAquick PCR purification kit (Qiagen) or with the E.Z.N.A. Cycle-Pure Kit (Omega Bio-tek, Doraville, GA). Four internal primers were used in sequencing: *mat*K-EF, *mat*K-E1F, *mat*K-8R and *mat*K-ER (Table 1, Fig. 1).

ITS1 and ITS2 regions were amplified and sequenced together with 5.8S gene by using primers ITS5, and ITS4 or ITS-ER (Table 1). The PCR profile and fragment purification were similar to those of *mat*K. Universal primers for ITS (ITS5 and ITS4) gave multiple bands on agarose gel for some taxa. To avoid this problem we designed a more specific primer (ITS-ER) that allowed us to get a clear single band in most cases. This primer failed in certain taxa (*Sagittaria*, *Alisma*, *Caldesia* and *Helanthium*), but in these cases ITS4 produced only one band and it was used instead.

Table 1
Primers used in PCR and sequencing

Primer	Sequence 5′ → 3′	Reference
MG1*	CTACTGCAGAACTAGTCGGATGGAGTAGAT	Liang and Hilu (1996)
MG15*	ATCTGGGTTGCTAACTCAATG	Liang and Hilu (1996)
matK-EF†	GAAGAATTCCAAAARGATTT	Present paper
matK-E1F†	ATTGCGATTTTTTCTATACGA	Present paper
matK-8R†	AAAGTTCTAGCACAAGAAAGTCGA	Ooi et al. (1995)
matK-ER†	TCCTTGATATCGAACATAATG	Present paper
ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)
ITS-ER	ATGCTTAAACTCRGCGGGTGA	Present paper
FLint2-F1	CTTCCACCTCTACGACCAGTG	Grob et al. (2004)
FLint2-R1	TCTTGGGCTTGTTGATGTAGC	Grob et al. (2004)
PI	TGGGAAGTCCTYGTGTTGCA	Cox et al. (1992)
PII	KTMGYGCTGGTATGATCGCA	Cox et al. (1992)

*Used only as PCR primers.
†Used only as sequencing primers.

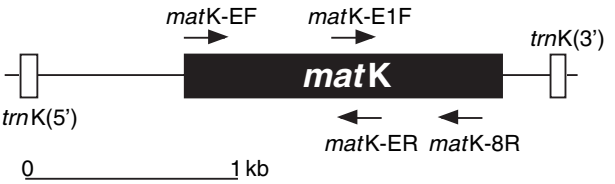

Fig. 1. Relative position of the primers used for sequencing *mat*K in this study.

The second intron of *LEAFY* gene was amplified and sequenced using two external primers, FLint2-F1 and FLint2-R1 (Table 1). The PCR profile and fragment purification were similar to *mat*K and ITS, except that 35 cycles were ran in PCR.

Primers used for 5S-NTS amplification and sequencing were PI and PII (Table 1). Numerous copies of 5S-NTS are present in plant genome, thus resulting in possibly incorrect statements of homology (Bailey et al., 2003). The pattern of DNA fragments run in agarose gel varied somewhat between groups of species and we cannot be certain that all sequenced fragments are actually truly orthologous. The PCR profile used for 5S-NTS was the same as for *LEAFY*, but fragments were run in 1% agarose gel and a single band was cut for purification by using the QIAquick Gel Extraction Kit (Qiagen).

We amplified the DNA in 25 μL reactions on a GeneAmp PCR System 9700. The reaction mixture contained Puretaq RTG PCR bead (Amersham Biosciences, Piscataway, NJ), 1 μL of each primer, 6 μL of DNA template, and 17 μL of ddH_2O. Sequencing was performed by using the BigDye Terminators v3.1 Cycle Sequencing Kit in an Applied Biosystems (AB) ABI PRISM® 377-XL DNA Sequencer (Applied Biosystems, Foster City, CA). The accuracy of sequences was ensured by sequencing and comparing the studied DNA regions in both directions; remaining ambiguities were coded using IUPAC ambiguity codes. Voucher specimens of the new sequences are deposited at TUR, UNA or AAU, and sequences in GenBank (Appendix 1).

Morphological data

Morphological characters from Lehtonen (2006) were partly recoded and supplemented with some new data and thus included 86 characters (Appendices 2 and 3). Coding is based on the study of both living plants in their natural habitats and herbarium specimens deposited in AAU, AMAZ, BM, FCQ, H, K, LPB, M, MEXU, MVJB, NY, QCA, QCNE, SI, TUR, U, UC, UNA and VEN (Appendix 4; herbarium acronyms according to Holmgren and Holmgren, 1998).

The analysis by Lehtonen (2006) was partly based on continuous overlapping characters, which were shown to be responsible for the finer-scale resolution in the resulting cladogram. In this study continuous data could not be used, as we analyzed our data with POY (Gladstein and Wheeler, 2001), which does not support the coding method used by Lehtonen (2006) as implemented in the program TNT (Goloboff et al., 2003). Many of these continuous characters were too variable to be recoded into discrete states in any reasonable way and were thus omitted. Others were recoded by using discontinuities as separation points for discrete character states. However, this led to some character states with wide variation. Characters were equally weighted, and they were coded as non-additive.

Phylogenetic analyses

We based our study on the concept of dynamic homology (Wheeler, 1996, 2001) by using direct optimization as implemented in POY (Gladstein and Wheeler, 2001).

Sequences were initially aligned with ClustalX (Thompson et al., 1997) using default parameters. Based on these alignments we divided both ITS and *LEAFY* sequences into three separate data partitions (ITS1, 5.8S, ITS2 and exon 2, intron 2, exon 3, respectively) to accelerate direct optimization (Giribet, 2001). This partition was done within regions that did not show variation between terminals. After data partitioning the gaps were removed and unaligned sequences were submitted to phylogenetic analyses.

The analyses were performed for five data combinations: morphology alone, chloroplast sequence alone (*mat*K), nuclear sequences combined (ITS, *LEAFY* and 5S-NTS), all molecular data combined, and combined total-evidence analysis of molecular and morphological data. Transitions, transversions and indels were given the same weight. Bremer support values (Bremer, 1988) were calculated for each data combination. Morphological data were analyzed with TNT (Goloboff et al., 2003), whereas molecular data and total-evidence analysis were analyzed with POY (Gladstein and Wheeler, 2001). The TNT-analysis was performed with 10 000 replicates, and by saving five trees per replicate. The command line used in the POY-analyses is given in Appendix 5. POY analyses were run in a parallel environment of eight processors of the IBMSC cluster in the CSC, the Finnish IT Center for Science.

In order to evaluate possible incongruence between different data sets and previously published results we calculated the minimum number of SPR moves required to convert a tree obtained from one data set into the other tree obtained from different data set, and corresponding tree-similarity measurements (100 sequences with five levels of stratification used throughout the analyses) (Goloboff et al., 2003). These minimum numbers of SPR moves were compared with moves required when 1000 pairs of random trees of equal number of terminals were compared. SPR moves were calculated

with TNT (Goloboff et al., 2003), and a simple script was used for random tree comparisons (loop 1 + 1 1000, rseed*, randtrees 2 0 0, sprdiff 0 1100 × 5, stop).

Results

Morphological data

Our morphological data yielded 1368 trees of 458 steps. The strict consensus tree (Fig. 2) is poorly resolved. Phylogenetic relationships of most Alismataceae genera remain unresolved, but *Helanthium* is resolved as a separate clade from *Echinodorus*. *Albidella nymphaeifolia* is sister to *Echinodorus*, and *E. berteroi* is sister to the rest of *Echinodorus*. Most groupings within *Echinodorus* are composed of populations of single species, but the species-level relationships are mostly unresolved. Nevertheless, *E. reticulatus*, *E. longipetalus*, *E. horizontalis* and *E. tunicatus* form a clade that is resolved with similar topology based on molecular data sets as well (note that no molecular data were available from *E. reticulatus*, but based on morphology its status as a species separate from *E. longipetalus* is highly questionable). This species group was placed in a clade together with several other species, including sp2, *E. uruguayensis–E. osiris* group, and a clade of *E. major*, *E. trialatus*, and *E. grisebachii* group. However, Bremer support values for these species-level groupings are mostly very low. The number of SPR moves required to change the obtained tree into *mat*K or total-evidence tree is equally low (Table 2), but a few more moves are needed for nuclear DNA tree.

Chloroplast data

The analysis of *mat*K data resulted in 12 equally parsimonious optimizations (trees) with a length of 829 steps (consensus tree represented in Fig. 3). *Alisma* is resolved as a basal species of the family, and *Limnocharis* is nested within Alismataceae. In this analysis *Helanthium* was resolved as a clade separate from *Albidella* and *Echinodorus*. *Caldesia* is a sister to *Sagittaria*, and this clade is a sister lineage to *Echinodorus–Albidella* clade. *Echinodorus berteroi* is resolved as a basal species of *Echinodorus*, and *Albidella* is nested within *Echinodorus sensu stricto*. Several clades are recognized in *Echinodorus sensu stricto*, the largest one without much internal resolution. The relatively derived position of *E. grisebachii* contradicts its placement in other analyses, but its position in the *mat*K tree is poorly supported based on Bremer values. Eight SPR moves are needed to convert the *mat*K tree into morphological, nuclear, or total-evidence tree (Table 2).

Nuclear data

The combined analysis of nuclear DNA data resulted in three equally parsimonious optimizations with a length of 3106 steps (Fig. 4). *Albidella* is resolved as a basal lineage in Alismataceae, and it is followed by the *Wiesneria–Sagittaria* clade. Interestingly, the *Alisma–Baldellia* clade separates *E. berteroi* from the main bulk of *Echinodorus*. Otherwise the topology in the main group of *Echinodorus* is congruent with the *mat*K tree, only the positions of the *E. bracteatus* and *E. grisebachii* are changed. The large clade that remained unresolved in *mat*K analysis is almost fully resolved by nuclear data, but in many cases populations belonging in separate species are mixed with each other.

Combined DNA analysis

The combined analysis of all molecular data resulted in three equally parsimonious optimizations with a length of 3956 steps, but a one-step shorter tree (3955 steps) was found during the Bremer support calculations (Fig. 5). The topology of the tree is mostly similar to the nuclear DNA topology, with the following exceptions: *E. berteroi* is resolved as a basal species in *Echinodorus sensu stricto*, the resolution in *E. major* (incorrectly named as *E. martii* in Haynes and Holm-Nielsen, 1994) and *E. grisebachii* clades is slightly changed, as well as the topology in the large *E. cordifolius* clade. The optimal combined DNA tree requires six SPR moves to be converted into nuclear DNA tree, or seven to be converted into *mat*K tree (Table 2). Twelve SPR moves are required to convert the tree into morphology-based tree.

Total evidence of molecular and morphological data

Simultaneous analysis of all molecular and morphological data resulted in a single parsimonious optimization of 4541 steps (Fig. 6). *Echinodorus sensu lato* is a polyphyletic group, as *Albidella* is resolved in a basal position to the family, *Helanthium* as a sister to *Ranalisma*, and *Echinodorus sensu stricto* is a separate clade. *Limnocharis* was resolved as a sister to *Caldesia* based on DNA, but this position changed in the simultaneous analysis, and *Limnocharis* is resolved within *Wiesneria–Sagittaria* clade. Within *Echinodorus* the topology differs only slightly from the topology of combined DNA analysis. Populations of *E. floribundus*, *E. longiscapus*, *E. cordifolius*, *E. ovalis*, sp1, sp3 and sp4 were mixed with each other in the combined DNA analysis, but when morphological data are included they form clades that correspond well with generally recognized taxa. Similarly, *E. heikobleheri* and *E. gracilis* are grouped within *E. grisebachii* in the

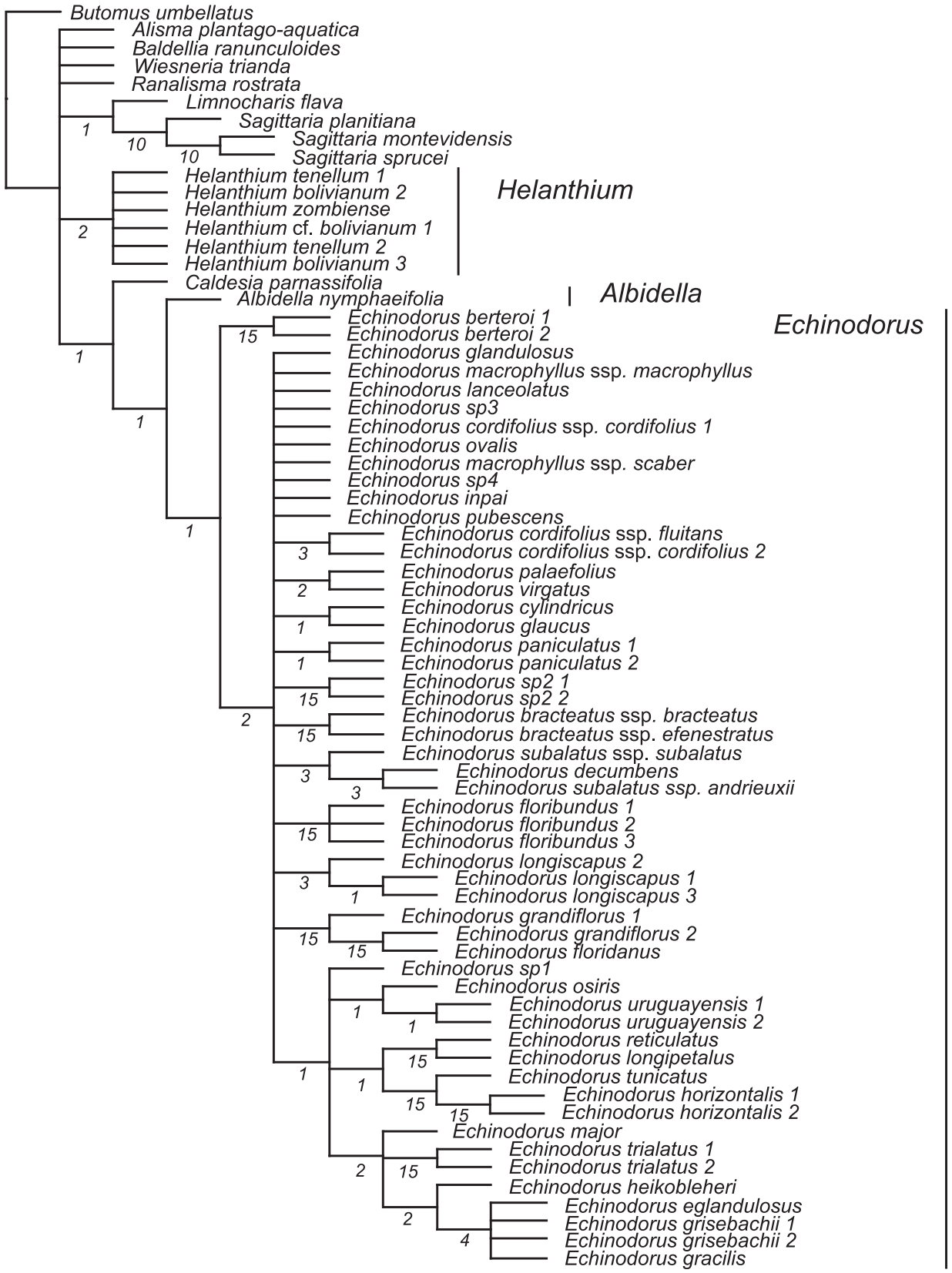

Fig. 2. Strict consensus tree of 1368 trees based on morphological characters. Bremer support values are indicated below branches.

Table 2
The minimum number of SPR moves required to convert a tree into another. The upper diagonal shows the number of SPR moves needed to convert trees obtained in this study, and the similarity measures. Lower diagonal shows the number of SPR moves expected on the basis of 1000 random tree pair comparisons with the same number of terminals

	Morphology	*mat*K	Nuclear DNA	Total DNA	Total evidence
Morphology	–	8 (0.8824)	11 (0.8382)	12 (0.8824)	8 (0.8824)
*mat*K	45–47	–	8 (0.8824)	7 (0.8824)	8 (0.8824)
Nuclear DNA	57	45–47	–	6 (0.8824)	8 (0.8824)
Total DNA	58	45–47	57	–	7 (0.8971)
Total evidence	68	45–47	57	58	–

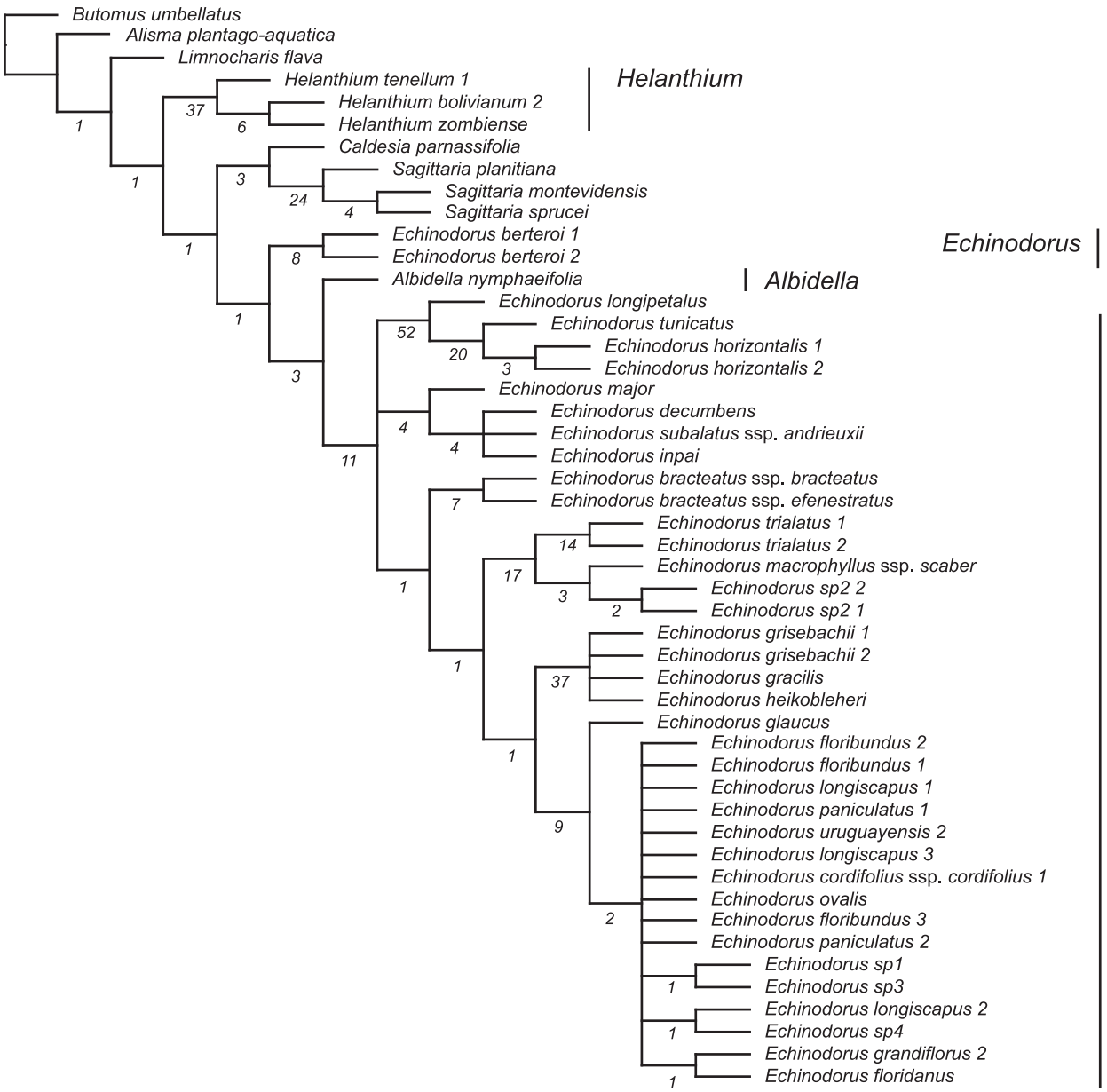

Fig. 3. Strict consensus tree of 12 trees based on the *mat*K DNA analysis. Bremer support values are indicated below branches.

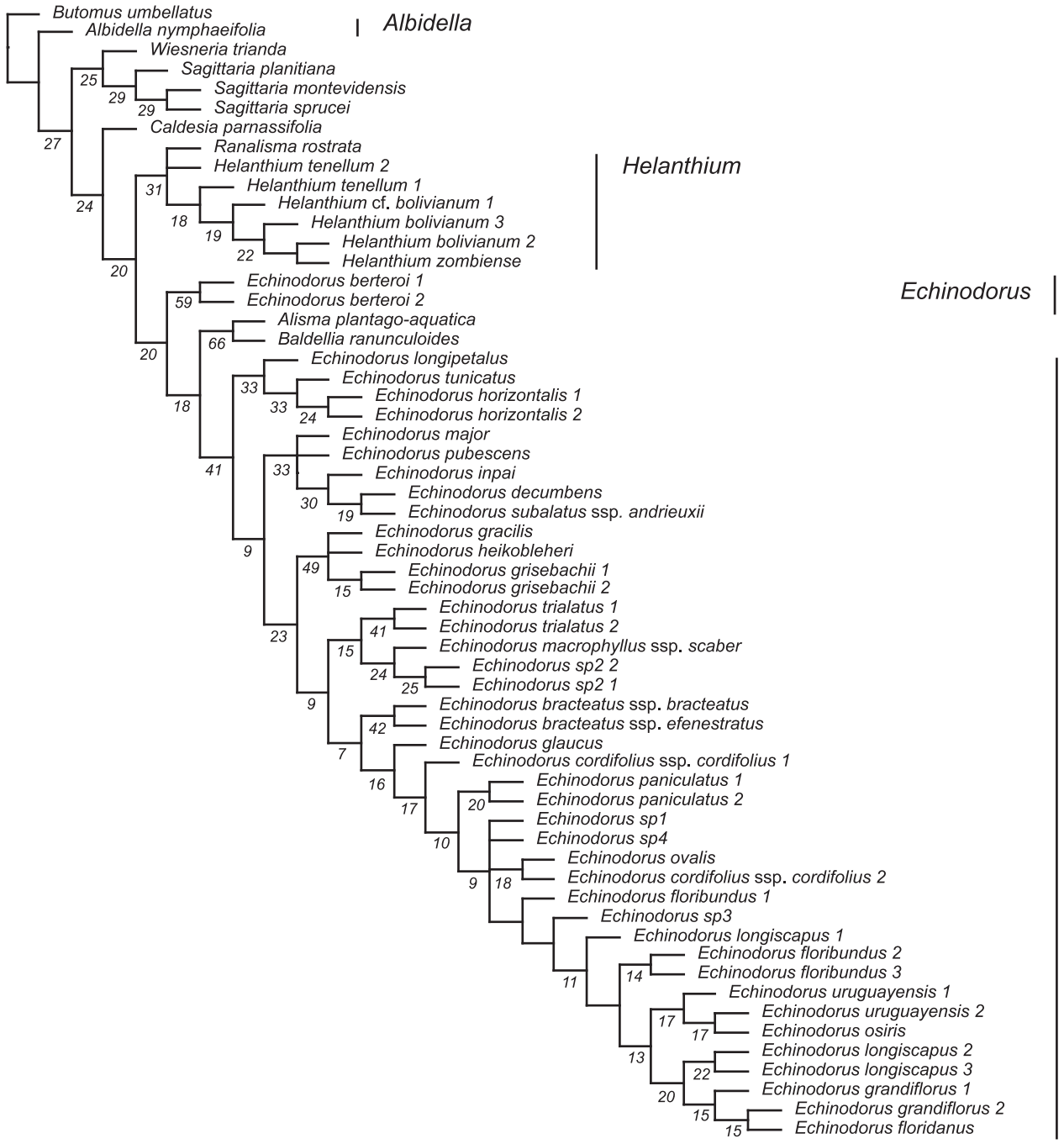

Fig. 4. Strict consensus tree of three trees based on the nuclear DNA analysis. Bremer support values are indicated below branches.

combined DNA analysis, but based on total evidence *E. heikobleheri* is sister to *E. gracilis–E. grisebachii* group.

Seven or eight SPR moves are required to change the total-evidence tree into any other tree produced in our analyses (Table 2), compared with 68 moves expected for random trees. However, when we transformed our total-evidence tree into the previously published tree that was based on discrete and continuous morphological characters (Lehtonen, 2006), a total of 15 SPR moves (similarity 0.5833) were required (29–33 moves expected on the basis of 1000 random tree pairs). Considerably more moves (21 SPR moves, similarity 0.4167) were required to convert the tree for continuous data (Lehtonen, 2006) into our total-evidence tree.

Fig. 5. Shortest tree found in a combined analysis of all molecular data. Bremer support values are indicated below branches.

Discussion

Comparison with previous studies

The phylogenetic analyses of Alismataceae made by Les et al. (1997) and Chen et al. (2004) were based on *rbc*L sequence only, but included representatives from over a half of the genera. Both analyses resolved *Limnocharis* together with *Hydrocleys* within Alismataceae (Les et al., 1997; Chen et al., 2004). Our analyses did not include *Hydrocleys*, but *Limnocharis* was placed within Alismataceae. However, we were unable to sequence nuclear DNA of *Limnocharis*. Furthermore, our analyses of different data sets resulted in varying topologies at the deeper phylogenetic level, and the total-evidence tree is incongruent with the published *rbc*L (Les et al., 1997; Chen et al., 2004) and mitochondrial (Petersen et al., 2006) phylogenies. Therefore we advocate further studies with emphasis on wider taxon and character sampling in currently recognized

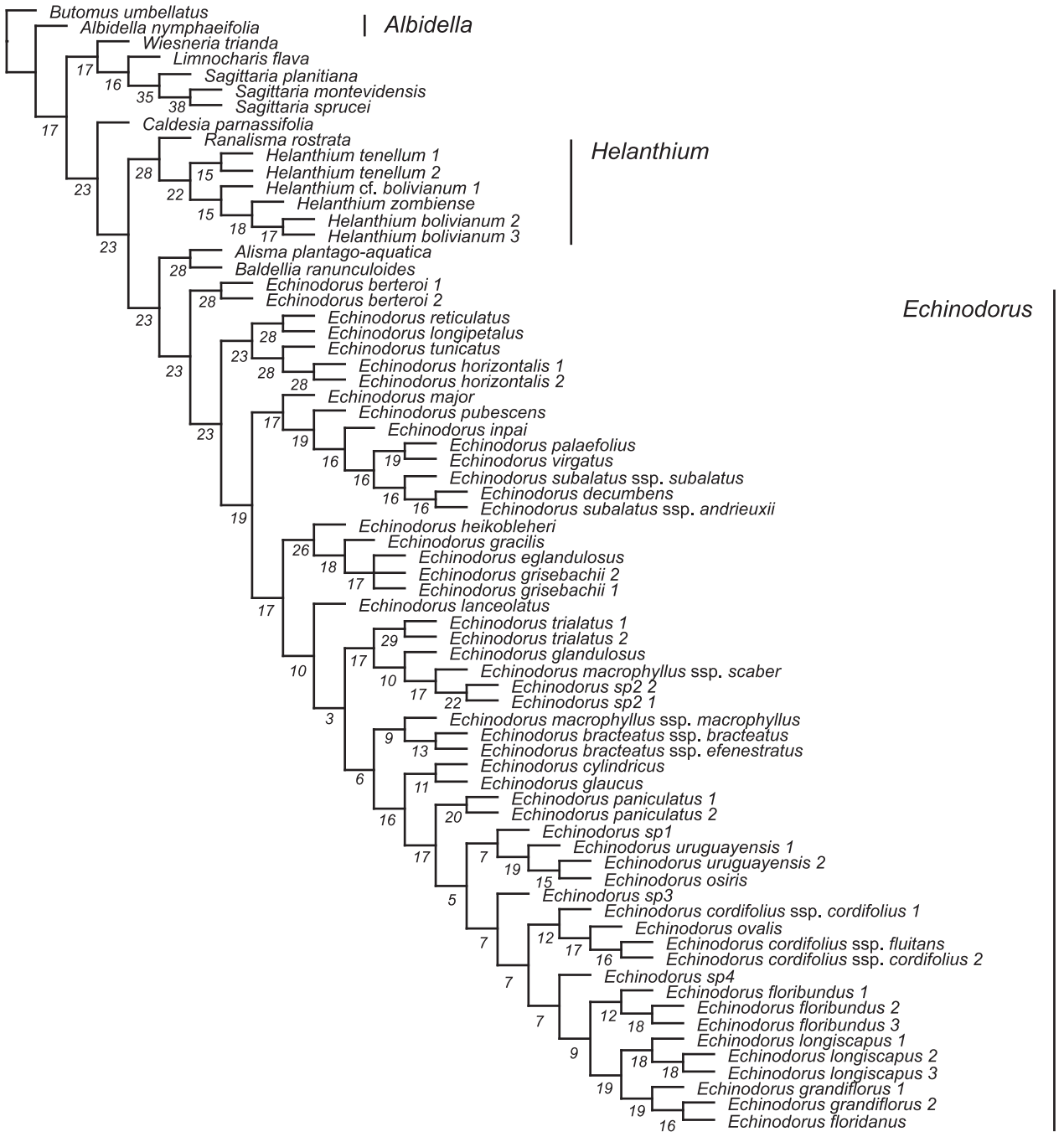

Fig. 6. Shortest tree found in the total-evidence analysis. Bremer support values are indicated below branches.

Alismataceae and Limnocharitaceae to resolve their ambiguous relationship.

However, our main goal in this study was not to investigate phylogenetics of the whole Alismataceae, but to concentrate in lower-level phylogenetic patterns within one of the largest genus of the family, *Echinodorus*. The phylogeny obtained here differs in many respects from the results of a previous analysis that was based on morphology (Lehtonen, 2006), but has some common features as well. The basal position of *E. berteroi* in *Echinodorus sensu stricto* is supported both in our total-evidence and in prior morphology-based analysis. *Echinodorus longipetalus* group is also resolved in the same way in the two analyses, but the phylogenetic position of the group is different. Close relations of many species hypothesized in the previous morphology-based

study (Lehtonen, 2006) are further confirmed here, although some other species are resolved in a totally different position.

Echinodorus as circumscribed by Fassett (1955) and later workers (Rataj, 1975; Haynes and Holm-Nielsen, 1994; Haynes and Hellquist, 2000; Rataj, 2004) is clearly a polyphyletic combination of three groups. On the other hand, the classification proposed by Pichon (1946) corresponds well with the phylogeny obtained in this study. In this classification *Albidella nymphaeifolia* forms a monotypic genus, and *Helanthium* and *Echinodorus* are independent genera. Several morphological characters distinguish these genera: the broadly paniculate compound inflorescence and crested fruits of *Albidella* are distinct from *Echinodorus* and *Helanthium*. *Helanthium*, on the other hand, has turgid fruits and it is the only pseudostoloniferous Alismataceae genus in the New World. Within *Echinodorus* the most distinct species *E. berteroi* differs from the other species by its clawed petals and fruits, which are two-keeled.

New combinations in Helanthium

Based on our results, *Helanthium* is sister to the Old World genus *Ranalisma*. Both *Helanthium* and *Ranalisma* have pseudostolons, which are inflorescences modified for vegetative reproduction under submerged conditions (Charlton, 1999). On the other hand, *Ranalisma* has quite distinctive fruits and fruiting heads, and a closer examination of the ITS sequences revealed remarkable differences when compared with *Helanthium* sequences. For these reasons we are not combining *Helanthium* and *Ranalisma*, but in order to achieve a monophyletic circumscription of *Echinodorus* we have to accept *Helanthium* as a genus of its own.

A detailed description of the genus *Helanthium* is given below.

Annuals or short-lived perennials, glabrous, scapose, pseudostoloniferous aquatic or semiaquatic plants. Leaves as basal rosette, erect, ascending or floating; emersed leaves petiolate, blades narrow to elliptic, one to three ribbed, with pellucid markings absent or present as lines, the margins entire, the apex acute to acuminate, the base attenuate; submersed leaves sessile phyllodes, the blades linear. Inflorescence erect to creeping, umbelliform or racemose of two to three whorls, vegetatively proliferating or transformed to pseudostolon in submerged conditions, bracts deltoid. Flowers perfect, pedicellate; pedicels spreading in fruit; sepals 3, erect; petals 3, clawed, white, larger than sepals; stamens (6–)9, the anthers short, basifixed, the filaments glabrous; carpels 10–20, separate, each with one ovule. Fruits achenes in a loose head, turgid, obovate, 3–4-ribbed, without keel, without glands, beaked, the beak erect.

Type species:

Helanthium tenellum (Martius) Britton

Basionym: *Alisma tenellum* Martius in: Schultes, J.A., Schultes, J.H. (Eds.), Syst. Veg. 7, 1600. 1830.

Type: Brazil, Minas Gerais, *Martius* s.n. (lectotype M!; selected by Rataj, 1975).

The following new combinations are proposed here:

Helanthium bolivianum (Rusby) Lehtonen & Myllys **comb. nov**.

Basionym: *Alisma boliviana* Rusby, Mem. New York Bot. Gard. 7, 208. 1927.

Type: Bolivia, Reyes, October 25, 1921. *White 1540* (lectotype NY [photograph in AAU!]; isolectotypes GH, K, NY, US [digital image!]; selected by Haynes and Holm-Nielsen, 1994).

Helanthium zombiense (Jérémie) Lehtonen & Myllys **comb. nov**.

Basionym: *Echinodorus zombiensis* Jérémie, Adansonia 23, 192. 2001.

Type: Guadeloupe, Basse-Terre, NE Trois Rivières, Étang Zombis, ≈420 m, June 15, 1994. *Jérémie 1989* (holotype P).

Taxonomy and classification have been even more controversial in genus *Helanthium* than in *Echinodorus sensu stricto*. Fassett (1955) recognized four species in the group, but Rataj (2004) listed nine species. Haynes and Holm-Nielsen (1994) accepted only two polymorphic and widely distributed species, *H. tenellum* and *H. bolivianum*. Jérémie et al. (2001) did not accept this segregation and treated *H. tenellum* and *H. bolivianum* as one species, but they described a new species *H. zombiense* from Guadeloupe. Our phylogenetic analyses do not support the conclusions made by Jérémie et al. (2001), but if *H. zombiense* is understood to be conspecific with *H. bolivianum* the obtained phylogeny corresponds with the classification proposed by Haynes and Holm-Nielsen (1994). However, we believe that the variation within the clade comprising *H. bolivianum* and *H. zombiense* is great enough for accepting more than just one species. If this view is accepted, *H. bolivianum* is paraphyletic and thus should be split further. Haynes and Holm-Nielsen (1994) listed 10 heterotypic synonyms under *H. bolivianum*, but unfortunately none of the plants in the population represented by our terminal *H.* cf. *bolivianum* 1 were flowering, making it practically impossible to match the terminal with any proposed name, or to decide whether it is yet undescribed. Therefore we are not able to verify the correct name for the specimen and more thorough sampling is needed before a reliable taxonomy of this morphologically highly plastic genus can be achieved.

Phylogenetic position of Echinodorus berteroi

Echinodorus berteroi seems to be quite distantly related to the other species of the genus. This species

was resolved as a sister to the rest of the genus in the chloroplast DNA, combined DNA and total-evidence analyses, but in the nuclear DNA analysis *Alisma–Baldellia* clade was placed between *E. berteroi* and other *Echinodorus*. As Small (1909) selected *E. berteroi* as the type species of the genus, its phylogenetic position determines the generic name of rest of the species. If *E. berteroi* does not form a monophyletic group with the rest of the *Echinodorus*, most of the species traditionally classified as *Echinodorus* will need a new generic name. However, we consider our total-evidence analysis to be the most reliable hypothesis of the phylogenetic relationships for *Echinodorus* available at the moment, and therefore we consider the monophyletic clade with *E. berteroi* as a basal species to represent a single genus, *Echinodorus*.

Other taxonomic implications

Rataj (1975, 2004) divided the genus *Echinodorus* (as circumscribed here) into nine sections. With the exception of sections *Berteroii* and *Uruguayensii* none of the sections appear to be monophyletic. Even though several clades are recognized within *Echinodorus*, we cannot see any reason for formal subdivision of the genus, especially because morphological characters are widely overlapping between the clades. Thus, we follow Haynes and Holm-Nielsen (1994) and reject all proposed sections.

Our study throws light on the many existing problems in *Echinodorus* classification. *Echinodorus decumbens* is resolved together with *E. subalatus* ssp. *subalatus* and *E. subalatus* ssp. *andrieuxii*, but we lack molecular data from *E. subalatus* ssp. *subalatus*. The two subspecies of *E. macrophyllus* are resolved as two distinct lineages suggesting that they should be recognized as separate species. *Echinodorus grisebachii*, *E. gracilis*, *E. eglandulosus* and *E. heikobleheri* are mixed in one clade with varying topology and position in phylogeny depending on the data set. *Echinodorus cylindricus* is not grouped together with *E. paniculatus* as Haynes and Holm-Nielsen (1994) suggested, but as a sister to *E. glaucus* (*E. teretoscapus*). *Echinodorus osiris* is nested within two populations of *E. uruguayensis* and apparently should be considered to be conspecific (as done by Haynes and Holm-Nielsen, 1994). *Echinodorus ovalis* is nested within *E. cordifolius*, and also the subspecific classification of *E. cordifolius* seems unnatural.

Species delimitation in the *E. grandiflorus* complex has been controversial (Rataj, 1969; Haynes and Holm-Nielsen, 1986). While Haynes and Holm-Nielsen (1986, 1994) recognized only one species (*E. grandiflorus*) with two subspecies, and Haynes and Burkhalter (1998) two species (*E. grandiflorus* and *E. floridanus*), Rataj (1969, 1975, 2004) has accepted *E. floribundus*, *E. grandiflorus* and *E. longiscapus* as separate species (although he used incorrect names for two of them: *E. grandiflorus* for *E. floribundus*, and *E. argentinensis* for *E. grandiflorus*). These taxa formed a clade in the total-evidence analysis, but not in the DNA-based analyses or in the previous morphology-based analysis (Lehtonen, 2006). Therefore it seems reasonable to recognize *E. floribundus* (*E. grandiflorus* ssp. *aureus sensu* Haynes and Holm-Nielsen, 1986) at the species level.

Echinodorus grandiflorus, *E. longiscapus* and *E. floridanus* form a monophyletic group of two morphologically distinctive subgroups. Haynes and Holm-Nielsen (1986, 1994) treated *E. grandiflorus* and *E. longiscapus* as *E. grandiflorus* ssp. *grandiflorus*, and later Haynes and Burkhalter (1998) described *E. floridanus* as a distinct species. This classification seems incorrect, and due to the lack of any molecular or morphological differences between *E. grandiflorus* and *E. floridanus* they should not be recognized as separate species. *Echinodorus grandiflorus–E. floridanus* group is resolved monophyletic in every separate analysis, except in chloroplast analysis where the resolution is poor at that level. *Echinodorus longiscapus* group is also mostly resolved in separate analyses. Molecular evidence together with morphological and ecological differences (*E. grandiflorus* occurs along rivers in coastal areas, while *E. longiscapus* is widely distributed in temporary pools of standing water on savannas) indicates the existence of two separate species (*E. longiscapus* and *E. grandiflorus*) instead of one subspecies (*E. grandiflorus* ssp. *grandiflorus*).

The four morphologically distinct populations, sp1, sp2, sp3 and sp4 could not be identified as members of any described species. Sp1 is resolved as a sister to *E. uruguayensis*, but they are clearly distinguishable both on molecular and morphological level. Several morphologically similar populations of sp1 are known from Paraguay, North Argentina and South Brazil, and they seem to represent an undescribed species. Two populations of sp2 (one from Peru and another from Bolivia) are resolved as a sister group to *E. macrophyllus* ssp. *scaber*. The sister group position of these lineages is clearly supported by all data, but the lineages can be separated by different flower and fruit morphology, and by differences in ITS and 5S-NTS sequences. They also differ biogeographically (sp2 present in western Amazonia, *E. macrophyllus* ssp. *scaber* in central Brazil and northern South America) and therefore, sp2 most probably represents an undescribed species as well.

Sp3 was collected from western Paraguay and it is morphologically quite distinct from the other species. However, as only one small population growing in an unusual habitat for the group (large plants were growing submerged in flowing stream) was found, it is not clear whether it represents species of its own, or just extreme phenotypic plasticity of some other species. Sp3 is resolved as a distinct lineage in the total-evidence

analysis, but sequence data do not provide a clear picture and the position in the total-evidence analysis may be confounded by the unusual morphology caused by submerged growth. Sp4 was collected from the Chaco region in Paraguay, and it has a mixture of morphological characters of *E. longiscapus* and *E. floribundus* with a unique character state, a creeping inflorescence. In the *mat*K tree this specimen is grouped together with one *E. longiscapus* specimen, but nuclear DNA gives contradicting results.

Morphological and molecular evidence

Morphology-based studies of aquatic plants encounter many difficulties due to the plasticity, reduced structures and presumed convergence (Sculthorpe, 1967; Björkqvist, 1968; Wooten, 1986; Les and Haynes, 1995), and this may explain the contradicting results between the morphology-based (Lehtonen, 2006) and combined studies. In order to overcome limitations in morphological data the use of supporting molecular-level data is thus highly desirable. On the other hand, relatively small data sets in molecular systematics have repeatedly produced phylogenies contradicting old classifications, and the need for a wide character sampling is generally acknowledged (Doyle, 1992; Soltis et al., 2005). Our study supports these conclusions: phylogenies based on single genes should be considered cautiously, and it may be wise not to make dramatic changes in existing classifications without further sampling.

In this study we used various loci from chloroplast and nuclear genomes. Separate analysis of these two genomes resulted in topologies with incongruence at the deeper phylogenetic level. This may be due to the fact that taxon sampling has been recognized to play a crucial part in the phylogenetic inference (e.g., Rydin and Källersjö, 2002; Zwickl and Hillis, 2002) and in our study outgroup sampling varied between the analyses: we had much more outgroup taxa in the nuclear genome analysis. In addition, we had taxa with a high amount of missing data in the total-evidence analysis as we lacked *LEAFY* and 5S-NTS sequences of most outgroup taxa. Although incomplete taxa may have a positive influence on phylogeny reconstruction by cutting long branches it is still possible that the negative effects overcome these benefits (Wiens, 2006). Relationships between the studied genera may have been further obscured by the mistaken orthology of ITS and 5S-NTS (Álvarez and Wendel, 2003). It is possible that we failed to identify homologous copies of distantly related groups, but in lower-level nodes the risk to mistake orthology should be less likely, although still possible.

Despite the obvious incongruence in some of the deeper level nodes, the topology of *Echinodorus sensu stricto* is remarkably similar between our molecular data sets, although morphological data yielded very little resolution at this level. This apparent congruence of molecular data sets is even more evident in the comparison of SPR moves required to transform trees into another (Table 2). Transforming *mat*K tree into nuclear DNA tree required only eight SPR moves, compared with 45–47 moves required for random trees. We believe that this congruence indicates that contradiction between our results and an earlier morphology-based study (Lehtonen, 2006) is a result of homoplasy in the continuously overlapping morphological characters. Our conclusion is based on the fact that transforming the most parsimonious tree of Lehtonen's (2006) study into our total-evidence tree required a total of 15 SPR moves (tree similarity 0.5833), but conversion of the tree for continuous characters required 21 SPR moves (similarity 0.4167). Although these numbers are clearly lower than the number of SPR moves required for random trees (29–33) of equal size, they are still much higher than required in any comparison of our trees. Because the resolution within *Echinodorus* was largely due to continuous overlapping characters in Lehtonen's (2006) study, it seems that those characters are responsible for a large part of the incongruence. We mostly rejected overlapping characters in this study, and therefore our morphology-based tree is more congruent with the molecular data sets.

The addition of morphological data into the molecular analysis did not affect the deeper level topology, but it resulted in a topology that is more in accordance with morphology and former classification in the otherwise disordered *E. grandiflorus* group. It seems that in the case of *Echinodorus* morphology is most useful in studying recently diverged taxa, while molecular evidence is far more important in resolving nodes deeper in the history. This could be due to introgression or conserved ancient polymorphism (Bailey et al., 2003) in the studied sequences of the *E. grandiflorus* group. Therefore we strongly recommend the use of morphological data in the studies of phylogenetic inference, and this should be done simultaneously (total evidence; Kluge, 1989).

Conclusions and future work

The aim of our study was to obtain a reliable hypothesis of *Echinodorus* phylogeny. We believe that our results fulfill this goal by providing a solid basis for rejecting previously proposed subgeneric classifications of *Echinodorus*. A more detailed discussion of the taxonomy and nomenclature of *Echinodorus* will be given elsewhere.

While our study resolved many long-standing problems in *Echinodorus* systematics, it also raised new questions about the phylogenetic relationships in

Alismataceae. Therefore a simultaneous analysis of the whole family using all the species as terminals is needed. Our results also confirm that taxonomic conclusions based on phylogenetic analyses with a single gene, or only single specimen of each species, should be considered cautiously.

Acknowledgments

The directors of the mentioned herbaria are acknowledged. S. Lehtonen is grateful to all the assistants who helped in the field studies. Maarten Christenhusz, Helmut Hromadnik, Claus-Dieter Junge, Helmut Mühlberg, Curt Quester and Dierk Wanke kindly provided additional material for the study. We thank Pablo Goloboff for his kind advice in the SPR move calculations. This work was funded by Societas pro Fauna et Flora Fennica, and the foundations of Otto A. Malmi, Jenny and Antti Wihuri, Turku University, Emil Aaltonen, and Oskar Öflund. Jaakko Hyvönen, Hanna Tuomisto and two anonymous reviewers gave valuable comments to the manuscript.

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Appendix 1: Terminals employed in the analyses with GenBank accession numbers

Terminal; voucher specimen for sequences	*mat*K	ITS	*LEAFY*	5S-NTS
Albidella nymphaeifolia (Griseb.) Pichon; Mexico, *Lehtonen & Rámirez 399* (TUR)	EF088125	EF088077	EF088171	EF088026
Alisma plantago-aquatica L.; n.a.	AF542573	DQ339085	EF088141	n.a.
Baldellia ranunculoides Parl.; n.a.	n.a.	DQ339092	n.a.	n.a.
Butomus umbellatus L.; n.a.	AY952416	DQ339094	n.a.	n.a.
Caldesia parnassifolia (L.) Parl.; Austria, *Hromadnik s.n.* (TUR)	EF088140	n.a.	EF088189	EF088043
Echinodorus berteroi (Spreng.) Fassett 1; Guadeloupe, *Christenhusz 4081* (TUR)	EF088121	EF088073	EF088167	EF088022
E. berteroi (Spreng.) Fassett 2; Mexico, *Lehtonen & Rámirez 412* (TUR)	EF088134	EF088087	EF088181	EF088036
E. bracteatus Micheli ssp. *bracteatus* (Fasset) Haynes & Holm-Niels.; Ecuador, *Lehtonen & Navarrete 491* (TUR)	EF088124	EF088076	EF088170	EF088025
E. bracteatus Micheli ssp. *efenestratus* (Fasset) Haynes & Holm-Niels.; Ecuador, *Lehtonen & Navarrete 494* (TUR)	EF088137	EF088091	EF088185	EF088040
E. cordifolius (L.) Griseb. ssp. *cordifolius* Haynes & Holm-Niels. 1; Venezuela, *Lehtonen 457* (TUR)	EF088126	EF088078	EF088172	EF088027
E. cordifolius (L.) Griseb. ssp. *cordifolius* Haynes & Holm-Niels. 2; USA, *Keener 275* (UNA)	n.a.	n.a.	EF088190	EF088044
E. cordifolius (L.) Griseb. ssp. *fluitans* (Fassett) Haynes & Holm-Niels.	n.a.	n.a.	n.a.	n.a.
E. cylindricus Rataj	n.a.	n.a.	n.a.	n.a.
E. decumbens Kasselm.; Cultivated, *Lehtonen 392* (TUR)	EF088117	EF088069	EF088163	EF088018
E. eglandulosus Holm-Niels. & Haynes	n.a.	n.a.	n.a.	n.a.

Appendix 1: *Continued*

Terminal; voucher specimen for sequences	*mat*K	ITS	*LEAFY*	5S-NTS
E. floribundus (Seub.) Seub. 1; Bolivia, *Lehtonen 161* (TUR)	EF088106	EF088057	EF088153	EF088007
E. floribundus (Seub.) Seub. 2 ; Bolivia, *Lehtonen 188* (TUR)	EF088103	EF088054	EF088150	EF088004
E. floribundus (Seub.) Seub. 3; Venezuela, *Lehtonen & Pacheco 485* (TUR)	EF088129	EF088081	EF088175	EF088030
E. floridanus Haynes & Burkhalter; Cultivated, *Lehtonen 393* (TUR)	EF088118	EF088070	EF088164	EF088019
E. glandulosus Rataj	n.a.	n.a.	n.a.	n.a.
E. glaucus Rataj; Cultivated, *Mühlberg s.n.* (TUR)	EF088132	EF088084	EF088178	EF088033
E. grandiflorus (Cham. Schltdl.) Micheli 1; Uruguay, *Lehtonen & Delfino 358* (TUR)	n.a.	EF088086	EF088180	EF088035
E. grandiflorus (Cham. Schltdl.) Micheli 2; Argentina, *Lehtonen 391* (TUR)	EF088113	EF088065	EF088160	EF088014
E. gracilis Rataj; Cultivated, *Mühlberg s.n.* (TUR)	EF088131	EF088083	EF088177	EF088032
E. grisebachii Small 1; Peru, *Lehtonen & Rodríguez 74* (TUR)	EF088095	EF088046	EF088142	EF087996
E. grisebachii Small 2; Bolivia, *Lehtonen 151* (TUR)	EF088102	EF088053	EF088149	EF088003
E. heikobleheri Rataj; Cultivated, *Quester s.n.* (TUR)	EF088139	EF088093	EF088187	EF088041
E. horizontalis Rataj 1; Peru, *Lehtonen & Rodríguez 99* (TUR)	EF088096	EF088047	EF088143	EF087997
E. horizontalis Rataj 2; Peru, *Lehtonen & Rodríguez 58* (TUR)	EF088099	EF088050	EF088146	EF088000
E. inpai Rataj; Cultivated, *Mühlberg s.n.* (TUR)	EF088138	EF088092	EF088186	n.a.
E. lanceolatus Rataj	n.a.	n.a.	n.a.	n.a.
E. longipetalus Micheli; Paraguay, *Lehtonen & Burguez 271* (TUR)	EF088115	EF088067	EF088162	EF088016
E. longiscapus Arechav. 1; Argentina, *Lehtonen & Dematteis 204* (TUR)	EF088108	EF088059	n.a.	EF088009
E. longiscapus Arechav. 2; Uruguay, *Lehtonen 341* (TUR)	EF088112	EF088063	EF088158	EF088012
E. longiscapus Arechav. 3; Uruguay, *Lehtonen & Delfino 334* (TUR)	EF088116	EF088068	n.a.	EF088017
E. major (Micheli) Rataj; Cultivated, *Lehtonen 394* (TUR)	EF088119	EF088071	EF088165	EF088020
E. macrophyllus (Kunth) Micheli ssp. *macrophyllus* Haynes & Holm-Niels.	n.a.	n.a.	n.a.	n.a.
E. macrophyllus (Kunth) Micheli ssp. *scaber* (Rataj) Haynes & Holm-Niels.; Venezuela, *Lehtonen & Pacheco 440* (TUR)	EF088128	EF088080	EF088174	EF088029
E. osiris Rataj; Cultivated, *Quester s.n.* (TUR)	n.a.	EF088094	EF088188	EF088042
E. ovalis Wright *ex* Sauvalle; Mexico, *Lehtonen & Rámirez 417* (TUR)	EF088127	EF088079	EF088173	EF088028
E. palaefolius (Nees & Mart.) J.F.MacBr.	n.a.	n.a.	n.a.	n.a.
E. paniculatus Micheli 1; Bolivia, *Lehtonen 168* (TUR)	EF088097	EF088048	EF088144	EF087998
E. paniculatus Micheli 2; Venezuela, *Lehtonen & Pacheco 469* (TUR)	EF088130	EF088082	EF088176	EF088031
E. pubescence (Mart.) Seub. *ex* Warm.; Brazil, *Harley* et al. *20019* (AAU)	n.a.	n.a.	EF088193	EF088045
E. reticulatus Haynes & Holm-Niels.	n.a.	n.a.	n.a.	n.a.
E. sp1; Paraguay, *Lehtonen & Burguez 261* (TUR)	EF088110	EF088061	EF088156	EF088010
E. sp2 1; Peru, *Lehtonen 140* (TUR)	EF088100	EF088051	EF088147	EF088001
E. sp2 2; Bolivia, *Lehtonen 190* (TUR)	EF088098	EF088049	EF088145	EF087999
E. sp3; Paraguay, *Lehtonen & Burguez 275* (TUR)	EF088111	EF088062	EF088157	EF088011
E. sp4; Paraguay, *Lehtonen & Burguez 309* (TUR)	EF088133	EF088085	EF088179	EF088034
E. subalatus (Mart.) Griseb. ssp. *andrieuxii* (Hook. & Arn.) Haynes & Holm-Niels.; Venezuela, *Lehtonen & Pacheco 472* (TUR)	EF088123	EF088075	EF088169	EF088024
E. subalatus (Mart.) Griseb. ssp. *subalatus* Haynes & Holm-Niels.	n.a.	n.a.	n.a.	n.a.
E. tunicatus Small; Peru, *Lehtonen 133* (TUR)	EF088107	EF088058	EF088154	EF088008
E. trialatus Fassett 1; Venezuela, *Lehtonen & Pacheco 441* (TUR)	EF088122	EF088074	EF088168	EF088023
E. trialatus Fassett 2; Venezuela, *Lehtonen & Pacheco 444* (TUR)	EF088136	EF088089	EF088183	EF088038
E. uruguayensis Arechav. 1; Uruguay, *Lehtonen & Delfino 364* (TUR)	n.a.	EF088064	EF088159	EF088013
E. uruguayensis Arechav. 2; Argentina, *Lehtonen* et al. *237* (TUR)	EF088114	EF088066	EF088161	EF088015
E. virgatus (Hook. & Arn.) Micheli	n.a.	n.a.	n.a.	n.a.
Helanthium bolivianum (Rusby) Lehtonen & Myllys 1; Venezuela, *Lehtonen & Pacheco 482* (TUR)	n.a.	EF088090	EF088184	EF088039
H. bolivianum (Rusby) Lehtonen & Myllys 2; Argentina, *Lehtonen* et al. *213* (TUR)	EF088109	EF088060	EF088155	n.a.
H. bolivianum (Rusby) Lehtonen & Myllys 3; Ecuador, *Øllgaard* et al. *57161* (AAU)	n.a.	n.a.	EF088192	n.a.
H. tenellum (Mart.) Britton 1; Bolivia, *Lehtonen 156* (TUR)	EF088105	EF088056	EF088152	EF088006
H. tenellum (Mart.) Britton 2; USA, *MacDonald 11345* (UNA)	n.a.	n.a.	EF088191	n.a.
H. zombiense (Jérémie) Lehtonen & Myllys; Guadeloupe, *Christenhusz 4040* (TUR)	EF088120	EF088072	EF088166	EF088021
Limnocharis flava Buchenau; n.a.	AB088778	n.a.	n.a.	n.a.
Ranalisma rostrata Stapf; n.a.	n.a.	AY395986	n.a.	n.a.
Sagittaria planitiana Agostini; Venezuela, *Lehtonen & Pacheco 428* (TUR)	EF088135	EF088088	EF088182	EF088037
S. montevidensis Cham. Schltdl.; Bolivia, *Lehtonen 180* (TUR)	EF088101	EF088052	EF088148	EF088002
S. sprucei Mich.; Peru, *Lehtonen & Rodríguez 31* (TUR)	EF088104	EF088055	EF088151	EF088005
Wiesneria trianda (Dalzell) Mich.; n.a.	n.a.	AY335953	n.a.	n.a.

Appendix 2: Morphological characters

0 Rhizome: (0) erect; (1) horizontal.
1 Rhizome: (0) short; (1) long.
2 Rhizome: (0) thin; (1) thick.
3 Submersed leaves: (0) not differentiated; (1) morphologically differentiated.
4 Leaf blades: (0) not undulating; (1) undulating.
5 Leaf blades: (0) 1–5 cm long; (1) 5–8 cm long; (2) 8–15 cm long; (3) over 15 cm long.
6 Leaf shape: (0) widest at the central 1/3 of the blade; (1) widest at the basal 1/3 of the blade.
7 Leaf blade: (0) more than 3 times as long as wide; (1) less than 2.5 times as long as wide.
8 Base of the leaf blade: (0) attenuate; (1) truncate.
9 Basal lobe: (0) absent; (1) less than 6% of the length of the blade; (2) 6–25% of the length of the blade; (3) more than 25% of the length of the blade.
10 Basal lobe: (0) not sagittate; (1) sagittate.
11 Apex of the blades: (0) acute; (1) obtuse; (2) retuse.
12 Leaf veins: (0) weak; (1) strong.
13 Number of parallel leaf veins: (0) 1–3; (1) 3–7; (2) 7–15; (3) 15–25.
14 Angle of secondary veins: (0) less than 38°; (1) 38–65°; (2) over 65°.
15 Leaves: (0) without waxy cover; (1) covered with bluish wax.
16 Leaves: (0) not pseudopenninerved; (1) pseudopenninerved.
17 Pellucid markings: (0) absent; (1) present.
18 Pellucid dots: (0) absent; (1) present.
19 Pellucid lines: (0) absent; (1) present.
20 Pellucid network: (0) absent; (1) present.
21 Cross-section of petiole: (0) round; (1) triangular.
22 Petiole: (0) not angled; (1) angled.
23 Petiole: (0) not channeled; (1) channeled.
24 Petiole: (0) no bulb under the blade; (1) bulb under the blade.
25 Hairs: (0) absent; (1) few; (2) abundant.
26 Hair type: (0) simple; (1) stellate.
27 Petiole: (0) not alate; (1) alate.
28 Cross-section of peduncle: (0) round; (1) triangular.
29 Inflorescence: (0) not branching; (1) 1–2 branches; (2) more branches.
30 Inflorescence: (0) without secondary branches; (1) with secondary branches.
31 Peduncle: (0) not ridged; (1) ridged.
32 Peduncle: (0) not angled; (1) angled.
33 Pseudostolons: (0) absent; (1) present.
34 Inflorescence vegetatively proliferating: (0) no; (1) yes.
35 Inflorescence: (0) creeping; (1) erect.
36 Length of inflorescence: (0) less than 30 cm; (1) 30–100 cm; (2) 100–150 cm; (3) over 200 cm.
37 Whorls in inflorescence: (0) less than 5; (1) 5–13; (2) more.
38 Internodes between whorls: (0) less than 10 cm long; (1) over 10 cm long.
39 Flowers per whorl: (0) 3; (1) more.
40 Inflorescence: (0) glabrous; (1) scabrous.
41 Cross-section of rachis: (0) round; (1) triangular.
42 Rachis: (0) not alate; (1) alate.
43 Pedicels: (0) spreading in fruit; (1) recurved in fruit.
44 Pedicels: (0) not growing after flowering; (1) growing after flowering.
45 Pedicels: (0) less than 1 cm long; (1) 1–4 cm long; (2) over 4 cm long.
46 Anther: (0) 0.5–1 mm long; 1.5–2 mm long.
47 Petals: (0) with spot at the base; (1) not spotted.
48 Petals: (0) not claved; (1) claved.
49 Petals: (0) not overlapping; (1) widely overlapping.
50 Apex of the petals: (0) round; (1) retuse.
51 Apex of the petals: (0) divided; (1) entire.
52 Flowers: (0) without strong odor; (1) with strong odor.
53 Flower diameter: (0) less than 1.5 cm; (1) over 2 cm.
54 Sepals: (0) not surrounding fruiting aggregate; (1) surrounding mature fruiting aggregate.
55 Sepals: (0) spreading; (1) erect.
56 Sepal veins: (0) without papillae; (1) with papillae.
57 Sepal veins: (0) 8–12; (1) 15–21; (2) 24–30.
58 Sepal veins: (0) weak; (1) strong.
59 Bract veins: (0) weak; (1) strong.
60 Bract veins: (0) 0–9; (1) 11–20.
61 Bracts: (0) free; (1) deeply connate.
62 Apex of bracts: (0) rounded; (1) acute; (2) acuminate.
63 Bracts: (0) less than 1 cm long; (1) much longer.
64 Bracts: (0) less than twice as long as wide; (1) two to five times as long as wide; (2) more than five times as long as wide.
65 Bracts: (0) delicate; (1) strong.
66 Bracts: (0) persistent; (1) deciduous.
67 Anthers: (0) basifixed; (1) versatile.
68 Stamens: (0) 6; (1) 9; (2) 12; (3) 15–20; (4) 20–35; (5) 3.
69 Carpels: (0) about 15; (1) about 50; (2) about 150; (3) about 500.
70 Carpels: (0) open at anthesis; (1) closed.
71 Fruiting head: (0) dense; (1) loose.
72 Flowers: (0) perfect; (1) imperfect.
73 Fruits: (0) 1–2 mm long; (1) 2–4 mm long; (2) about 10 mm long.
74 Fruit: (0) follicle; (1) achene.
75 Fruit beak: (0) horizontal; (1) erect.
76 Fruit beak length: (0) less than 20% of the fruit body; (1) 20–50% of the fruit body; (2) over 50% of the fruit body.
77 Fruit glands: (0) small; (1) large.
78 Fruit glands: (0) absent; (1) 1 or 2; (2) more.
79 Fruit ribs: (0) weak; (1) strong.
80 Fruit ribs: (0) absent; (1) 1–3; (2) more.
81 Fruits: (0) round; (1) oblanceolate.
82 Fruits: (0) flattened; (1) not flattened.
83 Air chambers in fruit: (0) absent; (1) present.
84 Fruits: (0) not winged; (1) winged.
85 Fruits: (0) not keeled; (1) keeled; (2) 2-keeled.

Appendix 3: Morphological character matrix

Polymorphic entries: a = [01], b = [12], c = [012]. Inapplicable data = ”-”. Missing data = ”?”.

Butomus umbellatus	1111030000-003?000---10000-000-0000120-10--002100001?10100001011110010010 2011-0-001000
Limnocharis flava	?0-1020101-1022001001100000-010-0000110-10--00210100101110200100100004000020---00100010
Alisma plantago-aquatica	00010101a100011000---00000-00210000111010000010010000001000010111100000011---0-000000
Baldellia ranunculoides	?001010000-0001000---?0000-000-0011a10110000020010 01?0000100111000000011011 10-01111001
Wiesneria trianda	???103?000-000?000---??000-0?0-0000a11010?0000???0???00?????????0????50??1?1??-00101107
Ranalisma rostrata	????00?000-1?0?000---??000-0?0-0011a?0000?00?1???????00?????????????11??0?111?1-00001?
Caldesia parnassifolia	?00001111301 02b000---00000-00200001111010000010010 00?0000100001011000011011 11-01111000
Sagittaria planitiana	0000020100-102200???00000-000-0000110000100011?10010101 0?00?0?00000031011101-0001001?
S. montevidensis	1111031113110320010010 0000-000-0000110000001001010010101020011000000431011101-00010011
S. sprucei	0000020113110320010010 0000-000-0000111000001001110010001010010000010131011101-00010011
Albidella nymphaeifolia	0001010113010220010100 0000-0021000011101010001 0??0???000000000111100 00?100110111101001
Helanthium cf. *bolivianum* 1	0001000000-0000000---00000-000-0011a000100000101101100110000001010001 0?100110-01101000
H. bolivianum 2	0001000000-0001000---00000-000-0011a000100000101101100110000001000001 0?100110-01101000
H. bolivianum 3	0001000000-0001001010000 00-000-0011a000100000101101 1?011000001000001 0?100112-00101000
H. tenellum 1	?0-1000000-0000000---00000-00100011a000100000101101100110000001000001 0?100110-01101000
H. tenellum 2	?0-1000000-0000000---00000-000-0011a000100000101101100110000001000001 0?100110-01101000
H. zombiense	?0-1000000-0000000---00000-000-0011a101100000201101100110000001010001 0?100110-01101000
Echinodorus berteroi 1	0001011101-1111001010010 00-001001001100101000101100100000000001111012210011121112 11002
E. berteroi 2	0001011111011210010100 1000-002001001110101000101100100000000001111012210011121112 11002
E. bracteatus ssp. *bracteatus*	1110131112011220011100000110020100112201111100110101010101111021110131100111011 1211001
E. bracteatus ssp. *efenestratus*	111013111201122000---000011002010011220111110011010101010111102111013110011101112 11001
E. cordifolius ssp. *cordifolius* 1	1110021112011220011100000110020100102111100021101010100101110211104210011101211 11001
E. cordifolius ssp. *cordifolius* 2	1110021112011220010100000110010100102111010002110101010010111021110142?001101211 11001
E. cordifolius ssp. *fluitans*	111002111200122000---00000-0010?001021110100021101010100?011102111014210011101211 11001
E. cylindricus	1110030000-0111100---00000-0010000012101000000110101010102111021010142100111111 1211001
E. decumbens	1110030000-0111001010111 00-001010010211101100000000100000111112121012?1001111111 1211001
E. eglandulosus	11011200a0-0112011010100 00-01100001a11010101000100010000 00?0121210111100011 0???111001
E. floribundus 1	1110031112021320011000000110020000113101100001110101010101111021110142100111012 0111001
E. floribundus 2	111003111202132000---000011002000 0?13101100001110101010101111021110142100111012 0111001
E. floribundus 3	1110031112021320011000000110020000113101100001110101010101111021110142100111012 0111001
E. floridanus	1110030101011210011100001110020000012101110012110101010101111021110142100111012 0111001
E. glandulosus	1?100211a2011220010010 0?01?0020?00?12101011001 0?0001?00100??102111013?1001111111 1111001
E. glaucus	1110031112001221 00---00000-0010000012101000000110101010102111021010142100111111 1211001
E. grandiflorus 1	1110030111011210011100001110020000?12101110011110101010101111021110142100111012 0111001
E. grandiflorus 2	1110030101011210011100001110020000?12101110012110101010101111021110142100111012 0111001
E. gracilis	1101110000-0112011010100 00-010-0001111010101000100010000000110?2001011110001100211 11001
E. grisebachii 1	11011200a0-0112011010100 00-011000010110101010001000100000011012121011110001100211 11001
E. grisebachii 2	1101120000-0112011010100 00-012000011110101010001000100000011012121011110001100211 11001
E. heikobleheri	?1?1130000-0111010---10000-010-0001010010101000100010 00?0??002??1011?1000111021111 11001
E. horizontalis 1	0100031112001220010010 0010-000-0001010110001010100010011010110211111431001100020111 11001
E. horizontalis 2	0100031112001220010010 0010-000-0001010110001010100010011010110211111431001100020111 11001
E. inpai	?1?0030000-1111001010101 00-1010?0001110101000001000100000011??2011012?1001112111111 1001
E. lanceolatus	???0030000-0110000---00?00-0020000?12101010000?????????0100111021110??2100111021211 11001
E. longipetalus	1110030000-0111011001100 00-100-0100131010101011101 01?1110211102111014310011 00-00210011
E. longiscapus 1	1110020112011220011100000110 00-1001111011100001101010101011110211101421001110120111 11001
E. longiscapus 2	1110020112011220011100000110 00-000?1110111000011010101010111102111014210011101201111 11001
E. longiscapus 3	1110020112011220011100000 00-0010100?1110111000011010101010111102111014210011101201111 11001

Appendix 3: *Continued*

E. major	?1?1130000-1111010---10010-000-000111101010000l?000100010011102111012?1000110021211001
E. macrophyllus ssp. *macrophyllus*	111003111300122000---000011002010011210110000011?0101?100011110211101321001111121211001
E. macrophyllus ssp. *scaber*	111003111201122000---000021002010001320110000101000100010111102111013210011111111211001
E. osiris	0100120000-111101101000000-000-000101101010001110??111??010110211101 3?10011???2??1100?
E. ovalis	1110020000-111100111000001 1000-100102111110002110101010010111021110132100111012121 1001
E. paniculatus 1	1110031000-0111000---10000-012000001210101000111010101000011102111014210011 10-01211001
E. paniculatus 2	111003101000111000---10000-012000010210101000111010101000011102111014210011 10-01211001
E. palaefolius	11100301a2011220010101110c00010?00012201a11a001000010000a011112111012210011111111211001
E. pubescence	11100300a1011210010100000200010000012201110000000001000111111121110122100111111 1211001
E. reticulatus	1110030000-011101100110000-100-010013101010101110101?111021110211101431001100-00210011
E. sp1	1100010000-011100101010000-000-000101001010002110001010001111020110 13?1001110021?11001
E. sp2 1	111003111201122000---000021001010011220110000001010101010111102001013210011 11-01211001
E. sp2 2	11100311120112200 0---000021000-100?122011000000101 01?10101111020010132100111 1-01211001
E. sp3	1110030000-011?001010000011002000 01a21111100?111010101 0?001110212101421001 11?????1100?
E. sp4	111002011201122001110000011002000010210111000111010101011110211101421001110120111001
E. subalatus ssp. *andrieuxii*	1110030000-1111000---111010001010011210111100000000010000001111212101221001112111211001
E. subalatus ssp. *subalatus*	1110030000-111100101011100-0010?001121010110000000001?000a011112111012210011111112 11001
E. tunicatus	010003111200122001001000 10-000-000111001010101010001001101011021111143100110002021 1001
E. trialatus 1	1101030000-0112010---10000-010000001110101110001000100010111102111012210011 10-01211001
E. trialatus 2	1101030000-0112010---10000-010000001110101110001000100010111102111012210011 10-01211001
E. uruguayensis 1	1101020000-1111011010a0000-000-000111001010001110111110100111021110132100111 0111111001
E. uruguayensis 2	1101020000-1111010---10000-000-000101001010001110??111010011102111013210011 10111111001
E. virgatus	111003011201121001010101 0a00010?00?12101a11a001?0?????0??0111?21110?2?1001111121211001

Appendix 4: List of examined specimens

Albidella nymphaeifolia, Mexico: 2 km N of China; Lehtonen & Rámirez 399 (TUR, MEXU), Lehtonen & Rámirez 400 (TUR, MEXU), Lehtonen & Rámirez 401 (TUR, MEXU), Lehtonen & Rámirez 402 (TUR, MEXU), Lehtonen & Rámirez 403 (TUR, MEXU), Lehtonen & Rámirez 404 (TUR, MEXU), Gutierrez 5608 (MEXU).

Alisma plantago-aquatica, Finland; Lehtonen 395 (TUR).

Baldellia ranunculoides, Netherlands; Bodlaender s.n. (TUR), Berge s.n. (H).

Butomus umbellatus, Estonia; Kari s.n. (TUR).

Caldesia parnassifolia, Austria; Hromadnik s.n. (TUR).

Echinodorus berteroi 1, Guadeloupe; Christenhusz 4081 (TUR), Stehle 1555 (UC).

E. berteroi 2, Mexico: close to Ebona; Lehtonen & Rámirez 412 (TUR), Lehtonen & Ramírez-García 413 (TUR, MEXU), Lehtonen & Ramírez-García 414 (TUR, MEXU), Lehtonen & Ramírez-García 415 (TUR, MEXU), Oliva & Rámon 1086 (MEXU).

E. bracteatus ssp. *bracteatus*, Ecuador: close to Daule; Lehtonen & Navarrete 491 (TUR, QCA), Lehtonen & Navarrete 492 (TUR, QCA), Lehtonen & Navarrete 493 (TUR, QCA).

E. bracteatus ssp. *efenestratus*, Ecuador: between Balzar and Palenque; Lehtonen & Navarrete 494 (TUR, QCA).

E. cordifolius ssp. *cordifolius* 1, Venezuela: between Maicillal and Piritu; Lehtonen 456 (TUR, VEN), Lehtonen 457 (TUR, VEN), Lehtonen 458 (TUR, VEN), Lehtonen 459 (TUR, VEN), Lehtonen 460 (TUR, VEN), Lehtonen 461 (TUR, VEN), Lehtonen 462 (TUR, VEN), Lehtonen 463 (TUR, VEN), Lehtonen 464 (TUR, VEN), Lehtonen 465 (TUR, VEN), Steyermark & Braun 94514 (VEN).

E. cordifolius ssp. *cordifolius* 2, USA: Alabama; Keener 275 (UNA).

E. cordifolius ssp. *fluitans*, Colombia: near Riohacha; Haught 4450 (UC).

E. cylindricus, Brazil: Pantanal; Pott et al 406 (UNA), Pott et al. 402 (UNA), Pott et al. 3925 (UNA).

E. decumbens, Brazil: Kasselmann 205 (M), Cultivated; Lehtonen 392 (TUR).

E. eglandulosus, Ecuador: Holm-Nielsen et al. 19996 (AAU, UNA), Holm-Nielsen et al. 19844 (AAU, QCA).

E. floribundus 1, Bolivia: Laguna Suarez; Lehtonen 160 (TUR, LPB), Lehtonen 161 (TUR, LPB), Lehtonen 162 (TUR, LPB), Lehtonen 163 (TUR, LPB), Lehtonen 164 (TUR, LPB), Lehtonen 165 (TUR, LPB), Sanjines et al. 40 (LPB), Sanjines & Orellano 347 (LPB).

E. floribundus 2, Bolivia: Laguna Normandia; Lehtonen 187 (TUR, LPB), Lehtonen 188 (TUR, LPB).

E. floribundus 3, Venezuela: Moquete river; Lehtonen & Pacheco 485 (TUR, VEN), Lehtonen & Pacheco 486 (TUR, VEN).

E. floridanus, Cultivated; Lehtonen 393 (TUR), USA; Junge s.n. (M), Reese 1 (UNA), Reese 2 (UNA), Reese 24 (UNA), Haynes & Burkhalter 9617 (UNA).

E. glandulosus, Brazil: Pickel 64a (SP).

E. glaucus, Cultivated: Mühlberg s.n. (TUR), Brazil; da Silva 411 (SP).

E. grandiflorus 1, Uruguay: Maldonado; Lehtonen & Delfino 357 (TUR, MVJB), Lehtonen & Delfino 358 (TUR, MVJB).

E. grandiflorus 2, Argentina: Gualeguaychú; Lehtonen 386 (TUR), Lehtonen 387 (TUR), Lehtonen 388 (TUR), Lehtonen 389 (TUR), Lehtonen 390 (TUR) Lehtonen 391 (TUR), Burkhart & Troncoso 26118 (SI).

E. gracilis, Cultivated; Mühlberg s.n. (TUR).

E. grisebachii 1, Peru: close to Iquitos; Lehtonen & Rodríguez 72 (AMAZ), Lehtonen & Rodríguez 73 (AMAZ), Lehtonen & Rodríguez 74 (TUR), Lehtonen & Rodríguez 75 (AMAZ), Lehtonen & Rodríguez 76 (AMAZ), Lehtonen & Rodríguez 77 (TUR), Lehtonen & Rodríguez 78 (TUR), Lehtonen & Rodríguez 79 (AMAZ), Lehtonen & Rodríguez 80 (TUR), Lehtonen & Rodríguez 81 (AMAZ), Lehtonen & Rodríguez 82 (AMAZ), Lehtonen & Rodríguez 83 (TUR), Lehtonen & Rodríguez 84 (AMAZ).

E. grisebachii 2, Bolivia: Chimore; Lehtonen 148 (TUR, LPB), Lehtonen 149 (TUR, LPB), Lehtonen 150 (TUR, LPB), Lehtonen 151 (TUR, LPB), Lehtonen 152 (TUR, LPB), Lehtonen 153 (TUR, LPB).

E. heikobleheri, Cultivated; Quester s.n. (TUR).

E. horizontalis 1, Peru: Nuevo Horizonte; Lehtonen & Rodríguez 98 (TUR), Lehtonen & Rodríguez 99 (TUR), Lehtonen & Rodríguez 100 (AMAZ), Lehtonen & Rodríguez 101 (TUR) Lehtonen & Rodríguez 102 (TUR), Lehtonen & Rodríguez 103 (TUR).

E. horizontalis 2, Peru: Tahuyao river; Lehtonen & Rodríguez 52 (TUR), Lehtonen & Rodríguez 53 (AMAZ), Lehtonen & Rodríguez 54 (TUR), Lehtonen & Rodríguez 55 (AMAZ), Lehtonen & Rodríguez 56 (TUR), Lehtonen & Rodríguez 57 (AMAZ), Lehtonen & Rodríguez 58 (TUR), Lehtonen & Rodríguez 59 (AMAZ).

E. inpai, Cultivated; Mühlberg s.n. (TUR).

E. lanceolatus, Brazil: São Paulo; Burchell 4158 (BR, K).

E. longipetalus, Paraguay: Caaguazú; Lehtonen & Burguez 270 (TUR, FCQ), Lehtonen & Burguez 271 (TUR, FCQ), Mereles & Soloaga 7452 (FCQ).

E. longiscapus 1, Argentina: close to Ituzaingó; Lehtonen & Dematteis 204 (TUR), Lehtonen & Dematteis 205 (TUR).

E. longiscapus 2, Uruguay: Chuy; Lehtonen 341 (TUR, MVJB), Lehtonen 341 (TUR, MVJB).

E. longiscapus 3, Uruguay: Ruta14; Lehtonen & Delfino 334 (TUR, MVJB), Lehtonen & Delfino 335 (TUR, MVJB), Lehtonen & Delfino 337 (TUR, MVJB).

E. major, Cultivated; Lehtonen 394 (TUR), Brazil; Bleher 114918/62 (K).

E. macrophyllus ssp. *macrophyllus*, Brazil; Miers s.n. (BM), Sellow 194 (BM), Bowie & Cunningham s.n. (BM), Gardner 700 (BM, K), Harley et al. 18210 (AAU, K), Luetzelberg 222 (M).

E. macrophyllus ssp. *scaber*, Venezuela: Guárico; Lehtonen & Pacheco 433 (TUR, VEN), Lehtonen & Pacheco 434 (TUR, VEN), Lehtonen & Pacheco 436 (TUR, VEN), Lehtonen & Pacheco 440 (TUR, VEN).

E. osiris, Cultivated; Quester s.n. (TUR).

E. ovalis, Mexico: close to Pánuco; Lehtonen & Rámirez 417 (TUR, MEXU), Lehtonen & Rámirez 418 (TUR, MEXU), Lehtonen & Rámirez 419 (TUR, MEXU), Lehtonen & Rámirez 422 (TUR, MEXU), Lehtonen & Rámirez 423 (TUR, MEXU).

E. palaefolius, Brazil: 6 km NE of Mossamedes; Anderson 10174 (UNA).

E. paniculatus 1, Bolivia: Laguna Suarez; Lehtonen 166 (TUR, LPB), Lehtonen 167 (TUR, LPB), Lehtonen 168 (TUR, LPB), Lehtonen 169 (TUR, LPB), Lehtonen 170 (TUR, LPB), Lehtonen 171 (TUR, LPB), Lehtonen 172 (TUR, LPB), Lehtonen 173 (TUR, LPB), Lehtonen 174 (TUR, LPB), Lehtonen 175 (TUR, LPB).

E. paniculatus 2, Venezuela: close to Barcelona; Lehtonen & Pacheco 469 (TUR, VEN), Lehtonen & Pacheco 470 (TUR, VEN), Lehtonen & Pacheco 471 (TUR, VEN).

E. pubescence, Brazil; Harley et al. 20019 (AAU, K).

E. reticulatus, Suriname: Sipaliwini; Oldenburger et al. 292 (NY, U).

E. sp1, Paraguay: Villa Florida; Mereles et al. 8512 (FCQ), Lehtonen & Burguez 260 (TUR, FCQ), Lehtonen & Burguez 261 (TUR, FCQ), Lehtonen & Burguez 262 (TUR, FCQ), Lehtonen & Burguez 263 (TUR, FCQ), Lehtonen & Burguez 264 (TUR, FCQ), Lehtonen & Burguez 265 (TUR, FCQ), Lehtonen & Burguez 266 (TUR, FCQ), Lehtonen & Burguez 267 (TUR, FCQ), Lehtonen & Burguez 268 (TUR, FCQ).

E. sp2 1, Peru: Sungachicocha; Lehtonen 135 (TUR, AMAZ), Lehtonen 136 (TUR, AMAZ), Lehtonen 137 (TUR, AMAZ), Lehtonen 138 (TUR, AMAZ), Lehtonen 139 (TUR, AMAZ), Lehtonen 140 (TUR, AMAZ), Lehtonen 141 (TUR, AMAZ), Lehtonen 142 (TUR, AMAZ).

E. sp2 2, Bolivia: close to Laguna Normandia; Lehtonen 190 (TUR, LPB), Lehtonen 191 (TUR, LPB).

E. sp3, Paraguay: close to Yhú; Lehtonen & Burguez 274 (TUR, FCQ), Lehtonen & Burguez 275 (TUR, FCQ).

E. sp4, Paraguay: Pte. Hayes; Lehtonen & Burguez 305 (TUR, FCQ), Lehtonen & Burguez 306 (TUR, FCQ), Lehtonen & Burguez 308 (TUR, FCQ), Lehtonen & Burguez 309 (TUR, FCQ).

E. subalatus ssp. *andrieuxii*, Venezuela: close to Barcelona; Lehtonen & Pacheco 472 (TUR, VEN), Lehtonen & Pacheco 477 (TUR, VEN).

E. subalatus ssp. *subalatus*, Bolivia: Perseverancia; Vargas 639 (UNA, LPB).

E. tunicatus, Peru: Sungachi; Lehtonen 104 (TUR, AMAZ), Lehtonen 105 (TUR, AMAZ), Lehtonen 106 (TUR, AMAZ), Lehtonen 107 (TUR, AMAZ), Lehtonen 108 (TUR, AMAZ), Lehtonen 109 (TUR, AMAZ), Lehtonen 110 (TUR, AMAZ), Lehtonen 111 (TUR, AMAZ), Lehtonen 112 (TUR, AMAZ), Lehtonen 113 (TUR, AMAZ), Lehtonen 114 (TUR, AMAZ), Lehtonen 115 (TUR, AMAZ), Lehtonen 118 (TUR, AMAZ), Lehtonen 119 (TUR, AMAZ), Lehtonen 120 (TUR, AMAZ), Lehtonen 121 (TUR, AMAZ), Lehtonen 122 (TUR, AMAZ), Lehtonen 123 (TUR, AMAZ), Lehtonen 124 (TUR, AMAZ), Lehtonen 125 (TUR, AMAZ), Lehtonen 126 (TUR, AMAZ), Lehtonen 127 (TUR, AMAZ), Lehtonen 128 (TUR, AMAZ), Lehtonen 129 (TUR, AMAZ), Lehtonen 130 (TUR, AMAZ), Lehtonen 131 (TUR, AMAZ), Lehtonen 132 (TUR, AMAZ), Lehtonen 133 (TUR, AMAZ), Lehtonen 134 (TUR, AMAZ), Lehtonen 147 (TUR, AMAZ).

E. trialatus 1: Venezuela: Espino; Lehtonen & Pacheco 441 (TUR, VEN), Lehtonen & Pacheco 442 (TUR, VEN), Lehtonen & Pacheco 443 (TUR, VEN).

E. trialatus 2: Venezuela: Espino; Lehtonen & Pacheco 444 (TUR, VEN), Lehtonen & Pacheco 445 (TUR, VEN), Lehtonen & Pacheco 446 (TUR, VEN), Lehtonen & Pacheco 447 (TUR, VEN).

E. uruguayensis 1, Uruguay: Arroyo del Soldado; Lehtonen & Delfino 360 (TUR, MVJB), Lehtonen & Delfino 361 (TUR, MVJB), Lehtonen & Delfino 362 (TUR, MVJB), Lehtonen & Delfino 363 (TUR, MVJB), Lehtonen & Delfino 364 (TUR, MVJB), Lehtonen & Delfino 365 (TUR, MVJB), Lehtonen & Delfino 366 (TUR, MVJB).

E. uruguayensis 2, Argentina: Arroyo Apuaray mi; Lehtonen et al. 231 (TUR), Lehtonen et al. 232 (TUR), Lehtonen et al. 233 (TUR), Lehtonen et al. 234 (TUR), Lehtonen et al. 235 (TUR), Lehtonen et al. 236 (TUR), Lehtonen et al. 237 (TUR).

E. virgatus, Mexico: Tepic; Beechy s.n. (K).

Helanthium cf. *bolivianum* 1, Venezuela: Moquete river; Lehtonen & Pacheco 481 (TUR, VEN), Lehtonen & Pacheco 482 (TUR, VEN), Lehtonen & Pacheco 483 (TUR, VEN), Lehtonen & Pacheco 484 (TUR, VEN).

H. bolivianum 2, Argentina: Montecarlo; Lehtonen et al. 213 (TUR), Lehtonen et al. 214 (TUR), Lehtonen et al. 215 (TUR), Lehtonen et al. 216 (TUR), Lehtonen et al. 217 (TUR), Lehtonen et al. 218 (TUR), Lehtonen et al. 219 (TUR), Lehtonen et al. 220 (TUR), Lehtonen et al. 221 (TUR), Lehtonen et al. 222 (TUR), Lehtonen et al. 223 (TUR).

H. bolivianum 3, Ecuador: Laguna Añangu; Øllgaard et al. 57161 (AAU QCA, QCNE).

H. tenellum 1, Bolivia: San Ignacio; Lehtonen 154 (TUR, LPB), Lehtonen 155 (TUR, LPB), Lehtonen 156 (TUR, LPB), Lehtonen 157 (TUR, LPB), Lehtonen 158 (TUR, LPB), Lehtonen 159 (TUR, LPB).

H. tenellum 2, USA: Alabama; MacDonald 11345 (UNA), MacDonald 11187 (UNA).

H. zombiense, Guadeloupe: Jérémie 2062 (UNA), Christenhusz & Katzer 4040 (TUR).

Limnocharis flava, Peru: close to Iquitos; Lehtonen & Arévalo 33 (TUR), Lehtonen & Arévalo 34 (TUR).

Sagittaria planitiana, Venezuela: close to Las Vegas; Lehtonen & Pacheco 428 (TUR, VEN).

S. montevidensis, Bolivia: San Ignacio de Moxos; Lehtonen 180 (TUR, LPB).

S. sprucei, Peru: close to Iquitos; Lehtonen & Arévalo 31 (TUR), Lehtonen & Arévalo 32 (TUR).

Appendix 5: Command line used in POY analyses

poy –parallel –solospawn 7 –molecularmatrix 111 –maxtrees 5 –holdmaxtrees 30 –random n* –multibuild 5 –ratchettbr 3 –ratchettrees 2 –treefuse –fuselimit 25 –fusingrounds 1 –slop 2 –checkslop 5 –seed −1 –fitchtrees –noleading –norandomizeoutgroup –indices –diagnose –impliedalignment

*number of random addition sequence searches was 40 for total-evidence analysis, and 50 for combined DNA, nuclear DNA, and *mat*K analyses, and 20 for Bremer support analyses.