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Molecular phylogenetics of an aquatic plant lineage, Potamogetonaceae

Charlotte Lindqvist[1,*], Jan De Laet[2], Robert R. Haynes[3], Lone Aagesen[4], Brian R. Keener[3,5] and Victor A. Albert[1]

[1]*Natural History Museum, University of Oslo, PO Box 1172 Blindern, 0318 Oslo, Norway;* [2]*Leuvenstraat 20, B-3020 Veltem-Beisem, Belgium;* [3]*Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA;* [4]*Instituto de Botánica Darwinion, C.C. 22, 1642 San Isidro, Argentina;* [5]*Department of Biological and Environmental Sciences, University of West Alabama, Livingston, AL 35470, USA*

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Abstract

Like most aquatic plants, the pondweeds (Potamogetonaceae) are among the most phenotypically reduced and plastic of all angiosperms. As such, hypotheses of structural homology present difficulties for morphological phylogenetic reconstruction. We used non-coding nuclear and plastid DNA data to address Potamogetonaceae relationships and accompanying issues in character evolution and biogeography. Genera currently assigned to Potamogetonaceae, plus *Zannichellia*, formed a strongly supported monophyletic group. *Potamogeton* and *Stuckenia* (*Potamogeton* subg. *Coleogeton*) were both resolved as monophyletic. Within *Potamogeton* proper, two major clades followed the traditional split between broad- and narrow-leaved species, with the latter condition optimized as basal. Heterophylly (submerged plus floating leaves) has evolved several times, and the ancestral distribution for *Potamogeton* appears to be Northern Hemispheric. Our phylogenetic results have provided a useful genetic framework from which to interpret morphological, cytological and biogeographical evolution.

Aquatic plants exhibit striking morphological diversity despite constituting only a relatively small fraction of angiosperms (less than 2%; Cook, 1990). They are characterized by extreme morphological reductions, both losses of features that are assumed to be otherwise adaptive to terrestrial life, and repeated gains among unrelated species of attributes perceived as adaptive for the aquatic habitat (Arber, 1920; Sculthorpe, 1967). Furthermore, extensive phenotypic plasticity, which is largely influenced environmentally as opposed to genetically, is commonplace among aquatic plants. The challenges these attributes pose for general evolutionary hypothesis construction and for the classification of aquatic plants were recognized early by Arber (1920; see also Sculthorpe, 1967; Barrett et al., 1993). Problems with homology assessments of traits associated with aquatic life, which may convergently define specialized characters or extensive phenotypic plasticity that is not genetically based, have presented a great challenge when attempting phylogenetic inferences (Les and Haynes, 1995).

The pondweeds, *Potamogeton* L. (Potamogetonaceae), represent one of the most important plant genera in the aquatic environment, especially as food or habitat for aquatic animals (Haynes, 1974). Studies have also shown some species to be important in stabilizing substrates, removing particulate matter from the water column, and as indicators for water quality (e.g., Dierberg et al., 2002; Fritioff and Greger, 2003). This cosmopolitan genus, which is generally thought to comprise approximately 80–100 species, displays heterophylly both between and within species. Furthermore,

*Corresponding author:
E-mail address: charlotte.lindqvist@nhm.uio.no

several species exhibit extensive growth phase, seasonal, and geographic morphological plasticity. Hybridization is thought to be relatively frequent, and complex series of (even infraspecific) polyploidy and aneuploidy are present within the genus (e.g., Haynes, 1974, 1978; Les, 1983; Wiegleb, 1988; Hollingsworth et al., 1998; Kaplan, 2002; Fant et al., 2003). Consequently, *Potamogeton* has been considered to be taxonomically difficult, and the large number of known phenotypes has historically led taxonomists to create a complex infrageneric classification with varying numbers of subsections and infraspecific taxa, which has resulted in considerable nomenclatural confusion. Traditionally, *Potamogeton* has been separated into two subgenera (Raunkiær, 1896), although recent suggestions have been made to elevate subgenus *Coleogeton* (Rchb.) D.H.Les & R.R.Haynes to the generic level, giving it the correct name *Stuckenia* Börner (Les and Haynes, 1996; Holub, 1997; Haynes et al., 1998). Within subgenus *Potamogeton*, two morphological groups have been recognized, the broad-leaved species and the linear-leaved species (Fernald, 1932; Ogden, 1943), although it has been questioned if these groupings represent monophyletic entities (Les, 1983).

Potamogeton (including *Stuckenia*) belongs to the family Potamogetonaceae, which as currently circumscribed also includes the monotypic genus *Groenlandia* J.Gay. The delimitation of Potamogetonaceae and assumed allies has, however, changed throughout history: e.g., the family has included the genus *Ruppia* L., and members of the family have been placed in the Najadaceae and have even been combined with members of Zannichelliaceae and Zosteraceae (also see Haynes, 1978 and references therein; Les and Haynes, 1995). Furthermore, various hypotheses as to the position of Potamogetonaceae within the largely aquatic subclass Alismatidae (Alismatales *sensu* Angiosperm Phylogeny Group, 2003) have been suggested, highlighting the problems of systematic studies of aquatic plants based on morphological data (Les and Haynes, 1995).

Few molecular phylogenetic studies of Potamogetonaceae and relatives have been published, of which only the work by Les and coworkers (Les et al., 1993; Les and Haynes, 1995; Les et al., 1997) has included sufficient taxa to provide valuable insight into phylogenetic relationships within Alismatidae. Based on *rbcL* sequence data representing all recognized families and 83% of the genera, Potamogetonaceae was placed in a derived position within the subclass, together with members of Zannichelliaceae, in a strongly supported clade sister to Zosteraceae (Les et al., 1997). However, the *rbcL* sequences provided poor phylogenetic resolution within this lineage and only few taxa of Potamogetonaceae were included. Therefore, although the overall position of Potamogetonaceae within Alismatidae seems relatively unambiguous, family and generic level relationships are still left incomplete.

Our principal purpose with the present study is to ascertain major infrageneric groupings and phylogenetic relationships within *Potamogeton sensu stricto*. A small but relevant study among *Potamogeton* species was recently published (Iida et al., 2004), but the sampling was restricted to Japanese taxa. Although North American taxa are emphasized in our present study, we have included a large number of taxa from outside this area since we wanted to address not only gross morphological evolutionary patterns in *Potamogeton* but also biogeographical relationships. In addition, we wished to evaluate: (1) higher-order relationships to other Alismatidae; (2) the phylogenetic position of *Potamogeton* within Potamogetonaceae; and (3) the monophyly of taxa assigned to *Stuckenia* and their position within or outside *Potamogeton*.

To investigate phylogenetic relationships on the infrageneric level, we analyzed variation within the rapidly evolving non-transcribed spacer of the 5S nuclear ribosomal array (5S-NTS), which has been used for a number of molecular phylogenetic and species-level studies in plants (Cox et al., 1992; see Lindqvist et al., 2003). In order to address interrelationships among the members assigned to Potamogetonaceae, we used the non-coding chloroplast DNA regions, *trnL* intron and *psbA-trnH* spacer (Taberlet et al., 1991; Sang et al., 1997).

Materials and methods

Plant material

Most Potamogetonaceae accessions used in this study were obtained from herbarium material held at the University of Alabama. One *Groenlandia* accession and all other outgroup taxa included were obtained from either herbarium material held at University of Oslo or fresh material grown in the Botanical Garden at the Natural History Museum, University of Oslo. Included in the study were extracted DNAs from herbarium and fresh, silica-dried material from a total of 70 accessions representing 58 accessions of *Potamogeton*, eight of *Stuckenia*, two of the monotypic *Groenlandia densa*, and two of *Zannichellia* L. (see Table 1). In the 5S-NTS study, 57 *Potamogeton* accessions representing 44 species, six *Stuckenia* accessions representing four species, as well as one accession each of *Groenlandia densa* and *Zannichellia* were included. In the *psbA-trnH* spacer analysis, 41 *Potamogeton* sequences representing 33 species, three *Stuckenia* species, and one accession each of *Groenlandia* and *Zannichellia* were used. Of *trnL* intron sequences, 23 *Potamogeton* accessions representing 20 species, four *Stuckenia* species, two *Zannichellia*

species, and one *Groenlandia* accession were included. In order to investigate intergeneric phylogenetic relationships among *Potamogeton*, *Groenlandia*, *Stuckenia* and *Zannichellia*, we attempted to isolate DNAs and determine *psbA-trnH* spacer and *trnL* intron cpDNA sequences for several putative outgroup alismatid taxa. Sequences from the following genera were successful: *Butomus* L., *Echinodorus* Rich. ex Engelm., *Ruppia*, *Sagittaria* L., *Scheuchzeria* L., *Triglochin* L., *Zostera* L. as well as the arid genus *Alocasia* (Schott) G. Don. Furthermore, *trnL* intron sequences from *Magnolia* L., *Orontium* L., and *Tofieldia* Huds. were obtained from GenBank, NCBI. See also Table 1 for a listing of the accessions and their voucher information.

Molecular methods

Dried leaf tissue was in most cases ground using the FastPrep instrument (Qbiogene, Carlsbad, CA) and DNA extracted as described in Lindqvist and Albert (2002). In case of the outgroup taxa, tissue was ground using a Mixer Mill MM 301 (Retsch GmbH & Co. KG, Haan, Germany) and the DNA was isolated using the DNeasy Plant Mini kit (Qiagen, Valencia, CA) following the manufacturer's instructions. Polymerase chain reaction (PCR) amplifications and automated DNA sequencing were performed as described in Lindqvist and Albert (2002). The 5S-NTS region was amplified using the primers PI and PII described by Cox et al. (1992) and the following program: hold 94 °C 2 min; 27 cycles of 94 °C 1 min, 60 °C 1 min, 72 °C 1 min; extend 72 °C 4 min. The *trnL* intron was amplified using primers "c" and "d" of Taberlet et al. (1991), and the *psbA-trnH* region was amplified using the primers *psbA*F and *trnH*R of Sang et al. (1997). The same primers were used for sequencing. Forward and reverse sequences were edited and aligned for each accession and each locus using the software program Sequencher, version 3.1 (GeneCodes, Ann Arbor, MI), and the consensus sequences were deposited in GenBank, NCBI (see Table 1).

Phylogenetic analyses

Instead of performing *a priori* multiple alignments, we optimized nucleotide sequences directly on to trees, an approach first described by D. Sankoff in 1975 and further developed by, a.o., W. Wheeler (e.g., Wheeler, 1996; Wheeler, 2003; Wheeler, 2005). A major practical problem with the algorithm of Sankoff (1975) and other exact algorithms to determine the length of a sequence character on a given tree is their computational complexity. A direct consequence is that for most real-world data sets, heuristic approximations of these algorithms have to be applied. So, in addition to heuristics to explore tree space (such as branch swapping, ratcheting, drifting; see, e.g., Goloboff, 1999; Nixon, 1999), the generalized tree alignment problem requires an additional level of heuristics to explore, for any tree to be examined, the space of all its possible tree alignments. Examples of such heuristic algorithms to calculate the length of a sequence character on a given tree are, e.g., lifted alignments (Jiang and Lawler, 1994; see also Wheeler, 1999) or Wheeler's (Wheeler, 1996) algorithm for optimization alignment.

We analyzed our data using POY (Wheeler et al., 2003), a computer program that offers and integrates several heuristics at both levels (see De Laet and Wheeler, 2003). In all analyses, we used optimization alignment (Wheeler, 1996) as tree alignment heuristic. The basic tree search strategy, embedded in a jackknifing approach, consisted of building initial trees using a random addition sequence of taxa, followed by SPR branch swapping. The cost regime used was substitution 2, gap opening 3, and gap extension 1 (see detailed justification in De Laet, 2005b; Giannini and Simmons, 2005).

Evidential support for our results was assessed through jackknife analysis (Farris et al., 1996), a statistical resampling method that aims to identify groups that are well supported by the data. This approximation strategy is equivalent in its aim to that of Farris et al. (1996), which similarly uses parsimony as its optimality criterion. We performed a jackknife analysis (Farris et al., 1996) as adapted by De Laet (2005b) for use in Sankoff (1975) style analyses: pseudoreplicates are generated by randomly turning 37% of the nucleotides of the observed sequences into "N", indicating the presence of a further unspecified base. This was done using the program *(G)oechel* (De Laet, 2005a). The resulting pseudoreplicates were analyzed using POY. Two approaches were used to summarize the results of the jackknife analysis: (1) construction of a majority rule consensus tree of the strict consensus trees of the individual pseudoreplicates, as in Farris et al. (1996), and (2) construction of a frequency difference consensus tree (Goloboff et al., 2003) of the strict consensus trees of the individual pseudoreplicates. In both cases, this was done using TNT (Goloboff et al., 2002). For the majority rule consensus trees, all groups exceeding 50% are shown; for the frequency difference trees, a cutoff value of 25% was used. The difference between these two approaches for summarizing the jackknife is best explained using a simple example. Assume a jackknife analysis in which 100 pseudoreplicates have been performed and consider the resulting 100 strict consensus trees. Next assume that a particular clade A occurs in 55 of those trees. Using the majority rule way of summarizing jackknifing results, clade A gets a jackknife support value of 55. Using the frequency difference way, the support value for clade A depends on the

structure of the 45 trees that do not display that clade. In the worst case, all 45 could display a same clade B that contradicts A. The support for A would then become $55 - 45 = 10$. Using a cutoff of 50, clade A would be considered unsupported. This correctly reflects that the difference in support (as measured by the frequency in the jackknife profile) for the best hypothesis (clade A) and its runner-up (clade B) is small indeed. At the other extreme, all 45 would display a clade that contradicts clade A, but this contradicting clade would in each case be a different one. In this case, the frequency of the second best hypothesis would be only 1 out of 100, and the frequency difference between the best hypothesis and its runner-up $55 - 1 = 54$. Still using 50 as a cutoff, clade A will now be considered as slightly supported by the data.

Simultaneous optimization and jackknife analyses

Phylogenetic analyses were executed of: (1) the 5S-NTS data alone; (2) the chloroplast data combined; and (3) all available (cpDNA and 5S-NTS) data combined. Within each of these basic data sets, no orthologous subsequences were indicated *a priori*.

For data set 1, 100 pseudoreplicates were analyzed, whereas 50 pseudoreplicates were performed for data sets 2 and 3. The resampled data sets (pseudoreplicates) and POY scripts to analyze these were generated through *(G)oechel* (De Laet, 2005a) using seeds 1 … 100 (or 1 … 50) and exclusion percentage 37 (after Farris et al., 1996). Each pseudoreplicate was analyzed using two replicates of initial tree creation by means of a random addition sequence of taxa, followed by SPR branch swapping. Next, the strict consensus of the best trees over the two replicates was calculated, using *(G)oechel*. Finally, the consensus trees of all individual pseudoreplicates were used as input trees for the summary via the majority rule or frequency difference approaches in TNT (Goloboff et al., 2002). Exemplar scripts for data set 1, pseudoreplicate 100

POY (first replicate for pseudoreplicate 100)

```
poy data set1.jack.100.1 -change 2 -gap
3 -extensiongap 1 -enabletmpfiles
-maxtrees 1 -sprmaxtrees 1 -notbr -spr
-nooneasis -approxbuild -slop 0
-nodiscrepancies -terminalsfile
terms.5S -nooneasis -deletegapsfrom
input -seed 1
```

POY (second replicate for pseudoreplicate 100)

```
poy data set1.jack.100.2 -change 2 -gap
3 -extensiongap 1 -enabletmpfiles
-maxtrees 1 -sprmaxtrees 1 -notbr -spr
-nooneasis -approxbuild -slop 0
-nodiscrepancies -terminalsfile
terms.5S -nooneasis -deletegapsfrom
input -seed 2
```

Character-state optimizations

Geographical and morphological character state optimizations were performed using WinClada (Nixon, 2002). Optimizations were performed directly on jackknife consensus trees, as these were the only tree output available using our approximation method. Of course, optimizations on trees that are not most-parsimonious (or even soft-polytomous most-parsimonious trees) may themselves not be most parsimonious, but our intention with these was to provide suggestive character-state trends for groups that are arguably well-supported by our approximate tree-search method. Data were treated as non-additive or additive (leaf width), and unambiguous character-state optimizations are reported for the majority rule jackknife tree since this tree was less resolved than the frequency difference tree (see Fig. 2) and therefore gave a more conservative estimate.

Results

Sequence data

The 5S-NTS sequence data matrix comprised a total of 66 taxa. The lengths of the DNA sequences varied in *Potamogeton* from 281 bp (*P. tricarinatus*) to 315 bp (*P. epihydrus*), in *Stuckenia* they varied from 273 bp (*S. vaginata*) to 284 bp (*S. pectinata*), and the *Groenlandia* and *Zannichellia* sequences were 340 and 271 bp long, respectively. Using direct sequencing, only very few intraindividual polymorphic sites were found, and these seemed to appear in a random phylogenetic pattern as opposed to representing, e.g., paralogs. Therefore, no effort was made to clone individual repeat elements to assess their diversity.

The *psbA-trnH* spacer sequence data matrix comprised 55 taxa. The lengths of the sequences varied in *Potamogeton* from 324 bp (*P. ochreatus*) to 420 bp (*P. robbinsii*), and in *Stuckenia* from 356 bp (*S. pectinata*) to 395 bp (*S. striata*). Among the outgroup taxa the lengths varied from 384 bp in *Triglochin maritima* to 579 bp on *Alocasia odora*.

The *trnL* intron sequence data matrix comprised 39 taxa. The lengths of the sequences varied in *Potamogeton* from 486 bp (*P. lucens*) to 563 bp (*P. diversifolius* and *P. spirillus*), and in *Stuckenia* from 558 bp (*S. vaginata* and *S. filiformis*) to 576 bp (*S. striata*). Among the outgroup taxa, the lengths varied from 480 bp in *Magnolia sieboldii* to 745 bp in *Echinodorus muricatus*.

Phylogenetic analyses of chloroplast DNA data

The *trnL* intron and *psbA-trnH* spacer regions were not analyzed separately but were combined into one "plastid DNA" data matrix, which comprised 61 taxa. Of the 61 accessions included, 27 were non-overlapping

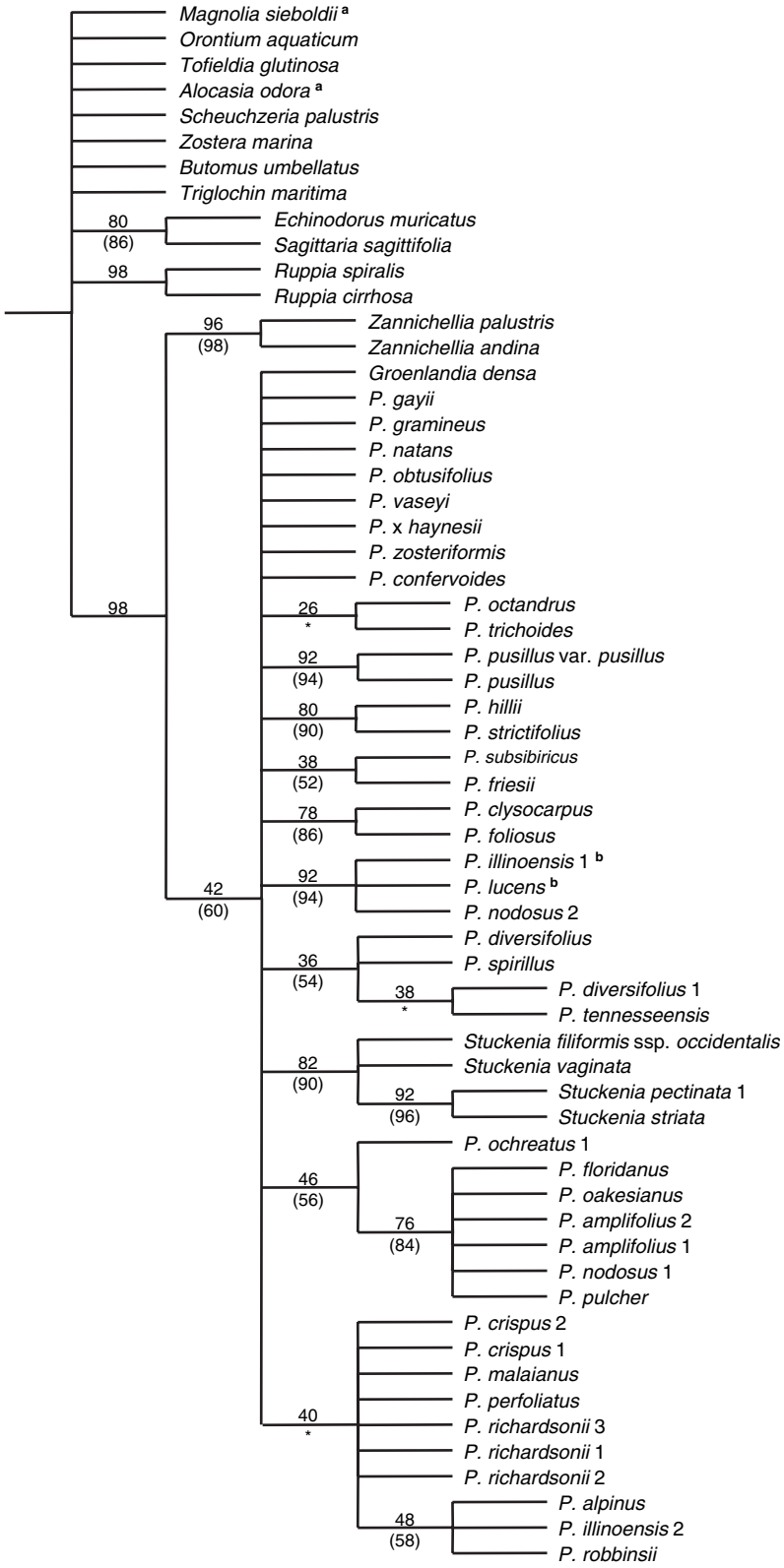

Fig. 1. cpDNA combined frequency difference jackknife consensus tree. Frequency difference jackknife support values shown above branches, and majority rule jackknife support values shown in parentheses below branches if different from frequency difference tree ([a]supported at 54%, [b]supported at 52%, *node collapsed in majority rule tree).

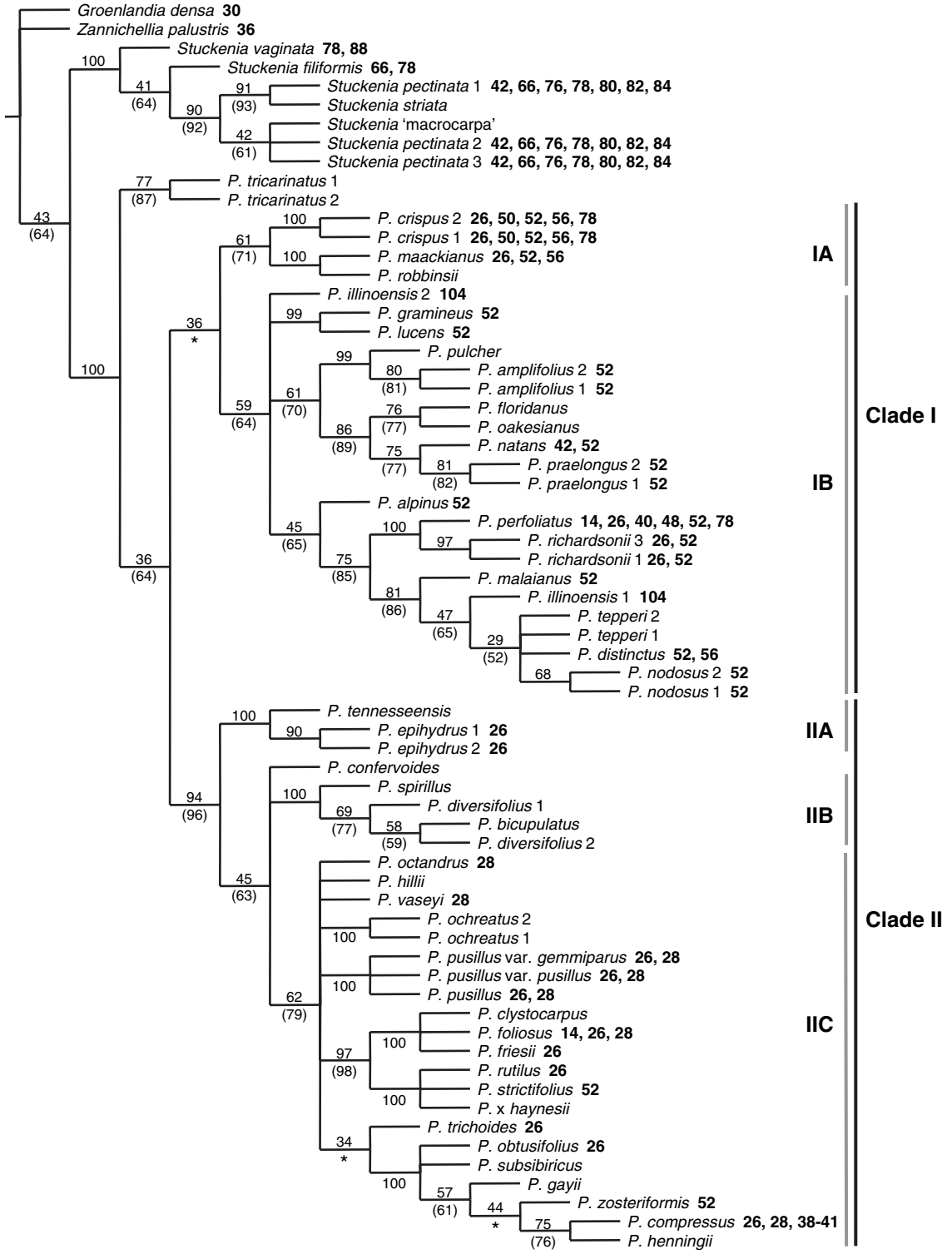

Fig. 2. 5S-NTS frequency difference jackknife consensus tree. Frequency difference jackknife support values shown above branches, and majority rule jackknife support values shown in parentheses below branches if different from frequency difference tree (*node collapsed in majority rule tree). Chromosome numbers (2n) indicated in bold next to taxon name.

(i.e., represented either an "orphan" *trnL* intron or *psbA-trnH* spacer sequence). The majority rule and frequency difference jackknife trees were highly congruent in topology with only minor differences (Fig. 1; see Methods for an explanation of the two different approaches). In both the majority rule and frequency difference jackknife trees, Potamogetonaceae plus *Zannichellia* constituted a strongly supported, monophyletic clade, and Potamogetonaceae itself was supported as a monophyletic group at 60% and 42%, respectively (Fig. 1). Within Potamogetonaceae, the four *Stuckenia* taxa included were strongly supported as a monophyletic group. However, the relationship of this group and the single *Groenlandia* accession to the remaining taxa of Potamogetonaceae was unresolved. Nevertheless, several well-supported subclades within *Potamogeton* were recognized (see Fig. 1), e.g., (1) *P. illinoensis*, *P. lucens*, and *P. nodosus*, and (2) *P. floridanus*, *P. oakesianus*, *P. amplifolius*, *P. nodosus* and *P. pulcher*.

Phylogenetic analyses of 5S-NTS data

The 5S-NTS sequence data matrix comprised 66 accessions. The 5S-NTS jackknife trees were far better resolved than the cpDNA trees (Fig. 2). *Potamogeton* constituted a well-supported, monophyletic clade sister to *Stuckenia*. Within *Potamogeton*, *P. tricarinatus* was supported as sister to the remaining taxa, which largely grouped into two separate clades I and II, although clade I was not supported in the majority rule jackknife tree and only marginally supported in the frequency difference tree. Clade I comprised two well-supported clades: one that included *P. crispus*, *P. maackianus*, and *P. robbinsii* (clade IA) and one that included predominantly "broad-leaved" taxa, e.g., *P. illinoensis*, *P. lucens*, and *P. nodosus* (clade IB). Clade II comprised a basal clade of *P. tennesseensis* and *P. epihydrus* (clade IIA) sister to a clade consisting of: (1) *P. confervoides*; (2) a clade of *P. spirillus* and allies (clade IIB); and (3) a clade of largely *P. pusillus* and allies, and *P. compressus* and allies (clade IIC).

Phylogenetic analyses of all data combined

The matrix of all data combined comprised 80 accessions. As with the chloroplast data combined, in the "total (available) evidence" results Potamogetonaceae plus Zannichellia constituted a strongly supported, monophyletic clade (Fig. 3). However, several differences in tree topology were found among the analyses of combined data versus the analyses of the chloroplast and nuclear DNA data alone. In the majority rule jackknife tree, *Groenlandia* was found to be sister to a poorly supported (51%) clade of *Zannichellia* plus *Stuckenia* and *Potamogeton*, and *Zannichellia* in turn was supported as sister to *Stuckenia* and *Potamogeton*. In the frequency difference jackknife tree, *Groenlandia* and *Zannichellia* were collapsed and weakly supported as sister to *Stuckenia* plus *Potamogeton* (see Fig. 3). Also inside *Potamogeton* several differences were present between the 5S-NTS analysis alone and the analysis of combined data, of which the most notable were: (1) *P. tricarinatus* was found as sister to "clade I" instead of as sister to all other *Potamogeton*, and (2) clade IA in the 5S-NTS tree was embedded inside clade IB of the combined analysis.

Discussion

Circumscription of Potamogetonaceae and infrafamilial relationships

The definition of Potamogetonaceae has historically varied among different authors. The family has, e.g., included the genus *Ruppia* and has been variously combined with members of the Zosteraceae, Cymodoceaceae, Zannichelliaceae and Najadaceae (e.g., Haynes, 1978; Les and Haynes, 1995). However, more recently the family has been considered to consist of three genera, *Potamogeton*, *Stuckenia* and *Groenlandia*. Based on morphology, Potamogetonaceae can be separated from these other families by their perfect flowers, lack of spathe-like bracts, and in some species, the presence of turions. The major difference between Zannichelliaceae and Potamogetonaceae is that the former has an envelope around the inflorescence, a character that is absent in Potamogetonaceae. Both Ruppiaceae and Potamogetonaceae bear drupelets, but those of the Potamogetonaceae are sessile, whereas those of Ruppiaceae are stipitate. Based on DNA sequence data from the chloroplast gene *rbcL*, Les et al. (1993) found Potamogetonaceae to be closely related to Zosteraceae but not to Ruppiaceae, which was placed as the sister group to the marine Cymodoceaceae, nor to Najadaceae, which was more closely related to Hydrocharitaceae than to other families of the previously circumscribed Najadales. Expanding the sampling to include more Alismatidae taxa, Les et al. (1997) showed Potamogetonaceae, together with members of Zannichelliaceae, to be placed in a relatively derived position within the subclass in a strongly supported clade sister to Zosteraceae. However, the *rbcL* sequences only provided poor phylogenetic resolution within this clade, and only 10 taxa of Potamogetonaceae were included.

Our phylogenetic results of both the chloroplast data only and all data combined (chloroplast and 5S-NTS) show Potamogetonaceae plus *Zannichellia* to be a strongly supported group (Figs 1 and 3). In turn, however, the relationship of this group to other outgroup taxa included remains unresolved. Also, hypotheses on the interrelationships of the genera within

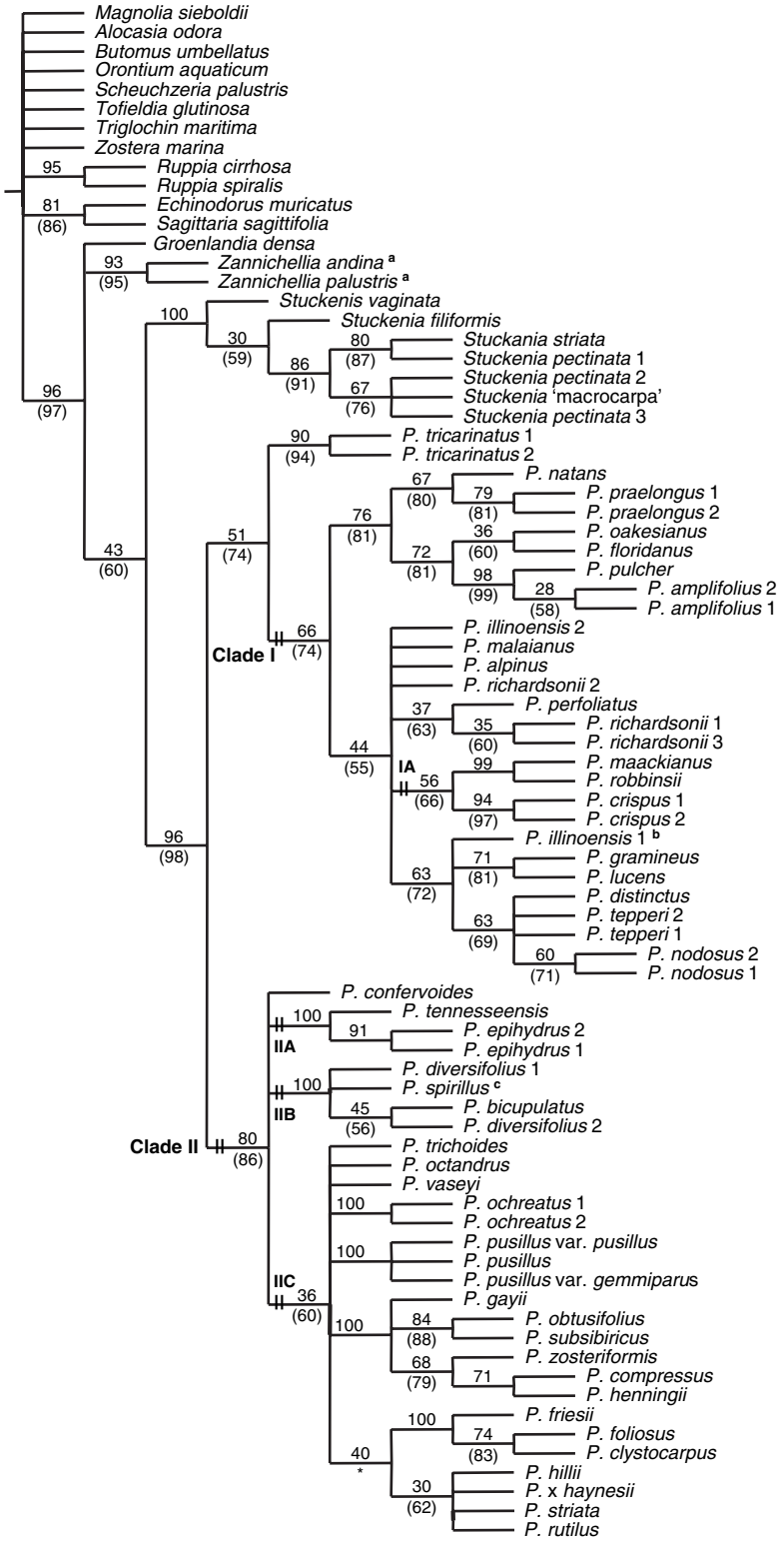

Fig. 3. Frequency difference jackknife consensus tree of 5S-NTS plus cpDNA combined. Frequency difference jackknife support values shown above branches, and majority rule jackknife support values shown in parentheses below branches if different from frequency difference tree ([a]the *Zannichellia* clade is supported at 51% as sister to *Potamogeton* plus *Stuckenia* in majority rule tree, [b]supported at 54% as sister to *P. distinctus* clade, [c]supported at 51% as sister to *P. bicupulatus* clade, *node collapsed in majority rule tree). Bars on branches refer to clade names in the 5S-NTS tree (Fig. 2).

this group are inconclusive. The cpDNA data alone show *Zannichellia* to be sister to the remaining taxa of Potamogetonaceae, whereas the chloroplast and nuclear DNA data combined show, while only poorly supported, *Groenlandia* either to be (1) sister to a clade of *Zannichellia*, which is in turn sister to *Stuckenia* plus *Potamogeton* (majority rule tree), or (2) unresolved with the *Zannichellia* clade as sister to the latter two genera (frequency difference tree). These inconsistencies between the regular and the frequency difference jackknife consensus trees are reflected in the low support values (see Fig. 3). Nevertheless, our data including considerably more Potamogetonaceae taxa as well as an additional *Zannichellia* accession still does not contradict the findings by Les et al. (1997) that Zannichellia(ceae) may be weakly embedded inside Potamogetonaceae. As a consequence of those findings the APG II system (Angiosperm Phylogeny Group, 2003) and Stevens (2004) included Zannichelliaceae within Potamogetonaceae. However, as none of the additional genera in Zannichelliaceae (*Pseudalthenia* (Graebn.) Nakai, *Althenia* Petit, and *Lepilaena* J.Drum. ex Harv.) were included here, our data may only support a transfer of *Zannichellia* alone.

Stuckenia—*a separate genus?*

Infrageneric classifications of *Potamogeton* have traditionally placed the species *Potamogeton pectinatus* and allies in separate units, either as a section (e.g., *Coleophylli*; Ascherson and Graebner, 1907) or subgenus (e.g., *Coleogeton*; Raunkiær, 1896), or more recently, as a species group (Wiegleb, 1988). Börner (1912) was the first author to propose this group as a separate genus, *Stuckenia*, although his endeavor was neglected by later works on *Potamogeton* taxonomy (Holub, 1997). Also Hagström (1916) recognized the separation of subgenus *Coleogeton* from subgenus *Potamogeton* and viewed it as occupying an isolated position within the genus (see also Les and Sheridan, 1990). More recently, several authors have attempted to elevate this group of taxa to generic level (Les and Haynes, 1996; Holub, 1997; Haynes et al., 1998). Among the main diagnostic features separating *Stuckenia* from *Potamogeton s.str.* (and *Groenlandia*) are long stipular sheaths, opaque submerged leaves, channeled and turgid flexible peduncles, and elongate stigmatic papillae (Les and Haynes, 1996; Holub, 1997). However, Wiegleb and Kaplan (1998) did not consider these characters as sufficient for distinguishing *Stuckenia* as a separate genus and adopted the traditional concept of treating the group at the rank of subgenus.

In our phylogenetic results based on nuclear and chloroplast plus nuclear data (Figs 2 and 3), all included *Stuckenia* species group into a strongly supported monophyletic clade sister to all included *Potamogeton* taxa, and therefore, corroborate the earlier view that this group of taxa represent a distinct lineage. Consequently, *Stuckenia* does not represent a highly specialized group derived from the *Potamogeton pusillus* group as proposed by Les and Sheridan (1990) but perhaps rather an ancestral lineage within *Potamogeton s.l.* as originally suggested by Hagström (1916; see also Les and Sheridan, 1990). As the *Stuckenia* and *Potamogeton* clades together form a monophyletic group, our results do not refute the inclusion of *Stuckenia* within the genus *Potamogeton*. However, we do argue for a continued recognition of the genus *Stuckenia* based on morphological, and anatomical as well as molecular data; these taxa clearly occupy an isolated position within Potamogetonaceae separate from *Potamogeton sensu stricto*.

Infrageneric relationships and character evolution in Potamogeton

Several attempts have been made to generate an infrageneric classification of *Potamogeton*, and species have been separated into either more formal sections and subsections or more informal groupings based on affinities (e.g., Raunkiær, 1903; Ascherson and Graebner, 1907; Hagström, 1916; Wiegleb, 1988). However, these groupings have often been criticized (see e.g., Les, 1983; Les and Sheridan, 1990), and in more recent taxonomic work on *Potamogeton* (Wiegleb and Kaplan, 1998), no infrageneric groupings were adopted but only notes on systematic affinities were included. Also, Haynes and Hellquist (2000) did not include an infrageneric classification in their treatment for the Flora of North America, as they believed that recognition of the many infrageneric categories was not of much utility. Taxonomic subdivision of *Potamogeton* is encumbered by the extensive phenotypic and genotypic variability, which may not only be expressed as a response to seasonal and environmental changes, but can also differ in different parts of the distributional range of a species (see e.g., Wiegleb, 1988; Kaplan, 2002).

Surprisingly, with our 5S-NTS sequence data we found *Potamogeton tricarinatus* to be supported as a sister taxon to the remaining *Potamogeton* (see Fig. 2). The epithet *tricarinatus* has been applied to almost any broad-leaved species of *Potamogeton* from Australia (Wiegleb and Kaplan, 1998), and it has been known for a long time that plants identified as *P. tricarinatus* include specimens that may represent a diversity of species (Papassotiriou, 1998). However, the two individuals included here, which were collected by Jacobs and Hellquist from Queensland and New South Wales and apparently represent a relatively rare plant (see Wiegleb and Kaplan, 1998), do indeed hold a very interesting position in *Potamogeton*, and obviously deserve species status. Wiegleb (1988) included *P. tricarinatus* in his "*P. amplifolius* group" but did

question whether this taxon deserved a species status of its own. Unfortunately, it was not possible at this time to include *P. tricarinatus* in our cpDNA analyses, but in the combined data analyses this species was supported as sister to clade I (Fig. 3). Such incongruences are not unexpected giving the possible maternal inheritance of chloroplast DNA and presumed existence of hybridization within *Potamogeton*. Nevertheless, further studies of this interesting taxon are clearly needed. It may be that *P. tricarinatus* represents a relic taxon within *Potamogeton*.

None of the major taxonomic groups described in Wiegleb (1988) are found to be monophyletic in our analyses, although three small groups are supported as monophyletic: the monotypic "*P. crispus*" and "*P. confervoides*" groups as well as the "*P. robbinsii* group", which consist of *P. maackianus* and *P. robbinsii*, only. Other studies have pointed out the distinct positions these taxa hold in the genus. Most recently, a molecular phylogenetic study of Japanese *Potamogeton* (Iida et al., 2004) found *P. crispus* to be unique by having a long deletion and several autapomorphic substitutions in the *trnT-trnL* sequence. Also, allozyme analyses have shown this easily distinguished taxon to be distinct from other species in the genus (Hettiarachchi and Triest, 1991). Furthermore, studies of fruit characters have shown similarities between *P. robbinsii* and *P. crispus* (Aalto, 1974), which provide additional support to clade IA (Fig. 2). *Potamogeton confervoides* is regarded as an isolated species within *Potamogeton* (see e.g., Wiegleb and Kaplan, 1998).

Wiegleb (1988) combined *Potamogeton perfoliatus* and *P. praelongus* into his "*P. perfoliatus* group" (which also included *P. richardsonii*) although this group previously had been separated into two subsections (e.g., Raunkiær, 1903; Hagström, 1916). The members of these two subsections are the only species in the genus with clasping leaves. In addition to morphological features, the flavonoid chemistry support combining the two subsections into one (Haynes, 1985). However, in our phylogenetic analyses, the taxa of the two subsections are found in two different subclades of clade IB (Fig. 2), and therefore the data do not support combining the subsections. Instead, *P. praelongus* is supported as sister to *P. natans* (and is nested inside Wiegleb's "*P. natans* group" in the 5S-NTS analyses; Fig. 2). This latter relationship was also found by Iida et al. (2004). The close relationship of *P. perfoliatus* and *P. richardsonii* is supported here.

The species *Potamogeton epihydrus*, *P. tennesseensis*, *P. bicupulatus*, *P. diversifolius*, and *P. spirillus* have all been suggested to belong to one group, the "*P. epihydrus* group" (Wiegleb, 1988). In our study they are separated into two clades, which are not monophyletic with respect to each other (clade IIA and IIB, Fig. 2), although their interrelationship is unresolved in the combined analyses (see Fig. 3). In fact, clade IIA is found to be sister to all other taxa in clade II, which are all linear-leaved taxa (Fig. 2). Taxa in clades IIA and IIB are the only heterophyllous species (see also below) in clade II, apart from *P. octandrus* and *P. vaseyi*, which are very closely related taxa and polymorphic with respect to presence of floating leaves (see Fig. 4). Some of the morphological differences between clade IIA and IIB are that taxa in clade IIB have adnate stipules of the submerged leaves, di-trimorphic inflorescences, and the dorsal keel of the fruits is distinct. Members of clade IIB were also suggested by Wiegleb and Kaplan (1998) to be closely related. In fact, they noted that *Potamogeton bicupulatus* may be an extreme morphotype of *P. diversifolius*, and indeed in our 5S-NTS phylogenetic analyses we found this taxon to be nested inside *P. diversifolius* (Fig. 2).

Most of the members of our clade IIC (see Fig. 2) are members of the *P. pusillus* complex or subsection *Pusilli* Graebner. This clade is also the least resolved in our phylogenetic analyses. The *P. pusillus* complex has long been considered to be taxonomically difficult and uncertainty still exists as to the number of taxa that should be included (Haynes, 1974). Some of the reasons for the taxonomic confusion in this group may be that they are physically small and the morphological characters are therefore difficult to observe (Haynes, 1974). Moreover, they are extremely variable phenotypically, although this variation seems to be mostly environmentally induced (Kaplan, 2002). Haynes (1974) recognized 15 species in subsection *Pusilli*, and of these, eight North American species, including *P. groenlandicus*, were described and discussed. All eight species except *P. groenlandicus* are included in the present study. Haynes's (1974) phylogenetic hypothesis is not supported here, as he suggested that *P. friesii* and *P. strictifolius* represent ancestral *Pusilli* from which two main lines have evolved: (1) those taxa with dorsal and lateral keels absent (*P. pusillus* and *P. groenlandicus*), and (2) those with dorsal and/or lateral keel present (*P. obtusifolius*, *P. hillii*, *P. clystocarpus* and *P. foliosus*). In our present analyses: (1) *P. friesii* is found to be closely related to *P. clystocarpus* and *P. foliosus*; (2) *P. obtusifolius* is strongly supported as belonging to a subclade including taxa of the "*P. compressus* group" (*sensu* Wiegleb, 1988; see also Wiegleb and Kaplan, 1998); and (3) *P. hillii* is unresolved within clade IIC (see Fig. 2). Wiegleb and Kaplan (1998) noted that *P. obtusifolius* is the most distinct species in the *P. pusillus* group.

A general trend from our phylogenetic results, when more than one accession of a species has been included, is that they are either exclusive lineages or group together (unresolved) within a particular clade. One exception is *P. illinoensis*, and clearly more work is needed on the *P. illinoensis* group, which also includes *P. lucens* and *P. malaianus*.

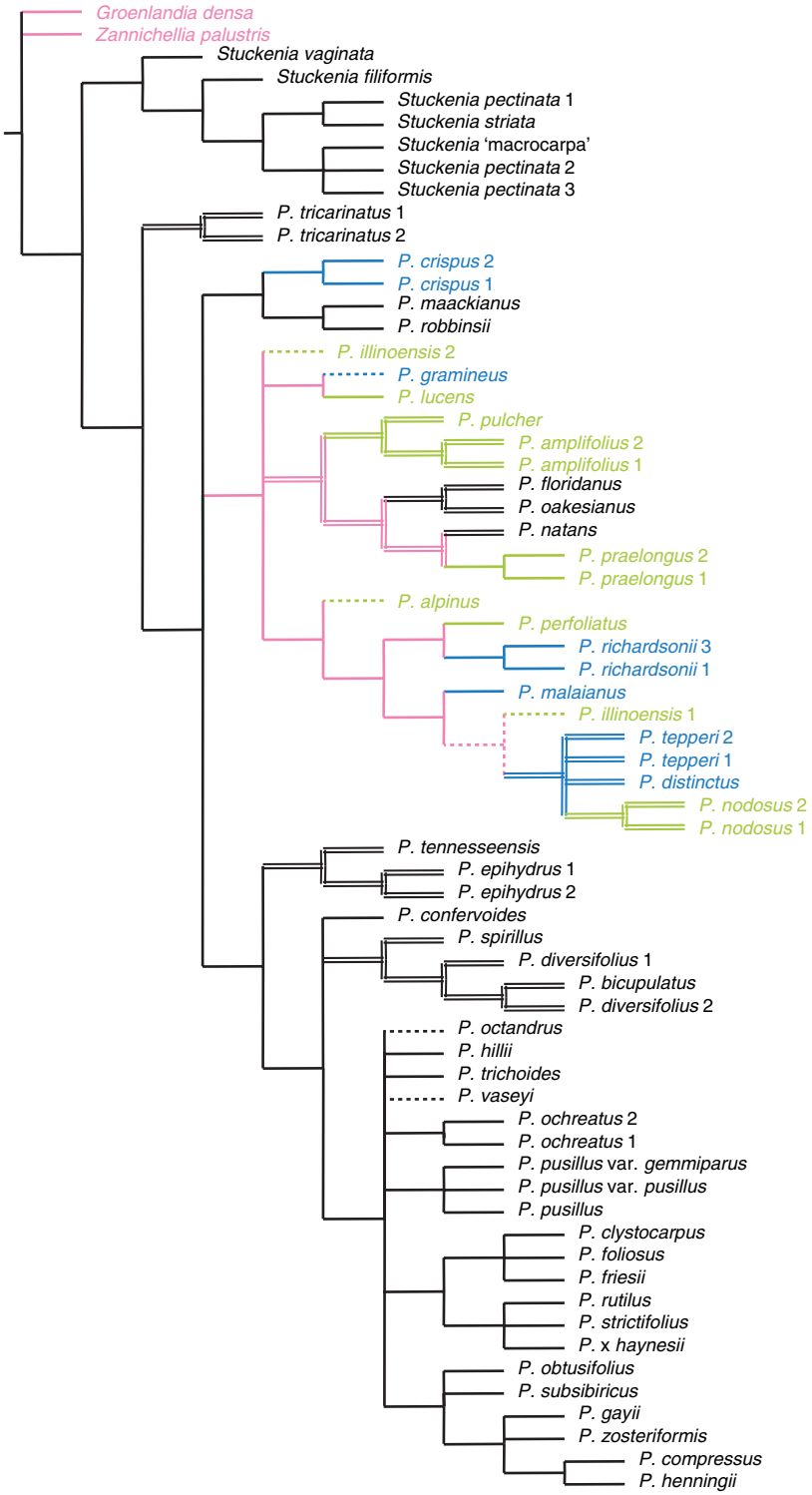

Fig. 4. Leaf width and heterophylly. 5S-NTS jackknife majority rule tree with maximum submerged leaf width and heterophylly unambiguously optimized. Leaf width optimization colors: black = < 15 mm, blue = 16–25 mm, green = > 30 mm, pink = ambiguity. Optimization of heterophylly: solid line = absence and open line = presence of floating leaves, dotted line = polymorphic.

Leaf evolution

Like many other aquatic plants, several *Potamogeton* species are characterized by heterophylly. In *Potamogeton*, the heterophyllous taxa typically exhibit much broader, coriaceous floating leaves in addition to the translucent, membranous, narrower submerged leaves. Several authors have speculated on the evolutionary relationships of heterophyllous and homophyllous species (e.g., Raunkiær, 1903; Hagström, 1916; Wiegleb, 1988; Les and Sheridan, 1990; see also below). When we optimized presence of floating leaves on to our 5S-NTS phylogenetic result, homophylly was clearly the ancestral state in *Potamogeton* (see Fig. 4). As a consequence, heterophylly has evolved several times in different lineages in the genus by parallel evolution. This trend supports the molecular phylogenetic results obtained from Japanese *Potamogeton* species (Iida et al., 2004).

Conveniently, two morphological groups in *Potamogeton* have been recognized, the "broad-leaved" species and the "linear-leaved" species, referring to the width of the submerged leaves (Fernald, 1932; Ogden, 1943), although it has been questioned if these groupings represent natural (monophyletic) entities (Les, 1983). In our molecular phylogenetic results with 5S-NTS nuclear DNA sequence data, the *Potamogeton* taxa grouped largely into a "broad-leaved" lineage (Clade I, Fig. 2) versus a "linear-leaved" lineage (Clade II, Fig. 2). Optimization of the maximum widths of the submerged leaves on to the 5S-NTS majority rule jackknife consensus tree (Fig. 4) indicated that the ancestral state in *Potamogeton* is leaves less than 15 mm broad, that the evolution of broader leaves has happened at least once, and at least one reversal back to more narrow leaves has occurred in Clade IB (*P. floridanus*, *P. oaksianus*, and *P. natans*, although the submerged leaf lamina of the latter is considered to be reduced to linear phyllodes). The maximum recorded leaf width (according to Wiegleb and Kaplan, 1998 and own observations) was used rather than the more spurious terms "broad" and "narrow". The findings by Iida et al. (2004) showed a similar pattern, although *P. natans* and the broad-leaved *P. praelongus* were found in their Group II, which otherwise constituted the linear-leaved taxa. The maximum widths of the submerged leaves of the included taxa in our study shows that there is a curvilinear continuum from the most narrow to the broadest recorded width (see Fig. 5), although the broader leaves (e.g., width > 30 mm) exhibit a greater heterogeneity in minimum and maximum recorded width.

Evolutionary hypotheses on the origin of homophyllous versus heterophyllous and linear-leaved versus broad-leaved *Potamogeton* have been discussed frequently by several authors (e.g., Raunkiær, 1903; Hagström, 1916; Wiegleb, 1988; Les and Sheridan, 1990). For example, Raunkiær (1903) hypothesized that heterophylly evolved from homophyllous, broad-leaved species as a consequence of adaptation to the aquatic habitat, whereas Hagström (1916) suggested that the genus arose from homophyllous, linear-leaved ancestors. More recently it has been argued that homophyllous, linear-leaved species arose from heterophyllous *Potamogeton* (e.g., Les, 1983; Wiegleb, 1988). With our molecular phylogenetic findings, an ancestral state in *Potamogeton* is indicated as being homophyllous with linear leaves. Broad, submerged leaves have evolved at least once in *Potamogeton*, whereas heterophylly has arisen several times in parallel within the genus (Fig. 4). It has been shown that in a number of aquatic plants, exogenous

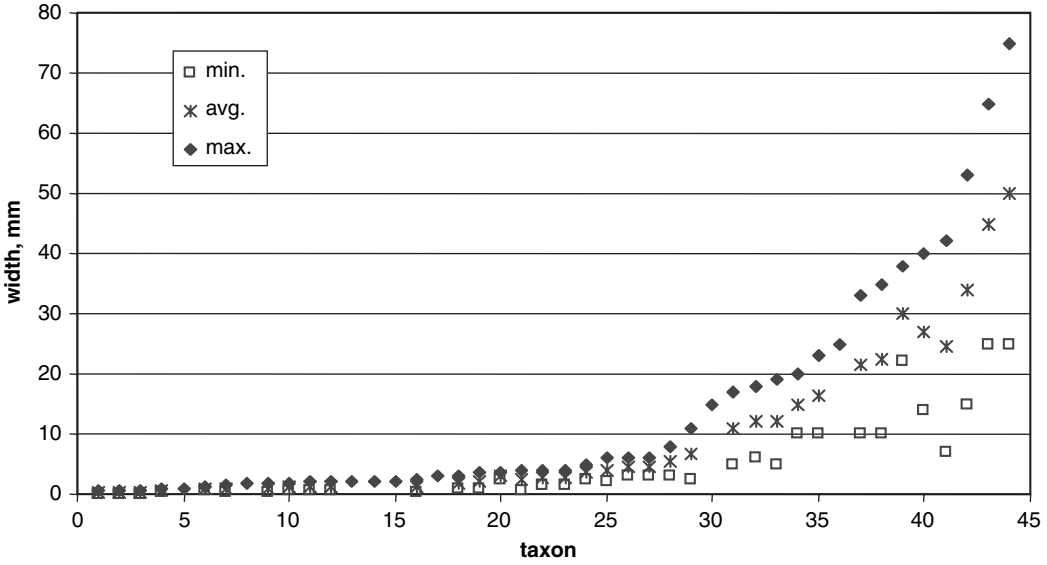

Fig. 5. Widths (maximum, minimum, and calculated average) of the submerged leaves plotted for each *Potamogeton* taxon included in the present study. Measurements according to Wiegleb and Kaplan (1998) and own observations.

application of the plant hormone abscisic acid (ABA) triggers a phase change to heterophylly during the adult vegetative stage, and that ABA in these plants induces the formation of aerial-type morphology that is distinct from its counterpart submersed in water, particularly in the size and shape of the leaf (e.g., Lin, 2002). This ABA effect has been seen in phylogenetically derived taxa, including *Potamogeton nodosus* (Anderson, 1978). Thus, the genetic mechanism responsible for heterophylly may be easily triggered and could explain the repeated derivation of this trait in *Potamogeton*.

Chromosomal evolution

The chromosome numbers in *Potamogeton sensu lato* (i.e., including *Stuckenia*) exhibit extensive variation, not only between species, but also within many species. In *Stuckenia*, which in general show high ploidy levels and considerable aneuploidy, at least seven different numbers have been counted for *S. pectinata* (see Fig. 2). Also within *Potamogeton*, large chromosomal variability seems evident, with two chromosomal series present, one based on $x = 7$ and the other on $x = 13$. Several investigators have speculated as to chromosomal evolution and its correlation with *Potamogeton* taxonomy (see e.g., Les, 1983). However, varying classification, incorrect plant identification, technical difficulties in counting the small chromosomes, and use of misleading sources of chromosome counts are just some of the potential problems in this kind of endeavor. As a consequence of the latter, Hollingsworth et al. (1998) published a review of chromosome numbers in *Potamogeton s.l.* as an attempt to sort out previous errors and confusion in the literature. Although this review does not necessarily solve the three first issues, we have relied on their review here and used it in our interpretations based on our phylogenetic results. Of the total number of species, for which chromosome counts are reported in Hollingsworth et al. (1998), over 70% (29 of 41 species) are represented in the present study. Of these, *Potamogeton* is represented by 26 species. Most of the ploidal and aneuploidal variation exists within our clade I (see Fig. 2), as both chromosomal series and ploidy levels from $2n = 14$ (*P. perfoliatus*) to $2n = 104$ (*P. illinoensis*) are found here. In addition, most of the intraspecific variation is found here, particularly represented by the species *P. crispus* and *P. perfoliatus*. In clade II, lower ploidy levels are evident, and it is only here that taxa with chromosome number $2n = 28$ are found. Consequently, species with floating leaves in clade I have for the most part $2n = 52$ or higher (*P. natans* being the only exception with $2n = 42$ as well as $2n = 52$), whereas in clade II, species with floating leaves apparently have lower ploidy levels, i.e., $2n = 26$ or $2n = 28$. Within clade I and II no clear pattern is evident, and neither chromosome numbers nor base numbers seem to assemble into monophyletic groups in our phylogenetic tree. Furthermore, a correlation between morphology and chromosome/base number has previously been hypothesized, but our study corroborate the earlier observation (see Les, 1983; Les and Sheridan, 1990; Hollingsworth et al., 1998) that many of the suggested morphological groups are not homogeneous with respect to base chromosome number.

In reference to the apparent widespread occurrence of aneuploidy in *Potamogeton* (even when omitting *Stuckenia*) it has been proposed that $x = 7$ represents the ancestral base number and that $x = 13$ species arose from multiple origins through aneuploidy (“multiple origin hypothesis”; see Les, 1983; Les and Sheridan, 1990). The presence of $x = 7$ numbers in almost all the taxa exhibiting chromosomal variation seems to support this hypothesis (see Les and Sheridan, 1990). However, if the two different chromosomal series ($x = 7$ and $x = 13$) are mapped on to our phylogenetic tree, $x = 13$ is optimized as the basal number (not shown), as was suggested by Wiegleb (1988). Other members in Potamogetonaceae and close relatives exhibit the numbers $n = 6$ (*Zannichellia*), $n = 15$ (*Groenlandia*), and in the sister group to Potamogetonaceae, *Zostera*, $n = 6$ has been reported. It is important to note here that many *Potamogeton* taxa exhibit “missing” data (with either no counts reported, or counts suspicious and therefore excluded). Whether $x = 7$ or $x = 13$ represents the base number in *Potamogeton* does not entirely explain the extensive chromosomal variation and existence of both series within many species. It seems evident that the dynamics of chromosomal evolution in *Potamogeton* bear further study.

Biogeographical inferences

It has long been known that aquatic angiosperms, in general, have a wider distribution than their terrestrial relatives and that their remarkably wide distribution has caused great difficulties in speculations on their geographic origins (e.g., Arber, 1920; see also Les et al., 2003). *Potamogeton* is no exception, and in fact, not only are several species found throughout the Northern Hemisphere or found on numerous continents, but some species are even near-cosmopolitan (e.g., *P. nodosus* and *P. perfoliatus*; see also Fig. 7). Nevertheless, we will here attempt to infer some distributional trends based on our results.

When the actual geographic localities of the individual accessions used in this study are optimized on to the 5S-NTS tree, the ancestral geographic area for *Potamogeton* (plus *Stuckenia*) is North America (Fig. 6). In order to account for the predominant sampling of North American populations for this study, we also optimized the entire geographic distributions known for each species represented by the accessions, and again, North America was most-parsimoniously interpreted as the ancestral area (see Fig. 7). Based on fossil evidence, it

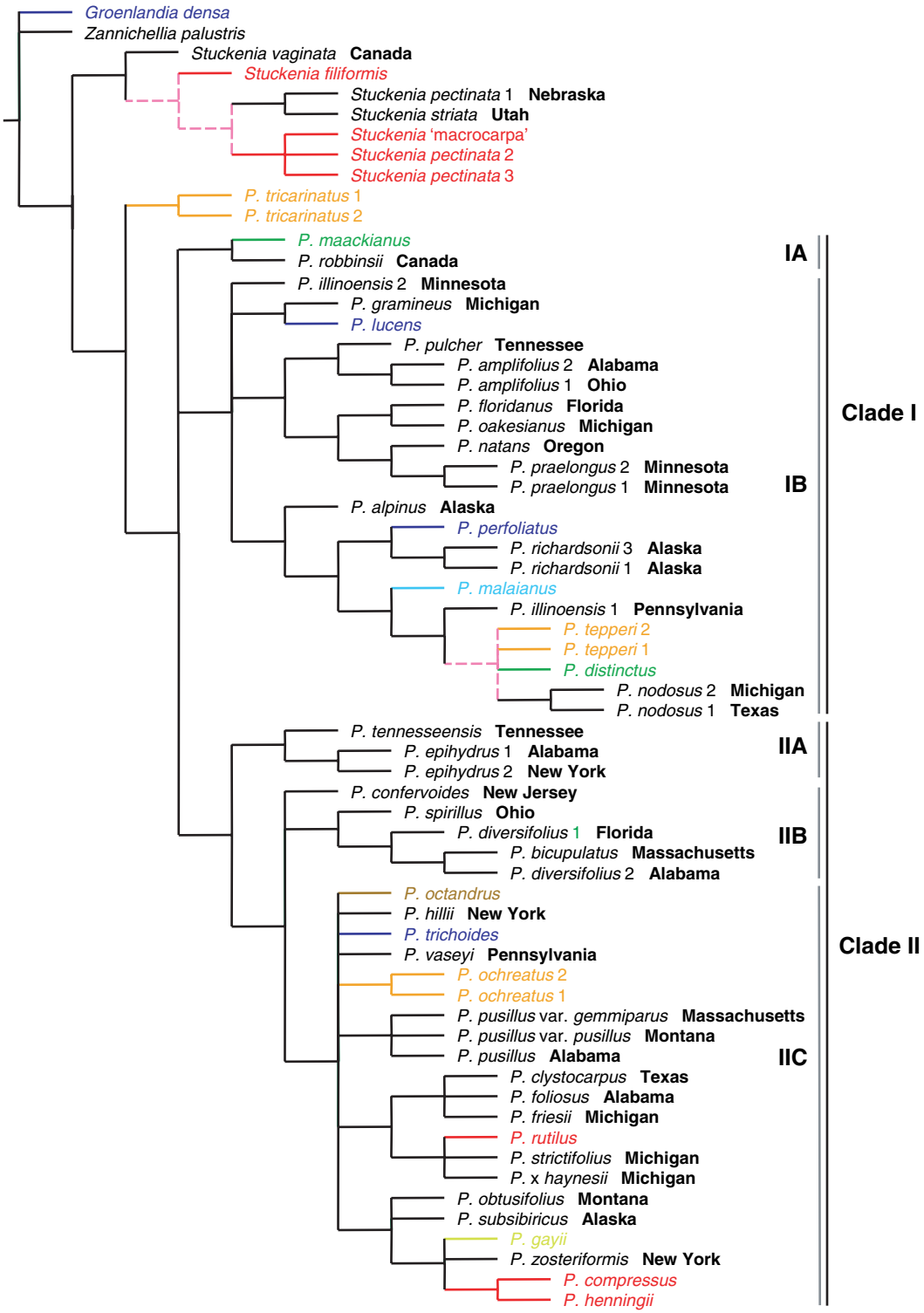

Fig. 6. Biogeography I. 5S-NTS jackknife majority rule tree with geographic collection locality of each accession unambiguously optimized (for North American taxa, the country or specific US state is indicated in bold after the taxon name; see also Table 1). Optimization colors: black = North America, light green = South America, blue = Europe, red = Siberia, green = China, brown = Africa, orange = Australia, light blue = Papua New Guinea, pink and stippled = ambiguity. Two accessions of *Potamogeton crispus* were omitted from the optimization analysis since they represent recent introductions to North America. Experiments with different coding schemes within the actual distribution of these accessions (see (Fig. 7) did not change the overall optimization of the *Potamogeton* lineage (not shown).

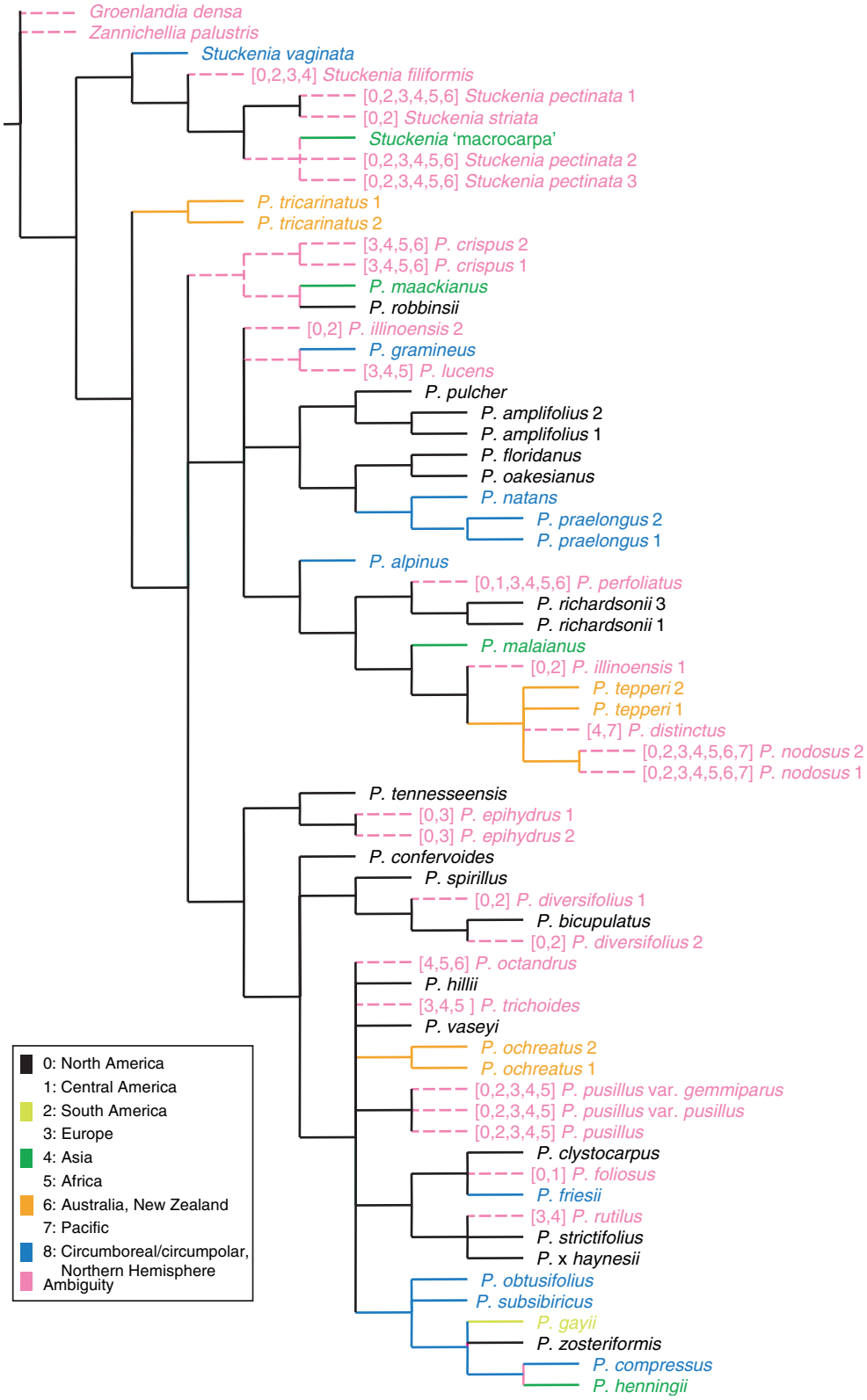

Fig. 7. Biogeography II. 5S-NTS jackknife majority rule tree with total geographic distribution area for each species unambiguously optimized. Colors on optimized tree: black = North America, light green = South America, green = Asia, orange = Australia and New Zealand, blue = circumboreal/circumpolar/Northern Hemisphere, pink and stippled = ambiguity.

Table 1
Voucher information and GenBank accession numbers for the Potamogetonaceae and outgroup taxa included in the study

Taxon	Voucher information*	Geographic locality	GenBank accession no.		
			trnL	*psbA-trnH*	5S-NTS
Potamogetonaceae					
Groenlandia densa Fourr.	1. P. Garcia Murillo & J. Herrera s.n., 18 Aug 1985 (UNA 34612)	Zaragosa, Spain	DQ786416	DQ786521	
	2. K.A. Lye 20182 (O)	Randers, Denmark			DQ786446
Potamogeton alpinus Balb.	S.S. Talbot 21 (UNA 28595)	Simeonof Island, AK, USA	DQ786423	DQ786526	DQ786461
P. amplifolius Tuckerm.	1. J.K. Bissell 1977:109 (UNA 34565)	Geauga Co., OH, USA		DQ786564	DQ786477
	2. M. Birk 694 (UNA 26499)	Bibb Co., AL, USA	DQ786424	DQ786563	DQ786476
P. bicupulatus Fernald	C.B. Hellquist 15389 (UNA 34579)	Windham Co., MA, USA			DQ786487
P. clystocarpus Fernald	C.B. Hellquist 16574 (UNA 57090)	Jeff Davis Co., TX, USA		DQ786558	DQ786492
P. compressus L.	G.E. Crow et al. 93-300 (UNA)	Altai Territory, Siberia, Russia			DQ786493
P. confervoides Reichb.	C.B. Hellquist 11328 (UNA 34671)	Ocean Co., NJ, USA	DQ786425		DQ786486
P. crispus L.	1. R.R. Haynes 10212 (UNA 54705)	Marshall Co., AL, USA	DQ786426	DQ786528	DQ786458
	2. A. Tiehm 3814 (UNA 34611)	Washoe Co., NV, USA		DQ786527	DQ786457
P. distinctus A.Benn.	C.B. Hellquist 15722 (UNA)	Wuhan, China			DQ786470
P. diversifolius Raf.	1. R.R Haynes 10165 (UNA 54775)	Santa Rosa Co., FL, USA	DQ786427	DQ786530	DQ786489
	2. L.J. Davenport 1255 (UNA 34607)	Clay Co., AL, USA		DQ786529	DQ786488
P. epihydrus Raf.	1. B.R. Keener 2081 (UNA 58054)	Baldwin Co., AL, USA			DQ786484
	2. G.C. Tucker & N.G. Miller 10064 (UNA 26113)	Rensselaer Co., NY, USA			DQ786485
P. floridanus Small	R.R. Haynes 10166 (UNA 54776)	Santa Rosa Co., FL, USA	DQ786428	DQ786561	DQ786478
P. foliosus Raf.	R.R. Haynes 10216 (UNA 54701)	Blount Co., AL, USA	DQ786429	DQ786559	DQ786494
P. friesii Rupr.	R.R. Haynes 6217 (UNA 34803)	Emmet Co., MI, USA		DQ786560	DQ786495
P. gayii A.Benn.	N. Ritter et al. 3432 (UNA 08962)	Santa Cruz, Prov. of Florida, Bolivia	DQ786430	DQ786533	DQ786496
P. gramineus L.	R.R. Haynes 5068 (UNA 34889)	Cheboygan Co., MI, USA		DQ786534	DQ786473
P. henningii A.Benn.	G.E. Crow et al. 93-334 (UNA)	Altai Territory, Siberia, Russia			DQ786497
P. hillii Morong	C.B. Hellquist 15409 (UNA 34939)	Dutchess Co., NY, USA	DQ786431	DQ786535	DQ786498
P. illinoensis Morong	1. J.K. Bissell 1985:254 (UNA 34981)	Erie Co., PA, USA	DQ786433	DQ786537	DQ786466
	2. V.E. McNeilus 87-816 (UNA 34921)	Becker Co., MN, USA	DQ786432	DQ786536	DQ786467
P. lucens L.	R.R. Haynes 8736 (UNA 35081)	Silkeborg, Denmark	DQ786434	DQ786538	DQ786474
P. maackianus A.Benn.	C.B. Hellquist 15699 (UNA)	Wuhan, China			DQ786459
P. malaianus Miq.	G. Leach 7805 (UNA 35570)	Kandep Enga Prov., Papua New Guinea		DQ786539	DQ786465
P. natans L.	J.S. Williams s.n., 26 June 1981 (UNA 35003)	Douglas Co., OR, USA		DQ786540	DQ786480
P. nodosus Poir.	1. C.B. Hellquist 12906 (UNA 35456)	Grayson Co., TX, USA		DQ786565	DQ786472
	2. R.R. Haynes 6256 (UNA 35195)	Ogemaw Co., MI, USA	DQ786435	DQ786541	DQ786471
P. oakesianus Robbins ex A.Gray	L.J. Davenport 1414 (UNA 35156)	Kalkaska Co., MI, USA	DQ786436	DQ786562	DQ786479
P. obtusifolius Mert. & Koch.	A.E. Schuyler 5147 (UNA 35131)	Glacier Co., MT, USA		DQ786542	DQ786499
P. ochreatus Raoul	1. R.R. Haynes 8471 (UNA 35118)	New South Wales, Australia		DQ786543	DQ786501
	2. C.B. Hellquist 16213 (UNA)	Tasmania, Australia			DQ786500
P. octandrus Poir.	G.E. Gibbs Russell & H.M. Biegel 1485 (UNA 35121)	Northern Distr., Botswana		DQ786544	DQ786491
P. perfoliatus L.	R.R. Haynes 8739 (UNA 36208)	Silkeborg, Denmark		DQ786545	DQ786462
P. praelongus Wulfen	1. V.E. McNeilus 87-829 (UNA 35231)	Clearwater Co., MN, USA			DQ786482
	2. V.E. McNeilus s.n., 7 Aug 1987 (UNA)	Clearwater Co., MN, USA			DQ786481

Table 1
Continued

Taxon	Voucher information*	Geographic locality	GenBank accession no.		
			trnL	*psbA-trnH*	5S-NTS
P. pulcher Tuckerm.	V.E. McNeilus 87-840 (UNA 35248)	Scott Co., TN, USA		DQ786566	DQ786475
P. pusillus L.	R.R. Haynes 10213 (UNA 54704)	Marshall Co., AL, USA	DQ786439	DQ786548	DQ786505
P. pusillus var. *gemmiparus* Robbins	C.B. Hellquist 13803 (UNA 35397)	Franklin Co., MA, USA			DQ786503
P. pusillus var. *pusillus*	B. McCune & A.E. Schuyler 4980 (UNA 35367)	Flathead Co., MT, USA	DQ786438	DQ786547	DQ786504
P. richardsonii Rydb.	1. S. & S. Talbot 95-1351 (UNA 28659)	Izembek NWR, AL, USA		DQ786550	DQ786464
	2. S. & S. Talbot 95-1373 (UNA 28661)	Izembek NWR, AL, USA	DQ786441	DQ786551	
	3. S. & S. Talbot 079 (UNA 28593)	Simeonof Island, AL, USA	DQ786440	DQ786549	DQ786463
P. robbinsii Oakes	J.S. Pringle 1726 (UNA 35449)	Sydenham, Canada	DQ786442	DQ786552	DQ786460
P. rutilus Wolfg.	G.E. Crow et al. 93-335 (UNA)	Altai Territory, Siberia, Russia			DQ786506
P. spirillus Tuckerm.	J.K. Bissell 1982:203 (UNA 35439)	Ashtabula Co., OH, USA	DQ786443	DQ786531	DQ786490
P. strictifolius A.Benn.	R.R. Haynes 5286 (UNA 35432)	Cheboygan Co., MI, USA	DQ786444	DQ786553	DQ786507
P. subsibiricus Hagstr.	S. & S. Talbot 262 (UNA 23361)	Izembek NWR, AL, USA	DQ786437	DQ786546	DQ786502
P. tennesseensis Fernald	V.E. McNeilus 87-843 (UNA 35417)	Polk Co., TN, USA		DQ786532	DQ786483
P. tepperi A.Benn.	1. S.W.L. Jacobs, C.B. Hellquist & J.H. Wiersema 8241 (UNA)	Queensland, Australia			DQ786469
	2. S.W.L. Jacobs & C.B. Hellquist 8280 (UNA)	Queensland, Australia			DQ786468
P. tricarinatus F.Muell. & A.Benn. ex A.Benn	1. S.W.L. Jacobs & C.B. Hellquist 8310 (UNA)	Queensland, Australia			DQ786456
	2. S.W.L. Jacobs & C.B. Hellquist 8349 (UNA)	New South Wales, Australia			DQ786455
P. trichoides Cham. & Schlecht.	M. Bernues & P. Garcia s.n., 11 April 1987 (UNA 35406)	Huelva, Spain		DQ786554	DQ786508
P. vaseyi J.W.Robbins	J.K. Bissell 1988:106 (UNA 35599)	Erie Co., PA, USA		DQ786555	DQ786509
P. x *haynesii* Hellquist & Crow	R.R. Haynes 3800 (UNA 34994)	Cheboygan Co., MI, USA	DQ786445	DQ786556	DQ786510
P. zosteriformis Fern.	R.R. Haynes 3344 (UNA 35582)	Columbia Co., NY, USA		DQ786557	DQ786511
Stuckenia filiformis Persson	G.E. Crow et al. 93-237 (UNA)	Khakasia Republic, Siberia, Russia			DQ786448
Stuckenia filiformis ssp. *occidentalis* (J.W. Robbins) R.R. Haynes, D.H. Les & M. Král	R.R. Haynes 9854 (UNA 41638)	Wasatch Co., UT, USA	DQ786419		
Stuckenia "macrocarpa" (Dobrocz) Tzvelev	G.E. Crow et al. 93-050 (UNA)	Novosibirsk Region, Siberia, Russia			DQ786449
Stuckenia pectinata (L.) Börner	1. R.R Haynes 9671 (UNA 41708)	Pawnee Co., Nebraska, USA	DQ786420	DQ786523	DQ786450
	2. G.E. Crow et al. 93-042 (UNA)	Novosibirsk Region, Siberia, Russia			DQ786451
	3. G.E. Crow et al. 93-236 (UNA)	Khakasia Republic, Siberia, Russia			DQ786452
Stuckenia striata (Ruiz & Pav.) J.Holub	R.R Haynes 9785 (UNA 41721)	Utah Co., UT, USA	DQ786421	DQ786524	DQ786453
Stuckenia vaginata (Turcz.) J.Holub	C.B. Hellquist & J. Wiersema 16156 (UNA 28564)	Saskatchewan, Canada	DQ786422	DQ786525	DQ786454
Zannichellia andina Holm-Niels.&R.R.Haynes	T. Franken 247 (UNA 36252)	Sajama Rio, Bolivia	DQ786417		
Zannichellia palustris L.	R.R. Haynes 10214 (UNA 54703)	Marshall Co., AL, USA	DQ786418	DQ786522	DQ786447
Outgroup taxa					
Alocasia odora K.Koch	Botanic Garden, Natural History Museum, UiO, 1976-112	Botanisk hage, UNM, Norway	DQ786411	DQ786512	
Butomus umbellatus L.	H. Solstad & R. Elven 145126 (O)	Finnmark, Norway	DQ786413	DQ786517	
Echinodorus muricatus Griseb.	Botanic Garden, Natural History Museum, UiO, 1993-30	Botanisk hage, UNM, Norway	DQ786412	DQ786514	

Table 1
Continued

Taxon	Voucher information*	Geographic locality	GenBank accession no.		
			trnL	*psbA-trnH*	5S-NTS
Magnolia sieboldii K.Koch †	Wang et al., unpub.	N/A	AY158162		
Orontium aquaticum L. †	Borsch et al. (2003)	N/A	AY145338		
Ruppia cirrhosa Grandr.	J.I. Båtvik & O.E. Stabbetorp 160496 (O)	Østfold, Norway		DQ786520	
Ruppia spiralis Dum.	A. Lundberg & K. Rydgren 59879 (O)	Østfold, Norway		DQ786519	
Sagittaria sagittifolia L.	O.E. Stabbetorp & T.E. Brandrud 218029 (O)	Østfold, Norway		DQ786515	
Scheuchzeria palustris L.	N. Ørderud 260398 (O)	Trøgstad, Norway	DQ786414	DQ786513	
Tofieldia glutinosa Pers. †	Borsch et al. (2003)	N/A	AY145337		
Triglochin maritima L.	R. Elven 291704 (O)	Nordland, Norway	DQ786415	DQ786518	
Zostera marina L.	K.A. Lye 252330 (O)	Bærum, Norway		DQ786516	

* Herbaria abbreviations follow Holmgren et al. (1990). Herbarium database number shown in parenthesis, if available. † Sequences obtained from GenBank.

was previously suggested that *Potamogeton* (including *Stuckenia*) originated in East Asia (Miki, 1937). This suggestion was supported by Les (1983), who inferred an eastern Asian affinity based on the hypothesis of an $x = 7$ ancestral base number. Although the position of *Potamogeton tricarinatus*, an Australian native, renders the ancestral origin of *Potamogeton* ambiguous if analyzed separately, i.e., excluding *Stuckenia*, it still seems likely that the entire lineage (*Potamogeton* plus *Stuckenia*) may have a North American origin. As many taxa in this lineage are also found in Eurasia or throughout the Northern Hemisphere, it is possible that a wider taxon and population sampling would expand the ancestral area to a general Northern Hemispheric distribution. This hypothesis can easily be supported by the presumed age of the lineage. Kato et al. (2003) suggested the divergence time between Potamogetonaceae and Zosteraceae to be approximately 100 million years (My). However, based on several reference fossils and comprehensive sampling of monocot taxa, Janssen and Bremer (2004) estimated the split between these two lineages to be less than 65 Myr old and the stem node age of Potamogetonaceae to be 47 Myr old. An early Tertiary origin would coincide with the existence of the North Atlantic land bridge, which connected the floras of North America and Eurasia (e.g., Tiffney, 1985a). Eocene records of *Potamogeton* have been found in both Europe and North America (see Sculthorpe, 1967). If the diversity of species in particular geographic areas could be some indication of the geographic origin, a North American or Northern Hemisphere ancestry for *Potamogeton* is supported by the species densities in these areas. For example, Wiegleb (1988) divided the *Potamogeton* taxa into distribution types and found that the largest species group was North American. In fact, it has been noted that the highest density of species is found in a small area in the eastern portion of the USA and adjacent Canada. High species concentrations have also been reported from central and western USA, temperate Europe, north China and Japan (see Wiegleb, 1988).

Looking closer at the distributions of individual *Potamogeton* accessions mapped on to our phylogenetic tree, it is clear that the *Potamogeton–Stuckenia* lineage demonstrate a complex biogeographical history with suggestions of North American disjuncts, amphiatlantic and amphipacific patterns, and several migration events to the Southern Hemisphere (Fig. 6). However, these patterns may become obscured as more populations from throughout the distribution area of the widespread taxa were to be included. Nevertheless, based on our data, examples of possibly *trans*-Beringian disjuncts can be seen, e.g., in the *Stuckenia* clade and in clade IA of *Potamogeton*, where collects occupying (northern) Asian and North American localities show sister relationships. The connection between Asia and North America across

Beringia remained a viable route for temperate–deciduous plant interchange through the Miocene and into the Quaternary (e.g., Hopkins, 1967; Tiffney, 1985a,b; Elias et al., 1996; Tiffney and Manchester, 2001). Particularly interesting are the Southern Hemisphere species, which are found in several places throughout the phylogenetic tree. For example, the three Australian taxa included (*P. tricarinatus*, *P. tepperi* and *P. ochreatus*) are found within different, well-supported clades, of which *P. tricarinatus* holds the most basal position in the genus (also see above). Only one taxon each from South America (*P. gayii*) and Southern Africa (*P. octandrus*) are included in this study, both of which are found in clade IIC. *Potamogeton malaianus* from Papua New Guinea is sister to a clade of accessions otherwise from USA, China and Australia.

The ability of hydrophytes to expand such wide geographic ranges is intriguing, and it has been suggested that waterfowl are important agents in the long-distance dispersal of aquatic plants (e.g., Figuerola and Green, 2002; Santamaría and Klaassen, 2002; Les et al., 2003). Indeed, pondweeds have been considered to belong to the most valuable food source for ducks in the USA (see Haynes, 1974), and it is possible that ducks and other waterfowl are significant vectors of seed and plant dispersal across continents (see also Mader et al., 1998). However, other factors have also been suggested to account for the wide distribution of aquatic plants, e.g., clonal growth, plasticity and selection for stress-tolerant taxa (see Barrett et al., 1993; Santamaría, 2002).

Conclusions

Aquatic plants are often characterized by extreme morphological reductions and extensive phenotypic plasticity, which have greatly challenged attempts of classical taxonomy and phylogenetic inference. *Potamogeton* is one of the most important plant genera in aquatic environments, yet until now, no comprehensive molecular phylogenetic study of this large genus has been published. We showed that the genera currently assigned to Potamogetonaceae, plus the genus *Zannichellia* (Zannichelliaceae), form a strongly supported monophyletic group and that *Potamogeton* and *Stuckenia* (*Potamogeton* subg. *Coleogeton*) are resolved as monophyletic sister clades. Within *Potamogeton* itself, two major clades largely follow the traditional split between broad- and narrow-leaved species, whereas heterophylly (submerged plus floating leaves) apparently evolved several times.

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