

Phylogeny of *Potamogeton* (Potamogetonaceae) Revisited: Implications for Hybridization and Introgression in Argentina

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Abstract *Potamogeton* is a cosmopolitan genus of 90–95 species in which numerous hybridization events have occurred worldwide. A plant recently collected from Argentina exhibited ambiguous morphology that does not match any species of the genus. We aimed to assess if the plant coexisting with another *Potamogeton* species is a product of reticulate evolution. A concatenated plastid DNA (*psbA-trnH*, *trnL* intron, and *trnL-trnF*) and nuclear ribosomal DNA (5S-NTS) data sets, primarily based on previous studies sample set mainly consisting of American and Asian species, were analyzed using Bayesian inference. Nuclear ribosomal ITS sequences were also obtained from five Argentina materials. We recovered similar topologies from both the plastid DNA and nuclear ribosomal 5S-NTS analyses in which most specimens are consistently placed. The specimen of primal interest from Argentina strongly clustered with co-occurring linear-leaved species in the 5S-NTS tree, but was genetically identical to broad-leaved ones in the plastid DNA analysis. The ITS sequence of the specimen was the same as that of the linear-leaved species and no polymorphisms were observed. Considering the discrepant phylogenetic positions between the trees and lack of ITS infra-individual variations, the origin of the specimen from Argentina is better explained by hybridization and subsequent introgression.

Key words: Alismatales, aquatic plants, Bayesian inference, hybridization, introgression, molecular phylogeny, plastid DNA, *Potamogeton*, topological conflicts, 5S-NTS.

Introduction

Potamogeton L. is one of the three genera of the aquatic plant family Potamogetonaceae. The number of the species varies depending on literature, but 90–95 species are in general accepted in the world (Haynes and Holm-Nielsen, 2003; WCSP, 2014). This cosmopolitan genus has its

center of species diversity in temperate regions of the northern hemisphere (Kaplan *et al.*, 2013), with nearly 70% of the world's species occurring in either Asia, Europe, or North America (Wiegleb and Kaplan, 1998). In contrast, the species in Southern South America, comprising Argentina, Chile, and Uruguay, has been scarcely studied. Following Tur (1982), Wiegleb and Kaplan

(1998), and Haynes and Holm-Nielsen (2003), nine well-recognized species were recorded in southern South America: i) *P. crispus* L. (cosmopolitan); ii) *P. ferrugineus* Hagstr. (South America); iii) *P. gayi* A.Benn. (South America); iv) *P. illinoensis* Morong (North and South America); v) *P. linguatus* Hagstr. (South America); vi) *P. montevidensis* A.Benn. (South America); vii) *P. polygonus* Cham. (South America); viii) *P. pusillus* L. (cosmopolitan); and ix) *P. spirilliformis* Hagstr. (South America). As reported in other regions, difficulties of morphological identification of *Potamogeton* species are known in South America. A plant recently collected from Argentina (Y. Ito YI1992 & *al.*; Fig 1; Appendix 1) exhibited ambiguous morphology that does not match any above-mentioned species of the genus (Fig. 1; Table 1).

Potamogeton is a genus in which numerous

hybridization events have occurred; therefore, it is recognized as a “classic” example of hybridization in aquatic plants (Les and Philbrick, 1993). In their morphology-based monographic work, Wiegleb and Kaplan (1998) reported the number of hybrids that are approximately the same as the number of non-hybrid species. Subsequent molecular studies have detected and documented further cases of hybridization in *Potamogeton*, including ones that do not exhibit obvious morphological characteristics (Les *et al.*, 2009). A case of triple hybridization (a hybrid arisen from crosses of a primary hybrid with a third species) has been also reported in *Potamogeton* (Kaplan and Fehrer, 2007), implying that the genus has undergone rather complicated evolution. The aforementioned morphologically ambiguous plant from Argentina may be a product of hybridization, because it coexists with *P. pusillus*.



Fig. 1. Habitat of *Potamogeton* sp. YI1992_TNS in Córdoba, Argentina.

Table 1. Vegetative and reproductive morphology of *Potamogeton* sp. (obtained in this study) and its two inferred parental species (adapted from Wiegleb and Kaplan, 1998)

	Submerged leaves						Floating leaves		Inflorescences		
	shape	petiolate (mm)	length (mm)	width (mm)	length-width ratio	margins	no. veins	apex	Floating leaves	carpel no.	flower no.
<i>P. illinoensis</i>	narrowly oblong/oblanceolate/elliptical	short petiolate/ sessile	(50-)70-180(-220)	(4-)15-40(-53)	3.5-7	minutely denticulate/ entire	(7-)9-17(-19)	mucronate	absent/ present	(2-)4	numerous
<i>P. pusillus</i>	linear	sessile	(15-)20-85(110)	(0.3-)0.8-2.5(-3.1)	(15-)20-60(-90)	entire	(1-)3(-5)	acuminate/ acute/ subob- tuse	absent	(3-)4(-7)	2-7
<i>Potamogeton</i> sp.	linear-lanceolate	sessile	20-85	2.5-9	8-16	entire	(3-)5	acuminate	?absent	4	<12

Molecular phylogenetic analyses on *Potamogeton* have been independently conducted by different research groups based on their own unique sample and data sets that scarcely overlapped each other (e.g., Iida *et al.*, 2004; Lindqvist *et al.*, 2006; Wang *et al.*, 2007; Zhang *et al.*, 2008; Les *et al.*, 2009; Ito and Tanaka, 2013; Kaplan *et al.*, 2013). Therefore, it remains unclear which phylogeny is the most reliable one, as significant topological incongruences have occasionally been found among the studies (Ito and Tanaka, 2013). Lindqvist *et al.* (2006) provided a relatively well-resolved phylogeny of the genus, comprising broad-leaved and linear-leaved lineages based on the 5S non-transcribed spacer [hereinafter called 5S-NTS of the nuclear ribosomal DNA (nrDNA)], followed by Kaplan *et al.*'s (2013) 5S-NTS tree. Still, a question remains as to whether the same or similar topology can be recovered with a plastid DNA (hereinafter called ptDNA) data set because Lindqvist *et al.* (2006) failed to reconstruct a resolved phylogeny in their ptDNA analysis using two fast-evolving markers, *psbA-trnH* and *trnL* intron, and no analyses using ptDNA sequences were performed by Kaplan *et al.* (2013). However, Lindqvist *et al.*'s (2006) data set itself appears to be useful if 19 outgroups from the other genera of Potamogetonaceae and other distantly related monocots and non-monocot families, e.g., Araceae, Alismataceae, Juncaginaceae, and Magnoliaceae, are excluded from the analysis. Comparative data are available by Zhang *et al.* (2008, unpublished data), which using *trnT-trnL*, *trnL* intron, and *trnL-trnF*, recovered roughly the same broad-leaved and linear-leaved lineages (Zhang *et al.*, 2008).

To assess whether the unidentified *Potamogeton* (*Y. Ito YII992 & al.*) with ambiguous morphology from Argentina is a product of hybridization, we employed simultaneous molecular phylogenetic analyses of nrDNA and ptDNA based on data sets of Lindqvist *et al.* (2006) and Zhang *et al.* (2008) and comparable data from GenBank, which occasionally contains *trnL-trnF* from ptDNA (Kaplan and Fehrer, 2007; Zhang *et al.*,

2008). The data set of Lindqvist *et al.* (2006) is particularly relevant for our purpose because it mainly consisted of North American species that either are distributed in South America or apparently have South American relatives (Wiegleb and Kaplan, 1998; Haynes and Holm-Nielsen, 2003). Topological incongruence between reconstructed phylogenetic trees were compared, with a particular focus on the taxa from Argentina. In addition, DNA sequences of the internal transcribed spacer (ITS) region of nrDNA were generated to seek another line of evidence of hybridization.

Materials and Methods

Taxon sampling

The data set for molecular phylogenetic analyses mainly consists of sequences deposited in GenBank: 41 samples (Lindqvist *et al.*, 2006); 26 (Zhang *et al.*, 2008, unpublished data); four (Kaplan and Fehrer, 2007; Kaplan *et al.*, 2013). Seventeen out of samples used in Lindqvist *et al.* (2006) that lack either ptDNA or 5S-NTS were not included in this study. Five samples from Argentina were added, which were *P. gayi* (one specimen), *P. pusillus* (three), and the unidentified *Potamogeton* sp. YII992_TNS (one). The data set included 76 samples, which were equivalent to 46 species including one putative hybrid, *P. × haynesii* Hellq. & G.E.Crow (Appendix 1).

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the five newly collected *Potamogeton* specimens from Argentina following the method outlined in Ito *et al.* (2010) and their sequences of ptDNA and nrDNA regions were determined by PCR amplification and direct sequencing. The following primer pairs were used for PCR and sequencing: *psbAF* and *trnHR* (Sang *et al.*, 1997) for *psbA-trnH*; "c" and "d" (Taberlet *et al.*, 1991) for *trnL* intron; "e" and "f" (Taberlet *et al.*, 1991) for *trnL-trnF*; and PI and PII (Cox *et al.*, 1992) for 5S-NTS, and ITS-4 and ITS-5 for nrITS (Bald-

win, 1992). The PCR amplification was conducted using TaKaRa Ex Taq polymerase (TaKaRa Bio, Japan), and PCR cycling conditions were 94°C for 60 s; then 30 cycles of 94°C for 45 s, 52°C for 30 s, 72°C for 60 s; and finally 72°C for 5 min. The PCR products were cleaned using illustra ExoProStar (GE Healthcare, Piscataway, USA) and then reacted using ABI Big Dye Terminator ver. 3.1 (Applied Biosystems, USA) with the same primers as those used for the PCR amplifications. DNA sequencing was performed with an ABI PRISM 3130xl DNA sequencer (Applied Biosystems). Automatic base calling was checked by eye using Genetyx-Win ver. 3 (Software Development Co., Japan). All sequences generated in the present study have been submitted to the DNA Data Bank of Japan (DDBJ), which is linked to GenBank, and their accession numbers and voucher specimen information are presented (Appendix 1).

Data analysis

We assembled two datasets from the 76 samples, which included the aforementioned five samples from Argentina: i) ptDNA (*psbA-trnH*, *trnL* intron, and *trnL-trnF*) and ii) nuclear 5S-NTS. Missing data found in ptDNA were retained because “it should generally be possible to accurately place incomplete taxa in phylogenies, if enough informative characters are sampled” (Wiens and Morrill, 2011); those are used as equivocal characters (N). Following Kaplan *et al.* (2013), one of the basal-most *Potamogeton* taxa in their ITS tree, namely *P. spirillus*, were chosen as an outgroup for addressing the intrageneric relationships, because this region was too variable to allow a reliable alignment with the other two genera. Sequences were aligned using MAFFT ver. 7.058 (Kato and Standley, 2013) using “leave gappy regions” option and then inspected manually.

Phylogenetic inference was performed using Bayesian inference (BI; Yang and Rannala, 1997). Analyses were conducted with MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck, 2003; Ronquist *et al.*, 2012) run on the CIPRES portal

(Miller *et al.*, 2010) after the best models had been determined in MrModeltest ver. 3.7 (Nylander, 2002); these models were GTR + I + G for ptDNA and GTR + G for 5S-NTS. For gap characters, the datatype = standard option of MrBayes was used. Analyses were run for 8,150,000 million and 460,000 million generations for the ptDNA and 5S-NTS data sets, respectively, until the average standard deviation of split frequencies dropped below 0.01, sampling every 1,000 generations and discarding the first 25% as burn-in. The convergence and effective sampling sizes (ESS) of all parameters were checked in Tracer ver. 1.6 (Rambaut *et al.*, 2014). All trees were visualized using FigTree ver. 1.3.1 (Rambaut, 2009). The data matrices and the MrBayes trees are available at Treebase (TB2:S18639).

Results

Molecular phylogeny

The ptDNA data set of *Potamogeton* included 1,391 aligned characters (*psbA-trnH*: 364 bp; *trnL* intron: 591 bp; *trnL-trnF*: 436 bp) and 17 indels, of which 146 characters including the binary-coded indels are polymorphic. The topology is resolved, yet the support values are mostly low (Fig. 2a).

The 5S-NTS data set of *Potamogeton* included 310 aligned characters and six indels, of which 275 characters including the binary-coded indels are polymorphic. We obtained a well-resolved tree, which topology followed that of the ptDNA tree (Fig. 2b).

Respective four groups, that belonged to linear-leaved and broad-leaved lineages detected in Lindqvist *et al.* (2006) and Kaplan *et al.* (2013), were recovered in either or both ptDNA and 5S-NTS trees; those are numbered from groups L1–L4 and groups B1–B4, respectively: group L1 (*P. diversifolius*–*P. spirillus*); group L2 (*P. compressus*–*P. gayi*–*P. obtusifolius*–*P. subsibiricus*–*P. trichoides*); group L3 [*P. clystocarpus*–*P. foliosus*–*P. friesii*–*P. × haynesii*–*P. pusillus* (China)–*P. strictifolius*]; group L4 [*P. oxyphyllus*–*P. pusillus*

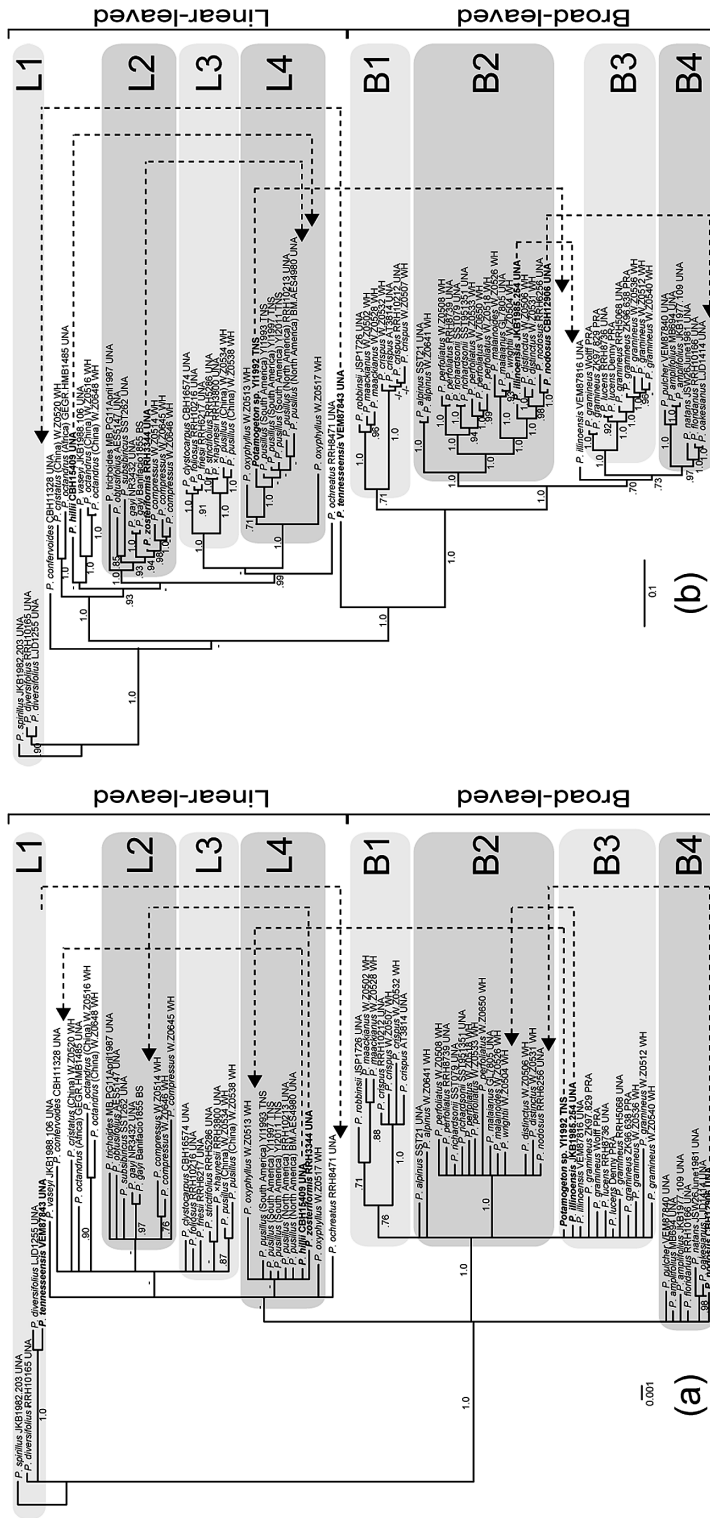


Fig. 2. MrBayes trees for *Potamogeton* based on a) the concatenated plastid DNA (*psbA-trnH*, *trnL* intron, and *trnL-trnF*) and b) the nuclear 5S-NTS data sets. Letters refer to groups noted in the text. The six accessions of which phylogenetic positions are inconsistent between the data sets are emphasized in bold, for which the corresponding phylogenetic positions in the other analysis are indicated by dotted arrows. Numbers above or below the branches indicate Bayesian posterior probabilities (PP). Clades with PP < 0.7 are indicated by hyphens.

(North and South America)]; group B1 (*P. crispus*–*P. maackianus*–*P. robbinsii*); group B2 [*P. alpinus*–*P. distinctus*–*P. malaianus*–*P. malainoides*–*P. nodosus* (RRH6256 UNA)–*P. perfoliatus*–*P. richardsonii*–*P. wrightii*]; group B3 [*P. gramineus*–*P. illinoensis* (VEM87816 UNA)–*P. lucens*]; group B4 (*P. amplifolius*–*P. floridanus*–*P. oakesianus*–*P. natans*–*P. pulcher*).

Of samples from Argentina, *Potamogeton* sp. YI1992_TNS was positioned significantly differently between ptDNA and 5S-NTS trees (group B3 in ptDNA; group L4 in 5S-NTS; Fig. 2).

Nuclear DNA (ITS) sequence comparisons

ITS sequences obtained from the five specimens from Argentina were 713 bp in length. No intra-individual variation were found in any of the specimens, except the 1-bp intra-individual variation observed in *Potamogeton pusillus* YI1997. No sequence differences were found between *P. pusillus* and *Potamogeton* sp. Y1992_TNS, from which *P. gayi* is distinguishable by two nucleotide substitutions.

Discussion

The present study reconstructed phylogenies of *Potamogeton* based on ptDNA and nuclear 5S-NTS of nrDNA data sets, in which six accessions show significant inconsistency between the trees (Fig. 2). Such topological incongruences resulting from ptDNA and nrDNA markers are often reported in phylogenetic studies (Wendel and Doyle, 1998, Degnan and Rosenberg, 2009). Although some causes of phylogenetic incongruence, e.g. gene choice and incomplete lineage sorting, are suggested, hybridization and introgression are likely to be attributed to the topological conflicts in *Potamogeton*, as is concluded by Hamzeh and Dayanandan (2004), Fehrer *et al.* (2007), Tippery and Les (2011), Ito *et al.* (2013), Ren *et al.* (2015), Soto-Trejo *et al.* (2015). The six specimens discrepantly resolved between the trees may indicate such reticulate evolution. Here, out of the six accessions, we discussed only for *Potamogeton* sp. YI1992_TNS, that was

collected by ourselves.

Potamogeton sp. (Y. Ito YI1992 & al.) has ptDNA close to those of broad-leaved species, such as *P. illinoensis* and *P. lucens* (group B3: Fig. 2a) and nrDNA identical or closely related to that of the co-occurring linear-leaved *P. pusillus* (group L3: Fig. 2b). Considering the incongruence of phylogenetic position and the lack of ITS intra-individual polymorphisms, which has been used as a line of evidence to identify *Potamogeton* hybrids (e.g. Kaplan and Fehrer 2007, Du *et al.*, 2010), not simple hybridization but introgression following multiple hybridizations between linear-leaved and broad-leaved species better explain the origin of *Potamogeton* sp. YI1992_TNS. With the empirically confirmed maternal inheritance of chloroplast DNA in *Potamogeton* (Kaplan and Fehrer, 2006), hybridization between a paternal *P. pusillus* and a maternal broad-leaved *Potamogeton* species is likely. The maternal parent is most probably *P. illinoensis* because this American species is also distributed in Argentina (Haynes and Holm-Nielsen, 2003), where it once may have occurred with *P. pusillus* (which yet co-exists in the river in Córdoba, Argentina) and have repeatedly hybridized with its pollen, termed “cytoplasmic introgression” (Rieseberg, 1997).

Conclusions

We performed simultaneous molecular phylogenetic analyses of *Potamogeton* based on previous studies' data sets and our newly collected samples. The topological comparison between ptDNA and 5S-NTS clearly exhibited significant incongruences. A single accessions from Argentina that was inconsistently positioned between the trees may be a product of hybridization or introgression. Future phylogenetic researches may aim at i) improving the support values by adding more valuable ptDNA regions, ii) seeking alternative nrDNA markers, such as low-copy nuclear DNA loci, and iii) applying alternative methods such as AFLP and RADseq.

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Appendix 1. List of the GenBank accessions (*psbA-trnH*, *trnL* intron, *trnL-trnF*, 5S-NTS, and ITS) of *Potamogeton* used in the present study. Also provided are: Voucher (Herbaria in parentheses); Code; Locality; Reference

Taxon	Voucher	Code	Locality	Reference	<i>psbA-trnH</i>	<i>trnL</i> intron	<i>trnL-trnF</i>	5S-NTS
<i>P. alpinus</i> Balb.	S. S. Tallbot 21 (UNA)	SST121_UNA	USA: Alaska	Lindqvist <i>et al.</i> (2006)	DQ786526	DQ786423		DQ786461
<i>P. alpinus</i> Balb.	Wang & Zhang 0641 (WH)	WZ0641_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428411		FI495487
<i>P. amplifolius</i> Tuckerm.	M. Birk 694 (UNA)	MB694_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786563	DQ786424		DQ786476
<i>P. amplifolius</i> Tuckerm.	J. K. Bissell 1977:109 (UNA)	JKB1977:109_UNA	USA: Ohio	Lindqvist <i>et al.</i> (2006)	DQ786564			DQ786477
<i>P. chysocarpus</i> Fernald	C. B. Hellquist 16574 (UNA)	CBH16574_UNA	USA: Texas	Lindqvist <i>et al.</i> (2006)	DQ786558			DQ786492
<i>P. compressus</i> L.	Wang & Zhang 0514 (WH)	WZ0514_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428389	EF432079	FI495491
<i>P. compressus</i> L.	Wang & Zhang 0645 (WH)	WZ0645_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428414		FI495492
<i>P. compressus</i> L.	Wang & Zhang 0646 (WH)	WZ0646_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428415		FI495494
<i>P. confertifolius</i> Reicheb.	C. B. Hellquist 11328 (UNA)	CBH11328_UNA	USA: New Jersey	Lindqvist <i>et al.</i> (2006)	DQ786528	DQ786425		DQ786486
<i>P. crispus</i> L.	R. R. Haynes 10212 (UNA)	RRH10212_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)		DQ786426		DQ786458
<i>P. crispus</i> L.	Wang & Zhang 0507 (WH)	WZ0507_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428382	EF432072	FI495473
<i>P. crispus</i> L.	Wang & Zhang 0532 (WH)	WZ0532_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428404	EF432094	FI495474
<i>P. crispus</i> L.	A. Triehm 3814 (UNA)	AT3814_UNA	USA: Nevada	Lindqvist <i>et al.</i> (2006)	DQ786527			DQ786457
<i>P. cristatus</i> Regel & Maack	Wang & Zhang 0520 (WH)	WZ0520_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428394	EF432084	FI495503
<i>P. distinctus</i> A. Benn.	Wang & Zhang 0506 (WH)	WZ0506_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428381	EF432071	FI495471
<i>P. distinctus</i> A. Benn.	Wang & Zhang 0531 (WH)	WZ0531_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428403	EF432093	FI495472
<i>P. diversifolius</i> Raf.	R. R. Haynes 10165 (UNA)	RRH10165_UNA	USA: Florida	Lindqvist <i>et al.</i> (2006)	DQ786530	DQ786427		DQ786489
<i>P. diversifolius</i> Raf.	L. J. Davenport 1255 (UNA)	LJD1255_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786529			DQ786488
<i>P. floridanus</i> Small	R. R. Haynes 10166 (UNA)	RRH10166_UNA	USA: Florida	Lindqvist <i>et al.</i> (2006)	DQ786561	DQ786428		DQ786478
<i>P. foliosus</i> Raf.	R. R. Haynes 10216 (UNA)	RRH10216_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786559	DQ786429		DQ786494
<i>P. friessii</i> Rupr.	R. R. Haynes 6217 (UNA)	RRH6217_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786560			DQ786495
<i>P. gottii</i> A. Benn.	N. Ritter <i>et al.</i> 3432 (UNA)	NR3432_UNA	Bolivia	Lindqvist <i>et al.</i> (2006)	DQ786533	DQ786430		DQ786496
<i>P. gottii</i> A. Benn.	Banifacio 1855 (SI)	Banifacio1855_SI	Argentina	this study	KT634263	KT634278	KT634283	KT634288
<i>P. gramineus</i> L.	Z. Kaplan 96/638 (PRA)	ZK96.638_PRA	Czech Republic	Kaplan & Fehrer (2007); Kaplan <i>et al.</i> (2013)		EF174575	EF174575	EF174575
<i>P. gramineus</i> L.	P. Wolff x. n. (PRA)	Wolff_PRA	France	Kaplan & Fehrer (2007); Kaplan <i>et al.</i> (2013)		EF174576	EF174576	KF270963
<i>P. gramineus</i> L.	Wang & Zhang 0512 (WH)	WZ0512_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428387	EF432077	FI495481
<i>P. gramineus</i> L.	Wang & Zhang 0536 (WH)	WZ0536_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428408	EF432098	FI495482
<i>P. gramineus</i> L.	Wang & Zhang 0540 (WH)	WZ0540_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428410	EF432100	FI495484
<i>P. gramineus</i> L.	Z. Kaplan 97/829 (PRA)	ZK97.829_PRA	Czech Republic	Kaplan & Fehrer (2007); Kaplan <i>et al.</i> (2013)	EF174574			KF270960
<i>P. gramineus</i> L.	R. R. Haynes 5068 (UNA)	RRH5068_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786534			DQ786473
<i>P. gramineus</i> L.	R. R. Haynes 3800 (UNA)	RRH3800_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786556	DQ786445		DQ786510
<i>P. × haynesii</i> Hellq. & G. E. Crow	R. R. Haynes 3800 (UNA)	RRH3800_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786535	DQ786431		DQ786498
<i>P. hillii</i> Morong	C. B. Hellquist 15409 (UNA)	CBH15409_UNA	USA: New York State	Lindqvist <i>et al.</i> (2006)	DQ786536	DQ786432		DQ786466
<i>P. illinoensis</i> Morong	V. E. McNeill 87-816 (UNA)	VEM87816_UNA	USA: Minnesota	Lindqvist <i>et al.</i> (2006)	DQ786537	DQ786433		DQ786466
<i>P. illinoensis</i> Morong	J. K. Bissell 1985:254 (UNA)	JKB1985:254_UNA	USA: Pennsylvania	Lindqvist <i>et al.</i> (2006)	DQ786538	DQ786434		DQ786474
<i>P. lucens</i> L.	R. R. Haynes 8736 (UNA)	RRH8736_UNA	Denmark	Lindqvist <i>et al.</i> (2006)				
<i>P. lucens</i> L.	P. Denny s. n. (PRA)	Denny_PRA	The Netherlands	Kaplan & Fehrer (2007)	EF174573	EF174578	EF174578	KF270967
<i>P. maackianus</i> A. Benn.	Wang & Zhang 0502 (WH)	WZ0502_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428377	EF432067	FI495464
<i>P. maackianus</i> A. Benn.	Wang & Zhang 0528 (WH)	WZ0528_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428401	EF432091	FI495465
<i>P. malaiensis</i> Miq.	G. Leach 7805 (UNA)	GL7805_UNA	Papua New Guinea	Lindqvist <i>et al.</i> (2006)	DQ786539			DQ786465
<i>P. malainoides</i>	Wang & Zhang 0526 (WH)	WZ0526_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428400	EF432090	FI495513
<i>P. natans</i> L.	J. S. Williams s.n., 26 June 1981 (UNA)	JSW26June1981_UNA	USA: Oregon	Lindqvist <i>et al.</i> (2006)	DQ786540			DQ786480
<i>P. nodosus</i> Poir.	R. R. Haynes 6256 (UNA)	RRH6256_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786541	DQ786435		DQ786471
<i>P. nodosus</i> Poir.	C. B. Hellquist 12906 (UNA)	CBH12906_UNA	USA: Texas	Lindqvist <i>et al.</i> (2006)	DQ786565			DQ786472
<i>P. oedocianus</i> Robbins ex. A. Gray	L. J. Davenport 1414 (UNA)	LJD1414_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786562	DQ786436		DQ786479
<i>P. obtusifolius</i> Mert. & Koch.	A. E. Schuyler 5147 (UNA)	AES5147_UNA	USA: Montana	Lindqvist <i>et al.</i> (2006)	DQ786542			DQ786499
<i>P. ochreatius</i> Raoul	R. R. Haynes 8471 (UNA)	RRH8471_UNA	Australia: NSW	Lindqvist <i>et al.</i> (2006)	DQ786543			DQ786501

Appendix 1. Continued

Taxon	Voucher	Code	Locality	Reference	<i>psbA-trnH</i>	<i>trnL</i> intron	<i>trnL-trnF</i>	5S-NTS
<i>P. octandrus</i> Poir.	Wang & Zhang 0516 (WH)	W.Z0516_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF432080	EF432080	FJ495497
<i>P. octandrus</i> Poir.	Wang & Zhang 0648 (WH)	W.Z0648_WH	China	Zhang <i>et al.</i> (Unpubl.)		EF432107	EF432107	FJ495495
<i>P. octandrus</i> Poir.	G. E. Gibbs Russell & H. M. Biegel 1485 (UNA)	GEGR.HMB1485_UNA	Botswana	Lindqvist <i>et al.</i> (2006)	DQ786544			DQ786491
<i>P. oxyphyllus</i> Miq.	Wang & Zhang 0513 (WH)	W.Z0513_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428388	EF432078	FJ495488
<i>P. oxyphyllus</i> Miq.	Wang & Zhang 0517 (WH)	W.Z0517_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428391	EF432081	FJ495489
<i>P. perfoliatus</i> L.	Wang & Zhang 0508 (WH)	W.Z0508_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428383	EF432073	FJ495475
<i>P. perfoliatus</i> L.	Wang & Zhang 0533 (WH)	W.Z0533_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428405	EF432095	FJ495477
<i>P. perfoliatus</i> L.	Wang & Zhang 0650 (WH)	W.Z0650_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428419		FJ495478
<i>P. perfoliatus</i> L.	R. R. Haynes 8739 (UNA)	RRH8739_UNA	Denmark	Lindqvist <i>et al.</i> (2006)				DQ786462
" <i>P. praelongus</i> "*	Wang & Zhang 0518 (WH)	W.Z0518_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)	DQ786545	EF428392	EF432082	FJ495479
<i>P. pulcher</i> Tuckerm.	V. E. McNeillus 87-840 (UNA)	VE87840_UNA	USA: Tennessee	Lindqvist <i>et al.</i> (2006)	DQ786566			DQ786475
<i>P. pusillus</i> L.	Y. Ito Y11993 & al. (TNS)	Y11993_TNS	Argentina	this study	KT634259	KT634274	KT634279	KT634284
<i>P. pusillus</i> L.	Y. Ito Y11997 & al. (TNS)	Y11997_TNS	Argentina	this study	KT634260	KT634275	KT634280	KT634285
<i>P. pusillus</i> L.	Y. Ito Y12011 & al. (TNS)	Y12011_TNS	Argentina	this study	KT634261	KT634276	KT634281	KT634286
<i>P. pusillus</i> L.	R. R. Haynes 10213 (UNA)	RRH10213_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786548	DQ786439	EF432096	DQ786505
<i>P. pusillus</i> L.	Wang & Zhang 0534 (WH)	W.Z0534_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428406		FJ495499
<i>P. pusillus</i> L.	Wang & Zhang 0538 (WH)	W.Z0538_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428409	EF432099	FJ495500
<i>P. pusillus</i> var. <i>pusillus</i>	B. McCune & A. E. Schuyler 4980 (UNA)	BM.AES4980_UNA	USA: Montana	Lindqvist <i>et al.</i> (2006)	DQ786547	DQ786438		DQ786504
<i>P. richardsonii</i> Rydb.	S. & S. Talbot 079 (UNA)	SST079_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786549	DQ786440		DQ786463
<i>P. richardsonii</i> Rydb.	S. & S. Talbot 95-1351 (UNA)	SST951351_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786550			DQ786464
<i>P. robbinsii</i> Oakes	J. S. Pringle 1726 (UNA)	JSP1726_UNA	Canada	Lindqvist <i>et al.</i> (2006)	DQ786552	DQ786442		DQ786460
<i>P. spirillius</i> Tuckerm.	J. K. Bissell 1982:203 (UNA)	JKB1982:203_UNA	USA: Ohio	Lindqvist <i>et al.</i> (2006)	DQ786531	DQ786443		DQ786490
<i>P. stricifolius</i> A. Benn.	R. R. Haynes 5286 (UNA)	RRH5286_UNA	USA: Michigan	Lindqvist <i>et al.</i> (2006)	DQ786553	DQ786444		DQ786507
<i>P. subsibiricus</i> Hägstr.	S. & S. Talbot 262 (UNA)	SST262_UNA	USA: Alabama	Lindqvist <i>et al.</i> (2006)	DQ786546	DQ786437		DQ786502
<i>P. tenuisensis</i> Fernald	V. E. McNeillus 87-843 (UNA)	VE87843_UNA	USA: Tennessee	Lindqvist <i>et al.</i> (2006)	DQ786532			DQ786483
<i>P. trichoides</i> Cham. & Schlecht.	M. Bernues & P. Garcia s. n., 11 April 1987 (UNA)	MB.PG11April1987_UNA	Spain	Lindqvist <i>et al.</i> (2006)	DQ786554			DQ786508
<i>P. vaejii</i> J. W. Robbins	J. K. Bissell 1988:106 (UNA)	JKB1988:106_UNA	USA: Pennsylvania	Lindqvist <i>et al.</i> (2006)	DQ786555			DQ786509
<i>P. wrightii</i> L.	Wang & Zhang 0504 (WH)	W.Z0504_WH	China	Zhang <i>et al.</i> (2008; Unpubl.)		EF428379	EF432069	FJ495466
<i>P. zosteriformis</i> Fern.	R. R. Haynes 3344 (UNA)	RRH3344_UNA	USA: New York State	Lindqvist <i>et al.</i> (2006)	DQ786557			DQ786511
<i>Potamogeton</i> sp.	Y. Ito Y11992 & al. (TNS)	Y11992_TNS	Argentina	this study	KT634262	KT634277	KT634282	KT634287

* Originally named as *P. praelongus* in Zhang *et al.* (2008) but is re-identified (Ito and Tanaka 2013).