Erroneous identities of *Potamogeton* hybrids corrected by molecular analysis of plants from type clones

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Abstract Hybridization is one of the main sources of taxonomic complexity in *Potamogeton*. In spite of long and intensive research, the total hybrid diversity and distribution of many hybrids are still insufficiently known. Identities of two currently recognized hybrids were tested using molecular analyses of plants from the original clones growing at the type localities of their correct names. Additive characters in direct sequencing of the ITS region and the placement of cloned sequences in a Bayesian consensus tree were used to identify the exact identity of these hybrids. *Potamogeton ×lanceolatifolius* is widely recognized as a hybrid *P. gramineus × P. polygonifolius*. However, the molecular analyses and cytological investigation identified this Swedish hybrid as *P. gramineus × P. nodosus* although *P. nodosus* currently does not occur in the whole of Scandinavia. This hybrid provides some insight into the former distribution area of *P. nodosus* as affected by past climatic changes. The recently detected *P. ×vepsicus* was originally described as *P. alpinus × P. natans* but the molecular investigation revealed that its correct identity is *P. natans × P. praelongus*. In both cases, the actual identities could hardly have been unequivocally deduced from the morphology of the hybrids. The existence of these hybrid combinations is here confirmed for the first time. The maternally inherited chloroplast *rpl20-5'rps12* sequences were used to identify the female parental species. The name "*P. ×argutulus*", previously, but erroneously, used for a hybrid *P. gramineus × P. nodosus*, is typified and reduced to the synonymy of *P. gramineus*.

Keywords determination; hybridization; molecular identification; nomenclature; *Potamogeton*; taxonomy

■ INTRODUCTION

Interspecific hybridization is a frequent natural phenomenon and an important source of plant diversity (e.g., Stace, 1975; Grant, 1981; Harrison, 1993; Arnold, 1997; Rieseberg & Carney, 1998). In Potamogetonaceae, the occurrence of hybridization is known for more than a century (see the reviews in Wiegleb & al., 2008 and Kaplan & al., 2009) and is currently recognized as one of the main sources of taxonomic complexity (e.g., Preston, 1995; Wiegleb & Kaplan, 1998; Kaplan, 2010a).

Understanding of the identities of many hybrids has changed greatly in the course of time. For example, the majority of European hybrids were first described as species (Kaplan, 1997; Kaplan & Zalewska-Gałosz, 2004; Wiegleb & al., 2008; Kaplan & al., 2009). Most hybrids were identified on the basis of morphological intermediacy, in many cases associated with observations of sterility (e.g., Fryer, 1890; Fischer, 1907; Hagström, 1916; Ogden, 1943; Dandy, 1975; Preston, 1995; Wiegleb & Kaplan, 1998; Kaplan & Zalewska-Gałosz, 2004). Some authors also used anatomical features, which helped to resolve some intricate taxonomic problems and contributed to the detection of hybrids between species with different types of stem anatomy (e.g., Fischer, 1904; 1905; 1907; Hagström, 1916; Ogden, 1943; Symoens & al., 1979; Wiegleb, 1990a, b; Kaplan, 2001, 2005a, b; Kaplan & Symoens, 2004, 2005; Kaplan & Wolff, 2004; Alix & Scribailo, 2006; Zalewska-Gałosz & al., 2009, 2010).

The advent and widespread use of molecular methods in plant science over the last two decades substantially contributed to understanding of hybrid diversity and processes associated with hybridization (e.g., Rieseberg & Ellstrand, 1993; Avise, 1994; Arnold & al., 2003; Rieseberg & al., 2003; Hegarty & Hiscock, 2005; Krahulec & al., 2005, 2008; Fehrer & al., 2009). Hybrid origin was proved and exact identities of parental species were revealed in several hybrids of Potamogetonaceae by isozyme electrophoresis (e.g., Hollingsworth & al., 1995, 1996; Preston & al., 1998b; Fant & al., 2001a, b; Iida & Kadono, 2002; Kaplan & al., 2002; Fant & Preston, 2004; Kaplan & Wolff, 2004; Kaplan, 2007) or DNA-based analyses (King & al., 2001; Fant & al., 2003, 2005; Kaplan & Fehrer, 2004, 2006, 2009; Ito & al., 2007; Wang & al., 2007; Du & al., 2009; Zalewska-Gałosz & al., 2009). Direct DNA sequencing and RFLPs have recently contributed to the discovery and/or exact identification of several entirely new hybrid combinations (Kaplan & al., 2009, 2011; Zalewska-Gałosz & al., 2010) and even confirmed the existence of a natural triple hybrid (Kaplan & Fehrer, 2007).

As in many other aquatic plants, phenotypic plasticity plays a substantial role in plant morphology in Potamogetonaceae (Kaplan, 2002). Morphological characteristics can vary considerably according to growing conditions. The extensive range of phenotypic plasticity obscures morphological differences between taxa. Because our previous studies (Kaplan, 2002; Kaplan & Fehrer, 2004; Kaplan & al., 2009) demonstrated that some extreme phenotypes of true species may mimic hybrids, and vice versa, it is advisable to have molecular proof of the identity of questionable plants (Kaplan & Fehrer, 2007, 2009; Kaplan & al., 2009, 2011).

A key component of the current taxonomy is the concept of the type specimen that serves as a standard for the identity

of the plant name and as the reference for comparative systematic studies. Together with scientific names, nomenclatural types constitute an essential basis for biological communication about organisms. In hybrids, a name associated with the type specimen applies to all individuals derived from the crossing of respective parental species (McNeill & al., 2006) regardless of how many times they evolved. The correct interpretation of the identity of type specimens is therefore of key importance.

The taxonomic identity of many *Potamogeton* L. hybrids can be revealed only by molecular analysis (Kaplan & al., 2009; Zalewska-Gałosz & al., 2010). However, this is difficult to conduct when interpreting the identity of type specimens. Effective extraction and amplification from old herbarium specimens of Potamogetonaceae mostly fails. In addition, sampling is associated with damage of the specimen, which is hardly acceptable for type specimens. In such cases, plants from the original type localities that are in agreement with the original description of the taxon play an irreplaceable role in the determination of parental species. These "topotypes" can effectively provide the basis for the exact identification of the origin of the hybrid taxon.

As a part of our long-term multidisciplinary research on hybridization in Potamogetonaceae, *Potamogeton* plants morphologically corresponding to the type material were sampled from the respective type localities. Investigation of *P.* ×*torssanderi* (Tiselius) Dörfler from its type locality revealed that it is a triple hybrid of *P. gramineus* × *P. lucens* × *P. perfoliatus* (Kaplan & Fehrer, 2007). In this paper we report our results of molecular investigation of plants from the type localities of two currently recognized hybrids, *P.* ×*lanceolatifolius* and *P.* ×*vepsicus*, which are widely believed to have evolved from crosses of *P. gramineus* × *P. polygonifolius* and *P. alpinus* × *P. natans*, respectively.

■ MATERIALS AND METHODS

Plant material. — The original locality of *P.* ×*lanceolatifolius* was identified from a herbarium label of the type collection (for typification see Kaplan, 2010c). The original site was visited in 1998. It hosted a morphologically uniform colony of plants, which perfectly matched those from the type collection of *P.* ×*lanceolatifolius*. No other *Potamogeton* taxon was found at the type locality. Plants from the type clone of *P.* ×*vepsicus* were provided in 2005 by A. Bobrov who discovered the taxon.

These hybrids were cultivated in the experimental garden at the Institute of Botany, Průhonice, Czech Republic, in 1998–2010 or 2005–2010, respectively, and used for tests of fertility and phenotypic plasticity. Considering the fact that both hybrids are consistently sterile—like the great majority of hybrids involving broad-leaved *Potamogeton* species (Hagström, 1916; Dandy, 1975; Preston, 1995; Wiegleb & Kaplan, 1998; Kaplan & Fehrer, 2007; Kaplan & al., 2009; Bobrov & Chemeris, 2006; Kaplan, 2010a)—and no recombination or backcrossing is therefore possible, the morphologically matching plants analysed were considered as genetically identical with the herbarium types of their respective names.

The putative parental species of the hybrids, all similar species and all other European broad-leaved species of sect. *Potamogeton* were subjected to phylogenetic analyses along with cloned sequences of the hybrids. Several accessions of each species were collected from as distant geographic origin as possible to determine intraspecific genetic variation. A recently discovered hybrid similar to *P.* ×*vepsicus* was also included. Accessions used for the molecular analyses are summarized in the Appendix. Voucher specimens are preserved in the herbarium of the Institute of Botany, Průhonice (PRA). Taxonomic delimitations of species and nomenclature of all taxa follow Wiegleb & Kaplan (1998).

The majority of the listed species are tetraploids, whereas *P. coloratus* and *P. polygonifolius* are diploids and *P. schweinfurthii* is octoploid (Z. Kaplan and V. Jarolímová, unpub. data; Hollingsworth & al., 1998). The chromosome numbers established on the samples included in this study are indicated in the Appendix.

Molecular analyses. — Total genomic DNA was isolated from fresh or CTAB-conserved leaves according to the sorbitol extraction method of Štorchová & al. (2000). PCR-amplification and sequencing of the internal transcribed spacer (ITS) of nuclear ribosomal DNA and of the chloroplast *rpl20-5'rps12* intergenic spacer were done as described in Kaplan & Fehrer (2004, 2006). In case of intraspecific sequence identity in the more variable ITS region, *rpl20-5'rps12* was only sequenced for a geographically representative selection of samples. Sequence electropherograms were proofread manually and aligned in Bio-Edit (Hall, 1999). Intra-individual polymorphisms in the ITS region were represented by the IUPAC ambiguity codes. The ITS region of hybrid samples was at first directly sequenced and then cloned to verify their identity as inferred from additivity patterns (superimposed peaks and frameshift mutations due to diagnostic indels). Cloning was done as described in Fehrer & al. (2009), several clones per sample were sequenced. Polymerase errors (substitutions occurring in only one clone and differing from all other sequences in the alignment) were corrected prior to analysis. One recombinant sequence was observed and excluded from the analyses. GenBank accession numbers are given in the Appendix.

Maximum parsimony (MP; PAUP* v.4.0b10; Swofford, 2002) and Bayesian analyses (MrBayes v.3.1.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) were performed for the ITS and *rpl20-5'rps12* datasets. As the purpose of these analyses was to identify the origin of the hybrids rather than to establish a molecular phylogeny of the genus, the most divergent species, *P. polygonifolius* and *P. coloratus* (MP) or one sequence of either (Bayesian) were used as outgroup. Insertions/deletions (indels) were treated as single mutations. MP analyses: Heuristic searches were performed with 1000 random sequence addition replicates, saving no more than 100 trees of length greater than or equal to 1 per replicate and TBR branch swapping. Bootstrapping was done with the same settings for 1000 replicates, but without branch swapping. Bayesian analysis: The model of molecular evolution best fitting to the data was determined with Modeltest v.3.5 (Posada & Crandall, 1998). A HKY+G/F81 model (ITS/*rpl20-5'rps12*)

was found in hierarchical Likelihood Ratio Tests of which the basic model parameters (two/one substitution rate(s) and gamma/equal distribution) were used as priors. Two replicate analyses with four chains each were performed with the default parameters and computed for 1.8/1.2 million generations, sampling every 1000th tree. All statistical parameters indicated that convergence was reached. The first 20% of the trees per run were discarded as burn-in, and the remaining 2882/1922 trees were summarized.

Directions of the crosses were identified from the chloroplast *rpl20-5'rps12* sequences. Maternal transmission of cpDNA in *Potamogeton* was confirmed by Kaplan & Fehrer (2006).

■ RESULTS

Intraspecific variation. — Phylogenetic analyses included presumed parents, morphologically similar species and all other European broad-leaved species of sect. *Potamogeton*. The species were represented by 3 to 26 samples each, depending on morphological variability and geographic distribution. The ITS tree (Fig. 1) shows that intraspecific variation is low except in *P. gramineus* which consists of two divergent variants (see also Kaplan & Fehrer, 2006, 2007; Kaplan & al., 2009) that are morphologically hardly distinguishable. We refer to them as *P. gramineus* genotype 1 and genotype 2; the former is very widespread while the latter was so far only found in Central and Southern Europe. They also differ in their chloroplast DNA haplotype (Fig. 2). The recently described and morphologically divergent species *P. sarmaticus* nests within *P. gramineus* genotype 1 in the ITS tree (Fig. 1). Thus, *P. gramineus* genotype 1 is paraphyletic and *P. sarmaticus* seems to have evolved from this variable species. Nevertheless, the potential contribution of *P. sarmaticus* to a hybrid can be distinguished from *P. gramineus* genotype 1 by an autapomorphic substitution in the former (A → C), by morphology, and by its geographic distribution, since *P. sarmaticus* is endemic to Kazakhstan, southern and eastern Ukraine and small adjacent regions of southern European Russia (Mäemets, 1979a, b; Belavskaya, 1994; Lisicyna & Papchenkov, 2000; Klinkova, 2006).

Origin of *P. ×lanceolatifolius*. — Direct sequencing of the ITS region of this hybrid showed a high number of superimposed peaks and additional indel mutations. The additivity patterns were not consistent with the presumed origin of *P. ×lanceolatifolius* from *P. gramineus × P. polygonifolius*, but unexpectedly from *P. gramineus × P. nodosus*. This was confirmed by cloned sequences. Those of the European hybrid accession 1005 clustered with both parental species (Fig. 1, genotype 1 of *P. gramineus*). For the American hybrid accession 1974, only *P. nodosus* ribotypes were found among three sequenced clones, but direct sequencing showed the same additivity patterns and also that the *P. nodosus* ribotypes were more abundant and therefore more likely to be retrieved. Even particular geographic variants of *P. nodosus* were detected: At two positions, *P. nodosus* exhibited intraspecific polymorphism

that distinguished European and American samples. The European hybrid accession showed a substitution/intra-individual polymorphism present only in European *P. nodosus* samples (A/A or G; American accessions: G; hybrid: A) whereas the American hybrid accession showed an intra-individual polymorphism (C or T; C: one clone, T: two clones) that also occurred in half of the American samples (others: C). The impact of the latter polymorphisms is not reflected in the tree, because T does not occur as the only character state in any of the American accessions. The chloroplast DNA of the American hybrid corresponded to *P. gramineus* (also genotype 1) while the European hybrid accession had *P. nodosus* as its maternal parent (Fig. 2).
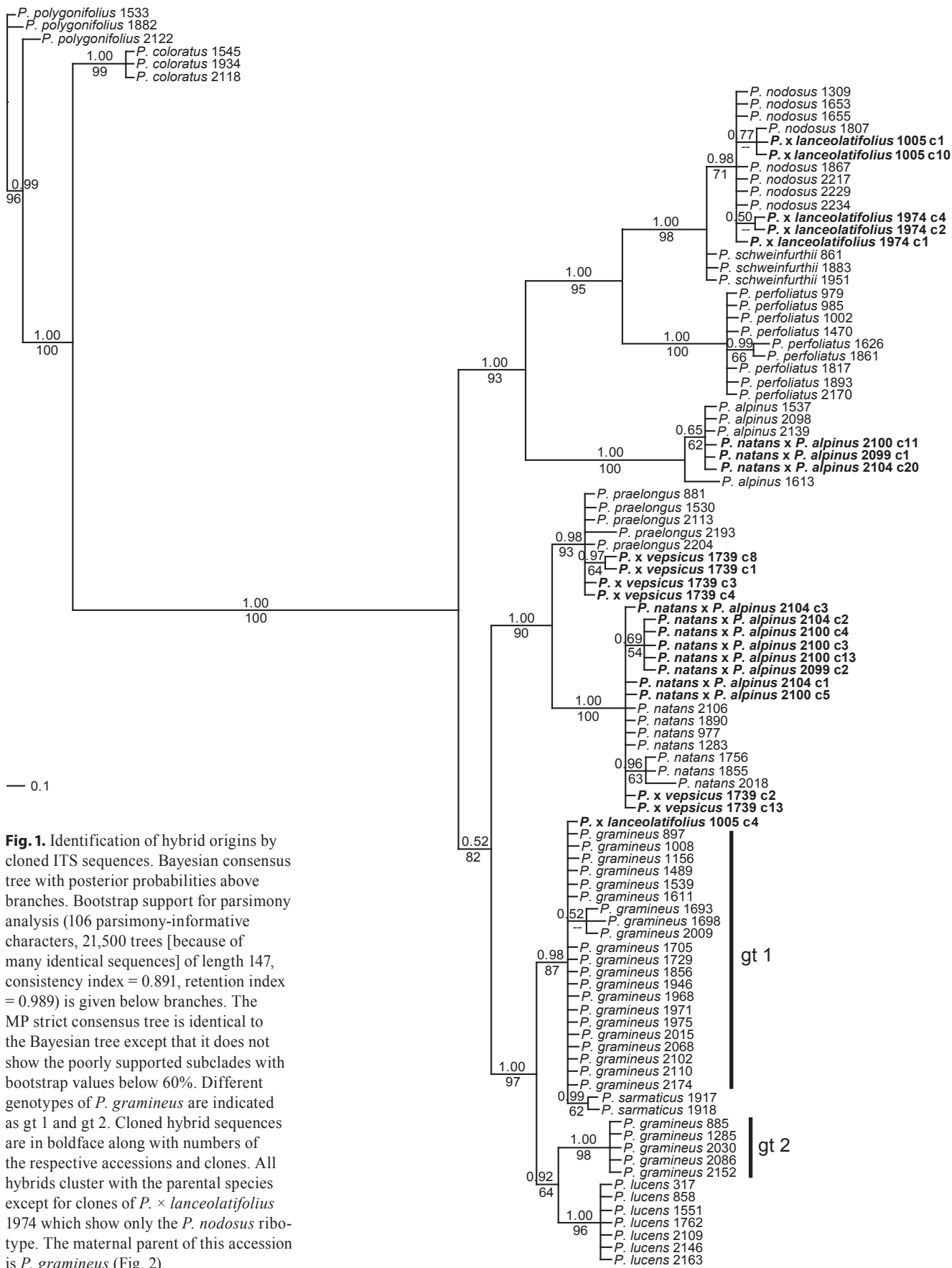
Origin of *P. ×vepsicus*. — The presumed parentage of *P. ×vepsicus* as *P. alpinus × P. natans* also could not be confirmed by the molecular analyses. Instead, additive characters in direct sequencing of the ITS region as well as the placement of the cloned sequences in the tree (Fig. 1) showed that the true parentage of *P. ×vepsicus* accession 1739 is *P. natans × P. praelongus*. The former species is the maternal parent (Fig. 2). The contribution of *P. praelongus* was also readily inferred from a unique 3 bp-deletion in direct sequencing of the ITS. The involvement of *P. alpinus* can easily be excluded as it differs from *P. praelongus* by 27 substitutions and three indels.
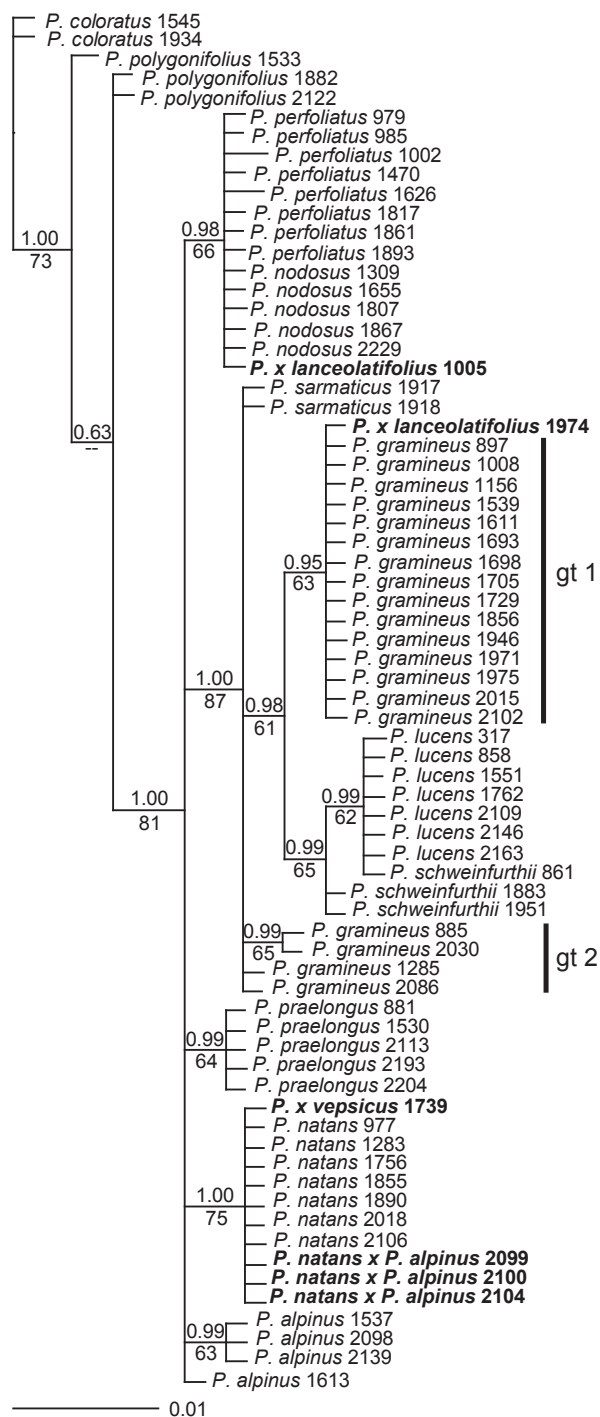
In contrast, three hybrid accessions from Finland morphologically similar to *P. ×vepsicus* were determined by molecular analyses as *P. natans × P. alpinus* with the former as their maternal parent. American accessions of *P. natans* (1756, 1855, and 2018; Fig. 1) differ slightly from European ones, and an intra-individual polymorphism of one accession from Finland (2106), is reflected by the cloned sequences of the hybrids from the same region.

■ DISCUSSION

Potamogeton ×lanceolatifolius was first described as one of several forms of *P. gramineus* (Tiselius, 1894–1897; Kaplan, 2010c). Based on careful morphological investigation of the type collection, Hagström (1916) correctly noted that these plants have sterile pollen, suggested their hybrid origin and interpreted them as a hybrid *P. gramineus × P. polygonifolius*. This view was adopted by later researchers (e.g., Dandy, 1975; Preston, 1987; Wiegleb & Kaplan, 1998). Because the type of the name *P. ×seemenii* Aschers. & Graebn. previously used for this hybrid combination almost certainly belonged to *P. gramineus*, Preston (1987) replaced it with the binomial *P. ×lanceolatifolius*. This nomenclature was adopted by Stace (1991, 2010), Preston (1995, 1996) and Wiegleb & Kaplan (1998).

Morphology of *P. ×lanceolatifolius* indicates that it is a hybrid between *P. gramineus* and either *P. polygonifolius* or *P. nodosus*. Of these two, only *P. polygonifolius* currently occurs in Sweden where *P. ×lanceolatifolius* was first detected, whereas *P. nodosus* has never been recorded from the whole of Scandinavia (e.g., Hagström, 1916, 1922; Pedersen, 1976; Dandy, 1980; Hultén & Fries, 1986; Jonsell & Jonsell, 1994;

Fig. 1. Identification of hybrid origins by cloned ITS sequences. Bayesian consensus tree with posterior probabilities above branches. Bootstrap support for parsimony analysis (106 parsimony-informative characters, 21,500 trees [because of many identical sequences] of length 147, consistency index = 0.891, retention index = 0.989) is given below branches. The MP strict consensus tree is identical to the Bayesian tree except that it does not show the poorly supported subclades with bootstrap values below 60%. Different genotypes of *P. gramineus* are indicated as gt 1 and gt 2. Cloned hybrid sequences are in boldface along with numbers of the respective accessions and clones. All hybrids cluster with the parental species except for clones of *P. × lanceolatifolius* 1974 which show only the *P. nodosus* ribotype. The maternal parent of this accession is *P. gramineus* (Fig. 2).

Fig. 2. Maternal origin of hybrids according to chloroplast *rpl20-5'rps12* sequences. Bayesian consensus tree with posterior probabilities above branches. Bootstrap support for parsimony analysis (16 parsimony informative characters, eight trees of length 20, consistency index = 0.950, retention index = 0.994) is given below branches. The MP strict consensus tree is identical to the Bayesian tree apart from the single branch that did not receive any bootstrap support. Not all species can be distinguished with this marker due to low variation. *Potamogeton gramineus* shows differentiation into the same two genotypes (gt 1, gt 2) as detected by ITS. Hybrids are in boldface. The assignment of the maternal parents is unequivocal in combination with the ITS data (Fig. 1).

Mossberg & Stenberg, 2010). This is probably the main reason why *P. nodosus* has never been suggested as the parental species of *P. ×lanceolatifolius* and was identified as such only by molecular analysis.

Although *P. nodosus* and *P. polygonifolius* are morphologically very similar and differ almost exclusively in quantitative characters (e.g., Preston, 1995; Kaplan, 2010b), they are not at all closely related (see Figs 1 & 2). They also differ in ecological requirements: *P. nodosus* prefers warmer climate and mesotrophic waters whereas *P. polygonifolius* mostly occurs in oligotrophic to dystrophic cold waters (e.g., Preston, 1995; Kaplan & Symoens, 2005; Kaplan, 2010b). In addition, they have different chromosome numbers, which provides further indirect evidence for the true hybrid identity. Although heteroploid crosses are possible in *Potamogeton*, these result in triploid plants (Kaplan & al., 2009; Kaplan and Jarolímová, unpub. data). If *P. ×lanceolatifolius* was a hybrid between *P. gramineus* ($4x$) and *P. polygonifolius* ($2x$), it could be assumed to be triploid. However, *P. ×lanceolatifolius* is tetraploid, which is consistent with the ploidy level of its actual parents, *P. gramineus* and *P. nodosus* (both $4x$).

Occurrences of Potamogetonaceae hybrids persisting vegetatively in the absence of the parental species, presumably being relics after their parents disappeared, have been repeatedly documented (e.g., Dandy & Taylor, 1946; Hollingsworth & al., 1996; Preston & al., 1998a, b, 1999; King & al., 2001; Kaplan & Fehrer, 2004; Kaplan & al., 2009; Kaplan, 2010b). Another *P. nodosus* hybrid, *P. ×schreberi* G. Fisch. (= *P. natans × P. nodosus*), is known to often occur in the absence of the parental species (Hollingsworth & al., 1995; Kaplan & Wolff, 2004; Kaplan & Fehrer, 2009; Kaplan, 2010b). Cases of ancient relic occurrences of hybrids comparable to *P. ×lanceolatifolius* in Sweden were described in the closely related *Stuckenia* Börner (for taxonomy of this genus see Kaplan, 2008). Great Britain hosts *S. ×bottnica* (Hagstr.) Holub (= *S. pectinata* (L.) Börner × *S. vaginata* (Turcz.) Holub) while one of its parents, *S. vaginata*, is now restricted in Europe only to Scandinavia (Preston & al., 1998b; King & al., 2001). Another hybrid, *S. ×fennica* (Hagstr.) Holub (= *S. filiformis* (Pers.) Börner × *S. vaginata*), also occurs in East Europe outside the current distribution of *S. vaginata* (Bobrov, 2007). These *Stuckenia* hybrids have apparently persisted there since the time of the last glaciation. Thus, in relation to climatic changes, one of the parental species adapted to different ecological conditions disappeared from a particular region and its former occurrence can be documented by the molecular signature it has left in its hybrids that were better adapted to the new conditions.

So far, *P. ×lanceolatifolius* (as *P. gramineus × P. polygonifolius*) was recorded only from three European countries: Sweden, Great Britain and Russia (Hagström, 1916; Dandy, 1975; Preston, 1995; Papchenkov, 1997, 2007). The Swedish material was shown here to be *P. gramineus × P. nodosus*. The exact taxonomic status of the plants recorded from the other two countries remains to be clarified. In contrast, our study revealed the occurrence of this hybrid in North America, where *P. ×lanceolatifolius* has never been reported. This American sample represents the opposite direction of the same cross.

The supposed hybrid between *P. gramineus* and *P. nodosus* has several times been recorded (Hagström, 1908, 1916; Ascherson & Graebner, 1913; Ogden, 1943) and was named *P. ×argutulus* Hagstr. Hagström (1908) recorded two specimens in the protologue but described and illustrated this new taxon based on a specimen from France whereas the morphology of the other specimen was indicated as "will be described in another work". The French type specimen shows morphology and stem anatomy typical of *P. gramineus* and the spikes contain well-developed fruit, indicating that this plant was fertile, in contrast to the confirmed specimens of *P. ×lanceolatifolius*, which are consistently sterile. The type specimen, preserved in C, was amply annotated by Hagström. Here it is used for simultaneous lectotypification of the name of the supposed nothospecies and for that of one of the subordinated formae published at the same time (the herbarium sheet was labelled by ZK in 2002). Both names below are reduced to the synonymy of *P. gramineus*:

Potamogeton argutulus Hagstr. in Bot. Not. 1908: 106. 1908 (pro hybr.) – Type: France: "Potamogeton heterophyllum DC. var., Gallia: Vienne: in stagno dicto "de la Puye", 10 Julio 1900, E. Simon" (lectotype designated here: C!).

Potamogeton argutulus f. ***puyensis*** Hagstr. in Bot. Not. 1908: 107. 1908 – Type: France: "Potamogeton heterophyllum DC. var., Gallia: Vienne: in stagno dicto "de la Puye", 10 Julio 1900, E. Simon" (lectotype designated here: C!).

The specimen is preserved in the separately kept *Potamogeton* collections of I. Baagöe. A fragment was preserved by Hagström in his personal herbarium, which is now incorporated in LD.

Because the name "*P. ×argutulus*" is actually based on a specimen of *P. gramineus*, the correct binomial for the hybrid *P. gramineus × P. nodosus* is *P. ×lanceolatifolius*. Unequivocal proof for the existence of this hybrid combination is provided here for the first time.

Potamogeton ×vepsicus was detected only recently as a new taxon (Bobrov & Chemeris, 2006). Detailed morphological and anatomical analysis, and comparison with other similar hybrids led its authors to the conclusion that this plant is a hybrid between *P. alpinus* and *P. natans*. However, the molecular analysis unequivocally identified *P. natans* and *P. praelongus* as its actual parents. As in *P. ×lanceolatifolius*, the identity of the hybrid could hardly be deduced from morphology. The actual parental species of *P. ×vepsicus* have a considerably different kind of foliage: *P. natans* has well-developed floating leaves but submerged leaves reduced to bladeless phyllodes whereas *P. praelongus* lacks floating leaves but has robust submerged leaves. Most of the structures that show characters traditionally used in the morphological distinction of *Potamogeton* hybrids are missing in one of the parental species (floating leaves in *P. praelongus*, laminar submerged leaves in *P. natans*). These divergent morphologies do not allow direct comparison of corresponding characters and searching for intermediate states. Without artificial hybridization, which is very difficult to achieve in *Potamogeton* (Kaplan & Fehrer, 2006), the morphology of a plant combining such divergent kinds of foliage therefore cannot be anticipated. This may also be the reason why the existence of the hybrid combination *P. natans × P. praelongus* has not been recorded previously. Detailed morphological comparison of the Russian *P. ×vepsicus* with similar Finnish plants, which are true hybrids *P. natans × P. alpinus*, will be published elsewhere.

Widespread hybrids, such as *P. ×angustifolius* J. Presl, *P. ×nitens* Weber or *P. ×salicifolius* Wolfg., have often been studied and the range of their variation is relatively well known (for detailed treatments see Hagström, 1916). In addition, their identity has recently been proven by isozyme electrophoresis and DNA analysis (Fant & Preston, 2004; Kaplan & Fehrer, 2006; Kaplan, 2007, 2010a; Kaplan & al., 2009). In contrast, rare hybrids are in higher risk to be misunderstood because of lack of adequate observations and inaccessibility of fresh plant material for molecular confirmation. Hybrids such as *P. ×babingtonii* auct., *P. ×nericius* Hagstr., *P. ×nerviger* Wolfg., *P. ×prussicus* Hagstr. and *P. ×vilnensis* Galinis are occasionally recorded in the literature but the scarce information on them is usually copied from one author to another. The identities of many of the new hybrids described by Papchenkov (1997, 2001, 2007) based on morphology only, must be considered doubtful and may just represent extreme forms of natural species or of other widespread hybrids. Molecular investigation of a hybrid recently recorded from Poland by Kraska & al. (2004) as *P. ×nericius* (= *P. alpinus × P. gramineus*) led to the discovery of the new, undescribed hybrid *P. nodosus × P. perfoliatus* (Zalewska & al., 2010). Plants from a German population recorded by Wiegleb & al. (2008) as *P. ×nerviger* (= *P. alpinus × P. lucens*) proved to be a slender form of *P. ×salicifolius* (= *P. lucens × P. perfoliatus*; Kaplan & Fehrer, unpub.). Recent molecular studies proved the existence of several so far unknown hybrids (Kaplan & al., 2009, 2011). These examples indicate that in spite of the long and intensive research on hybridization in *Potamogeton*, the total hybrid diversity and distribution of many hybrids are still insufficiently known and much work remains to be done.

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Appendix. Accessions used for the molecular analyses and GenBank accession numbers. Numbers in superscript given at some reference numbers indicate chromosome numbers established on that particular sample: [a]$2n = 28$; [b]$2n = 52$; [c]$2n = 104$ (Kaplan & Jarolímová, unpub.).

Taxon name (given only once for multiple accessions of the same taxon), **reference number:** origin, voucher collection records [collector's name, collection number], ITS accession number, *rpl20-5'rps12* accession number [samples not sequenced for this region are represented by a dash].

P. alpinus Balb., **1537:** Czech Republic: distr. Mělník, Vojtěchov, stream Pšovka, *Z. Kaplan 04/204*, FJ151201, HQ263435; **1613:** U.S.A.: Vermont, Washington Co., Maple Corner, Curtis pond, *Z. Kaplan & C.B. Hellquist 05/354*, FJ151202, HQ263436; **2098:** Finland: North Ostrobothnia, Pudasjärvi, Keinäsperä, brook Syrjäoja, *Z. Kaplan & P. Uotila 09/309*, HQ263485, HQ263437; **2139:** Russia: Prov. Irkutsk, Ziminskiy distr., Ignay, *Z. Kaplan & V. Chepinoga 09/365*, HQ263486, HQ263438. *P. coloratus* Hornem., **1545:** Austria: Niederösterreich, Reisenberg, rivulet Fürbach, coll. *H. Hromadnik*, cult. & coll. *Z. Kaplan 1545*, HQ263487, HQ263439; **1934:** Germany: Nordrhein-Westfalen, Dorsten, coll. *K. van de Weyer*, cult. & coll. *Z. Kaplan 1934*, HQ263488, HQ263440; **2118:** Germany: Nordrhein-Westfalen, Kempen, *Z. Kaplan & al. 09/325*, HQ263489, –. *P. gramineus* L., **885**[b]**:** Czech Republic: distr. Náchod, Šeřeč, Rozkoš Reservoir, *Z. Kaplan 97/829*, EF174589, DQ468864; **897**[b]**:** Czech Republic: distr. Česká Lípa, Hradčany u Mimoně, Držák fishpond, *Z. Kaplan 96/638*, DQ468866, DQ468866; **1008:** Sweden: Prov. Södermanland, Vårdinge, Lake Sillen, *Z. Kaplan 98/346*, HQ263490, HQ263441; **1156**[b]**:** France: Bretagne, Morbihan, Lorient, Scorff River, coll. *J. Květ*, cult. & coll. *Z. Kaplan 1156*, HQ263491, HQ263442; **1285:** France: Lorraine, Moselle, Rémelfing, coll. *P. Wolff*, cult. & coll. *Z. Kaplan 1285*, DQ468861, DQ468865; **1489:** Czech Republic: distr. Jihlava, Vysoká u Jihlavy, Lužný fishpond, *Z. Kaplan 03/188*, HQ263492, –; **1539:** Czech Republic: distr. Pardubice, Hrobice, Baroch fishpond, *Z. Kaplan 04/259*, HQ263493, HQ263443; **1611:** U.S.A.: Vermont, Washington Co., Calais, Adamant, Bliss Pond, *Z. Kaplan & C.B. Hellquist 05/352*, EF174587, EF174590; **1693:** U.S.A.: New Hampshire, Carroll Co., West Ossipee, Ossipee Lake, *Z. Kaplan & C.B. Hellquist 05/416*, EU596943, EU596952; **1698:** U.S.A.: New Hampshire, Carroll Co., Center Ossipee, Ossipee Lake, *Z. Kaplan & C.B. Hellquist 05/421*, EF174585, EF174592; **1705:** U.S.A.: Maine, Aroostook Co., Houlton, New Limerick, Nickerson Lake, *Z. Kaplan & C.B. Hellquist 05/430*, EF174586, EF174591; **1729:** U.S.A.: Maine, Penobscot Co., Gould Landing, Pushaw Lake, *Z. Kaplan & C.B. Hellquist 05/455*, EF174593, EF174593; **1856:** U.S.A.: Vermont, Orleans Co., Derby, Lake Memphramagog, *C.B. Hellquist 16963 & R. Popp*, HQ263494, HQ263444; **1946:** Montenegro: distr. Nikšić, Mratinje, Maglić, Lake Trnovačko, *Z. Kaplan 08/585*, HQ263495, HQ263445; **1968:** Czech Republic: distr. Česká Lípa, Zahrádky, Novozámecký fishpond, coll. *J. Sádlo & P. Petřík*, cult. & coll. *Z. Kaplan 1968*, HQ263496, –; **1971:** U.S.A.: Maine, Washington Co., Cherryfield, Narraguagus River, *C.B. Hellquist 17084*, HQ263497, HQ263446; **1975:** U.S.A.: Maine, Penobscot Co., Bangor, Kenduskeag Stream, *C.B. Hellquist 17092*, HQ263498, HQ263447; **2009:** U.S.A.: Montana, West Yellowstone, Divide Lake, *C.E. Hellquist & C.B. Hellquist 441–08*, HQ263499, –; **2015:** U.S.A.: Wyoming, Teton Co., Lewis Falls, Lewis River, *C.E. Hellquist & C.B. Hellquist 575–08*, HQ263500, HQ263448; **2030:** Montenegro: distr. Bar, Seoca, Lake Skadar, *Z. Kaplan 08/577*, HQ263501, HQ263449; **2068:** Czech Republic: distr. Hradec Králové, Štít, Dolní Flajšar fishpond, *Z. Kaplan 09/54*, HQ263502, –; **2086:** Slovakia: distr. Trebišov, Veľké Trakany, Stará Tisa River, *Z. Kaplan 09/279*, HQ263503, HQ263450; **2102:** Finland: North Ostrobothnia, Oulu, Kiiminkijoki River, *Z. Kaplan & P. Uotila 09/313*, HQ263504, HQ263451; **2110:** Finland: South Häme, Hattula, Parolannummi, Lake Lehijärvi, *Z. Kaplan & P. Uotila 09/319*, HQ263505, –; **2152:** Czech Republic: distr. Žďár nad Sázavou, Mostiště, Mostišťský fispond, *Z. Kaplan 09/375*, HQ263506, –; **2174:** Germany: Mecklenburg-Vorpommern, Carwitz, Lake Dreetz, *Z. Kaplan 09/347*, HQ263507, –. *P. lucens* L., **317:** Czech Republic: distr. Pardubice, Hrobice, Baroch fishpond, *Z. Kaplan 96/627*, EF174584, EF174595; **858:** The Netherlands: Limburg, Arcen, coll. *P. Denny*, cult. & coll. *Z. Kaplan 858*, EF174583, EF174594; **1551:** Japan: Fukuoka Pref., Kitakyuusyuu, coll. *N. Tanaka*, cult. & coll. *Z. Kaplan 1551*, HQ263508, HQ263452; **1762**[b]**:** Japan: Chiba Pref., Tokyo, Tega River, coll. *N. Tanaka*, cult. & coll. *Z. Kaplan 1762*, HQ263509, HQ263453; **2109:** Finland: South Häme, Hollola, Lake Vesijärvi, *Z. Kaplan & P. Uotila 09/318*, HQ263510, HQ263454; **2146:** Russia: Prov. Irkutsk, Ziminskiy distr., Ignay, Zima River, *Z. Kaplan & V. Chepinoga 09/372*, HQ263511, HQ263455; **2163:** Germany: Mecklenburg-Vorpommern, Feldberg, Lake Schmaler Luzin, *Z. Kaplan 09/334*, HQ263512, HQ263456. *P. natans* L., **977**[b]**:** Switzerland: St. Gallen, Altenrhein, *Z. Kaplan 98/122*, FJ883537, FJ883542; **1283:** Germany: Saarland, Saarbrücken, coll. *F.-J. Weicherding*, cult. & coll. *Z. Kaplan 1283*, FJ151208, FJ883543; **1756:** U.S.A.: Massachusetts, Berkshire Co., Hancock, Kinderhook Creek pond, *Z. Kaplan & C.B. Hellquist 05/342*, FJ151209, FJ883541; **1855:** U.S.A.: Vermont, Orleans Co., Derby, Lake Memphramagog, *C.B. Hellquist 16962 & R. Popp*, HQ263513, HQ263457; **1890**[b]**:** Czech Republic: distr. České Budějovice, Třeboř, stream Stropnice, *Z. Kaplan 07/215*, FJ883536, FJ883544; **2018:** U.S.A.: Wyoming, Teton Co., Seven Mile Bridge, Madison River backwater, *C.E. Hellquist & C.B. Hellquist 592–08*, HQ263514, HQ263458; **2106:** Finland: North Häme, Viitasaari, Lake Keitele, *Z. Kaplan & P. Uotila 09/316*, HQ263515, HQ263459. *P. nodosus* Poir., **1309:** France: Lorraine, Moselle, Welferding, coll. *P. Wolff*, cult. & coll. *Z. Kaplan 1309*, FJ151210, FJ883547; **1653:** U.S.A.: Vermont, Addison Co., Brooksville, Otter Creek, *Z. Kaplan & C.B. Hellquist 05/386*, FJ151211, –; **1655:** U.S.A.: Vermont, Addison Co., Brooksville, Otter Creek, *Z. Kaplan & C.B. Hellquist 05/388*, FJ883539, FJ883548; **1807:** Czech Republic: distr. Pardubice, Stéblová, sand pit Oplatil, *Z. Kaplan 06/345*, FJ883538, FJ883546; **1867:** U.S.A.: Michigan, Cheboygan Co., Cheboygan, Black River, *C.B. Hellquist 16974*, HQ263516, HQ263460; **2217:** U.S.A.: Massachusetts, Berkshire Co., Stockbridge, Lily Brook, *C.B. Hellquist & al. 17123*, HQ263518, HQ263461; **2229:** U.S.A.: New York, Columbia Co., Beebe Pond, *C.B. Hellquist & al. 17112*, HQ263517, –; **2234:** U.S.A.: Vermont, Addison Co., Orwell, Whiting, Lemon Fair River, *C.B. Hellquist & al. 17130*, HQ263519, –. *P. perfoliatus* L., **979**[b]**:** Switzerland: St. Gallen, Altenrhein, Lake Constance, *Z. Kaplan 98/125*, AY529527, DQ468862; **985**[b]**:** Austria: Vorarlberg, Fußach, Lake Constance, *Z. Kaplan 98/131*, HQ263520, HQ263462; **1002**[b]**:** Sweden: Prov. Skåne, Björka, *Z. Kaplan 98/338*, AY529526, DQ468863; **1470**[b]**:** Germany: Bavaria, Ebing, Main River, coll. *L. Meierott*, cult. & coll. *Z. Kaplan 1470*, AY529525, EF174597; **1626:** U.S.A.: Vermont, Orleans Co., West Glover, Lake Parker, *Z. Kaplan & C.B. Hellquist 05/360*, EU596953, EU596944; **1817:** Bosnia-Herzegovina: Poklečani, Lake Blidinje, coll. *J. Pokorný*, cult. & coll. *Z. Kaplan 1817*, HQ263521, HQ263463; **1861:** U.S.A.: New York, Clinton Co., Ausable, Lake Champlain, *C.B. Hellquist 16968*, HQ263522, HQ263464; **1893:** Czech Republic: distr. Sokolov, Dasnice, Ohře River, *Z. Kaplan 09/342*, HQ263523, HQ263465; **2170:** Germany: Mecklenburg-Vorpommern, Carwitz, Lake Krüselin, *Z. Kaplan 09/343*, HQ263524, –. *P. polygonifolius* Pourr., **1533**[a]**:** Czech Republic: distr. Cheb, Hranice, Novosedly, Nový fishpond, *Z. Kaplan 04/168*, HQ263525, HQ263466; **1882:** Portugal: Prov. Algarve, distr. Faro, Rogil, coll. *U. Schwarzer*, cult. & coll. *Z. Kaplan 1882*, HQ263526, HQ263467; **2122:** Germany: Rheinland-Pfalz, Schopp, 2 VIII 2009 *P. Wolff s. n.*, HQ263527, HQ263468. *P. praelongus* Wulfen, **881:** Germany: Mecklenburg-Vorpommern, Güstrow, Dobbin, Lake Gültz, *Z. Kaplan 97/824*, HQ263528, HQ263469; **1530:** Italy: Trentino-Alto Adige, Prov. Bolzano, San Valentino alla Muta, Lake Muta, *Z. Kaplan & J. Štěpánková 04/62*, HQ263529, HQ263470; **2113:** Finland: South Häme, Hattula, Parolannummi, Lake Lehijärvi, *Z. Kaplan & P. Uotila 09/322*, HQ263530, HQ263471; **2193:** U.S.A.: Maine, Aroostook Co., St. John Plt., eastern Wallagrass Lake, *C.B. Hellquist & al. 17141*, HQ263531, HQ263472; **2204:** U.S.A.: Michigan, Cheboygan Co., Monro, Lancaster Lake, *C.B. Hellquist 17165*, HQ263532, HQ263473. *P. sarmaticus* Mäemets, **1917:** Russia: region Voronezh, Varvarino, coll. *E. Pechenyuk*, HQ263533, HQ263474; **1918**[b]**:** Russia: region Voronezh, Varvarino, coll. *E. Pechenyuk*, cult. & coll. *Z. Kaplan 1918*, HQ263534, HQ263475. *P. schweinfurthii* A. Benn., **861**[c]**:** Kenya, coll. *P. Denny*, cult. & coll. *Z. Kaplan 861*, HQ263535, HQ263476; **1883**[c]**:** Portugal: Prov. Algarve, distr. Faro, Rogil, coll. *U. Schwarzer*, cult. & coll. *Z. Kaplan 1883*, HQ263536, HQ263477; **1951**[c]**:** Italy: Tuscany, Prov. Arezzo, Anghiari, *Z. Kaplan & al. 08/608*, HQ263537, HQ263478. *P. × lanceolatifolius* (Tiselius) C.D. Preston [= *P. gramineus × P. nodosus*], **1005**[b]**:** Sweden: Prov. Småland, Kalmar, Ålem, Alsterån River, *Z. Kaplan 98/342*, HQ263538–HQ263540, HQ263479; **1974:** U.S.A.: Maine, Penobscot Co., Bangor, Kenduskeag Stream, *C.B. Hellquist 17091*, HQ263541–HQ263543, HQ263480. *P. × vepsicus* A.A. Bobrov & Chemeris [= *P. natans × P. praelongus*], **1739**[b]**:** Russia: Prov. Vologda, distr. Babaevo, Pyazhelka, Nozhema River, coll. *A. Bobrov & E. Chemeris*, cult. & coll. *Z. Kaplan 1739*, HQ263544–HQ263549, HQ263481. *P. natans × P. alpinus*, **2099:** Finland: North Ostrobothnia, Pudasjärvi, Keinäsperä, brook Syrjäoja, *Z. Kaplan & P. Uotila 09/310*, HQ263550–HQ263552, HQ263482; **2100:** Finland: North Ostrobothnia, Pudasjärvi, Keinäsperä, river Olvasoja, *Z. Kaplan & P. Uotila 09/311*, HQ263552–HQ263556, HQ263483; **2104:** Finland: North Ostrobothnia, Pudasjärvi, Keinäsperä, river Olvasoja, *Z. Kaplan & P. Uotila 09/314*, HQ263557–HQ263560, HQ263484.