New Hybrid Combinations Revealed by Molecular Analysis: The Unknown Side of North American Pondweed Diversity (*Potamogeton*)

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Abstract—The occurrence and diversity of *Potamogeton* hybrids was investigated in eastern North America, the region of the highest diversity of *Potamogeton* species in the world. For various reasons, however, the existence of hybrids in this area has been largely overlooked. ITS direct sequencing and RFLPs revealed four previously unknown hybrids, which are described as *Potamogeton* ×*aemulans* (*P. bicupulatus* × *P. epihydrus*), *P.* ×*mirabilis* (*P. gramineus* × *P. oakesianus*), *P.* ×*versicolor* (*P. epihydrus* × *P. perfoliatus*), and *P.* ×*absconditus* (*P. perfoliatus* × *P. richardsonii*). These are the first confirmed hybrids involving *P. bicupulatus*, *P. epihydrus*, *P. oakesianus*, and *P. richardsonii*. Another hybrid, *P.* ×*nitens* (*P. gramineus* × *P. perfoliatus*), is for the first time confirmed for North America with molecular markers. The hybrids' maternal parents were revealed by cpDNA sequencing. Phenotypically, two of the hybrids more closely resemble other taxa than their parents. Hybrid diversity and recognition in North America and in Europe are compared. Morphological versus molecular identification of hybrids, occurrence of hybrids in the absence of their parents, parental species relationships, long-term persistence of hybrid clones, vegetative dispersal, frequency of hybridization events, and hybrid distribution patterns are discussed.

Keywords—direct sequencing, hybridization, new taxa, relic occurrence, RFLP, taxonomy.

Potamogetonaceae are one of the families of aquatic plants with the highest diversity and markedly pronounced taxonomic difficulties (Wiegleb and Kaplan 1998). One of the most important sources of diversity and taxonomic complexity is the occurrence of hybrids. In Europe, recognition of hybrids has a long tradition. The first *Potamogeton* hybrids were identified as separate morphological entities as early as the 1780s (e.g. *Potamogeton* ×*nitens* Weber in 1787, *P.* ×*fluitans* Roth in 1788; for hybrid formulae see Wiegleb and Kaplan 1998), although at that time their hybrid origin was not identified (Kaplan 2005; Kaplan and Fehrer 2006). The existence of hybrids in Potamogetonaceae was recognized over a century ago in the European literature (e.g. Fryer 1890; Ascherson and Graebner 1897; Baagøe 1897; Raunkiaer 1903; Fischer 1904, 1905, 1907; Graebner 1907; Linton 1907; Hagström 1916). By 1916, 90% (28 of 31) of the currently recognized European hybrids were formally described. The existence of hybrids in Potamogetonaceae is now considered a well-documented fact (e.g. Dandy 1975, 1980; Wiegleb 1988; Preston 1995; Kaplan 2001; Kaplan and Zalewska-Gałosz 2004), which "is not likely to be doubted by anyone who is familiar with the morphology of the species" (Preston 1995:42). Recent isozyme (e.g. Hollingsworth et al. 1995, 1996; Preston et al. 1998b; Fant et al. 2001a,b; Iida and Kadono 2002; Kaplan et al. 2002; Fant and Preston 2004; Kaplan and Wolff 2004; Kaplan 2007) and DNA-based studies (King et al. 2001; Fant et al. 2003; Kaplan and Fehrer 2004, 2006, 2007) on hybridization in *Potamogeton* and *Stuckenia* (often included in *Potamogeton*) conducted in Europe confirmed previous morphological and anatomical observations and a frequent occurrence of hybrids in some European regions. On a worldwide scale, Wiegleb and Kaplan (1998) identified 50 sufficiently documented hybrids, some of which are locally frequent and represent clearly circumscribed biological entities.

The occurrence of *Potamogeton* hybrids in North America was first recorded by Bennett (1890, 1901, 1902, 1908) and Hagström (1916) who both had rich field and herbarium experience with European hybrids. Hagström was largely criticized for his view on the hybrid origin of many North American plants by St. John (1925). Although most species of Potamogetonaceae occurring in North America were already recognized by early authors (Rafinesque-Schmaltz 1811; Tuckerman 1843, 1848; Robbins 1867; Morong 1880, 1881, 1893; Morong and Taylor 1913; Hagström 1916; Fernald 1932; Ogden 1943), the diversity and frequency of hybrids in North America still remains largely unknown. Their occurrence was systematically investigated only among broad-leaved species by Ogden (1943), based on morphological and anatomical data. Les and Philbrick (1993:194) argued that "it is remarkable that hybridization in *Potamogeton* has become a dogmatic attribute of the genus despite the lack of supporting empirical evidence" and pointed out that "convincing evidence is lacking for most hybrid reports". Only recently, a few studies appeared that dealt in detail with selected North American putative hybrids; hybrid origin for two linear-leaved taxa was indicated in flavonoid studies (Haynes and Williams 1975; Hellquist and Hilton 1983), possible occurrence of a putative hybrid between *P. foliosus* Raf. and *P. pusillus* L. was recently recorded (Whittall et al. 2004), and a new hybrid, known only so far from Europe, was recently detected by detailed morphological and anatomical analysis (Alix and Scribailo 2006).

To shed more light on the diversity and frequency of Potamogetonaceae hybrids in North America, we conducted extensive fieldwork and sampling in New England, which is the region of the highest diversity of Potamogetonaceae species (Hellquist and Crow 1980; Wiegleb 1988). In this paper, we focus on several morphotypes that lie outside the normal range of variation for taxa known from the area and whose exact taxonomic identities were unclear.

Nuclear ribosomal DNA (nrDNA), especially the variable internal transcribed spacer (ITS) region, is frequently employed for the identification of hybrid and allopolyploid origin by RFLP, direct sequencing, cloning, or a combination of these (e.g. Soltis and Soltis 1991; Sang et al. 1995; O'Kane et al. 1996; Rauscher et al. 2002; Nieto Feliner et al. 2004; Guggisberg et al. 2006; Kaplan and Fehrer 2006, 2007). Nuclear ribosomal DNA data alone can provide direct evidence of hybridization if concerted evolution fails to act across the repeat units contributed by different parent species (e.g. Hughes et al. 2002, and references therein). This was expected to be the case in

Potamogetonaceae hybrids, because they are almost always consistently sterile, and concerted evolution is known to be retarded in asexually reproducing organisms (e.g. Campbell et al. 1997). Thus, we employed direct sequencing and RFLPs of the internal transcribed spacer (ITS) to identify the origin of putative hybrids and of taxonomically unclear plants. Analysis of chloroplast DNA was used to identify the direction of the cross in confirmed hybrids.

Materials and Methods

Plant Material—Most material for this study was collected during fieldwork conducted in six states of New England, U. S. A. All sites known from previous research that hosted taxonomically unclear morphotypes and putative unknown hybrids of Potamogetonaceae were visited. An effort was made to cover the entire New England diversity of Potamogetonaceae species to achieve representative sampling of diversity. Altogether 161 samples of 44 taxa (species and hybrids) from 33 sites were collected. Additional material was collected in other parts of North America and in various regions of Europe. Voucher specimens of all samples are preserved in the herbarium of the Institute of Botany, Průhonice (acronym PRA); duplicates of the newly discovered hybrids were deposited in GH and MASS. Leaf material was preserved in CTAB solution and used for DNA extraction (Štorchová et al. 2000).

Our previous studies showed low intraspecific variation in *Potamogeton* in comparison to interspecific differences. In most cases, conspecific samples produced identical RFLP banding patterns (Kaplan and Fehrer 2004, 2006). Therefore, only two samples from each putative parent, collected from as distant geographic origins as possible, were used in most cases because of the high number of candidate parents. In the case of *P. diversifolius* Raf., only one sample was available, and in the case of *P. gramineus* L., two divergent genotypes identified previously (Kaplan and Fehrer 2006, 2007) were represented by 2–3 samples each. If the putative parents occurred at the same site as the hybrids, one sample per parent from that location was included. Specimens included in RFLPs are summarized in Appendix 1.

The majority of the listed species are tetraploids with $2n = 52$; *Potamogeton epihydrus* Raf. is diploid with $2n = 26$, *Stuckenia pectinata* (L.) Börner is hexaploid with $2n = 78$ (Z. Kaplan and V. Jarolímová, unpubl. data; Hollingsworth et al. 1998). The chromosome numbers of *P. bicupulatus* Fernald, *P. diversifolius*, *P. oakesianus* J. W. Robbins, and *P. spirillus* Tuckerm. are unknown.

Morphological Evaluation—Detailed morphological observations were made on herbarium material. Abundant morphological data compiled for our previous studies (Hellquist and Crow 1980; Wiegleb and Kaplan 1998; Crow and Hellquist 2000; Kaplan 2002; Kaplan and Fehrer 2004, 2006) were considered to cover the morphological variation of the respective taxa as completely as possible. Numbers of leaf veins include all longitudinal veins present in the lamina; as these are sometimes faint (and their number is often under-recorded in the literature, e.g. *P. perfoliatus* L.), their number was scored under an herbarium stereomicroscope. Rare extremes were excluded from the quantitative data.

Identification of Hybrids—To identify parental species, the total North American diversity of Potamogetonaceae was considered. The majority of species could be excluded morphologically. For example, all linear-leaved species could be excluded when the hybrid belonged to the group of broad-leaved species. Although character expression in hybrids is generally unpredictable and some characters of parents may be missing, or transgressive phenotypes or new traits may appear, many previous molecular studies (see the references in the introduction) demonstrate that for most Potamogetonaceae hybrids, either the parental species themselves or at least their respective species group can be reliably identified morphologically as long as adequate inspection of a large set of key features is adopted. All species that could *not* be eliminated morphologically as putative parents were retained.

Molecular Analyses—For molecular identification, putative hybrid origin was first assessed by direct ITS sequencing and compared to our unpublished database of ITS sequences that includes multiple individuals of all North American Potamogetonaceae species, with the exception of three extremely rare and taxonomically doubtful taxa (*P. floridanus* Small, *P. subsibiricus* Hagstr., and *P. tennesseensis* Fernald), none of which occurs in New England or in the surrounding states. While intraindividual ITS variation of 'pure' species was generally low (irrespective of ploidy level), hybrids always showed additive patterns (superimposed peaks at diagnostic positions) and in most cases, frameshift mutations after which the

sequence became unreadable (Kaplan and Fehrer 2007, see also below). For final identification and documentation of hybrid identity, the larger set of putative parental species was included in the RFLP analyses. An alignment of ITS sequences of hybrids and all potential parents (except *S. pectinata* which was too divergent) is provided as Supplementary online material.

We used PCR-RFLPs of ITS to document hybrid origin by additive banding patterns of parental species, to confirm the results from direct sequencing (where in most cases only part of the sequence was readable, see Supplementary online material), and to distinguish between the closely related species *P. bicupulatus* and *P. diversifolius*.

Isolation of DNA, PCR amplifications, and sequencing of ITS followed the methods described previously (Kaplan and Fehrer 2004). For RFLPs to generate patterns that distinguished between all putative parents, restriction enzymes (Fermentas, St. Leon-Rot, Germany) were selected based on the sequences. Restriction digests were performed with 5 units of enzyme and approx. 200 ng of PCR product in overnight digests according to the manufacturer's instructions. Products were separated on up to 3% high-resolution agarose gels (USB, Cleveland, Ohio) in TTE buffer (Tris-Taurine-EDTA) with 350–400 ng of DNA size standard to visualize fragments as short as 50 bp. In all cases, two subsequent digests with different enzymes were necessary to exclude uninvolved species and to confirm the parents.

After both parental species had been identified, the *rpl20–5′rps12* intergenic spacer region of the chloroplast DNA was sequenced for the hybrid and its parents to infer the direction of the cross. Maternal transmission of cpDNA in *Potamogeton* was confirmed by Kaplan and Fehrer (2006). Amplification and sequencing of this region followed the methods described previously (Kaplan and Fehrer 2006).

Putative Hybrids and Candidate Parents—Shetucket River (Connecticut) and Lake Rohunta (Massachusetts)—A putative hybrid similar to *P. diversifolius*, but more robust, was sampled from the Shetucket River (sample numbers 1631 and 1633). One of the species belonging to the *P. diversifolius* group, i.e. *P. spirillus*, *P. bicupulatus*, and *P. diversifolius*, appeared on morphological grounds to be parental to the putative hybrid, and *P. epihydrus* was considered a likely candidate for the other parent. A *Potamogeton* plant resembling *P. nodosus* Poir. and found with the putative hybrid was sampled and investigated as well. As this turned out to be another hybrid (unpubl. data), *P. nodosus* was represented by material from two other sites. A sample from Lake Rohunta (sample number 1679) was first considered *P. diversifolius*, but was later identified by molecular analysis as a hybrid.

Ossipee (New Hampshire)—This putative hybrid (sample number 1687) was previously assumed to be an unusual form of *P. gramineus* with adnate stipules (Hellquist 1978), and is known only from a single site. Morphology suggested a contribution of either *P. natans* L. or *P. oakesianus*, two closely related species, and a species with adnate stipules such as *P. bicupulatus*, *P. spirillus* or *S. pectinata* as the second parent. Additionally, *P. epihydrus* (the only other species growing with the hybrid) and *P. gramineus* (because of the previous taxonomic treatment of the hybrid) were included in the analyses.

Pushaw Lake (Maine) and Otter Creek (Vermont)—One of the Pushaw Lake putative hybrids (sample numbers 1724 and 1731) resembled *P. ×nitens* (*P. gramineus × P. perfoliatus*) but differed from the local form of the latter hybrid and was tentatively thought to be a combination of either *P. epihydrus* and *P. perfoliatus* or of *P. alpinus* Balb. and *P. perfoliatus*. In addition to these taxa, *P. richardsonii* (A. Benn.) Rydb. was included because of its similarity to *P. perfoliatus*. One sample from Pushaw Lake and another from Otter Creek, were initially morphologically determined as *P. perfoliatus* (sample numbers 1730 and 1649), but were later resolved as hybrids based on molecular data. Another hybrid discovered in Pushaw Lake was identified as *P. ×nitens* (*P. gramineus × P. perfoliatus*) in the field as well as by molecular analysis.

Results

Molecular Analyses—Shetucket River and Lake Rohunta hybrid—In all three hybrid accessions, direct ITS sequencing revealed the same 13 polymorphic sites within the first 100 sequenced bases of ITS 1 after which a diagnostic 2 bp indel occurred. The alternative character states were additive and identified *P. epihydrus* as one of the parents and either *P. bicupulatus* or *P. diversifolius* as the second one. *Potamogeton spirillus* differed at one of those positions from

both *P. bicupulatus* and *P. diversifolius*. Accordingly, in the first restriction digest, *P. spirillus* and *P. nodosus* could be excluded, and *P. epihydrus* was confirmed as one of the hybrid's parents (Fig. 1). While *P. bicupulatus* or *P. diversifolius* were identified as the only candidates for the second parent, they could not be distinguished by this RFLP. The parentage of *P. diversifolius* was therefore excluded based on a second digest (Fig. 2). Thus, this hybrid arose from a combination *P. bicupulatus* × *P. epihydrus*.

In all three hybrid accessions, the maternal parent was *P. bicupulatus* as indicated by three nucleotide substitutions and a 5 bp deletion in the *rpl20-rps12* intergenic spacer. Two accessions from the Shetucket River show a mutation unique for *Potamogeton* species known so far (GenBank data and unpubl. data), suggesting that they belong to the same vegetative clone. The Lake Rohunta hybrid does not show this feature, but shares a unique substitution with a *P. bicupulatus* sample from the same locality, indicating that this could have been the actual parent. Thus, the hybrids found at different localities arose independently from the same parental combination.

OSSIPEE HYBRID—A total of 18 polymorphic sites in the hybrid ITS sequence (six in ITS 1, 12 in ITS 2) showed character additivity for *P. oakesianus* and a genotype of *P. gramineus* while all other combinations could be excluded as parents. No length differences occurred between these species. In RFLP analyses, four out of the seven potential parents of the broader set of species plus one genotype of *P. gramineus* (gram 2) were excluded in the first restriction digest (Fig. 3) while the hybrid pattern was identical to the rest of the samples. These were subjected to a second RFLP that excluded

FIG. 2. Shetucket River and Lake Rohunta hybrid, second RFLP (*Nco*I). This digest excludes *P. diversifolius* as a potential parent of this hybrid, confirming its identity as *P. epihydrus* × *P. bicupulatus*. Samples: 1–1770, 2–1680, 3–1627, 4–1631, 5–1633, 6–1679. For details, see Appendix 1.

P. natans and showed additive patterns for *P. gramineus* genotype 1 and *P. oakesianus* (Fig. 4), as already inferred from direct sequencing.

Although *P. oakesianus* did not show any autapomorphic character states in the *rpl20-rps12* region of the chloroplast DNA, *P. gramineus* could be excluded as the maternal parent, because the hybrid is lacking two inserts of 11 bp and 8 bp and two substitutions characteristic for that genotype of *P. gramineus*. Thus, the new hybrid combination is *P. oakesianus* × *P. gramineus* with *P. oakesianus* as the maternal parent.

PUSHAW LAKE AND OTTER CREEK HYBRIDS—The combination of *P. epihydrus* and *P. perfoliatus* was suggested by 28 additive positions (14 in each ITS 1 and ITS 2) and a diagnostic 2 bp indel polymorphism shortly before the end of the PCR product. The hybrid between the closely related species *P. perfoliatus* and *P. richardsonii* showed only four additive sites across the whole sequence (Table 1). The identity of *P.* ×*nitens* was implied by 12 additive sites in ITS 1 and four in ITS 2 after which a 1 bp difference occurs between the parents. From the complete set of candidate species, the first RFLP (Fig. 5) excluded *P. alpinus*, which occurred in two slightly different variants, and the same *P. gramineus* genotype as before (gram 2) as potential parents for all hybrid combinations (Fig. 5; according to ITS sequences, the band of 68 bp in gram 2 is cut into 30 and 38 bp fragments in gram 1, which are not visible on the gel).

A unique band allowed *P. perfoliatus* to be determined as one of the parents of all three hybrid combinations. In addition, Fig. 5 shows that *P. epihydrus* and *P. perfoliatus* are the parental combination of hybrid 1. Two accessions that were initially determined as *P. perfoliatus* were actually hybrids as indicated by their additive banding patterns (hybrid 2). Furthermore, *P. richardsonii* 1720 (line 12) appeared to be introgressed with
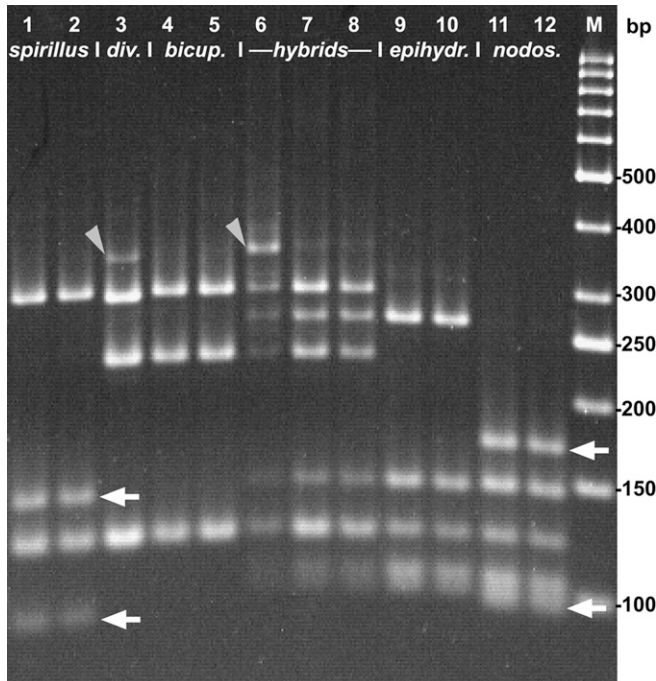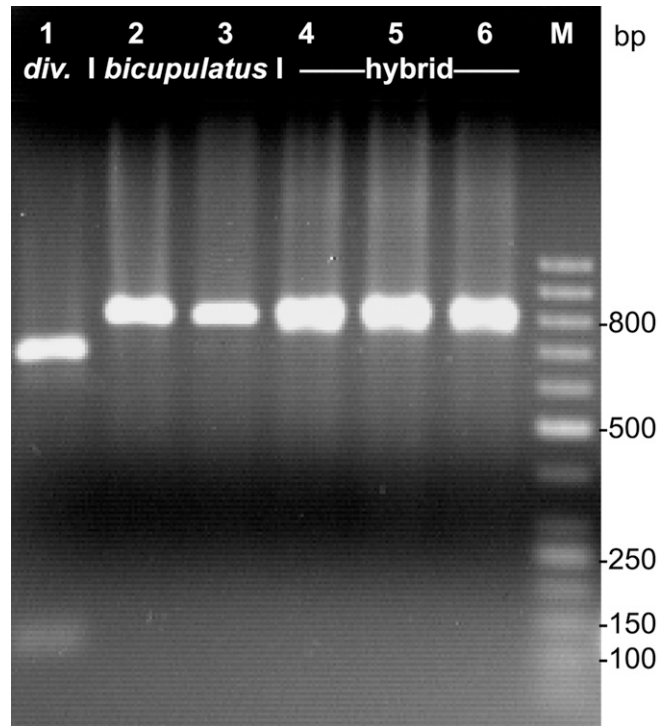

FIG. 1. Shetucket River and Lake Rohunta hybrid, first RFLP (*Mae*II = *Tai*I). According to this digest, *P. spirillus* and *P. nodosus* can be excluded as potential parents due to two bands neither of which is present in the hybrid samples (white horizontal arrows). Hybrid samples show additive patterns with *P. epihydrus* as one parent and either *P. bicupulatus* or *P. diversifolius* as the second parent. Grey arrowheads indicate PCR artifacts or partially lost restriction sites. Samples: 1–1632, 2–1722, 3–1770, 4–1680, 5–1627, 6–1631, 7–1633, 8–1679, 9–1635, 10–1700, 11–1653, 12–1309. For details, see Appendix 1.
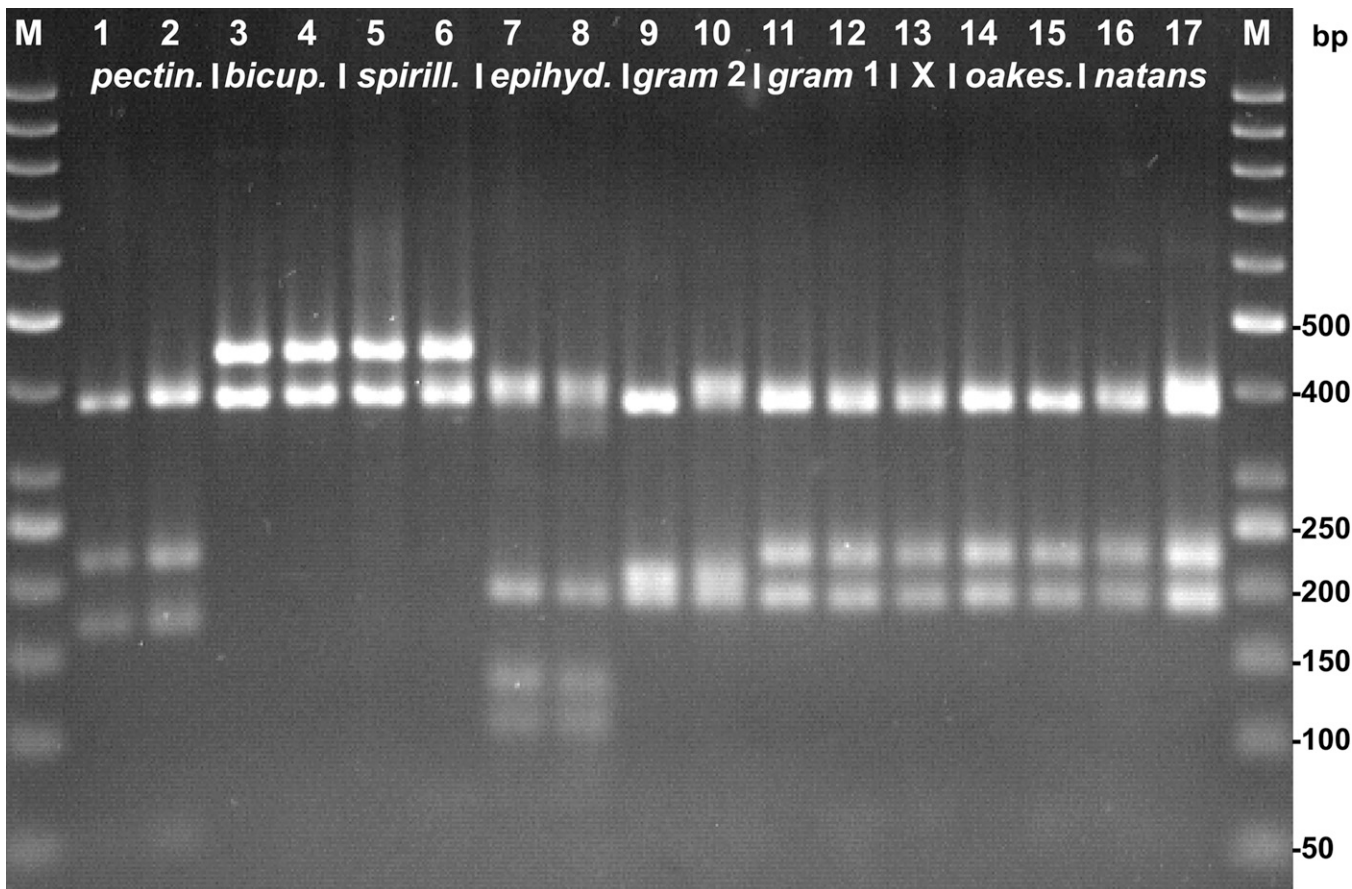
FIG. 3. Ossipee hybrid, first RFLP (*TasI* = *Tsp*509I). Digest with this enzyme excludes *S. pectinata*, *P. bicupulatus*, *P. spirillus*, *P. epihydrus* and one genotype of *P. gramineus* (gram 2) as potential parents. Another genotype of *P. gramineus* (gram 1) as well as *P. oakesianus* and *P. natans* are possible parents. Further samples of *P. spirillus* from New Hampshire and of *P. epihydrus* from the hybrid locality and from Massachusetts had identical profiles to the samples included here (now shown). Samples: 1–1652, 2–981, 3–1627, 4–1680, 5–1632, 6–1722, 7–1635, 8–1700, 9–1285, 10–885, 11–897, 12–1693, 13–1687, 14–1628, 15–1853, 16–1756, 17–1283. For details, see Appendix 1.

P. perfoliatus. A small amount (about 5–10% of the total signal) of *P. perfoliatus*-specific peaks was found in *P. richardsonii* 1720 at all four parent-discriminating sites, one of which (position 182) was responsible for the extra band ('+' in sample 12, Fig. 5) due to a partial loss of this restriction site.

Apart from an artifact (*) and the introgressed band (+), *P. richardsonii* produced the same RFLP profile as *P. gramineus* genotype 1 with this restriction enzyme. To distinguish the *P. richardsonii* pattern from *P. gramineus*, hybrids 2 and 3 were subjected to a second RFLP with the reduced set of potential parents. This second digest confirmed *P. gramineus* as the only other possible parent for hybrid 3, *P. × nitens* (Fig. 6). In this RFLP, *P. perfoliatus* had the same pattern as *P. richardsonii*. Consequently, the *P. perfoliatus*-like plants with additive patterns (hybrid 2, Fig. 5) are both a combination of these two very similar species and match the (identical) pattern of both parents in the second digest (Fig. 6; Table 1).

Thus, *P. perfoliatus* is involved in three different hybrid combinations at this locality, new ones with *P. epihydrus* and *P. richardsonii*, and another one with *P. gramineus*. The latter hybrid, *P. ×nitens*, is well known from Europe (Kaplan and Fehrer 2006) and also frequent in North America. Its identity is confirmed here for the first time for North America using molecular markers.

Chloroplast DNA sequencing showed that *P. epihydrus* was the maternal parent of both *P. perfoliatus × P. epihydrus* accessions, confirmed by a shared substitution with this species while *P. perfoliatus* could be excluded by another mutation. The maternal parent of both *P. × nitens* accessions was *P. gramineus* as shown by the four characteristic mutations mentioned above as well as by the absence of the *P. perfoliatus* autapomorphy. Distinguishing *P. perfoliatus* and *P. richardsonii* by their chloroplast DNA was difficult because of their high similarity and the lower variation of cpDNA. Sequences of *rpl20-rps12* were identical for the parental species except for a unique substitution occurring in both accessions of hybrid 2 which was also present in the introgressed *P. richardsonii* 1720 and in *P. perfoliatus* 1626, but not in other accessions of both parental species (nor in any other *Potamogeton* species sequenced so far). Thus, it is possible that all hybrid (or introgressed) accessions had their cpDNA derived from a haplotype similar to *P. perfoliatus* 1626, indicating maternal origin from such a variant. However, as no fixed differences were found between the parental species, this result is not entirely conclusive.

Morphological Evaluation and Description of the New Hybrids—Because four of the detected hybrids have not been previously described, the necessary records for validation of their respective new names are given here along with a brief diagnostic description.

Potamogeton ×aemulans Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. bicupulatus × P. epihydrus*)—TYPE: U.S.A. Connecticut: Windham Co., Shetucket River at N edge of South Windham, 0.75 km NW of bridge, 41°41′16″N,
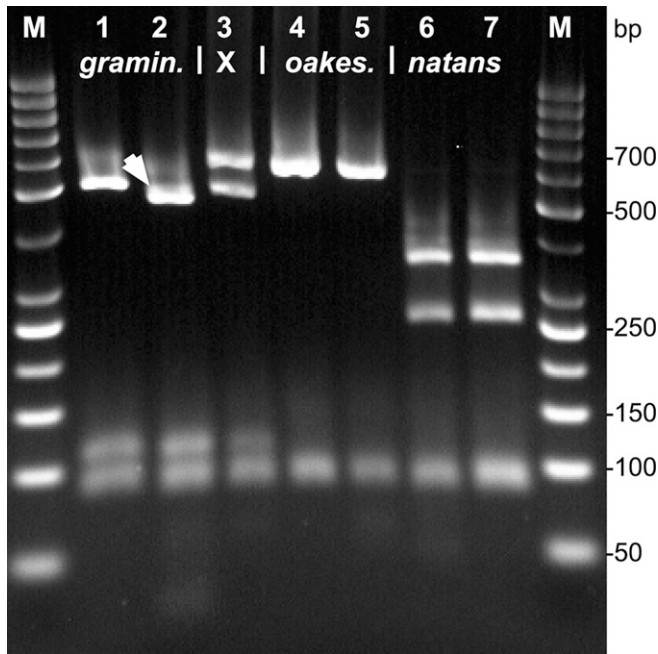
FIG. 4. Ossipee hybrid, second RFLP (*Nla*III = *Hin*1II). This digest excludes *P. natans* as one of the parents. The hybrid shows additive patterns of *P. oakesianus* and genotype 1 of *P. gramineus*. One of the samples (1693) has partly gained an additional restriction site due to an intraindividual polymorphism that was confirmed by sequencing. The short additional product (ca 30 bp long) is hardly visible; a rest of the uncut fragment is still present (arrow). Both *P. gramineus* samples show a band of ca. 120 bp which also appears in the hybrid. Samples: 1–897, 2–1693, 3–1687, 4–1628, 5–1853, 6–1756, 7–1283. For details, see Appendix 1.

72°10′24″W, 38 m, 24 July 2005, *Z. Kaplan & C. B. Hellquist 05/367* (holotype: PRA; isotypes: GH, MASS, PRA).

Plantae hybridae inter parentes intermediae, sed *P. diversifolio* maxime similes, a quo foliis submersis plerumque trinerviis, stipulis usque ad basin liberis vel solum breviter foliis adnatis, atque floribus semper abortivis differt.

Stem branched. Submerged leaves sessile, linear, 35–95 mm long, 0.2–1.8 mm wide, 45–175 times as long as wide, 3(–5)-veined, with or without a narrow to broad band of lacunae bordering the midrib. Floating leaves always present on adult flowering plants, petiolate; lamina oblong to elliptical, 18–35 mm long, 5–11 mm wide, 2.2–4.5 times as long as wide, subcoriaceous, 9–15-veined, subobtuse to rounded at apex; petiole 12–58 mm long. Stipules 3–34 mm long, mostly free

from the leaf base but some partially adnate, fused for up to 2.8 mm. Inflorescences dimorphic, mostly in axils of floating leaves; peduncle of inflorescences in axils of submerged leaves 3–4 mm long, spike globose to subglobose, 2–3 mm long; peduncle of emerged inflorescences in the axils of floating leaves 8–34 mm long, spike subglobose to cylindrical, 3–6 mm long. Flowers 1–4 in submerged spikes, 8–14 in emersed spikes, all with abortive carpels. Figure 7.

Potamogeton ×aemulans is intermediate between the parents, which is best expressed in the characters of the submerged leaves such as their width and length:width ratio, the number of veins and the development of lacunae (Table 2). Further combinations of parental characters include intermediate values of many quantitative characters, such as the length of the stipules, the length of the lamina and of the petiole of the floating leaves, the length of the peduncle, and the number of flowers of the emerged inflorescences. Submerged leaves resemble those of *P. bicupulatus* in shape, but are broader, mostly 3-veined and often with a narrow to broad band of lacunae bordering the midrib. The floating leaves are more similar to *P. epihydrus* in shape, but are narrower, more closely resembling those of *P. bicupulatus* in width. *Potamogeton ×aemulans* has dimorphic inflorescences, as does *P. bicupulatus*, although the small submerged ones are much less frequent and only found in some plants. In contrast, *P. epihydrus* has only robust emerged inflorescences. Unlike either parent, the hybrid has both types of stipule, i.e. axillary stipules free from the leaf base on most nodes as well as stipules adnate to the leaves on other nodes. This is a mixed state derived from the different stipule structures of its parental species.

Potamogeton ×aemulans resembles *P. diversifolius* (Table 2), with which it was formerly partly confused, more closely than either of its parents. The hybrid differs from this species in its submerged leaves being mostly 3-veined, having stipules mostly free or only shortly adnate to the leaf base, and being consistently sterile.

ETYMOLOGY—*aemulans* = imitating, emulating, because the hybrid imitates *P. diversifolius*, which is neither of its parental species.

PARATYPES—U.S.A. Connecticut: Windham Co., South Windham, shallow current of Shetucket River at Roger's Plastics factory, Rt. 32, 1/2 mile north of Rt. 203, 20 July 1987, *C. B. Hellquist 15616* (PRA, herb. C. B. Hellquist), 7 Aug. 1979, *C. B. Hellquist 13745* (NEBC), 7 Aug. 1979, *C. B. Hellquist 13751* (NEBC, herb. C. B. Hellquist), 7 Sep. 2007, *C. B. Hellquist 17081* (herb. C. B. Hellquist); Massachusetts: Franklin Co., northern part of Lake Rohunta near Route 2, 1.5 km SE of Orange, 42°33′47″N, 72°16′23″W, 162 m, 27 July 2005, *Z. Kaplan & C. B. Hellquist 05/402* (PRA), Lake Rohunta, 30 Sep. 2002, *M. G. Hickler s.n.* (MASS); Massachusetts:

TABLE 1. ITS polymorphism in the *P. perfoliatus* – *P. richardsonii* group. Numbers in boldface indicate sites with species-specific differences. Y = C or T, K = G or T, W = A or T, R = A or G, S = C or G. Small characters in brackets indicate that the second character state is strongly underrepresented. Notes: [1] Polymorphisms in parentheses indicate 5–10% of *P. perfoliatus*-specific peaks. The polymorphism at position 182 is responsible for the partial loss of the restriction site (sample 12, Fig. 5); [2] *P. richardsonii*-specific character states at polymorphic positions constitute about 30–40% of the total signal which is reflected by the weaker band of about 200 bp in the RFLP (sample 10, Fig. 5); [3] Additive sites show equal contribution from both parents; the polymorphism at position 56 shows a small amount of T, which may be derived from a *P. perfoliatus* genotype similar to accession 1626; [4] The T at position 56 is unique for ITS of all *Potamogeton* species sequenced so far (about 50, GenBank and unpublished data).

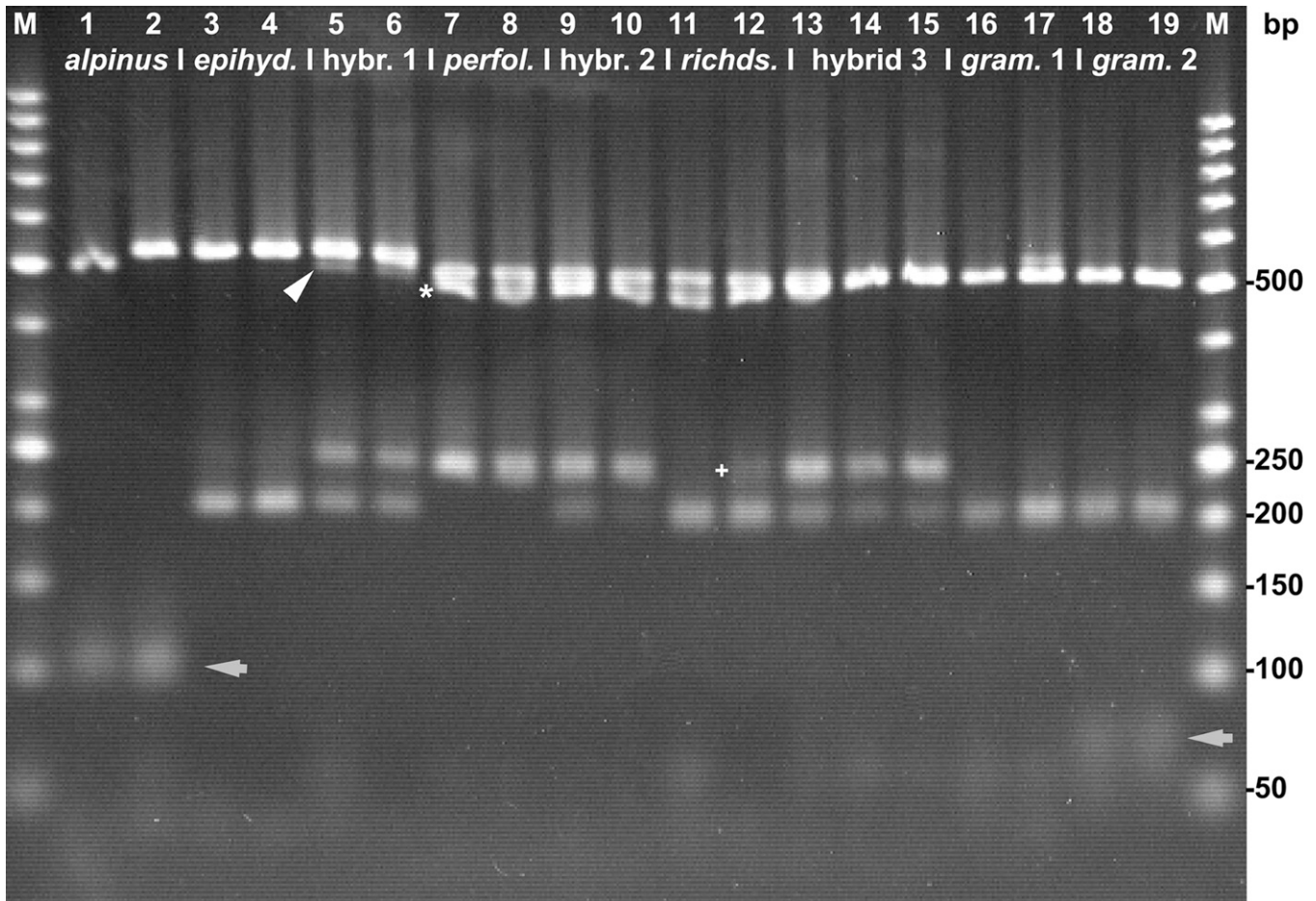| Species	Position in alignment										
	56	69	105	**120**	**133**	**182**	402	489	505	529	**658**
P. richardsonii 1056	A	G	G	C	G	C	C	A	G	C	C
P. richardsonii 1720[1]	A	G	K	C (y)	G (k)	C (y)	C	A	G	C	C (y)
P. perf. × *P. rich.* 1730[2]	A	G	G	Y	K	Y	C	A	G	C	Y
P. perf. × *P. rich.* 1649[3]	A (w)	G	G	Y	K	Y	C	A	G	C	Y
P. perfoliatus 1626[4]	T	k	G	T	T	T	C	T	G	C	T
P. perfoliatus 979	A	G	G	T	T	T	Y	A	R	S	T

Fig. 5. Pushaw Lake and Otter Creek hybrids, first RFLP (*Rsa*I). This digest excludes *P. alpinus* and *P. gramineus* genotype 2 as parents (grey arrows). It further shows *P. perfoliatus* to be involved in all three hybrids indicated by a band of about 250 bp and resolves hybrid 1 as a combination of *P. epihydrus* and *P. perfoliatus* (the largest band from *P. perfoliatus* being strongly underrepresented, white arrowhead). The asterisk (*) indicates a digestion or migration artifact in samples 7–12 which did not appear in previous test digests and is not accounted for by sequencing of these samples. The partial loss of a restriction site (sample 12) due to introgression of this accession is indicated by '+'. For more explanation, see text. Samples: 1–1537, 2–1613, 3–1700, 4–1635, 5–1731, 6–1724, 7–979, 8–1626, 9–1649, 10–1730, 11–1056, 12–1720, 13–1727, 14–1726, 15–1007, 16–1729, 17–897, 18–885, 19–1285. For details, see Appendix 1.

Franklin Co., Laurel Lake, 25 July 1975, *C. B. Hellquist 8982* (MASS, herb. C. B. Hellquist), 31 Aug. 1977, *C. B. Hellquist 12441* (herb. C. B. Hellquist), Franklin Co., Erving State Forest, Laurel Lake, 20 Aug. 1970, *W. J. L. Zubrin s.n.* (MASS).

Potamogeton ×mirabilis Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. gramineus × P. oakesianus*)—TYPE: U.S.A. New Hampshire: Carroll Co., Ossipee, small pond at crossroads of Route 16 and Brown Ridge Road 1 km NW of North Wakefield, 6 km SE of Ossipee, 43°38′27″N, 71°03′46″W, 189 m, 29 July 2005, *Z. Kaplan & C. B. Hellquist 05/412* (holotype: PRA; isotypes: GH, MASS, PRA).

Plantae hybridae inter parentes intermediae, *P. gramineo* habitu et ramificatione similes, sed foliis submersis longioribus, angustioribus et paucinervioribus, a *P. oakesiano* praeterea ramificatione etiam lamina foliorum submersorum bene evoluta (quae in illa specie in phyllodia reductae) differt.

Stem branched. Submerged leaves sessile, linear-oblong, reduced almost to phyllodes, 50–155 mm long, 0.3–1.6 mm wide, 90–170 times as long as wide, 3(–5)-veined. Floating leaves always present on adult flowering plants, petiolate; lamina narrowly lanceolate to narrowly elliptical, 19–58 mm long, 5–19 mm wide, 2.6–8.3 times as long as wide, subcoriaceous, (9–)11–19-veined; petiole 4–22 mm long. Transitional leaves often present. Stipules 7–30 mm long, mostly free from the leaf base but some partially adnate, fused for up to 1.2 mm. Peduncles 18–35 mm long. Spikes 22–26 mm long, flowers 16–18, with abortive carpels. Figure 8.

Potamogeton ×mirabilis resembles *P. gramineus* in general appearance and branching pattern. Submerged leaves are intermediate between those of the parental species; they are reminiscent of the phyllodes of *P. oakesianus*, but expanded into a narrow lamina as in *P. gramineus*. The length:width ratio of the submerged leaves is also intermediate (Table 3). *Potamogeton ×mirabilis* differs from *P. gramineus* in having longer and narrower submerged leaves with fewer longitudinal veins, and from *P. oakesianus* in having a richly branched stem and submerged leaves with a distinct lamina. Like many hybrids of *P. natans* (Kaplan and Wolff 2004), a species closely related to *P. oakesianus*, *P. ×mirabilis* often produces transitional leaves that are intermediate between submerged and floating leaves in their shape, size, structure and petiolation.

In *P. ×mirabilis*, some stipules of the submerged leaves are shortly adnate with the leaf bases, a character never observed in adult plants of either of the parental species. However, young plants of certain broad-leaved species can occasionally produce shortly adnate stipules (Wiegleb and Kaplan 1998). In *P. ×mirabilis*, this feature is also expressed in adult plants.
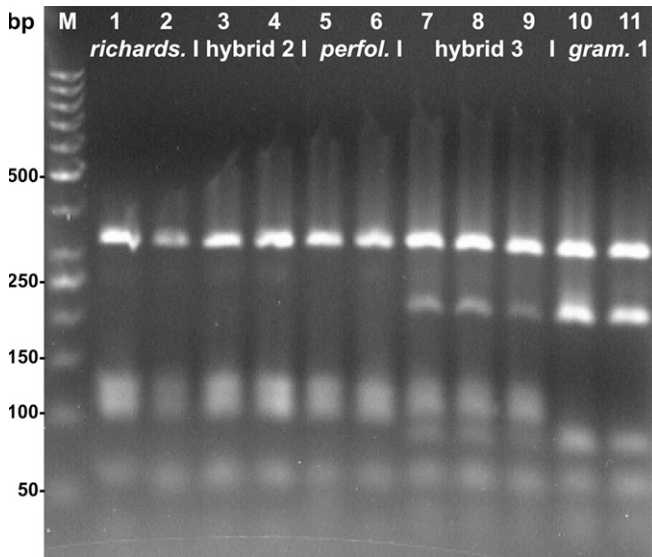
FIG. 6.　Pushaw Lake and Otter Creek hybrids, second RFLP (*Taq*I). The profiles of *P. perfoliatus* and *P. richardsonii* do not differ with this enzyme, hybrid 2 (additive in the first RFLP, Fig. 5) matches their pattern and thus confirms the parents. For hybrid 3, *P. gramineus* genotype 1 is shown to be the second parental species in addition to *P. perfoliatus* (see Fig. 5). For comparison, a sample of the same hybrid from Europe was included (line 9). Samples: 1–1056, 2–1720, 3–1730, 4–1649, 5–1626, 6–979, 7–1727, 8–1726, 9–1007, 10–1729, 11–897. For details, see Appendix 1.

Because the Ossipee population is the only one so far discovered of this hybrid, it cannot be anticipated if this stipule structure is a character peculiar to the hybrid as a whole or only to this clone. Because character expression in hybrids is largely unpredictable (Rieseberg and Ellstrand 1993), other clones of *P. ×mirabilis*, which may be eventually discovered, may not necessarily show this unusual trait.

ETYMOLOGY—*mirabilis* = remarkable, extraordinary, for having some stipules partially adnate to the leaf basis, unlike either of the parental species.

PARATYPES—U.S.A. New Hampshire: Carroll Co., Ossipee, pond on the north side of Brown Ridge Road at the junction of New Hampshire Route 16, 28 Aug. 1972, *C. B. Hellquist 4486* (MASS, herb. C. B. Hellquist), 28 Aug. 1979, *C. B. Hellquist 4659* (NEBC), 9 Sep. 1974, *C. B. Hellquist & E. C. Ogden 9939* (herb. C. B. Hellquist).

Potamogeton ×versicolor Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. epihydrus × P. perfoliatus*)—TYPE: U.S.A. Maine: Penobscot Co., Orono, southern edge of Pushaw Lake at Gould Landing 7 km W–WNW of Orono, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/448* (holotype: PRA; isotypes: GH, MASS, PRA).

Plantae hybridae inter parentes intermediae, sed *P. ×nitenti* maxime similes, a quo foliis submersis longioribus et angustioribus, foliis natantibus cum lamina parviore et petiolo alatiore differt.

Stem sparingly branched. Submerged leaves sessile, linear-oblong to narrowly lanceolate, 45–175 mm long (longer leaves mainly in vegetative shoots), 4–11 mm wide, 10–17 times as long as wide, translucent, bright green to olive green, sometimes with a brownish tinge, often yellowish green in lower vegetative shoots, older leaves turning to dark green or greyish green when dry, decaying with time, 5–17-veined, with a narrow band of lacunae bordering the midrib, broadly cuneate to rounded and semiamplexicaul at the base. Floating leaves sometimes present in adult flowering plants, petiolate;

lamina oblong, 22–32 mm long, 4–9 mm wide, 3–6 times as long as wide, subcoriaceous, opaque, yellowish green to olive green, sometimes deep green, 11–23-veined, gradually tapering to the base and forming wings along the petiole; petiole 4–22 mm long. Stipules 4–22 mm long, disappearing early. Peduncles 21–64 mm long. Spikes 8–32 mm long, flowers 20–24, with abortive carpels. Figure 9.

Potamogeton ×versicolor is readily distinguished from its parents. The submerged leaves are clearly intermediate between the long and narrow leaves of *P. epihydrus* and the short and broad leaves of *P. perfoliatus* (Table 4). The hybrid shares with *P. epihydrus* the ability to produce floating leaves, which are absent in *P. perfoliatus*, but the unwinged part of the petiole of these leaves is much shorter than in *P. epihydrus*. Besides the characters mentioned above, the hybrid also differs from *P. epihydrus* in having submerged leaves with up to 17 veins and only a narrow band of lacunae bordering the midrib, in contrast to the 5–9-veined leaves with expanded lacunae usually found in *P. epihydrus*. The submerged leaves of *P. ×versicolor* are semiamplexicaul, which is a trait inherited from *P. perfoliatus*, whereas the submerged leaves of *P. epihydrus* are cuneate and never amplexicaul at the base. The hybrid is unlikely to be confused with *P. perfoliatus*, which differs mainly in its general appearance, in the shape and size of the leaves, the markedly amplexicaul leaf bases, the number of leaf veins and the shorter stipules.

Potamogeton ×versicolor is more difficult to distinguish from another hybrid, *P. × nitens* (= *P. gramineus × P. perfoliatus*), than from its parents. The former can be distinguished by its longer but narrower submerged leaves and by the smaller laminae of the floating leaves with shorter and more pronouncedly flattened petioles (Table 4).

ETYMOLOGY—*versicolor* = variously colored, or changing color, because of color differences between submerged and floating leaves, between leaves on flowering and those on vegetative shoots, as well as between fresh and dry leaves.

PARATYPES—U.S.A. Maine: Penobscot Co., Orono, Pushaw Lake, Harrington Cove, in deep water, 16 July 1998, *C. B. Hellquist 16300* (NASC, PRA); Maine: Penobscot Co., Orono, southern edge of Pushaw Lake at Gould Landing 7 km W–WNW of Orono, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/457* (PRA).

Potamogeton ×absconditus Z. Kaplan, Fehrer and Hellq., hybr. nova (= *P. perfoliatus × P. richardsonii*)—TYPE: U.S.A. Vermont: Addison Co., Weybridge, fast flowing section of Otter Creek just at confluence with New Haven River 1 km W of Brooksville, 44°03′45″N, 73°10′39″W, 67 m, 25 July 2005, *Z. Kaplan & C. B. Hellquist 05/382* (holotype: PRA; isotypes: GH, PRA).

Potamogeto perfoliato habitu et foliorum forma valde similis sed cum stipulis non omnino emorientibus sed fibrae tenues albas relinquentibu, ut in *P. richardsonii*.

Stem unbranched to sparingly branched. All leaves submerged, sessile, lanceolate to ovate, 24–40 mm long, 9–21 mm wide, 1.6–3.4 times as long as wide, translucent, bright green, 19–27-veined, amplexicaul at the base. Stipules translucent, delicate, decaying early but leaving faint whitish fibers. Peduncles 15–62 mm long. Spikes 6–22 mm long, with 10–16 flowers. Figure 10.

Potamogeton ×absconditus is difficult to distinguish from its closely related and morphologically similar parents. The general appearance and particularly the shape and the length of the leaves of *P. ×absconditus* are more similar to *P. perfoliatus*. In contrast to this species, the stipules of the hybrid do not disappear completely with time, but disintegrate to fibers as

Fig. 7.   Holotype of *Potamogeton ×aemulans* Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. bicupulatus × P. epihydrus*). Scale bar = 5 cm.

TABLE 2. A comparison of the most important diagnostic characters of *Potamogeton epihydrus*, *P. bicupulatus*, their hybrid *P. ×aemulans*, and of *P. diversifolius*, the most similar species to the hybrid.

Character	P. epihydrus	P. ×aemulans	P. bicupulatus	P. diversifolius
Branching pattern	unbranched or sparingly branched	richly branched	sparingly to richly branched	richly branched
Length of submerged leaves (mm)	65–240	35–95	30–110	(10–)20–65(–110)
Width of submerged leaves (mm)	(1.0–)2.5–11.0	0.2–1.8	0.08–0.40(–0.50)	(0.2–)0.4–1.0(–1.5)
Length:width ratio of submerged leaves	18–30(–60)	45–175	(140–)190–500(–600)	20–180(–280)
Number of longitudinal leaf veins	5–9(–13)	3(–5)	1	1(–3)
Presence of lacunae along midrib	broad band of lacunae, particularly towards the base	narrow to broad band or no lacunae	no lacunae	narrow band or no lacunae
Length of lamina of floating leaves (mm)	35–80	18–35	6–23(–28)	(7–)13–40
Width of lamina of floating leaves (mm)	7–22	5–11	2–11	(2–)4–17(–20)
Length:width ratio of floating leaves	2.5–5.0	2.2–4.5	2–5	2–4
Number of longitudinal leaf veins	9–21	9–15	5–15	(3–)5–15(–17)
Shape of apex of floating leaves	subobtuse to rounded	subobtuse to rounded	acute	subobtuse to rounded
Length of petiole of floating leaves (mm)	20–60(–90)	12–58	5–35	2–40
Fusion of stipules with submerged leaves	free	mostly free but some adnate, fused for up to 2.8 mm	adnate, fused for 0.3–3.5 mm	adnate, fused for 0.3–4.0(–7.0) mm
Length of stipule (mm)	10–45	3–34	2–12	2–6(–18)
Types of inflorescences	monomorphic, in axils of floating leaves	dimorphic, most in axils of floating leaves, a few at submerged leaves	dimorphic, in axils of both floating and submerged leaves	dimorphic, in axils of both floating and submerged leaves
Length of peduncle of submerged inflorescence (mm)	–	3–4	1–10	1–8
Length of spike of submerged inflorescence (mm)	–	2–3	2–7	2–6
Length of peduncle of emerged inflorescence (mm)	23–90	8–34	3–22	3–32
Length of spike of emerged inflorescence (mm)	10–30	3–6	3–14	3–28
Number of flowers in submerged spikes	–	1–4	1–8	1–8
Number of flowers in emerged spikes	11–18	8–14	2–10	4–30

in *P. richardsonii*, though these fibers are faint and can easily be overlooked. The most important diagnostic characters of all three taxa are summarized in Table 5.

ETYMOLOGY—*absconditus* = hidden, concealed, because its hybrid status was not recognized morphologically in the field, but only from later molecular analysis.

PARATYPE—U.S.A. Maine: Penobscot Co., Orono, southern edge of Pushaw Lake at Gould Landing 7 km W–WNW of Orono, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/456* (PRA).

DISCUSSION

Morphological Versus Molecular Recognition of Hybrids— Three of the four new hybrids described here were suspected to be hybrids when they were collected, based on morphological characters, but their exact parentages were unclear. The semiamplexicaul base of the submerged leaves of *P. ×versicolor* (*P. epihydrus × P. perfoliatus*) coupled with its general appearance indicated a *P. perfoliatus* hybrid, whereas the other parental species was uncertain. Many features resembled *P. ×nitens*, another *P. perfoliatus* hybrid. The possibility that this plant was just another form of this extremely variable hybrid (for a detailed review of variation of *P. ×nitens* mainly in northern Europe see Hagström 1916) was therefore considered. The spectrum of hypothesized hybrid combinations included *P. epihydrus × P. perfoliatus* and *P. alpinus × P. perfoliatus*, as well

as corresponding hybrids with the morphologically similar *P. richardsonii* instead of *P. perfoliatus* as one of the parents. The definite resolution of the hybrid identity was provided only by DNA analysis.

Potamogeton ×mirabilis (*P. gramineus × P. oakesianus*) was for a long time considered to be an extreme form of *P. gramineus* (Hellquist 1978), rather than a hybrid. However, because of its similarity to *P. ×sparganiifolius* Laest. ex Fr. (= *P. gramineus × P. natans*), a hybrid well documented in Europe, the hybrid origin of this American population was suggested when this plant was collected for this study, and several different hybrid combinations were considered. The molecular analysis confirmed it to be a hybrid and provided evidence for crossing between two of the eight species considered after morphological elimination of other taxa.

Plants of *P. ×aemulans* (*P. bicupulatus × P. epihydrus*) in the Shetucket River were identified as similar to *P. diversifolius* but somewhat different and remarkable for being consistently sterile. Their morphology indicated they were either a species or a hybrid of the *P. diversifolius* group (corresponding to subsect. *Hybridi* as delimited by Fernald 1932; Klekowski and Beal 1965; Reznicek and Bobbette 1976). Because of the close similarity of the species within this group, the exact identity of the hybrid could be determined only by molecular analysis. Another population of this hybrid from Lake Rohunta
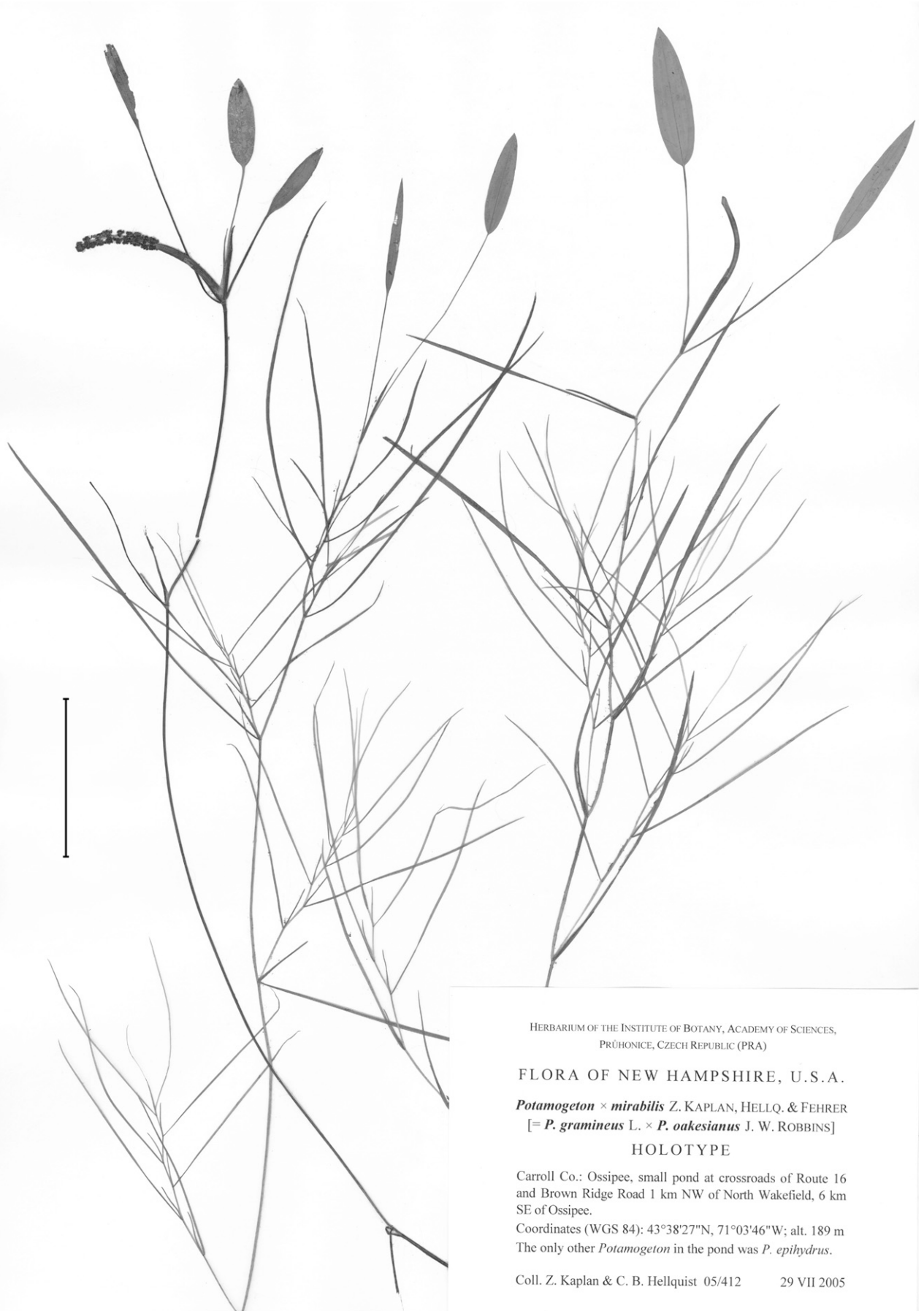
HERBARIUM OF THE INSTITUTE OF BOTANY, ACADEMY OF SCIENCES,
PRŮHONICE, CZECH REPUBLIC (PRA)

FLORA OF NEW HAMPSHIRE, U.S.A.

Potamogeton × *mirabilis* Z. KAPLAN, HELLQ. & FEHRER
[= *P. gramineus* L. × *P. oakesianus* J. W. ROBBINS]

HOLOTYPE

Carroll Co.: Ossipee, small pond at crossroads of Route 16
and Brown Ridge Road 1 km NW of North Wakefield, 6 km
SE of Ossipee.
Coordinates (WGS 84): 43°38'27"N, 71°03'46"W; alt. 189 m
The only other *Potamogeton* in the pond was *P. epihydrus*.

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FIG. 8.   Holotype of *Potamogeton* ×*mirabilis* Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. gramineus* × *P. oakesianus*). Scale bar = 5 cm.

TABLE 3. A comparison of the most important diagnostic characters of *Potamogeton gramineus*, *P. oakesianus*, and their hybrid *P. ×mirabilis*.

Character	*P. gramineus*	*P. ×mirabilis*	*P. oakesianus*
Branching pattern	mostly richly branched	richly branched	unbranched or sparingly branched
Development and shape of lamina of submerged leaves	well developed, linear-oblong to oblanceolate	reduced almost to phyllodes, linear-oblong	reduced to linear phyllodes
Length of submerged leaves (mm)	(17–)35–90(–135)	50–155	50–160
Width of submerged leaves (mm)	(2–)4–8(–12)	0.3–1.6	0.3–1.0
Length:width ratio of submerged leaves	5–12(–21)	90–170	120–300
Number of longitudinal leaf veins	(3–)7–9(–13)	3(–5)	1–3
Occurrence of transitional leaves	sometimes present	often present	absent
Length of lamina of floating leaves (mm)	15–70(–95)	19–58	(13–)22–40(–55)
Width of lamina of floating leaves (mm)	(5–)8–34	5–19	(5–)10–22(–29)
Number of longitudinal leaf veins	(7–)11–21(–23)	(9–)11–19	(7–)9–19(–23)
Fusion of stipules with leaves	free	mostly free but some partially adnate, fused for up to 1.2 mm	free

was for a long time considered to be *P. diversifolius* (one of two recorded sites of this species in Massachusetts, far from its main distribution), and only during this study identified as *P. ×aemulans*.

The hybrid origin of *P. ×absconditus* (*P. perfoliatus* × *P. richardsonii*) was not identified in the field because of its close similarity to *P. perfoliatus* and the overlapping and plastic morphological characters of the parents. It was detected during molecular analyses among samples taken for comparison with other hybrid samples.

The morphology of *P. ×nitens* from Pushaw Lake corresponded to the descriptions of this hybrid (Preston 1995; Kaplan and Fehrer 2006) and to our field experience from both Europe and North America, so its determination was straightforward.

The potential for correct morphological identification of Potamogetonaceae hybrids varies greatly between hybrid combinations. These range from the easily recognizable to hybrids that cannot be identified morphologically with certainty. The easiest identification is of hybrids between dissimilar species, such as those between a broad-leaved and a narrow-leaved species, or of hybrids between species with conspicuous species-specific features. For example, hybrids such as *P. ×angustifolius* J. Presl (= *P. gramineus* × *P. lucens* L.) or *P. ×nitens* are routinely recognized and ordinarily included in European Floras and plant identification guides. An example of a remarkable North American hybrid that is unlikely to be confused with any other *Potamogeton* taxon is *P. ×mysticus* Morong (= *P. berchtoldii* Fieber × *P. perfoliatus*). Previous molecular studies (Hollingsworth et al. 1995, 1996; Preston et al. 1998b; Fant et al. 2001a,b, 2003; King et al. 2001; Iida and Kadono 2002; Kaplan et al. 2002; Fant and Preston 2004; Kaplan and Fehrer 2004, 2006; Kaplan and Wolff 2004; Kaplan 2007) demonstrated that many *Potamogeton* hybrids can be reliably identified morphologically. In contrast, many hybrids can be identified morphologically only when careful examination of specific key structures is applied (Preston 1995; Preston et al. 1999; Kaplan 2008) or if the particular plant is optimally developed and shows diagnostic features of the species involved in hybridization (Kaplan and Wolff 2004; Kaplan and Fehrer 2007). Morphological recognition of some hybrids, such as those between linear-leaved species, may be impossible due to the overall similarity of the parental species and to their simple structure with highly reduced morphology, which does not provide sufficient characters.

The great majority of Potamogetonaceae hybrids are consistently sterile (e.g. Hagström 1916; Dandy 1975; Preston 1995;

Wiegleb and Kaplan 1998; Kaplan and Fehrer 2007), thus a rise of later generation recombinants is excluded. Only in a few cases, when the parental species are closely related, are their hybrids fertile; for example *P. ×angustifolius* (*P. gramineus* × *P. lucens*; Kaplan and Fehrer 2007). This may also be the case for some clones of *P. ×absconditus*. One of the *P. richardsonii* accessions investigated (1720) was apparently introgressed with *P. perfoliatus* and may represent a later generation backcross to *P. richardsonii*. It is also evident that the two *P. ×absconditus* accessions comprised different proportions of parental ITS variants, one in equal amounts and one in a roughly one-third/two-thirds ratio. These differences are not arbitrary as genomic DNA for RFLPs and sequencing was amplified independently, and the respective results match. Given that none of the *P. ×absconditus* hybrids, or possible introgressed forms, could be reliably identified morphologically, we cannot rule out the possibility that *P. perfoliatus* and *P. richardsonii* hybridize more frequently and the F_1's backcross to either parent. Taxonomically uncertain material of this complex can be relatively easily identified with the RFLP approach described above (see Fig. 5).

Morphological recognition of Potamogetonaceae hybrids requires some experience, and their identification must be always done with utmost care. Some extreme phenotypes of true species may mimic hybrids, and only careful and detailed examination of well developed material can reveal their true identity (Kaplan 2002; Kaplan and Fehrer 2004). The identification of underdeveloped, fragmentary, or inadequately preserved specimens may easily be misleading, and even external factors, such as the time of collection of plant material, and abiotic factors, such as temperature and nutrient conditions, can affect identification (Kaplan 2002; Kaplan and Wolff 2004). In uncertain cases, ITS sequencing provides a helpful tool.

Parental Species Relationships and Ploidy—All parental species involved in the hybrids described here were included in the molecular phylogenetic study of Lindqvist et al. (2006), which was based on two chloroplast regions and on the nuclear ribosomal 5S nontranscribed spacer (5S-NTS).

The most closely related parental species are apparently the morphologically similar *P. perfoliatus* and *P. richardsonii*. They are sister taxa based on the 5S-NTS, and their ITS sequences differ by only ~0.5%, which corresponds to four nucleotide positions (see Table 1). The fertility of their hybrid (*P. ×absconditus*) is indicated by signatures of introgression. All other hybrids are combinations of more distantly related parents, and are sterile.

FIG. 9.   Holotype of *Potamogeton ×versicolor* Z. Kaplan, Hellq. and Fehrer, hybr. nova (= *P. epihydrus × P. perfoliatus*). Scale bar = 5 cm.

TABLE 4.   A comparison of the most important diagnostic characters of *Potamogeton epihydrus*, *P. perfoliatus*, their hybrid *P.* ×*versicolor*, and of *P.* ×*nitens*, a taxon most similar to the new hybrid.

Character	*P. epihydrus*	*P.* ×*versicolor*	*P. perfoliatus*	*P.* ×*nitens*
Shape of submerged leaves	linear to ribbon-like	linear-oblong to narrowly lanceolate	narrowly lanceolate to orbicular-ovate	oblong-lanceolate to ovate
Length of submerged leaves (mm)	65–240	45–175	12–80(–110)	30–115
Width of submerged leaves (mm)	(1.0–)2.5–11.0	4–11	12–42	5–23
Length:width ratio of submerged leaves	18–30(–60)	10–17	1.3–5.3	3–9
Number of longitudinal leaf veins	5–9(–13)	5–17	(13–)19–33	7–17
Presence of lacunae along midrib	broad band of lacunae, particularly towards base	narrow band of lacunae	narrow band of lacunae	narrow band of lacunae
Shape of base of submerged leaves	cuneate, never amplexicaul	broadly cuneate to rounded, semiamplexicaul	rounded to cordate, amplexicaul	broadly cuneate to rounded, semiamplexicaul
Presence of floating leaves on adult flowering plants	present	sometimes present	absent	sometimes present
Length of lamina of floating leaves (mm)	35–80	22–32	–	27–65
Width of lamina of floating leaves (mm)	7–22	4–9	–	9–23
Number of longitudinal leaf veins	9–21	11–23	–	11–17
Winging of petioles	not winged	winged towards lamina	–	shortly winged or not winged
Length of unwinged part of petiole (mm)	20–60(–90)	4–22	–	12–40
Length of stipule (mm)	10–45	4–22	3–20	5–28

Potamogeton gramineus and *P. oakesianus* both belong to clade IB according to the 5S-NTS (Lindqvist et al. 2006). Their inter-specific ITS sequence divergence is 2.4–2.5%. As in *P.* ×*absconditus*, the direct sequence of the hybrid *P.* ×*mirabilis* was readable throughout its entire length due to the absence of indel mutations, which are usually found in Potamogetonaceae hybrids (this study, and unpubl. data).

With respect to the other three hybrid combinations, the phylogenetic placement of the parental species in Lindqvist et al. (2006) does not correspond well to the genetic differences (and to unpublished phylogenetic analyses) based on ITS. According to the 5S-NTS, *P. perfoliatus* and *P. gramineus* belong to the same subclade (IB) while *P. epihydrus* belongs to another major clade (II). However, sequence divergence based on ITS is similar for both parental combinations (*P. perfoliatus* – *P. gramineus*: 4.0–4.1% plus two indels; *P. perfoliatus* – *P. epihydrus*: 4.3–4.4% plus one indel). A similar discrepancy occurs with respect to the parental combination of *P. epihydrus* and *P. bicupulatus*. The ITS sequences show by far the largest pairwise divergence of all parental combinations described here (7.3%, plus five indels) while the 5S-NTS data place both parents as members of clade II, albeit separated into subclades IIA and IIB.

What is important to note here is that relatively large genetic or phylogenetic distances apparently do not prevent species from hybridizing. Hybrids are known to occur between species that belong to different major clades of *Potamogeton* (see Preston 1995, or Wiegleb and Kaplan 1998, for detailed accounts of a range of Potamogetonaceae hybrids).

Chromosome counts in Potamogetonaceae are technically difficult to obtain with their small and numerous chromosomes (see Hollingsworth et al. 1998). Therefore, unreliable counts are common in the literature. Nevertheless, there is clear evidence for homoploid as well as heteroploid hybrids. Three of the new hybrids' parents (*P. perfoliatus*, *P. gramineus*, *P. richardsonii*) are tetraploid and have produced the hybrids *P.* ×*nitens* and *P.* ×*abscondicus*, which are either certainly (in case of *P.* ×*nitens*) or very likely (*P.* ×*abscondicus*) tetraploid as well. *Potamogeton oakesianus*, for which no data are available,

may also be tetraploid, because it occurs in a species clade consisting almost exclusively of tetraploids (Lindqvist et al. 2006). This may also be the case for its hybrid with *P. gramineus*, *P.* ×*mirabilis*. On the other hand, the cross between *P. perfoliatus* and *P. epihydrus* is certainly one between a tetraploid and a diploid. Several other *Potamogeton* hybrids between different ploidy levels are known (Dandy 1975; Preston 1995; Wiegleb and Kaplan 1998). While we do not have chromosome counts from *P.* ×*versicolor*, we assume it to be triploid. A triploid count was obtained for *P.* ×*gessnacensis* G. Fisch., a hybrid between *P. natans* (tetraploid) and *P. polygonifolius* Pourr. (diploid), which shows that heteroploid crosses are possible and can result in triploid plants (Preston et al. 1998a; Jarolímová and Kaplan, unpubl. data).

Occurrence of Hybrids in the Absence of their Parents— Only two clones of the five hybrids discussed in this study were growing with both parental species. Both parental species were recorded with *P.* × *nitens* in Pushaw Lake. However, sites where this hybrid occurs with only one of its parents or in their absence were also recorded during fieldwork. *Potamogeton* ×*aemulans* was found growing with *P. epihydrus* at one site, and with both parents at the other. *Potamogeton* ×*versicolor* occurred alone in a rather deep area of the lake (ca 1.5–2 m), and was the only aquatic plant recorded at this depth, although one of its parents, *P. perfoliatus* was recorded at other sites in the same lake. *Potamogeton richardsonii* was not recorded in either of the two *P.* ×*absconditus* sites, whereas the occurrence of the other parent, *P. perfoliatus*, cannot be reliably commented on because this species was not distinguished from the hybrid during the field survey. *Potamogeton* ×*mirabilis* is certainly orphaned at its site as the only other *Potamogeton* growing in the small pond was *P. epihydrus*, which was not involved in the hybridization.

Previous studies on the occurrence of hybrids revealed that they do not always grow together with the parental species. The occurrence of a Potamogetonaceae hybrid in the absence of one or both parents has been repeatedly documented (e.g. Dandy and Taylor 1946; Hollingsworth et al. 1996; Preston et al. 1998a, 1998b, 1999; King et al. 2001; Kaplan and Fehrer

FIG. 10.   Holotype of *Potamogeton ×absconditus* Z. Kaplan, Fehrer and Hellq., hybr. nova (= *P. perfoliatus* × *P. richardsonii*). Scale bar = 5 cm.

TABLE 5.   A comparison of the most important diagnostic characters of *Potamogeton perfoliatus*, *P. richardsonii*, and their hybrid *P. ×absconditus*.

Character	*P. perfoliatus*	*P. ×absconditus*	*P. richardsonii*
Shape of submerged leaves	lanceolate to orbicular-ovate	lanceolate to ovate	narrowly lanceolate to ovate-lanceolate
Width of leaves (mm)	12–42	9–21	5–19
Length:width ratio of leaves	1.3–5.3	1.6–3.4	2.5–7.0
Consistency and persistence of stipules	translucent, delicate, without fibers, decaying and disappearing early	translucent, delicate, decaying but leaving faint whitish fibers	opaque, fibrous, decaying but remaining as whitish fibers

2004; Kaplan and Wolff 2004). This is associated with the ability of hybrid clones to persist at their sites vegetatively.

There are two alternative hypotheses for the fact that most hybrid clones included in this study occurred at their sites in absence of one or both parents: 1) these hybrid clones are relics surviving after their parents disappeared, or 2) established colonies of hybrids originated elsewhere and were transported to new sites either as plant fragments or as seeds. Since almost all Potamogetonaceae hybrids are sterile, the ability of adult hybrid plants to spread is confined to vegetative propagation. Fragmentation of stems is probably the main method of dispersal along the edges of lakes and rivers. In standing water, the hybrids can be dispersed only locally within the pond or the lake in which they arose. In rivers, they may be transported downstream, particularly during spring floods with rapid water flow (e.g. Kaplan et al. 2002; Kaplan and Wolff 2004). However, natural long-distance dispersal of hybrid plants outside a water body such as a reservoir or a river is likely to be rare. The frequency of dispersal of hybrid seeds is unknown. In our view, the most likely hypothesis is that the hybrid populations at different sites resulted from different hybridization events (which is in some cases evident from molecular signatures as in *P. ×aemulans*, see above) with the subsequent loss of parents from the locality.

Persistence of Hybrid Clones, Frequency of Hybrids and Their Distributions—Although *Potamogeton* hybrids are almost always sterile (Hagström 1916; Dandy 1975; Preston 1995; Wiegleb and Kaplan 1998; Kaplan and Fehrer 2007), hybrid clones can persist at a locality for a considerable period, even for hundreds or thousands of years (Hollingsworth et al. 1996; Preston et al. 1998b; King et al. 2001; Kaplan and Wolff 2004; Kaplan and Fehrer 2007), provided that the ecological conditions of the habitat remain suitable.

Our recent studies showed that cross-pollination between species with the same pollination system and the production of hybrid seeds are relatively frequent, if two or more *Potamogeton* species grow together (Kaplan and Fehrer 2004, 2006; Kaplan 2007). New individuals only establish from seed under optimal circumstances (Kaplan et al. 2002; Kaplan and Fehrer 2004). Eutrophic conditions of shallow fishponds with low water transparency and a bottom covered by thick organic-rich sediment (sapropel), which is often toxic because of anaerobic conditions, inhibit seed germination. In contrast, sufficient nutrients, weak competition, and clear (transparent) water that does not overheat in summer provide conditions for the germination of seeds, including hybrid seeds.

The recorded frequency and distribution of hybrids in Potamogetonaceae is extremely uneven on a worldwide scale (Wiegleb 1988; Wiegleb and Kaplan 1998). Habitats with the favorable environmental conditions described above, which catalyze the germination of hybrid seeds, become more frequent with increasing latitude. In Europe, there is an obvious gradient of hybrid occurrence towards higher latitude: both hybrid diversity and the number of localities with hybrids increase towards northern Europe (Kaplan 2007; Kaplan and Fehrer 2007), typically in areas associated with suitable biotopes in a previously glaciated landscape (Kaplan 2007). A similar situation to that in Europe may also prevail in North America where the diversity of Potamogetonaceae (and other aquatic plants) coincides with areas most severely affected by the Late Pleistocene glaciation as demonstrated by the hybrids found in this study. Future investigations in North America should search other glaciated, as well as nonglaciated, regions for the presence or lack of interspecific hybrids of *Potamogeton*.

Hybrid Identification in North America and in Europe— The existence of some North American *Potamogeton* hybrids may be obscured by the greater species diversity in North America and by the associated higher total morphological diversity within the genus relative to species in Europe. Some species groups that consist of only a single or a few species in Europe are represented by complexes of similar species in North America. For example, *P. perfoliatus* in Europe is a clearly defined and morphologically distinct species. In contrast, another similar species, *P. richardsonii*, occurs in North America in addition to *P. perfoliatus*. Two closely related species, *P. gramineus* and *P. lucens*, are always distinguishable in Europe, which makes identification of their hybrid possible. However, the situation is more complex in North America. The mainly Eurasian species *P. lucens* (with only submerged and shortly petiolate leaves) is replaced by the vicariant species *P. illinoensis* Morong in North America, which includes forms with some features of *P. gramineus* (such as floating leaves or sessile submerged leaves). In addition, *P. gramineus* shows higher morphological variation in North America than in Europe. This makes it difficult to determine the boundaries of variation between these two species, which makes morphological identification of their hybrid practically impossible. It is also possible that some of these differences might be due to overlooked hybrid accessions being assigned to particular species, the presence of cryptic species, or both.

Some North American species are best recognized if fruiting material is available. Haynes (1978) considered reproductive features as most important in separating species. For this reason, Haynes and Hellquist (2000:48) "strongly recommended that no one collect specimens of Potamogetonaceae that are lacking reproductive structures". However, almost all Potamogetonaceae hybrids are consistently sterile, and some species (such as *P. robbinsii* Oakes) flower only rarely. Following the above instruction, herbaria would be unrepresentative of the existing taxonomic diversity and would provide little information on ontogenetic variation, as pointed out by Preston (2001). If only fertile material was considered and vegetative material intentionally neglected, none of our new hybrids would have been discovered. The lack of

attention paid to the vegetative material of Potamogetonaceae in the field may thus be another reason why some hybrids are unrecorded in North America.

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APPENDIX 1. *Potamogeton* and *Stuckenia* specimens used in the molecular analyses. Records are presented in the following order: species or hybrid, samples: reference number (in boldface), origin, field collection records (date, collector's name, collection number), and GenBank accession numbers (*rpl20-rps12*, ITS; regions not sequenced for the sample are represented by a dash). Sequences published in earlier papers are indicated:[1] sequence from Kaplan and Fehrer (2004);[2] sequence from Kaplan and Fehrer (2006);[3] sequence from Kaplan and Fehrer (2007).

Potamogeton alpinus: **1537**, Czech Republic, distr. Mělník, Vojtěchov, 50°27′17″N, 14°35′10″E, 238 m, 22 Aug. 1997, *Z. Kaplan 04/204*, —, FJ151201. **1613**, U.S.A., Vermont, Washington Co., Maple Corner, Curtis Pond, 44°22′36″N, 72°30′03″W, 371 m, 22 July 2005, *Z. Kaplan & C. B. Hellquist 05/354*, —, FJ151202. *Potamogeton bicupulatus*: **1627**, U.S.A., Massachusetts, Berkshire Co., Savoy, Bog Pond, 42°38′26″N, 73°01′59″W, 566 m, 23 July 2005, *Z. Kaplan & C. B. Hellquist 05/363*, EU596937, FJ151203. **1680**, U.S.A., Massachusetts, Franklin Co., Orange, Lake Rohunta, 42°33′47″N, 72°16′23″W, 162 m, 27 July 2005, *Z. Kaplan & C. B. Hellquist 05/403*, EU596938, FJ151204. *Potamogeton diversifolius*: **1770**, U.S.A., Texas, Jeff Davis Co., Cherry Canyon Ranch, Cherry Creek, 30°51′10″N, 104°03′28″W, 31 May 2005, *C. B. Hellquist 16954*, —, FJ151205. *Potamogeton epihydrus*: **1635**, U.S.A., Connecticut, Windham Co., South Windham, Shetucket River, 41°41′16″N, 72°10′24″W, 38 m, 24 July 2005, *Z. Kaplan & C. B. Hellquist 05/369*, EU596939, FJ151206. **1700**, U.S.A., Maine, Washington Co., Cherryfield, Narraguagus River, 44°36′03″N, 67°55′36″W, 6 m, 1 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/422*, EU596940, FJ151207. *Potamogeton gramineus* (genotype 1): **897**, Czech Republic, distr. Ceská Lípa, Hradčany u Mimoně, Držák fishpond, 50°36′37″N, 14°43′23″E, 273 m, 18 Sep. 1996, *Z. Kaplan 96/638*, DQ468866[2], DQ468860[2]. **1693**, U.S.A., New Hampshire, Carroll Co., West Ossipee, Ossipee Lake, 43°48′33″N, 71°09′49″W, 124 m, 29 July 2005, *Z. Kaplan & C. B. Hellquist 05/416*, EU596943, EU596952. **1729**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/455*, EF174593[3], EF174588[3]. *Potamogeton gramineus* (genotype 2): **885**, Czech Republic, distr. Náchod, Šeřeč, Rozkoš Reservoir, 50°23′02″N, 16°05′14″E, 280 m, 22 Aug. 1997, *Z. Kaplan 97/829*, DQ468864[2], EF174589[3]. **1285**, France, Lorraine, Moselle, Rémelfing, 49°06′N, 07°04″E, 21 July 2001, *P. Wolff s.n.*, DQ468865[2], DQ468861[2]. *Potamogeton natans*: **1283**, Germany, Saarland, Saarbrücken, 49°14′37″N, 07°00′47″E, 21 July 2001, *F.-J. Weicherding s.n.*, —, FJ151208. **1756**, U.S.A., Massachusetts, Berkshire Co., Hancock, Kinderhook Creek pond, 42°34′40″N, 73°17′51″W, 385 m, 21 July 2005, *Z. Kaplan & C. B. Hellquist 05/342*, —, FJ151209. *Potamogeton nodosus*: **1309**, France, Lorraine, Moselle, Welferding, 49°06′53″N, 07°02′50″E, 14 June 2002, *P. Wolff s.n.*, —, FJ151210. **1653**, U.S.A., Vermont, Addison Co., Brooksville, Otter Creek, 44°03′45″N, 73°10′39″W, 67 m, 25 July 2005, *Z. Kaplan & C. B. Hellquist 05/386*, —, FJ151211. *Potamogeton oakesianus*: **1628**, U.S.A., Massachusetts, Berkshire Co., Savoy, Bog Pond, 42°38′26″N, 73°01′59″W, 566 m, 23 July 2005, *Z. Kaplan & C. B. Hellquist 05/364*, EU596941, FJ151212. **1853**, U.S.A., New Hampshire, Carroll Co., Ossipee, pond at Pine Hill Road, *C. B. Hellquist 16960*, EU596942, FJ151213. *Potamogeton perfoliatus*: **979**, Switzerland, St. Gallen, Altenrhein, Bodensee Lake, 47°30″N, 09°33″E, 396 m, 23 July 1998, *Z. Kaplan 98/125*, DQ468862[2], AY529527[1]. **1626**, U.S.A., Vermont, Orleans Co., West Glover, Lake Parker, 44°43′34″N, 72°13′53″W, 396 m, 22 July 2005, *Z. Kaplan & C. B. Hellquist 05/360*, EU596944, EU596953. *Potamogeton spirillus*: **1632**, U.S.A., Connecticut, Windham Co., South Windham, Shetucket River, 41°41′16″N, 72°10′24″W, 38 m, 24 July 2005, *Z. Kaplan & C. B. Hellquist 05/368*, —, FJ151214. **1722**, U.S.A., Maine, Hancock Co., Ellsworth, Graham Lake, 44°35′36″N, 68°24′40″W, 31 m, 3 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/446*, —, FJ151215. *Potamogeton richardsonii*: **1056**, Canada, Manitoba,

town of Snow Lake, Snow Creek, 54°53′36″N, 100°06′37″W, 20 Aug. 1998, *C. B. Hellquist s.n.*, EU596945, EU596954. **1720**, U.S.A., Maine, Aroostook Co., Washburn, oxbow of Pettingrill Brook, 46°44′20″N, 68°01′59″W, 130 m, 2 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/444*, EU596946, EU596955. *Stuckenia pectinata*: **981**, Switzerland, St. Gallen, Altenrhein, 47°29′08″N, 09°32′56″E, 396 m, 23 July 1998, *Z. Kaplan 98/127*, —, FJ151216. **1652**, U.S.A., Vermont, Addison Co., Brooksville, Otter Creek, 44°03′45″N, 73°10′39″W, 67 m, 25 July 2005, *Z. Kaplan & C. B. Hellquist 05/385*, —, FJ151217. *Potamogeton ×absconditus* = *P. perfoliatus* × *P. richardsonii*: **1649**, U.S.A., Vermont, Addison Co., Brooksville, Otter Creek, 44°03′45″N, 73°10′39″W, 67 m, 25 July 2005, *Z. Kaplan & C. B. Hellquist 05/382*, EU596949, EU596956. **1730**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/456*, EU596958, EU596957. *Potamogeton ×aemulans* = *P. bicupulatus* × *P. epihydrus*: **1631**, U.S.A., Connecticut, Windham Co., South Windham, Shetucket River, 41°41′16″N, 72°10′24″W, 38 m, 24 July 2005, *Z. Kaplan & C. B. Hellquist 05/367*, EU596933, —. **1633**, U.S.A., Connecticut, Windham Co., South Windham, Shetucket River, 41°41′16″N, 72°10′24″W, 38 m, 24 July 2005, *Z. Kaplan & C. B. Hellquist 05/367*, EU596934, —. **1679**, U.S.A., Massachusetts, Franklin Co., Orange, Lake Rohunta, 42°33′47″N, 72°16′23″W, 162 m, 27 July 2005, *Z. Kaplan & C. B. Hellquist 05/402*, EU596935, —. *Potamogeton ×mirabilis* = *P. gramineus* × *P. oakesianus*: **1687**, U.S.A., New Hampshire, Carroll Co., Ossipee, pond at Brown Ridge Road, 43°38′27″N, 71°03′46″W, 189 m, 29 July 2005, *Z. Kaplan & C. B. Hellquist 05/412*, EU596936, —. *Potamogeton ×nitens* = *P. gramineus* × *P. perfoliatus*: **1007**, Sweden, prov. Södermanland, Vårdinge, Sillen Lake, 59°01′19″N, 17°21′29″E, 13 Aug. 1998, *Z. Kaplan 98/344*, DQ468870[2], —. **1726**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/453*, EU596950, —. **1727**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/454*, EU596951, —. *Potamogeton ×versicolor* = *P. epihydrus* × *P. perfoliatus*: **1724**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/448*, EU596947, —. **1731**, U.S.A., Maine, Penobscot Co., Gould Landing, Pushaw Lake, 44°53′50″N, 68°47′16″W, 35 m, 4 Aug. 2005, *Z. Kaplan & C. B. Hellquist 05/457*, EU596948, —.