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First record of *Potamogeton* × *salicifolius* for Italy, with isozyme evidence for plants collected in Italy and Sweden

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Abstract
Potamogeton × *salicifolius*, a hybrid between *P. lucens* and *P. perfoliatus*, is identified as a new taxon for Italy. This is the first record of this hybrid from southern Europe. The Italian sample was studied in cultivation and compared with a living specimen of *P.* × *salicifolius* from Sweden, where the hybrid is rather widespread. In addition to morphological features, the most compelling evidence for the hybrid origin of these plants came from the isozyme analysis. The additive ''hybrid'' banding patterns of the five enzyme systems studied indicate inheritance from *P. lucens* and *P. perfoliatus*. The distribution of this hybrid in Europe coincides with the areas most severely affected by the Late Pleistocene glaciation. The relationships between environmental conditions, history of the habitat and rise of hybrids are discussed.

Key words: *Taxonomy, morphology, Mediterranean, electrophoresis, hybridization, postglacial lakes*

Introduction

Hybrids are an important component of *Potamogeton* diversity. However, the distribution and frequency of hybrids are considerably uneven. In Europe, there is an obvious gradient associated with the terrestrial latitude, both in hybrid diversity and number of localities with hybrids: many records for the British Isles (Dandy, 1975; Preston, 1995) and Scandinavia (Hagström, 1916), several for some countries in Central Europe (e.g., Fischer, 1907; Baumann, 1911; Wiegleb & Herr, 1984; Ploeg, 1990; Wolff et al., 1997; Wiegleb et al., 1998; Kaplan, 2001; Zalewska-Gałosz, 2002; Kaplan & Fehrer, 2004; Kaplan & Wolff, 2004), and no confirmed record from the Mediterranean part of Europe. The identity of several European hybrids was recently confirmed using molecular techniques, such as isozyme electrophoresis (e.g. Hollingsworth et al., 1995b, 1996b; Preston et al., 1998b; Fant et al., 2001a,b; Kaplan et al., 2002; Fant & Preston, 2004; Kaplan & Wolff, 2004) or DNA-based techniques (King et al., 2001; Fant et al., 2003, 2005; Kaplan & Fehrer, 2004, 2006).

Potamogeton × *salicifolius* Wolfg. is a sterile hybrid between *P. lucens* L. and *P. perfoliatus* L. Both its parental species belong to a group of broad-leaved homophyllous pondweeds, which produce only submerged leaves. *Potamogeton lucens* is characterized by robust stipules and shortly petiolate leaves with a large lamina, which has 9–11 veins and a prominent mucro at the apex. It is native in Europe and much of Asia, and rarely occurs in northern and eastern Africa (Wiegleb & Kaplan, 1998). The other parent, *P. perfoliatus*, has delicate and relatively small stipules and sessile leaves mostly with 19–33 longitudinal veins, an amplexicaul base and a rounded to subacute apex. It occurs mainly in the Northern Hemisphere, in Europe, northern and central Africa, Asia and eastern North America, but in some regions it penetrates further south down to Australia and Central America (Wiegleb & Kaplan, 1998). These species are sympatric over much of their ranges and as they occupy similar habitats, such as lakes, water reservoirs and rivers, they are sometimes found growing together at the same site. The hybrid between these two species, *P.* × *salicifolius*, is generally intermediate between its parents. The most important diagnostic characters are summarized in Table I.

Flowers of both *P. lucens* and *P. perfoliatus* are usually self-pollinated. However, as the flowers are

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Table I. A comparison of the most important diagnostic characters of *Potamogeton lucens*, *P. perfoliatus* and *P. × salicifolius*, compiled from the literature[a] and modified and supplemented based on personal experience.

	P. lucens	*P. × salicifolius*	*P. perfoliatus*
Shape of leaf lamina	Narrowly oblong to broadly elliptical	Narrowly lanceolate or oblong to elliptical	Narrowly lanceolate to orbicular-ovate
Length of leaf lamina (mm)	(30–)70–180(–240)	55–160(–200)	16–80(–115)
Width of leaf lamina (mm)	(15–)25–65	14–40	(7–)12–42
Length:width ratio of lamina	2–6(–10)	2.7–10	(1.0–)1.3–5.3(–10)
Number of longitudinal leaf veins	9–11	(7–)9–17	(11–)19–33
Shape of lamina apex	Acute to rounded and mucronate	Acute to rounded and indistinctly apiculate	Rounded to obtuse or subacute
Length of mucro (mm)	1–7(–25)	0(–1)	0
Shape of lamina base	Cuneate to rounded, gradually tapering or mostly abruptly narrowed to the petiole, never amplexicaul	Broadly cuneate to rounded, almost always sessile, sometimes semi-amplexicaul	Rounded to cordate, amplexicaul
Length of petiole (mm)	1–7(–18)	0(–2)	0
Persistence of stipules	Persistent	Persistent or present only on upper part of the stem and disappearing at older leaves	Fugacious, present mostly only at young leaves and disappearing early
Length of stipule (mm)	25–105	12–55	3–20
Length of spike (mm)	15–65	8–40	13–32
Capacity to produce fruits	Present	Absent	Present

[a]Dandy, 1975; Preston, 1995; Wiegleb & Kaplan, 1998; Fant & Preston, 2004.

markedly protogynous, they may occasionally permit cross-pollination. Both species are considered to be tetraploids with a chromosome number of $2n = 52$ (Kaplan & Jarolímová, unpublished), although different chromosome counts are exceptionally reported for *P. perfoliatus* (Hollingsworth et al., 1998).

This hybrid is reported from several countries in the northern half of Europe: Ireland, the United Kingdom, Denmark, Sweden, France, the Netherlands, Switzerland, Germany, Poland, Lithuania, Estonia and Russia (e.g. Magnin, 1897a,b; Fischer, 1907; Baumann, 1911; Ascherson & Graebner, 1913; Hagström, 1916; Galinis, 1963; Dandy, 1975; Pedersen, 1976; Mäemets, 1979, 1984; Wiegleb & Herr, 1984; Ploeg, 1990; Preston, 1995; Wiegleb et al., 1998; Zalewska-Gałosz, 2002). Although there are no publications on the occurrence of *P. × salicifolius* in southern Europe, I have identified it among herbarium material from Croatia and Greece.

A plant similar to *P. × salicifolius* was found in northern Italy in 1998. Only a single small detached fragment (ca. 7 cm long) of the terminal part of the stem of *Potamogeton* was located, floating just below the water surface close to the eastern shore of the northern part of Lake Como (Lago di Como), 0.8 km south-east of Gera Lario and 4 km north-east of Domaso. In spite of extensive searching in this part of the lake, no other individual of this appearance was found. The plant differed in appearance from any species known from Italy. The semi-amplexicaul leaf bases indicated a *P. perfoliatus* hybrid, the shape of leaves was most similar to *P. × salicifolius*. This was rather surprising because *P. × salicifolius* was not known from this part of Europe. Both putative parents, *P. lucens* and *P. perfoliatus*, also occurred at this site. The small fragment of the putative hybrid and plants of both its assumed parents were collected for cultivation and further detailed investigation.

Material and methods

Plant material

In addition to the putative hybrid (sample 972), living specimens of both putative parental species were collected from Lake Como. In order to define more accurately the isozyme diversity of the species, additional samples were collected in various regions, mainly in central Europe. A plant of *P. × salicifolius* from Sweden (sample 1017), where the hybrid is rather widespread, was also included in the study for comparison with the putative Italian hybrid. Because the genetic variation between populations is high, but low or absent within populations of *Potamogeton* species (van Wijk et al., 1988; Hettiarachchi & Triest, 1991; Hollingsworth et al., 1995a, 1996a; Kaplan & Štěpánek, 2003), only 1–3 individuals were taken from each population but more populations were sampled in order to cover most of the intraspecific isozyme variation. Specimens included in the study, their origins and reference numbers corresponding to numbers in Table III and to

numbers of herbarium vouchers are summarized in Table II.

Samples of the three taxa were cultivated in the experimental garden at the Institute of Botany, Průhonice, Czech Republic, and grown there from 1996 to 2004. Plants were cultivated in $180 \times 140 \times 80$ cm water-filled laminate tanks, which were sunk in the ground in order to prevent overheating of the water in summer. The samples were planted in submerged plastic pots containing previously desiccated pond mud.

The single sample of *P.* × *salicifolius* from Lake Como was first propagated vegetatively to a clone of more then 10 ramets and then grown up to the adult stage. This material was used for both morphological and isozyme analyses, and for preparation of herbarium vouchers for the occurrence of *P.* × *salicifolius* in Italy. Herbarium specimens from both the field and cultivation are preserved in the Herbarium of the Institute of Botany, Průhonice (acronym PRA). The specimen records are given in Table II.

Isozyme analysis

Leaf material was collected from cultivated plants early in the morning in the summers of 2002 and 2003 and immediately used for enzyme extraction. The leaves were dabbed free of water, marl and algae. Approximately 60 mg of leaf tissue was mechanically ground with Dowex-Cl (1-X8) and quartz sand, and homogenized on ice in 0.75 ml extraction buffer. Two different extraction buffer systems were used: (a) 'viola' (0.1 M tris-HCl pH 8.0, 70 mM 2-mercaptoethanol, 26 mM sodium metabisulfite, 11 mM ascorbic acid, 4% polyvinylpyrrolidone) was used to separate isozymes of AAT; and (b) 'luzula' (75 mM tris-H_3PO_4 pH 7.5, 13 mM 2-mercaptoethanol, 7.8 mM dithioerythritol, 2.8 mM L-ascorbic acid, 4% polyvinylpyrrolidone) for samples later stained for ADH, EST, GDH, LAP, PGM, SOD, and 6PGDH. The extracts were centrifuged for 10 min at 13,000 rpm and clear supernatants were stored in Eppendorf tubes at −75°C for up to 16 months until investigated in electrophoresis.

Electrophoresis was run on non-denaturing polyacrylamide gels in a Hoeffer vertical electrophoresis unit at 4°C. The gels consisted of a separating gel (8% acrylamide, 1.82 M tris-HCl buffer, pH 8.9) and a stacking gel (4% acrylamide, 0.069 M tris-HCl buffer, pH 6.9). The electrode buffer consisted of 0.02 M tris and 0.24 M glycine at pH 8.3. All enzymes migrated anodally. Visualized loci were numbered in order of decreasing anodal mobility.

The following eight enzymes were analyzed: aspartate aminotransferase (AAT, EC 2.6.1.1), alcohol dehydrogenase (ADH, EC 1.1.1.1), esterase (EST, EC 3.1.1.), glutamate dehydrogenase (GDH, EC 1.4.1.2), leucine aminopeptidase (LAP, EC 3.4.11.1), phosphoglucomutase (PGM, EC 2.7.5.1), superoxide dismutase (SOD, EC 1.15.1.1), 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44). The staining procedures to visualize ADH and 6PGDH followed Vallejos (1983), and Wendel & Weeden (1989) for PGM, EST, SOD, and GDH, with the following modifications: ADH (20 ml ethanol), 6PGDH (0.1 M tris-HCl pH 8.4, 30 mg 6-phosphogluconic acid), PGM (24 mg $MgCl_2$, 50 mg glucose-1-phosphate, 10 mg NADP), EST (Na-phosphate buffer pH 6.45; 25 mg β-naphthyl-phosphate, 50 mg Fast Blue BB), SOD (0.05 M tris-HCl pH 8.2, 4.5 mg EDTA, 5 mg NBT).

Results

Morphological evaluation

The cultivated plants of the presumptive *P.* × *salicifolius* hybrid from Lake Como showed the following combination of diagnostic characters: all leaves were

Table II. The origins and reference numbers of the *Potamogeton* samples included in this study.

Taxon	Reference no.	Origin + field collection records
P. × *salicifolius*	972	Italy, Lake Como, 18 VI 1998, coll. Z. Kaplan
	1017	Sweden, Runsa, 15 VIII 1998, coll. Z. Kaplan 98/361
P. lucens	316	Czech Republic, Hrobice, 9 IX 1996, coll. Z. Kaplan 96/627
	912	Czech Republic, Žehrov, 18 IX 1997, coll. Z. Kaplan 97/914
	966	Italy, Lake Como, 18 VI 1998, coll. Z. Kaplan 98/80
	978	Switzerland, Altenrhein, 23 VI 1998, coll. Z. Kaplan 98/123
	1150	Czech Republic, Žehrov, 29 VII 1999, coll. Z. Kaplan
	1284	France, Grosbliederstroff, 21 VII 2001, coll. P. Wolff s. n.
P. perfoliatus	840	Czech Republic, Ostrožská Nová Ves, 25 VI 1997, coll. Z. Kaplan 97/524
	967	Italy, Lake Como, 18 VI 1998, coll. Z. Kaplan 98/81
	973	Switzerland, Walenstadt, 22 VI 1998, coll. Z. Kaplan 98/102
	979	Switzerland, Altenrhein, 23 VI 1998, coll. Z. Kaplan 98/125
	985	Austria, Fußach, 23 VI 1998, coll. Z. Kaplan 98/131
	1002	Sweden, Björka, 12 VIII 1998, coll. Z. Kaplan 98/338

submerged, mostly oblong-elliptical in shape, 55–110 mm long, 14–25 mm wide, 3.4–7.9 times as long as wide, mature leaves 9–11(–15)-veined, young leaves 7–9-veined, acute to subacute and sometimes indistinctly apiculate at apex, sessile or rarely subsessile, with a short petiole 1 mm long, stipules 12–23 mm long, present only at young leaves on the upper part of the stem but soon disappearing; spikes were rarely produced, about 10 mm long.

This set of characters supports the hypothesis that the plant from Lake Como is a hybrid between *P. lucens* and *P. perfoliatus*. The cultivated plants are more *P. lucens*-like in general appearance and size and shape of the leaves. Also the number of longitudinal leaf veins is more similar to that of *P. lucens*, although some mature upper leaves have more (up to 15) than normally occur in this species, which is the effect of the genes of *P. perfoliatus*. The sessile leaves with semi-amplexicaul bases are clearly a feature of *P. perfoliatus*. The leaf apex is also similar to that of *P. perfoliatus*, lacking the characteristic mucronate termination of *P. lucens*. The stipules are intermediate in size and persistency between those of *P. lucens* and *P. perfoliatus*. All these characters when considered in conjunction clearly indicate that the plant is intermediate between the putative parents and was thus identified as *P.* × *salicifolius*. The Lake Como sample is a narrow-leaved form of this hybrid. The type collection of the name *P.* × *salicifolius* also has this phenotype (Kaplan & Zalewska-Gałosz, 2004). Also Fant & Preston (2004) noted that most of their samples of *P.* × *salicifolius* had on average, narrower leaves than either of its parents.

The few spikes produced in cultivation were shorter than those of the parental species. The tepals of flowers remained tightly closed. The entire spikes rotted soon instead of setting fruit. This is typical of sterile hybrids (Preston, 1995:46; Preston et al., 1998a; Kaplan & Fehrer, 2004; Kaplan & Wolff, 2004). In contrast, the tepals of fertile plants open to reveal the dehiscing anthers.

Isozyme analysis

Gels were stained for eight enzyme systems (AAT, ADH, EST, GDH, LAP, PGM, SOD and 6PGDH). PGM could not be interpreted because 6PGDH was unintentionally stained in the same place on the gel. The phenotypes of the enzyme GDH varied little between the taxa. Because of the variable staining of the bands between samples and the difficulty of reliably interpreting the phenotypes of this hexameric enzyme, the GDH results were not included in the analysis. LAP could not be analyzed because of low enzyme activity in some samples. The phenotypes of the remaining five enzyme systems were sufficiently legible and variable in at least one of their loci (Figure 1).

The pattern of AAT (dimer) consisted of three visualized loci. *Aat-1* was variable in the visualized samples but the bands of about half of the individuals were insufficiently stained and could not be consistently scored. Thus this locus was omitted from the analysis. The middle locus *Aat-2* showed species-specific isozyme phenotypes for *P. lucens* and *P. perfoliatus*, each consisting of a single band representing one homozygous allele (Figure 1). These contributed to the banding pattern of *P.* × *salicifolius*. Both samples of this hybrid (972 and 1017) showed an additive profile, composed of three bands consistent with a heterozygote pattern. Besides the two bands inherited from the parents, an additional novel band appeared in the middle between the two parental bands. This was interpreted as a heterodimer. The slowest locus *Aat-3* was invariable and exhibited the same triple-banded pattern in the hybrid and both parents (Figure 1).

The gel stained for ADH (dimer) showed a single continuous zone of activity. Three different isozyme phenotypes were detected corresponding to the three taxa studied (Figure 1). In many samples there was a second band of secondary origin just above the homomeric bands. These secondary bands were excluded from the interpretation (and not shown in the figure). A homozygous phenotype was present in *P. lucens*, a double-banded pattern was found in *P. perfoliatus*. The hybrid (in both samples 972 and 1017) showed an additive profile, which consisted of the three bands identified in the parental species with an additional heterodimer band between the fastest and middle bands.

EST (monomer or dimer) gave a very complicated pattern of bands of four colours (brown, violet, green, yellow) in three zones of activity consisting of a fully unresolved number of loci (at least five distinguished, four of them overlapping on the gels). Enzyme activity was variable both between and within samples. Most bands were insufficiently stained in the narrow slowest zone (consisting of about three bands). Thus, only the middle zone with a total of nine different bands and the fastest zone with up to three bands could be interpreted. The middle zone included six phenotypes (A–F), each specific to a given taxon (Figure 1). Three different phenotypes were present in *P. lucens*, two in *P. perfoliatus*. The hybrid (phenotype C) showed an additive profile, composed of five bands, three of which are present in phenotype C of *P. lucens* and the other two in phenotype E of *P. perfoliatus*. The fastest zone showed a single locus. The triple-banded pattern of both samples of *P.* × *salicifolius* was the same as that observed in all samples of *P. lucens*, and

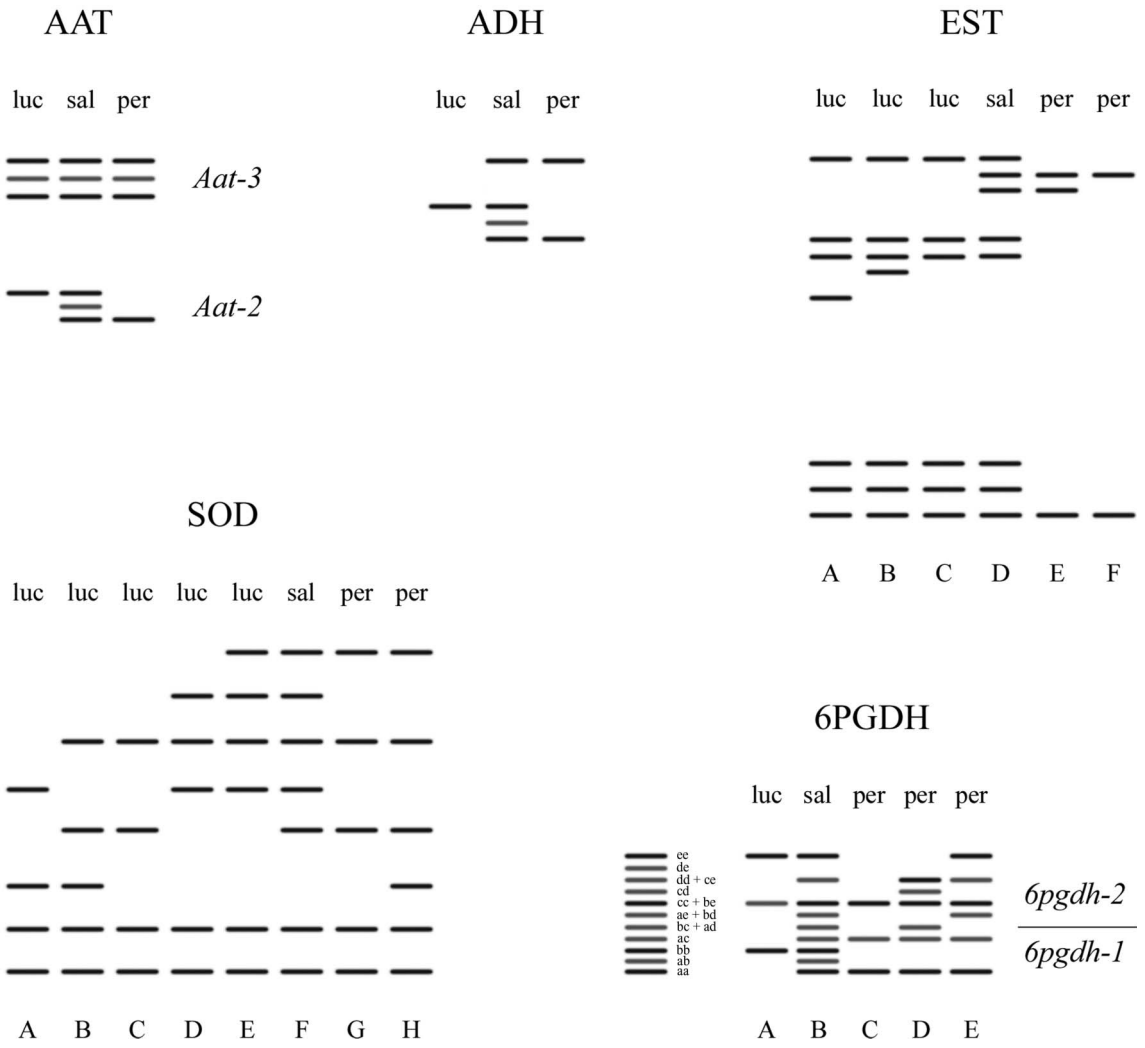

Figure 1. Enzyme phenotypes of AAT, ADH, EST, SOD, and 6PGDH observed in samples of *Potamogeton lucens* (luc), *P.* × *salicifolius* (sal) and *P. perfoliatus* (per). All enzymes migrated anodally (towards the bottom of the figure). Size of the bands and distances between them within an enzyme system are the same size as they were on the gel. In the dimeric systems AAT, ADH and 6PGDH, supposed homomeric bands are black, bands corresponding to supposed heterodimers are gray. The secondary bands that appeared in some samples at ADH, SOD and 6PGDH are not shown. Alphabetical codes below the banding patterns of EST, SOD and 6PGDH denote a different locus or enzyme phenotype (see text). For 6PGDH, a full structure of the bands composed of both all the homodimers and possible combinations of heterodimers is appended on the left, together with their allelic interpretations (homodimers of *6pgdh-1*: aa, bb; homodimers of *6pgdh-2*: cc, dd, ee; heterodimers intragenic: ab, cd, ce, de; heterodimers intergenic: ac, ad, ae, bc, bd, be).

is expected for a hybrid between *P. lucens* and the single-banded isozyme profile of *P. perfoliatus*.

A single more or less continuous zone of activity was revealed for SOD (dimer or tetramer) (Figure 1). Though it probably consisted of three loci, they were difficult to separate and the area of activity was therefore interpreted as one complex locus with a total of eight bands. Eight isozyme phenotypes were observed and each was specific to one of the three taxa. Among them, phenotype E of *P. lucens* and phenotype G of *P. perfoliatus* can explain the pattern observed in the putative *P.* × *salicifolius* (phenotype F).

6PGDH (dimer) was the most informative enzyme system, and therefore deserved an analysis of alleles. Two loci were detected in 6PGDH, which formed intergenic heterodimers giving rise to multibanded isozyme patterns (Figures 1 and 2). In some samples there were 1 – 3 additional faint bands of secondary origin just above the slowest homomeric band. These secondary bands were excluded from the interpretation (and not shown in Figure 1). Altogether 10 different bands appeared on the gel (Figures 1 and 2). Five of them represented homodimers, which could potentionally produce novel heterodimers, and altogether give rise to an 11-banded full pattern (see the column of bands shown left of the 6PGDH phenotype in Figure 1). Five different phenotypes could be distinguished in the samples studied (Figure 2). All samples of *P. lucens* were uniform.

Their enzyme pattern was homozygous in both loci, with an intergenic heterodimer in the middle between the homodimers (phenotype A). The samples of *P. perfoliatus* studied included three phenotypes, of which one was homozygous at both loci (phenotype C) and two were heterozygous for *6pgdh-2* (phenotypes D and E). *P.* × *salicifolius* is heterozygous in both loci (phenotype B), combining alleles of phenotype A of *P. lucens* and phenotype C of *P. perfoliatus*. The bands recorded for each phenotype and the interpretation of their allelic constitution is given in Table III.

Discussion

The results of the isozyme analysis support the morphological evidence indicating that the small fragment found in Lake Como is a hybrid between *P. lucens* and *P. perfoliatus*. This is the first record of *P.* × *salicifolius* for Italy. Another taxon, an African species *P. schweinfurthii*, was recently discovered in the Mediterranean part of Europe (Kaplan, 2005). This indicates that a more intensive research of aquatic communities in this region may result in additional new records. Besides the observations on the Italian sample of *P.* × *salicifolius*, this paper provides isozyme evidence also from the Swedish population of this hybrid.

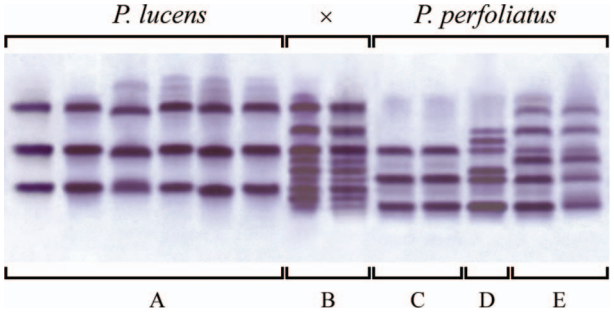

Figure 2. A part of the polyacrylamide gel stained for 6PGDH showing a complex structure of altogether 10 bands, which are composed of five homodimers and additional intra- and intergenic heterodimers. Five enzyme phenotypes can be distinguished, which belong to *P. lucens*, *P.* × *salicifolius* (designated '×') and *P. perfoliatus*. See text and Table III for details and phenotype codes, and Figure 1 for the interpretation of the bands.

Although the enzymes EST and SOD varied in *P. lucens* and EST, SOD and 6PGDH in *P. perfoliatus*, the Italian (972) and Swedish (1017) samples of *P.* × *salicifolius* shared the same multienzyme phenotype. This indicates that the same multienzyme phenotypes of the parental species took part in the two hybridization events. An alternative explanation, that both hybrid populations represent an offspring of a single hybridization event, is extremely unlikely. The plants of *P.* × *salicifolius* are sterile and can only spread by vegetative propagation over a relatively limited area (see also the discussion in Kaplan & Fehrer, 2004).

The fact that both parental species were found together with the hybrid in Lake Como indicates that it originated at this site, possibly recently. Hybrid *Potamogeton* plants are usually found together with their parents but exceptions are not rare. Colonies of *Potamogeton* hybrids can persist at a locality for a considerably long period, sometimes even in the absence of one or both of their parents (e.g. Hollingsworth et al., 1996b; Preston et al., 1998b; King et al., 2001; Kaplan & Fehrer, 2004; Kaplan & Wolff, 2004).

The available observations suggest that the transfer of pollen from a flower of one species to that of another with the same pollination system and subsequent cross-fertilization is a relatively frequent event (Kaplan & Fehrer, 2004). However, the ability of the seed to successfully germinate is critical. *Potamogeton* plants often propagate vegetatively by clonal growth and shoot fragmentation (Hollingsworth et al., 1995a, 1996a; Kaplan & Štěpánek, 2003). New individuals only establish from seed under optimal circumstances. Sufficient nutrients, little competition and clear water, which does not overheat in summer, seem to be the decisive factors.

Table III. 6PGDH phenotypes of *P. lucens*, *P. perfoliatus* and *P.* × *salicifolius* and their allelic interpretations.[a]

Taxon	Samples with the respective 6PGDH phenotype	6PGDH phenotype	Bands on the gel				Interpretation of allelic constitution	
			Total number	Homodimers	Heterodimers intragenic	Heterodimers intergenic	*6pgdh-1*	*6pgdh-2*
P. lucens	316 + 912 + 966 + 978 + 1150 + 1284	A	3	bb, ee		be	bb	ee
P. × *salicifolius*	972 + 1017	B	9[b]	aa, bb, cc, ee	ab, ce	ac, ae, bc, be	ab	ce
P. perfoliatus	967 + 973 + 979	C	3	aa, cc		ac	aa	cc
	840	D	6	aa, cc, dd	cd	ac, ad	aa	cd
	985 + 1002	E	6	aa, cc, ee	ce	ac, ae	aa	ce

[a]The bands are designated with letters 'a' to 'e' in order of decreasing anodal mobility; [b]The band corresponding to the homodimer cc comigrated to the same place on the gel as the intergenic heterodimer be. Therefore, instead of 10 bands there are only 9 on the gel.

Habitats with these features are rather frequent in northern (and northwestern) Europe. That is why Scandinavian and British lakes and rivers host *Potamogeton* hybrids relatively often. In contrast, similar habitats decrease southwards and are rare in the Mediterranean region. The perialpine lakes (such as those in northern Italy) are an exception. In contrast to lowland eutrophic fishponds with a toxic anaerobic bottom and low water transparency, which inhibit germination of *Potamogeton* seeds (Kaplan, 2001; Kaplan & Fehrer, 2004), the perialpine lakes generally have environmental features similar to those of the lakes in the northern half of Europe, which were formed after the retreat of the last continental glacier. They also often host a high diversity of aquatic plants, which, together with the environmental conditions described above, increase the probability of successful interspecific hybridization.

The association of hybrids with environments severely affected by the glacial cycles of the Late Pleistocene is well documented (Kearney, 2005). Hybridization creates new genetic combinations, some of which may be even more successful in that environment than their parents (e.g., Anderson & Stebbins, 1954; Lewontin & Birch, 1966; Arnold, 1992; Barton, 2001). The opportunities for the new hybrid genotypes are even greater in the open habitats of a postglacial landscape. *Potamogeton* hybrids overcome their sterility by vegetative propagation (e.g. Kaplan & Štěpánek, 2003; Kaplan & Fehrer, 2004; Kaplan & Wolff, 2004). This allows them to persist in localities even for thousands of years (Preston et al., 1998b; King et al., 2001), provided that the habitat remains suitable. Thus, although *P.* × *salicifolius* is not confined to lakes but sometimes occurs in running water, its present-day distribution in Europe (see earlier) coincides with the occurrence of postglacial lakes that have a favourable combination of environmental conditions.

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