An orphaned clone of *Potamogeton* ×*schreberi* in the Czech Republic

Nález křížence *Potamogeton* ×*schreberi* v jižních Čechách bez přítomnosti rodičovských druhů

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Kaplan Z. & Fehrer J. (2009): An orphaned clone of *Potamogeton* ×*schreberi* in the Czech Republic. – Preslia 81: 387–397.

A *Potamogeton* hybrid found growing in the absence of parental species in a South Bohemian stream, Czech Republic, was subjected to molecular analyses to identify its exact identity. RFLP of the ITS region confirmed its previous morphological identification as *P. natans* × *P. nodosus* (= *P.* ×*schreberi*). A comparison of its RFLP pattern with those of *P. gramineus*, *P. lucens* and *P. polygonifolius* unambiguously excluded the possibility that the investigated plants are specimens of other similar hybrids (*P.* ×*fluitans*, *P.* ×*sparganiifolius*, *P.* ×*gessnacensis*). The discovery of *P.* ×*schreberi* in South Bohemia is the first record of this hybrid for the Czech Republic. So far, it is known only from five countries and the Czech clone is one of a few extant clones of this hybrid in Central Europe. Chloroplast DNA sequencing identified *P. nodosus* as the maternal parent although at present this species neither occurs at the locality, nor upstream, nor in the entire drainage basin. The other species, *P. natans*, only occurs downstream of the locality in isolated side pools in a former stream bed and fishponds in an adjacent drainage basin. The available data indicate that this hybrid has persisted vegetatively at this locality for some time in the absence of its parents.

Keywords: *Potamogeton*, hybridization, taxonomy, morphology, vegetative propagation, relic occurrence, distribution, new records, rare taxon

Introduction

Although the occurrence of hybrids in *Potamogeton* was first reported almost 120 years ago by Fryer (1890), their diversity and distribution are still insufficiently known. For a long time, the identification of *Potamogeton* hybrids depended mainly on morphology, which required a detailed knowledge of the diagnostic characters and ranges of phenotypic variation of species. Some authors also used characters of stem anatomy, which helped to resolve some old intricate taxonomic problems and contributed to the identification of some hybrids (e.g., Raunkiær 1896, 1903, Fischer 1904, 1905, 1907, Hagström 1916, Ogden 1943, Symoens et al. 1979, Wiegleb 1990a, 1990b, Kaplan 2001, 2005a, b, Kaplan & Symoens 2004, 2005, Zalewska-Gałosz 2010). Identification of another hybrid was facilitated by a cytological investigation (Preston et al. 1998a). In spite of this, a worldwide revision of the genus by Wiegleb & Kaplan (1998) indicates that most recognizable hybrids are between rather dissimilar species or between species with different patterns of stem anatomy.

The taxonomy of *Potamogeton* species is generally considered to be difficult, mainly because of high species diversity (Wiegleb & Kaplan 1998), an extensive range of phenotypic plasticity (Kaplan 2002) and a considerably reduced morphology (e.g., Preston 1995a, Preston & Croft 1997, Kaplan & Štěpánek 2003). Even more difficult is the identification of hybrids, which has become the domain of a relatively few *Potamogeton* experts. Although Preston (1995a: 42) expressed his belief that "the existence of hybrids is not likely to be doubted by anyone who is familiar with the morphology of the species", botanists who have less empirical experience sometimes tend to doubt the occurrence of many *Potamogeton* hybrids and call for more convincing evidence (e.g., Les & Philbrick 1993). With the growing scepticism about the diversity of *Potamogeton* hybrids and the possibility of their morphological identification, potential hybrids were overlooked or even intentionally neglected, even in regions where they occur frequently and show high diversity, such as eastern North America (Kaplan et al. 2009).

With the advent of molecular methods over the last two decades, the identity of several (mainly European) *Potamogeton* hybrids was confirmed using molecular methods, such as isozyme electrophoresis (e.g., Hollingsworth et al. 1995, 1996, Preston et al. 1998b, Fant et al. 2001a, b, Iida & Kadono 2002, Kaplan et al. 2002, Fant & Preston 2004, Kaplan & Wolff 2004, Kaplan 2007) or DNA-based techniques (King et al. 2001, Fant et al. 2003, 2005, Kaplan & Fehrer 2004, 2006, Zalewska-Gałosz et al. 2009). Direct DNA sequencing and RFLPs have recently contributed to the discovery and/or exact identification of several entirely new hybrid combinations (Kaplan et al. 2009, Zalewska-Gałosz et al. 2010) and even the existence of a triple hybrid (Kaplan & Fehrer 2007).

Several detailed studies (e.g., Hollingsworth et al. 1995, Kaplan et al. 2002, Fant & Preston 2004, Kaplan & Fehrer 2004, 2006, Kaplan & Wolff 2004, Kaplan 2007) clearly demonstrate that many *Potamogetonaceae* hybrids can be reliably identified morphologically by experts. In contrast, other hybrids can be identified morphologically only after careful examination of specific key structures (Preston 1995a, Preston et al. 1999, Kaplan 2008) or if the particular plant is optimally developed and shows diagnostic features of the species involved in the hybridization (Kaplan & Wolff 2004, Kaplan & Fehrer 2007). Previously unknown rare hybrids, especially, are often almost impossible to identify morphologically, because character expression in hybrids is largely unpredictable (Rieseberg & Ellstrand 1993, Kaplan et al. 2009). The identification of extreme phenotypes or incomplete specimens is likely to be incorrect, and even external factors, such as the time of collection of the plant material, and abiotic factors, such as temperature and nutrient conditions, can affect identification (Kaplan 2002, Kaplan & Wolff 2004). Molecular proof of identification of *Potamogeton* hybrids is therefore always advisable (Kaplan & Fehrer 2007, Kaplan et al. 2009), and is even more important if a new hybrid is recorded for the first time from an area where it was not recorded previously, or it belongs to a group whose members are difficult to identify, which is the case of the recent discovery of a putative *Potamogeton* hybrid in the stream Stropnice in South Bohemia.

When these plants were collected for the first time in September 2006, their morphology allowed only a preliminarily identification as either an aberrant form of *P. natans* or a hybrid between *P. natans* and another broad-leaved species. Another observation of the population in June of the following year indicated that the plants are of hybrid origin and that the other parental species is likely to be *P. nodosus*. This hybrid, named *P.* ×*schreberi* (Figs 1 & 2), belongs to one of the most difficult complexes of the genus and can easily be confused not only with its parents, but also with other *P. natans* hybrids such as *P.* ×*gessnacensis* (= *P. natans* × *P. polygonifolius*), *P.* ×*fluitans* (= *P. lucens* × *P. natans*) and *P.* ×*sparganiifolius* (= *P. gramineus* × *P. natans*) (Kaplan & Wolff 2004).

Fig. 1. – An adult flowering specimen of *Potamogeton* ×*schreberi* from the stream Stropnice with both floating and upper submerged leaves.

Fig. 2. – Young shoots of *Potamogeton* ×*schreberi* with mainly submerged leaves, ranging in shape from phyllodial to transitional.

None of the putative parental species was detected in the stream together with the hybrid. The only associated *Potamogeton* species was *P. crispus*, which can be easily excluded as a potential parent due to its unique morphology. Another species, *P. natans*, was found only downstream in pools of stagnant water in a former stream bed. The other putative parental species, *P. nodosus*, is extremely rare in South Bohemia, with the nearest localities more than 50 km downstream from the site where the hybrid occurs or in different drainage basins (Kaplan, unpublished). The putative hybrid, *P.* ×*schreberi*, has not been previously recorded in the Czech Republic.

Because of the taxonomic difficulties associated with this group and the absence of both parents at the locality, DNA sequencing and RFLP based on the PCR-amplified internal transcribed spacer (ITS) region was used to investigate whether the Stropnice plant falls within the range of variation of *P. natans*, or shows additive patterns of *P. natans* and *P. nodosus*, or of any other candidate parental species.

Material and methods

Plant material used in the molecular analyses

Besides samples of the putative hybrid, the parental species of *P.* ×*schreberi* and of all similar hybrids were included in this study. Four accessions of *P. natans*, three of which were from Europe (one from the same area as the hybrid) and one from North America were used to determine intraspecific genetic variation. The alternative parents were represented by two accessions each, mostly of material from different continents. Two genotypes of *P. gramineus*, which were detected earlier (Kaplan & Fehrer 2006, 2007), were also represented by two samples each. Voucher herbarium specimens of all samples are preserved in the herbarium of the Institute of Botany, Průhonice (acronym PRA). Specimens included in the molecular analyses are summarized in Table 1. The majority of the listed species are tetraploids with 2n = 52, whereas *P. polygonifolius* is diploid with 2n = 28 (V. Jarolímová & Z. Kaplan, unpubl. data; Hollingsworth et al. 1998).

Taxonomic delimitations of species, hybrid formulas and nomenclature of all taxa follow Wiegleb & Kaplan (1998). A detailed description of the morphology and stem anatomy of *P.* ×*schreberi* was recently published in this journal by Kaplan & Wolff (2004).

Molecular analyses

DNA isolations, PCR amplifications and sequencing of the ITS region were done as described previously (Kaplan & Fehrer 2004). RFLPs were performed as described in Kaplan & Fehrer (2006) except that the enzyme *Hpy*CH4V (New England Biolabs) was used for the digests.

In order to determine the maternal parent of the hybrid, the *rpl*20-5’*rps*12 intergenic spacer region of chloroplast DNA was sequenced for both parents and for the hybrid as described previously (Kaplan & Fehrer 2006). GenBank accession numbers are given in Table 1.

Table 1. – Samples used in the molecular analyses. * indicates two genotypes of *Potamogeton gramineus*

Taxon	Ref. no.	No. in Fig. 1	Origin and field collection records	ITS GenBank no.	*rpl*20-5'*rps*12 GenBank no.
P. natans	977	1	Switzerland, Sankt Gallen, Rorschach, Altenrhein, 47°29'08"N, 09°32'56", 398 m, 23 June 1998, coll. Z. Kaplan 98/122	FJ883537	FJ883542
	1283	2	Germany, Saarland, Saarbrücken, 49°14'37"N, 07°00'47"E, 217 m, 21 July 2001, coll. F.-J. Weicherding s. n.	FJ151208	FJ883543
	1756	3	U.S.A., Massachusetts, Berkshire Co., Hancock, Kinderhook Creek pond, 42°34'40"N, 73°17'51"W, 385 m, 21 July 2005, coll. Z. Kaplan & C. B. Hellquist 05/342	FJ151209	FJ883541
	1890	4	Czech Republic, distr. České Budějovice, Třebeč, side pool of the stream Stropnice, 48°52'41"N, 14°41'17"E, 450 m, 29 June 2007, coll. Z. Kaplan 07/215	FJ883536	FJ883544
P. ×*schreberi*	1889	5	Czech Republic, distr. České Budějovice, Třebeč, stream Stropnice, 48°52'39"N, 14°41'32"E, 450 m, 29 June 2007, coll. Z. Kaplan 07/214	FJ883540	FJ883545
P. nodosus	1807	6	Czech Republic, distr. Pardubice, Stéblová, sand-pit Oplatil, 50°06'30"N, 15°44'54"E, 220 m, 23 July 2006, coll. Z. Kaplan 06/345	FJ883538	FJ883546
	1309	7	France, Lorraine, Moselle, Welferding, 49°06'53"N, 07°02'50"E, 194 m, 14 June 2002, coll. P. Wolff s. n.	FJ151210	FJ883547
	1655	8	U.S.A., Vermont, Addison Co., Weybridge, Brooksville, Otter Creek, 44°03'45"N, 73°10'39"W, 67 m, 25 July 2005, coll. Z. Kaplan & C. B. Hellquist 05/388	FJ883539	FJ883548
P. lucens	317	9	Czech Republic, distr. Pardubice, Hrobice, Baroch fishpond, 50°05'54"N, 15°46'58"E, 224 m, 9 Sep. 1996, coll. Z. Kaplan 96/627		
	1762	10	Japan, Chiba Pref., Tokyo, Tega River, ca. 35°51'N, 140°04'E, 1 m, 2006, coll. N. Tanaka s. n.		
P. gramineus 1*	897	11	Czech Republic, distr. Česká Lípa, Hradčany u Mimoně, Držák fishpond, 50°36'37"N, 14°43'23"E, 273 m, 18 Sep. 1996, coll. Z. Kaplan 96/638		
	1698	12	U.S.A., New Hampshire, Carroll Co., West Ossipee, Ossipee Lake, 43°48'33"N, 71°09'49"W, 124 m, 29 July 2005, coll. Z. Kaplan & C. B. Hellquist 05/416		
P. gramineus 2*	885	13	Czech Republic, distr. Náchod, Šeřeč, Rozkoš Reservoir, 50°23'02"N, 16°05'14"E, 280 m, 22 Aug. 1997, coll. Z. Kaplan 97/829		
	1285	14	France, Lorraine, Moselle, Rémelfing, ca. 49°05'N, 07°06'E, 200 m, 21 July 2001, coll. P. Wolff s. n.		
P. polygonifolius	1882	15	Portugal, prov. Algarve, distr. Faro, Rogil, ca. 37°22'N, 8°45'W, 150 m, 16 Jan. 2007, coll. U. Schwarzer s. n.		
	1533	16	Czech Republic, distr. Cheb, Hranice, Novosedly, Nový fishpond, 50°16'35"N, 12°10'24"E, 610 m, 13 July 2004, coll. Z. Kaplan 04/168		

Results

PCR-RFLPs of the amplified ITS region with the enzyme *Hpy*CH4V produced different patterns for most of the species (Fig. 3). Only the very closely related *P. gramineus* and *P. lucens* had identical patterns. The pattern of *P. polygonifolius* is similar, but some length variations are apparent. Both *P. natans* and *P. nodosus* have unique patterns and show no intraspecific variation. The questionable accession was indeed a hybrid between *P. natans* and *P. nodosus* as it showed additive patterns of these two species. The *P. natans* accession from the same area was most likely an individual from the actual parental population because it shared a unique intra-individual sequence polymorphism with the hybrid in direct sequencing. Both parental species differed by three substitutions in the chloroplast *rpl*20-5'*rps*12 region. The hybrid showed the *P. nodosus*-specific character states indicating that this was the maternal parent although it does not currently occur at the locality.

Discussion

The molecular analysis confirmed the previous morphological identification of the hybrid clone from the stream Stropnice as the hybrid *P. natans* × *P. nodosus*, which is the first record of *P.* ×*schreberi* for the Czech Republic. So far, this hybrid is documented only from Great Britain, France, Germany, Switzerland and the central part of European Russia (Fischer 1905, Fischer 1907, Koch 1933, 1934, Hollingsworth et al. 1995, Preston 1995a, b, Bobrov & Reshetnikova 2002, Kaplan & Wolff 2004). As shown by Kaplan & Wolff (2004), most past populations of this hybrid in Central Europe are now extinct, which is probably associated with long-term environmental changes and subsequent decline in the abundance of its parental species.

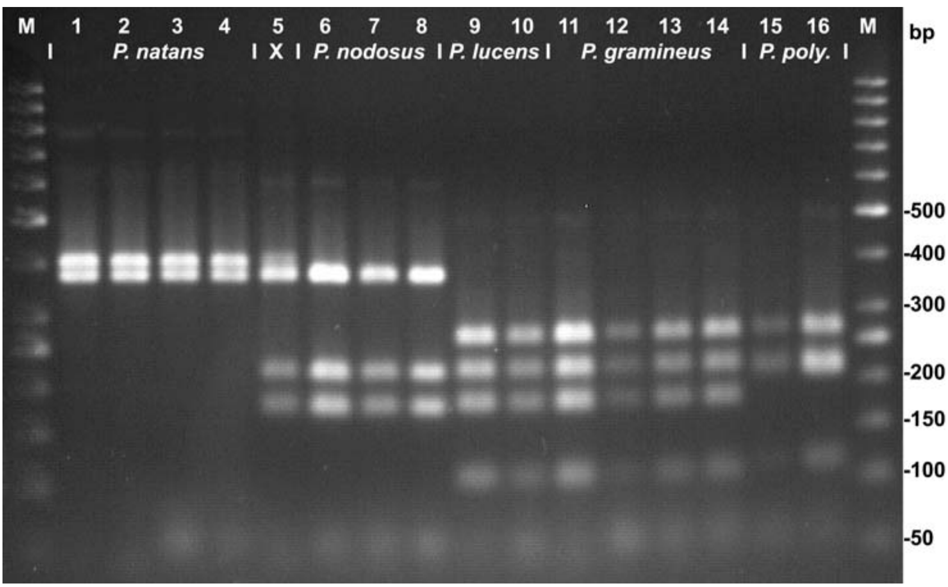

Fig. 3. – PCR-RFLP of the ITS region. The hybrid (line 5, 'X') shows additive banding patterns of *P. natans* and *P. nodosus*; the other three species can be excluded by two bands each (at ~280 bp and 100–120 bp). For the details of the samples, see Table 1.

Fig. 4. – A stretch of the stream Stropnice with stands of *Potamogeton* ×*schreberi*. The water level rises after heavy rain.

At the Stropnice locality, *P.* ×*schreberi* occurs in several patches in about a 550 m long stretch of this meandering stream (Fig. 4). Neither of the parental species occurs with *P.* ×*schreberi* at this site nor a few kilometres upstream. There is also no literature record of an occurrence of a heterophyllous broad-leaved *Potamogeton* species in the Stropnice, nor did a search of all the major Czech public herbaria reveal any collection from this site or upstream from this site. In addition, *P. nodosus* is currently unknown in the entire drainage basin. There is only one herbarium specimen recording a past solitary occurrence of *P. nodosus* in the Stropnice at Komařice (23 VIII 1962 Blažková, CB, with a remark: "one clump in the river, elsewhere missing"), which is about 16 km downstream of the site where *P.* ×*schreberi* occurs. All this suggests that the hybrid has persisted vegetatively at this locality for some time (presumably several decades or even more) in the absence of its parents.

Potamogeton hybrids mostly co-occur at their sites together with their parents, but there are exceptions. The occurrence of a *Potamogetonaceae* hybrid in the absence of one or both parents has been documented from several countries (e.g., Dandy & Taylor 1946, Hollingsworth et al. 1996, Preston et al. 1998a, 1998b, 1999, King et al. 2001, Kaplan & Fehrer 2004, Kaplan et al. 2009). Field studies conducted on *P.* ×*schreberi* in Great Britain, France and Germany (Hollingsworth et al. 1995, Kaplan & Wolff 2004) reveal a similar pattern of distribution as in the Czech Republic: neither of the parental species occurred at any of the sites with this hybrid nor were they present upstream.

Although *Potamogeton* hybrids are almost always sterile (e.g., Hagström 1916, Dandy 1975, Preston 1995a, Wiegleb & Kaplan 1998, Kaplan & 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, Kaplan & Wolff 2004, Kaplan & Fehrer 2007), provided the ecological conditions remain suitable. Thus, hybrids with old histories are mostly found in ecologically stable habitats, and this is likely to be also the case of the upper section of the Stropnice stream, which has never attracted any significant economic use, or been affected by direct human disturbance and is situated in a relatively well-preserved and unpolluted landscape.

Acknowledgements

The authors thank P. Wolff, U. Schwarzer, F.-J. Weicherding and N. Tanaka for additional plant material, C. B. Hellquist, I. Kaplanová and J. Štěpánková for their help with fieldwork, A. Yadollahi with labwork, M. Štech for valuable suggestions on the manuscript, and T. Dixon for improving the English. The research was supported by grants no. 206/09/0291 and 206/07/0706 from the Grant Agency of the Czech Republic, and the long-term institutional research plan no. AV0Z60050516 from the Academy of Sciences of the Czech Republic.

Souhrn

V jižních Čechách v říčce Stropnici u obce Třebeč byla nalezena kolonie rostlin patřící do jednoho z taxonomicky složitých komplexů v rámci rodu *Potamogeton*. Tyto rostliny jsou morfologicky poněkud odlišné od taxonů dosud známých z ČR. Ačkoliv rostliny vykazovaly intermediární znaky, což naznačovalo jejich hybridní původ, na lokalitě a ani proti proudu nebyly nalezeny žádné potenciální rodičovské druhy. Analýza úseku ITS jaderné DNA metodou PCR-RFLP potvrdila předchozí morfologickou identifikaci rostlin jako křížence *P. natans* × *P. nodosus* (= *P.* ×*schreberi*), a zároveň vyloučila identitu s morfologicky podobnými hybridy s účastí druhů *P. gramineus*, *P. lucens* a *P. polygonifolius*. Jedná se o první nález tohoto křížence rdestů v ČR. Velmi vzácný je i mimo naše území; dosud byl nalezen jen v pěti zemích (Velká Británie, Francie, Německo, Švýcarsko a evropská část Ruska). Lokalita v jižních Čechách je jedna z velmi mála existujících míst s výskytem *P.* ×*schreberi* ve střední Evropě. Analýzou sekvencí jeho chloroplastové DNA bylo prokázáno, že mateřskou rostlinou byl druh *P. nodosus*, který se však v současnosti nejenže nevyskytuje na studované lokalitě, ale ani v celém povodí. Druhý rodičovský druh se nyní vyskytuje pouze v tůni v mrtvém ramenu říčky Stropnice níže po proudu, nemálo lokalit má dále v rybnících na většině území Třeboňské pánve. Dostupná data z terénního pozorování a ze studia herbářů naznačují, že *P.* ×*schreberi* již po nějakou dobu na lokalitě přežívá bez přítomnosti rodičovských druhů.

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Received 5 April 2009
Revision received 1 October 2009
Accepted 3 October 2009