The first European record of *Potamogeton* ×*subobtusus* identified using ITS and cpDNA sequence data

První údaj o výskytu křížence *Potamogeton* ×*subobtusus* v Evropě s molekulárním potvrzením jeho identity analýzou jaderné a chloroplastové DNA

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Zalewska-Gałosz J., Ronikier M. & Kaplan Z. (2009): The first European record of *Potamogeton* ×*subobtusus* identified using ITS and cpDNA sequence data. – Preslia 81: 281–292.

A combined study of morphology, stem anatomy and DNA sequencing data (nuclear ribosomal ITS region and *rpl*32-*trn*L and *rps*12-*rpl*20 intergenic spacers of chloroplast DNA) was used to identify a putative *Potamogeton* hybrid from a river in NE Poland. Based on the morphological and anatomical characters the plants were tentatively identified as *P.* ×*subobtusus* Hagstr., a hybrid between *P. alpinus* Balb. and *P. nodosus* Poir. This identification was independently confirmed by the presence in hybrid individuals of an additive ITS sequence pattern from these two parental species. In all plants peaks corresponding to nucleotide states of both parents were clearly distinguishable, however the variants from *P. nodosus* dominated over those from *P. alpinus*. *P. nodosus* was also identified as the maternal parent of the hybrid based on cpDNA data and dominated the expression of morphological features in hybrid individuals. A detailed morphological description of *P.* ×*subobtusus* and the typification of the name are provided. As *P. nodosus* rarely hybridizes with other species, existence of other hybrids, as well as possible difficulties in recognizing these taxa are also discussed.

Keywords: hybridization, matroclinal variation, molecular identification, nomenclature, sequencing, taxonomy, typification

Introduction

Potamogeton is the richest in species and ecologically the most important genus of aquatic vascular plants. It is also one of the most taxonomically difficult plant groups. There are three important sources of taxonomic difficulty. First, the aquatic environment restricts potential morphology and physiology of many plant organs, thereby limiting the range of qualitative phenotypic differences (characters) among species (Niklas 1997). Second, *Potamogeton* taxa express remarkable phenotypic plasticity, which is a more important source of morphological variation within species than differences between genotypes (Kaplan 2002). Third, hybridization that commonly occurs between many species results in new entities, sometimes extremely difficult to accurately identify. Due to these factors, the taxonomy of *Potamogeton* is characterized by an extensive and complicated nomenclature (Wiegleb 1988). Over 200 hybrid combinations have been reported in the genus (Les & Philbrick 1993), but only about 50 hybrids are well established and distinguishable from their parental species (Wiegleb & Kaplan 1998).

Revision of extremely rare and poorly known hybrids is important as it will greatly increase our knowledge of the diversity and taxonomy of this group (Wiegleb & Kaplan 1998). Novel insights can be provided by molecular studies, such as surveys of selected DNA marker regions inherited from parental taxa. For this the analysis of the nrDNA ITS region appears to be a useful tool, especially when applied to broad-leaved *Potamogeton* taxa (e.g., Kaplan & Fehrer 2004, 2006, Zalewska-Gałosz et al. 2010).

One of the interesting taxonomic problems in *Potamogeton* is distinguishing the hybrid between *P. nodosus* Poir. and *P. alpinus* Balb. In 2005 striking, heterophyllous pondweeds were collected for the first time in the river Rospuda (NE Poland, Central Europe). They belonged to the group of broad-leaved *Potamogeton* taxa and were morphologically similar to *P. nodosus*. Besides this species, this group includes some *P. natans* hybrids (Kaplan & Wolf 2004). A detailed morphological and anatomical examination, however, indicated that these plants were neither typical *P. nodosus* or hybrids of *P. natans*. Instead, these plants were provisionally identified as a hybrid of *P. nodosus*. Long submerged leaves on relatively short petioles suggested that the second parental species is characterized by shortly petiolate or sessile, but not amplexicaul leaf bases, which indicates four taxa: *P. alpinus* Balb., *P. crispus* L., *P. gramineus* L. or *P. lucens* L. Based on all the features, *P. alpinus* was designated as the most probable second parental species. A hybrid between *P. alpinus* and *P. nodosus* was described by Hagström based on North American material and named *P.* ×*subobtusus* (Hagström 1916). Recently, this name was made a synonym of the American species *P. amplifolius* Tuckerm. (Wiegleb & Kaplan 1998), which, however, was not made intentionally but happened by mistake (G. Wiegleb, pers. comm.). Both putative parental species are relatively rarely involved in hybridization, thus the need to confirm and clarify reports of their common hybrids. Probable discovery of the first European locality of this extremely rare hybrid was the basis of the present paper.

The aims of this contribution are as follows: (i) to test, using molecular data, whether individuals from the newly discovered European locality are indeed hybrids between *P. alpinus* and *P. nodosus*, (ii) to provide a detailed survey of the morphological and anatomical characters of this hybrid, and (iii) to clarify the nomenclatural ambiguities connected with this hybrid.

Materials and methods

Plant material

Individuals of the putative *Potamogeton* hybrid analyzed were collected from the river Rospuda (NE Poland) in 2006 and 2008. Assuming a clonal population structure and taking advantage of a wide distribution of the population along the river, ramets were sampled at a distance of at least 15 m between plant clumps in order to increase the probability of collecting genetically different individuals. Both herbarium specimens and samples in silica gel were gathered. In total, thirty samples were collected for the morphological and anatomical analysis. Two samples were used for DNA extraction and subsequent genetic analysis. In addition to the putative hybrid a set of typical broad-leaved *Potamogeton* species were also included in the molecular analysis, as potential parental taxa. In order to detect potential intraspecific sequence polymorphism, all reference species were collected from several distant populations in different parts of Poland. Data on all the specimens

Table 1. – Samples of *Potamogeton* taxa included in the DNA study: origin, number of individuals studied and GenBank accession numbers of sequences

Taxon	Origin	No ind.	ITS	*rps*12-*rpl*20	*rpl*32-*trn*L
P. ×subobtusus	NE Poland, river Rospuda, 21 Jul. 2006, coll. J. Zalewska-Gałosz	2	FJ883625	FJ883624	FJ883623
P. alpinus	SW Poland, Prószków, 16 Oct. 2006, coll. A. Nowak	1	FJ883580	FJ883598	FJ883616
P. alpinus	NW Poland, Krzywy Róg, 7 Jul. 2004, coll. J. Zalewska-Gałosz	1	FJ883581	FJ883599	
P. alpinus	S Poland, Dulowa, 5 May 2005, coll. J. Zalewska-Gałosz	1	FJ883582	FJ883600	
P. nodosus	S Poland, Wierzchosławice, 13 Sept. 2006, coll. M. Nobis	1	FJ883593	FJ883611	FJ883620
P. nodosus	SE Poland, Ulanów, 27 Oct. 2006, coll. A. Nobis	1	FJ883594	FJ883612	
P. nodosus	SW Poland, Kantorowice, 16 Oct. 2006, coll. A. Nowak	1	FJ883595	FJ883613	
P. lucens	NE Poland, river Rospuda, 21 Jul. 2006, coll. J. Zalewska-Gałosz	1	FJ883591	FJ883609	FJ883618
P. lucens	NW Poland, river Drawa, 6 Jul. 2004, coll. J. Zalewska-Gałosz	2	FJ883592	FJ883610	
P. perfoliatus	NW Poland, lake Kramsko, 7 Aug. 2006, coll. J. Zalewska-Gałosz	1	FJ883583	FJ883601	FJ883619
P. perfoliatus	SE Poland, Zwolaki, 27 Oct. 2006, coll. A. Nobis	1	FJ883584	FJ883602	
P. perfoliatus	NW Poland, lake Sitno, 9 Jul. 2004, coll. J. Zalewska-Gałosz	1	FJ883585	FJ883603	
P. perfoliatus	NW Poland, river Drawa, 6 Jul. 2004, coll. J. Zalewska-Gałosz	1	FJ883586	FJ883604	
P. gramineus	NW Poland, lake Chądzie, 4 Aug. 2004, coll. J. Zalewska-Gałosz	1	FJ883587	FJ883605	FJ883617
P. gramineus	E Poland, lake Kleszczów, 4 Aug. 2004, coll. J. Zalewska-Gałosz	1	FJ883588	FJ883606	
P. gramineus	E Poland, lake Uściwierz, 15 Aug. 2004, coll. J. Zalewska-Gałosz	1	FJ883589	FJ883607	
P. crispus	NW Poland, Olpuch-Wdzydze, 3 Aug. 2006, coll. J. Zalewska-Gałosz	1	FJ883590	FJ883608	

included in the molecular analysis are summarized in Table 1. All herbarium specimens were deposited in the Herbarium of the Institute of Botany, Jagiellonian University, Krakow (KRA).

Morphological and anatomical analysis

Morphological characters of the stem, submerged and floating leaves, stipules, inflorescence, peduncles and flowers were measured or qualitatively described. Anatomical characters of all the specimens investigated were also assessed. Short pieces of stem (ca 2 mm) were cut from internodes of the main stem and placed in water for a few minutes. Then the stem fragments were cut transversally with a razor blade under a stereomicroscope to produce approximately 0.05 mm thick slices, which were stained in an aquatic solution of

toluidine blue for 1–3 minutes. Stained tissues were subsequently washed in distilled water. Stem anatomy was investigated using a transmitted light microscope at magnifications between 50× (general anatomical pattern) and 400× (for details). Forty five morphological and five anatomical features of 30 individuals were examined. The anatomical terminology used is that of Wiegleb (1990).

DNA isolation, PCR amplification and sequencing

Between 10–15 mg of dried plant material was used for DNA isolation. The plant tissue was ground to a fine powder using Mixer Mill 200 (Retsch) and 3-mm tungsten beads. The total genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen), following the manufacturer's protocol (final elution step used 2 × 50 µl elution buffer). Quality of DNA extractions was roughly verified by electrophoresis on 1% agarose gels.

The nuclear ribosomal Internal Transcribed Spacer region (including ITS1, 5.8S and ITS2) was amplified using universal primers ITS1A and ITS 4 (White et al. 1990, Fuertes Aguilar et al. 1999). The following reaction composition was applied in a total volume of 25 µl: 1× concentration of PCR AmpliTaq Buffer (Applied Biosystems), 2.5 mM Mg^{2+}, 0.11 mM of each dNTP (Roche Diagnostics), 0.2 µM of each primer, 1 µg of bovine serum albumine (BSA), 1 U of the AmpliTaq DNA Polymerase (Applied Biosystems) and 0.5 µL of DNA template. A touchdown cycling profile was applied, including 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C (with decrease of 0.4 °C per cycle and a constant temperature of 48 °C starting from cycle 15) and 1 min at 72 °C, and a final extension step of 10 min at 72 °C.

Two non-coding chloroplast DNA (cpDNA) regions, used in an earlier study to differentiate the taxa studied (Zalewska-Gałosz et al. 2010), were analysed: the *rps*12–*rpl*20 intergenic spacer (modified from Hamilton 1999 according to Shaw et al. 2005) and the *rpl*32-*trn*L intergenic spacer (Shaw et al. 2007). The same reaction mix composition as described above was used, except for the primer concentrations, which were 0.1 ěM. The PCR cycling conditions were: 5 min at 80 °C, 30 cycles of 1 min at 95 °C, 1 min at 50 °C, ramp of 0.3 °C/s to 65 °C and 4 min at 65 °C, followed by a final extension step of 5 min at 65 °C. PCR reactions were performed in a GeneAmp 9700 thermal cycler (Applied Biosystems) or a PTC200 thermal cycler (MJ Research).

PCR products were purified using a High Pure PCR Product Purification Kit (Roche Diagnostics) and sequenced in two directions using the primers used for amplification. Sequencing was performed using BigDye Terminator ver. 3.1 (Applied Biosystems) with supplied 5× sequencing buffer, according to the manufacturer's manual. Purified samples were resuspended in 12 µl formamide and separated on an ABI 3100 Genetic Analyzer using 50 cm capillaries and POP-6 polymer (Applied Biosystems). Raw sequencing profiles were analyzed using the DNA Sequencing Analysis Software version 5.1 (Applied Biosystems).

The sequences were manually verified/adjusted using Finch TV 1.4.0 software (Geospiza Inc.). Alignments of sequences of all regions were conducted manually using BIOEDIT 5.0.9. software (Hall 1999), based on consensus sequences from forward and reverse sequencing directions. Additive nucleotide polymorphisms were examined with two strands to ensure their consistency and coded using IUPAC nucleotide ambiguity codes.

All polymorphic variants of sequences were submitted to GenBank (for accession numbers see Table 1).

Investigation of the type material of Potamogeton ×subobtusus

The herbaria known to host the main sets of the original Hagström's material (LD, S and UPS, acronyms follow Holmgren & Holmgren 1990) were searched for the type collection of *P. ×subobtusus*. In addition, duplicates of the original material were located in other European herbaria (for the complete list of herbaria searched see Kaplan 2008). The morphology of all the specimens found was compared with the original protologue of *P. ×subobtusus* and Hagström's comments and handwritten remarks on herbarium labels were considered.

Results

Morphological and anatomical description of the Polish hybrid

Stem up to 1.5 m long, terete, unbranched. Submerged leaves petiolate, with the lamina 180–290 (–360) mm long, (10–) 22 mm wide, 11–23 (–30) times as long as wide, translucent, narrowly oblanceolate to narrowly elliptical, bright green, with reddish or brownish tinge when dried, very gradually tapering to cuneate base, acuminate, acute to narrowly obtuse at the apex, entire, midrib bordered by a band of lacunae reaching apex, the lateral veins 4–8 on each side, 2(–3) of them stronger because bordered by a band of lacunae, the inner one running along the midrib nearly up to the middle before bending out into the lamina (character inherited from *P. nodosus*), the stronger veins alternating with weaker ones, secondary veins numerous, more or less ascending, irregular at the base; petioles (7–) 25–60 (–90) mm long (Fig. 1a). Transitional leaves petiolate, with the lamina 160–224 mm long, 12–21 mm wide, 9.5–13.3 times as long as wide, translucent to semi-opaque, narrowly elliptical to elliptical, cuneate at base, narrowly obtuse at apex, entire, midrib bordered by a band of lacunae reaching apex, the lateral veins 5–8 on each side, 2 of them stronger, secondary veins numerous, more or less ascending; petioles 60–80 mm long. Floating leaves petiolate, lamina 60–170 mm long, 15–24 mm wide, 5–11 times as long as wide, coriaceous, opaque, narrowly elliptical to elliptical, green, brownish when dry, gradually tapering to the cuneate base, obtuse at apex, the lateral veins 4–8 on each side, secondary veins numerous, rather obscure, ascending in the centre of the leaf and transverse towards the margins; petioles 85–150 mm long (Fig. 1b). Stipules 50–100 mm long, open, translucent and hyaline, pale brown when dry, rounded at apex, fairly persistent, veins inconspicuous when dry, 2 of them slightly stronger than the others. Inflorescence 12–19 mm long, peduncles 75–100 mm long, terete, as thick as the stem and of uniform diameter throughout their length. Flowers numerous, in 8–14 whorls, contiguous. Fruits not observed and presumably not produced (plants sterile). Stem anatomy: Stele trio type, endodermis O-type, pseudohypodermis absent, subepidermal and cortical strands absent.

DNA sequence variability and analysis of hybrid individuals

The sequences of the ribosomal ITS region obtained by direct sequencing had 623–626 bp and included the entire ITS1, the 5.8S rDNA and a major part of ITS2. Sixty four polymorphisms were detected in the total data set, including 59 nucleotide substitutions and five single-base pair insertions/deletions (indels; Table 2). Based on the composition

Table 2. – Polymorphism of the ITS sequences from the *Potamogeton* species and hybrid individuals of *P.* ×*subobtusus* examined. * – Presence and absence of a single base in the additive pattern. Polymorphic nucleotide sites are coded using the IUPAC code.

Species	Position in the alignment																												
	22	25	37	48	51	53	62	80	87	97	149	169	178	189	200	201	214	216	228	230	241	370	415	420	421	425	426	428	431
P. alpinus	T	C	A	A	A	C	G	A	C	C	C	G	G	G	A	A	G	ACT	T	TT–	A	C	G	C	T	T	T	A	T
P. gramineus	T	C	A	A	G	A	G	A	C	T	C	G	T	G	A	T	G	ACT	C	–GA	G	C	G	C	A	T	C	A	C
P. crispus	C	C	T	A	G	A	G	A	C	T	C	A	G	C	A	T	T	TTA	T	–GA	G	T	A	T	C	T	T	T	C
P. lucens	T	C	A	A	G	A	G	A	C	T	C	G	G	G	A	T	G	ACT	C	–GA	G	C	G	C	A	T	C	A	C
P. perfoliatus	T	T	A	T	C	A	T	T	T	C	T	T	G	G	A	T	G	ACT	T	–GA	A	C	T	C	C	A	C	A	T
P. nodosus	T	T	A	A	C	A	T	A	C	C	C	T	G	G	T	T	G	ACT	T	TGA	A	C	C	C	C	T	C	A	T
P. nodosus	T	T	A	A	C	A	T	A	C	C	C	T	G	G	T	T	G	ACT	T	TGA	A	C	C	C	C	T	C	A	T
P. ×*subobtusus*	T	Y	A	A	M	M	K	A	C	C	C	K	G	G	W	W	G	ACT	T	TK*	A	C	S	C	Y	T	Y	A	T

Species	Position in the alignment																												
	432	434	437	438	440	445	447	458	470	481	487	491	496	499	502	518	532	535	545	563	569	570	571	574	576	581	586	609	610	623
P. alpinus	C	–	C	T	C	G	T	A	C	CC	G	–	C	C	A	T	A	T	G	T	T	C	T	G	A	C	T	G	T	G
P. gramineus	C	C	T	C	C	G	C	A	T	CC	T	–	C	C	A	C	A	T	G	C	C	C	T	A	A	C	G	G	C	G
P. crispus	T	–	T	T	C	A	C	A	T	TT	T	T	T	G	A	T	A	–	G	C	C	C	T	A	A	T	G	G	T	C
P. lucens	C	C	T	C	C	G	C	A	T	CC	T	–	C	C	A	T	A	T	T	C	C	C	C	A	A	C	G	G	C	G
P. perfoliatus	C	–	T	T	C	G	T	A	T	CC	T	–	C	C	G	T	A	T	G	T	C	T	T	G	A	C	G	A	T	G
P. nodosus	C	–	T	T	G	G	C	G	T	CC	T	–	T	C	R	T	T	T	G	T	C	T	T	A	G	C	A	G	T	G
P. nodosus	C	–	T	T	G	G	C	G	T	CC	T	–	T	C	A	T	T	T	G	T	C	T	T	A	G	C	A	G	T	G
P. ×*subobtusus*	C	–	Y	T	S	G	Y	R	Y	CC	K	–	Y	C	A	T	W	T	G	T	Y	Y	T	R	R	C	W	G	T	G

of polymorphisms and specific polymorphisms all the species analyzed were clearly differentiated. All representatives of each taxon had identical sequences and did not show any additive, intra-individual polymorphism, except for *P. nodosus*, where one accession had one intra-individual single nucleotide polymorphism (A/G) at alignment position 502, while other samples had A. Both of the accessions of the putative hybrid examined had identical sequences. They displayed an additive polymorphism at 25 sites: 24 single nucleotide polymorphisms and one indel causing a single-base pair shift in the overlapping ribotype sequences. The comparative analysis of the hybrid sequence with that of putative parental taxa unambiguously confirmed that the two parental taxa were *P. alpinus* and *P. nodosus* (Table 2). The hybrid taxa had a consistently additive sequence pattern derived from both these two taxa and displayed no polymorphisms at other sites on the sequences. Based on their specific polymorphisms, all the other *Potamogeton* species studied were excluded as potential parental taxa of the hybrid. In sequences from the hybrid plants the variants from *P. nodosus* dominated over those from *P. alpinus,* but both peaks were clearly distinguishable.

Both cpDNA regions examined were polymorphic. The sequences of *rps*12-*rpl*20 intergenic spacer were 734–771 bp long in the whole data set. This region contained six single nucleotide polymorphisms and four insertions/deletions (duplications). The sequences of the *rpl*32-*trn*L intergenic spacer were 732–758 bp long. In the samples of all the taxa examined 23 polymorphic sites were identified. They included 19 single nucleotide substitutions and four insertions/deletions: two A/T stretch length polymorphisms and two duplications of 7 and 14 bp (see also Zalewska-Gałosz et al. 2010). The polymor-

Fig. 1. – *Potamogeton* ×*subobtusus* from the river Rospuda, Poland; a – specimen with only submerged leaves, b – specimen with floating and submerged leaves; scale bar = 1 cm.

b


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HERBARIUM INSTITUTI ET HORTI BOTANICI UNIVERSITATIS JAGELLONICAE CRACOVIENSIS
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HERBARIUM INSTITUTI ET HORTI BOTANICI
UNIVERSITATIS JAGELLONICAE CRACOVIENSIS (KRA)
Potamogetonaceae
Potamogeton ×subobtusus Hagstr.
[= P. alpinus Balb. × P. nodosus Poir.]
NE Poland, Pojezierze Suwalskie lakeland, Rospuda River near Augustów below the site called Święte Miejsce
Coordinates (WGS 84): 53°56'56"N, 22°52'27"E
Date 25.07.2008
leg. J. ZALEWSKA-GAŁOSZ
det. J. ZALEWSKA-GAŁOSZ, M. RONIKIER

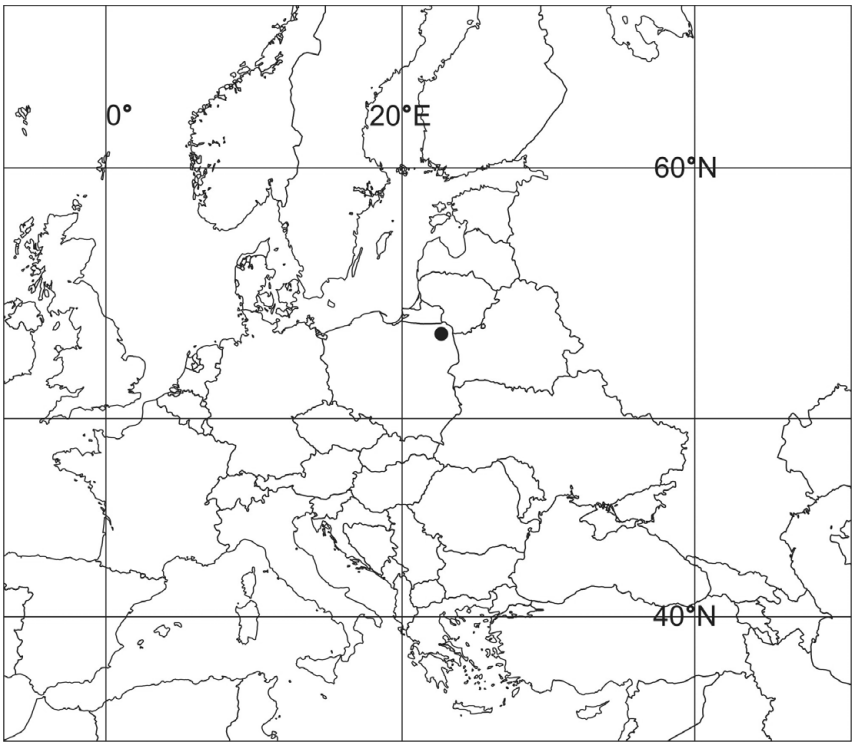

Fig. 2. – Location of the first European site of *Potamogeton ×subobtusus* in NE Poland.

phism was distributed among taxa, and no intraspecific variation was observed. In both regions examined the hybrid plants shared the identical cpDNA haplotype with *P. nodosus*, clearly indicating that this species is the maternal taxon. The distinction between *P. nodosus* and *P. alpinus* was based on two single nucleotide substitutions in *rps*12-*rpl*20, while there was as many as 14 polymorphisms in *rpl*32-*trn*L in the parental taxa: 11 single nucleotide substitutions, one 4-nucleotide length difference in a poly-A stretch and two duplications (14- and 7-bp long) in *P. alpinus*.

Locality and habitat

The new locality, the only one besides the locus classicus in North America, is in the river Rospuda, Pojezierze Suwalskie lakeland, NE Poland (Fig. 2). Individuals of *P. ×subobtusus* grow where there is a sandy bottom and running water from 0.40 to 1.5 m deep. Population is abundant and spread over a distance of about 8 km, with the highest density of individuals in and below the site called Święte Miejsce (N 53°56'56", E 22°52'27"). In that part of the river, the community is made up of different synusiae. Locally dominant taxa, besides *P. ×subobtusus*, include *P. pectinatus*, *P. perfoliatus*, *P. natans*, *Butomus umbellatus*, *Sagittaria sagittifolia* and *Batrachium fluitans*. Together with *P. ×subobtusus*, another very rare *P. nodosus* hybrid, *P. ×assidens* also grows in the river (Zalewska-Gałosz et al. 2010). None of the parental taxa are recorded from this part of the river Rospuda.

Nomenclature and typification

The only binomial proposed for the hybrid *P. alpinus* × *P. nodosus* is *P.* ×*subobtusus* (Hagström 1916). The taxon has been cited in the literature only exceptionally. It was listed by Ogden (1943) and Haynes & Hellquist (2000) but they did not investigate the original material and these sources only refer back to Hagström (1916). The identity of the name may therefore be considered doubtful.

Duplicates of the type collection of *P.* ×*subobtusus* were discovered in G, K, UPS and W. Each of the herbaria sheets bears one to several sterile plants, which belong to the hybrid, and one fruiting fragment of *P. alpinus*. Hagström (1916) clearly distinguished these two taxa and that is why the name *P.* ×*subobtusus* refers solely to the sterile specimens. We agree with Hagström that these sterile plants are the hybrid *P. alpinus* × *P. nodosus*. The duplicate preserved in UPS is cited in the protologue and was annotated by J. O. Hagström on 20th August 1906, i.e. prior to the publication of the name. Therefore, it is here selected as the lectotype (the herbarium sheet was labelled accordingly by ZK in 2003):

Potamogeton* ×*subobtusus Hagstr., Kungl. Svenska Vetenskapsakad. Handl. 55/5: 147, 1916. (= *P. alpinus* × *P. nodosus*)

Type: "Ex Herb. E. Tuckerman jun[is.], Potam. rufescens, Nov. Ebor." (**lectotype designated here**: UPS; isolectotypes: G, K, W).

The origin of the specimens is indicated by Tuckerman as "Nov. Ebor.", i.e. an abbreviation for Novum Eboracum, which is the Latinized designation for New York. By this Tuckerman meant the state of New York, U.S.A., not the city.

Discussion

Hybridization is an important source of diversity in the genus *Potamogeton*. Most *Potamogeton* hybrids are sterile but their ability to actively propagate vegetatively means they are persistent elements of ecosystems and important components of local floras. It is known that some hybrids have persisted at a locality for one hundred years (Kaplan & Fehrer 2007) or more, as in the case of *P.* ×*bottnicus* Hagstr. (= *P. pectinatus* × *P. vaginatus*) in Great Britain and *P.* ×*fennicus* Hagstr. (= *P. filiformis* × *P. vaginatus*) in continental Europe (Preston et al. 1998, Bobrov 2007).

Recognition of hybrid individuals of *Potamogeton*, based on morphological and anatomical characters, is sometimes relatively easy; in contrast, the reliable identification of their parental taxa is difficult, even for a taxonomist who specializes on *Potamogeton*. Biochemical and molecular techniques can be used to resolve such difficult cases. Recently, using this kind of approach, a few rare *Potamogeton* hybrids were recognized (e.g., Kaplan & Fehrer 2004, Kaplan & Wolf 2004), evidence for a natural triple hybrid obtained (Kaplan & Fehrer 2007) and hybrids new to science described based on data from direct sequencing (Kaplan et al. 2009, Zalewska-Gałosz et al. 2010). Sequence analysis also proved to be an appropriate tool to provide unequivocal evidence of the genetic composition also in the case of *P.* ×*subobtusus*. Thanks to several polymorphic sites and a clear additive pattern, the identification of the parental sequences was straightforward. Interestingly, in both individuals sequenced the ITS variants from *P. nodosus* markedly prevailed over those from *P. alpinus.* The former species was identified as the maternal parent of the

hybrid based on cpDNA data and it also dominated the expression of morphological features in the hybrid plants. This maternally driven expression at both the morphological and molecular levels confirms previous observations on another *Potamogeton* hybrid and supports our hypothesis that genetic mechanisms may exist that are responsible for the dominance of the maternal lineage in some hybrid taxa (Zalewska-Gałosz et al. 2010). It is likely that the wider use of molecular surveys in *Potamogeton* taxonomy will reveal not only new *Potamogeton* hybrids but also help to define the ranges in the variability of taxa and explain mechanisms controlling variation among taxa, such as introgression.

As already emphasized in the Introduction, *P. nodosus* is regarded as a species that rarely hybridizes. Only one hybrid, *P. ×schreberi* (= *P. natans* × *P. nodosus*), is widely recognized and recorded from Europe: Great Britain, Germany, France, Switzerland, Russia (Kaplan & Wolf 2004) and the Czech Republic (Z. Kaplan & J. Fehrer, unpublished data). Other confirmed hybrids of this species are *P. ×rectifolius* A. Benn. (= *P. nodosus* × *P. richardsonii*; Zalewska-Gałosz et al. 2010), *P. ×subobtusus* Hagstr. (Hagström 1916 and this study) and *P. ×subrufus* Hagstr. (= *P. lucens* × *P. nodosus*; Zalewska-Gałosz 2010). Recently, another *P. nodosus* hybrid was described, namely *P. ×assidens* (= *P. nodosus* × *P. perfoliatus*; Zalewska-Gałosz et al. 2010). All these hybrids are extremely rare, known only from one or few localities. It is known that some *Potamogeton* species may have a restricted tendency to cross interspecifically compared to other representatives of the genus, due to biological, ecological and genetic properties. Thus, they rarely produce hybrids. It is also possible that the great polymorphism of *P. nodosus* (Wiegleb & Kaplan 1998) makes it very difficult to recognize its hybrids. Therefore, it is essential that the identification of *P. nodosus* hybrids should be supported by biochemical or molecular studies. Such an approach will unequivocally enable botanists to define the number of *P. nodosus* hybrids.

Acknowledgements

We are greatly indebted to Maciej Kozak who collected the "strange" *Potamogeton nodosus* from the river Rospuda in 2005 and by this discovered the first locality of *P. ×subobtusus* in Poland. Piotr Osyczka took the photographs of herbarium specimens used in this study. Our thanks are also due to two anonymous reviewers for their critical comments of the manuscript. Tony Dixon kindly improved the English of the manuscript. J. Z-G. & M.R. were financially supported by grant no. N303 098 32/3404 from the Polish Ministry of Science and Higher Education. Z.K. was supported by grant 206/09/0291 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. The visits of Z.K. to collections and libraries of the Botanical Museum of the University of Copenhagen, the Naturhistorisches Museum Wien, the Royal Botanic Garden Edinburgh and the Nationaal Herbarium Nederland in Leiden and Wageningen were supported by the European Commission's Integrated Infrastructure Initiative programme SYNTHESYS.

Souhrn

K odhalení taxonomické identity předpokládaného křížence rdestů nalezeného v řece Rospuda v severovýchodním Polsku byla použita kombinace tří metod. Detailní analýza morfologických znaků a způsobu uspořádání anatomie lodyhy umožnila provizorní určení studovaných rostlin jako *Potamogeton ×subobtusus*, což je kříženec druhů *P. alpinus* a *P. nodosus*. Tato teorie byla potvrzena pomocí molekulárních analýz založených na sekvencích jaderné ribozomální a chloroplastové DNA. Analýza jaderné DNA potvrdila přítomnost specifických sekvencí obou předpokládaných rodičovských druhů a zároveň vyloučila účast dalších srovnávaných potenciálních rodičovských druhů. Analýza chloroplastové DNA odhalila jako mateřskou rostlinu studovaného křížence druh *P. nodosus*. Zjištěný kříženec byl dosud znám jen z jediné lokality v Severní Americe. Článek dále přináší detailní morfologický popis tohoto křížence a typifikaci jména *P. ×subobtusus*.

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Received 24 March 2009
Revision received 22 June 2009
Accepted 23 June 2009