Potamogeton ×maëmetsiae: a new hybrid between linear-leaved pondweeds from Central Europe

***Potamogeton ×maëmetsiae* – nový kříženec úzkolistých rdestů ze střední Evropy**

Joanna Zalewska-Gałosz[1] & Michał Ronikier[2]

[1]*Institute of Botany, Jagiellonian University, Kopernika 27, PL-31-501 Kraków, Poland, e-mail: joanna.zalewska-galosz@uj.edu.pl;* [2]*Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland, e-mail: m.ronikier@botany.pl*

Zalewska-Gałosz J. & Ronikier M. (2011): *Potamogeton ×maëmetsiae*: a new hybrid between linear-leaved pondweeds from Central Europe. – Preslia 83: 259–273.

The recognition of hybrids of linear-leaved taxa of *Potamogeton* (sect. *Graminifolii*) based on morphology is difficult and often debatable. As a consequence, currently only a few hybrid taxa are considered valid and many linear-leaved hybrids described in the past are not now recognized. On the other hand, the use of molecular tools has recently allowed more efficient tests of the origin of morphological forms and the tracking of hybridization events in *Potamogeton* systematics. In this paper, *Potamogeton ×maëmetsiae* Zalewska-Gałosz et M. Ronikier nothosp. nov. (*Potamogetonaceae*), a hybrid between two linear-leaved species, *P. friesii* and *P. rutilus*, is described and illustrated. Hybrid plants were collected from two Central-European populations growing in Lake Skaidrys (Lithuania) and Soitsjärv (Estonia). The hybrid origin of the new entity was identified based on a morphological survey and independently confirmed using nuclear (ITS, 5S-NTS) and chloroplast (*rpl*32-*trn*L intergenic spacer) DNA sequence data and AFLP analysis of genetic structure. Differences between *P. ×maëmetsiae* and similar taxa are outlined and other relevant details of the new hybrid discussed.

K e y w o r d s: 5S-NTS, AFLP, aquatic plants, holotype, hybridization, ITS, molecular identification, paratype, *Potamogeton*, sequencing, taxonomy

Introduction

Interspecific hybridization is one of the important factors shaping *Potamogeton* diversity (e.g. Preston 1995, Zalewska-Gałosz 2002, Wiegleb et al. 2008, Kaplan 2010). Populations of *Potamogeton* hybrids are often vigorous and long-persistent, even in the absence of one or both parental species (Preston et al. 1998, Kaplan & Fehrer 2009, Zalewska-Gałosz 2010). The modern taxonomy of the genus focuses in particular on the molecular assessment and unequivocal confirmation of the origin of *Potamogeton* hybrids (e.g. Kaplan & Wolf 2004, Kaplan & Fehrer 2006, Kaplan et al. 2009, Zalewska-Gałosz et al. 2010). The majority of these studies, however, are on broad-leaved hybrids. Only recently, a few studies on hybridization among linear-leaved *Potamogeton* species (section *Graminifolii*) were published (Whittall et al. 2004, Les et al. 2009, Zalewska-Gałosz & Ronikier 2010). Recognition of hybrids in this group, especially if based only on morphology, is difficult because there are few distinct differences between linear-leaved species and the differences are often obscured by the phenotypic plasticity observed in this genus (Kaplan 2002). As a consequence many linear-leaved hybrids described in the past (e.g. Ascherson & Graebner 1907, Hagström 1916) are currently not recognized or debatable

(Wiegleb & Kaplan 1998) and are not included in the modern taxonomy of the genus. The marked difference in the proportions of described and confirmed hybrids between broad-leaved and linear-leaved groups of *Potamogeton* indicates that the knowledge on the extent of hybridization and taxonomy of existing linear-leaved hybrids is rudimentary and a comprehensive assessment of hybridization in this group of species is required.

In the present paper, untypical morphotypes of linear-leaved pondweeds from Central Europe were analysed in order to determine their origin and character. The plants were discovered during the sampling of *Potamogeton rutilus* in Lake Skaidrys (Lithuania). A careful morphological examination indicated that they were not typical ("pure") *P. rutilus*. General habit, very strong lateral veins and bristle-like apices to at least some of the leaves (mostly those on the main stem), suggested an affinity with individuals of *P. rutilus* but the plants also displayed characters typical of *P. friesii* and/or *P. pusillus*, such as the presence of obscurely mucronate leaf apices and well developed nodal glands. The intermediate morphological appearance of the plants indicated they were of hybrid origin. However, it was impossible to demonstrate this and identify the parental species based on morphology because the putative parental taxa are very similar. During a revision of the genus *Potamogeton*, led by the first author in 2008 in Estonia (Herbaria TAA, TAM, TU, EMHC), another collection of plants morphologically intermediate between the species listed above was recognized. The putative hybrid was collected in Lake Soitsjärv (Estonia) by Aime Mäemets on 13 July 1972 and tentatively identified as: "P. friesii (?) (rutilus?)".

Among the species morphologically close to the putative hybrid phenotype, *Potamogeton friesii* and *P. rutilus* are relatively common in Baltic countries. In contrast, *P. pusillus* is a very rare species in Lithuania and rare in Estonia (Mäemets 1984, Trei et al. 2003). Moreover, it was not observed either at the localities of the plants studied or in their close vicinity (J. Zalewska-Gałosz, personal observation). Thus, it is unlikely that *P. pusillus* is a parental species of this putative hybrid, even though it could not definitively be discounted on morphological grounds. Taking into account the morphological features and probability of a hybridization event it was assumed that the deviating morphotypes found at the two localities represent a hybrid between *P. friesii* and *P. rutilus*. It is noteworthy that a hybrid between these taxa was earlier described by Galinis (1963) but the author did not provide any details of the material or designate the type specimen and therefore according to the Art. 37.1 of ICBN this taxon has not been validly published (McNeill et al. 2006).

The specific aims of the present study are: (i) to provide a detailed morphological analysis of the putative hybrid *Potamogeton friesii* × *P. rutilus* collected in Lithuania and Estonia, and (ii) to analyse a set of molecular characters of plants potentially of this hybrid taxon, together with those of individuals of all the potential parental species and to unequivocally resolve the taxonomic status of the plants studied.

Material and methods

Plant material used for molecular analyses

In the sequence analysis, two samples of putative hybrids, morphologically intermediate between *Potamogeton friesii*, *P. pusillus* and *P. rutilus*, were analysed. As a reference, 11 generative individuals of potential parental species were also sequenced. Data on all the

Table 1. – Samples of *Potamogeton* taxa included in the DNA study, their reference numbers and geographical origin. Samples sequenced are marked in bold; remaining numbers refer to samples included in the AFLP analysis.

Taxon	DNA sample no.	Geographical origin	Coordinates (N, E)
P. friesii	**143**; 141–144, 192, 193	E Lithuania, Lake Baltys, coll. J. Z-G.	55°21'9.9" 26°10'57.6"
P. friesii	87, 88, 124	E Poland, Lake Bikcze, coll. J. Z-G.	51°22'49.32" 23°3'3.37"
P. friesii	**85**; 85, 86, 123	E Poland, Lake Glinki, 18 Jun 2005, coll. J. Z-G.	51°30'13" 23°33'22"
P. friesii	79	E Lithuania, Lake Metelys, coll. J. Z-G.	54°17'59.8" 23°45'56.8"
P. friesii	90	NW Poland, Lake Kramsko, coll. J. Z-G.	54°3'13" 17°56'21"
P. friesii	173, 174	NE Poland, Lake Sajno, coll. J. Z-G.	53°49'24.6" 22°58'40.88"
P. friesii	106	NE Poland, Lake Studzieniczno, coll. J. Z-G.	53°51'56.9" 23°7'11.20"
P. friesii	141–143, 192, 193	E Lithuania, Lake Baltys, coll. J. Z-G.	55°21'9.9" 26°10'57.6"
P. friesii	**145**; 57, 80, 145, 146, 194	E Lithuania, Lake Ilgis, coll. J. Z-G.	55°20'54.9" 26°10'49.8"
P. friesii	**177**; 175–177	E Lithuania, Lake Lakajai, coll. J. Z-G.	55°13'00" 25°36'00"
P. obtusifolius	**216**	SE Estonia, Lake Viitina, coll. J. Z-G.	57°42'21.9" 27°03'24.8"
P. pusillus	**287**	NE Poland, river Rospuda, coll. J. Z-G.	54°3'49.60" 22°41'35.49"
P. pusillus	**288**	SE Poland, Nisko, coll. J. Z-G.	50°32'59.81" 22°7'14.20"
P. pusillus	**289**	W Ukraine, a peat bog near village Yastrubychi, coll. L Borsukievicz	50°17'55,1" 24°18'12,2"
P. rutilus	**136**; 134–137, 239–246	SE Estonia, Plaani, Küläjärv, J. Z-G.	57°40'44.1" 27°04'34.4"
P. rutilus	**93**; 91–93, 235–238	E Poland, Lake Rotcze, coll. J. Z-G.	51°22'26.02" 23°6'45.58"
P. rutilus	**158**; 59, 78, 157–160	E Lithuania, Lake Ilgis, coll. J. Z-G.	55°20'54.9" 26°10'49.8"
P. rutilus	**153**; 66, 128–130, 153–156	E Lithuania, Lake Baltys, coll. J. Z-G.	55°21'9.9" 26°10'57.6"
P. rutilus	65	E Lithuania, Lake Lakajai, coll. J. Z-G.	55°13'00" 25°36'00"
P. rutilus	107, 116, 226–230, 234, 253, 255,257, 258, 260	SE Estonia, Plaksi, Tuuljärv, coll. J. Z-G.	59°39'20.4" 25°40'19.0"
P. rutilus	113, 118, 225	S Estonia, Valgjärv, coll. J. Z-G.	58°05'09.1" 26°38'40.2"
P. rutilus	111, 117, 119,120, 249–252	SE Estonia, Lake Viitina, coll. J. Z-G.	57°42'21.9" 27°03'24.8"
P. ×maëmetsiae	**67**; 68–77	E Lithuania, Lake Skaidrys, coll. J. Z-G.	55°35'52.1" 26°21'15.5"
P. ×maëmetsiae	**218**	E Estonia, Lake Soitsjärv, coll. A. Mäemets	58°33" 26°41'00"

specimens studied are summarized in Table 1. Additionally, because of small morphological differences among the linear-leaved pondweed taxa, two other species representing this group not considered to be parental taxa, namely *P. obtusifolius* and *P. berchtoldii*, were included in this study as a comparison. In the case of *P. berchtoldii*, a sequence published in GenBank (accession number GQ 247403.1) was used.

In the AFLP analysis, 25 samples of *Potamogeton friesii* collected from nine populations, 59 samples of *P. rutilus* from eight populations and 11 samples of putative hybrid plants from Lake Skaidrys were analysed. Herbarium specimen from Estonia was not included because the DNA extracted from this material was of poor quality. In order to detect potential intra-specific genetic polymorphism, individuals of parental species were gathered from several distant populations in Poland, Lithuania, Estonia and Ukraine (Table 1).

The majority of the samples were of fresh material collected in the field and stored in plastic tubes with silica gel. Reference herbarium specimens were prepared for each DNA sample and deposited in the Herbarium of the Institute of Botany, Jagiellonian University, Krakow (KRA). The material used to obtain the extract of DNA of the Estonian individual

of the putative hybrid was taken directly from the herbarium specimen preserved in the Hydrobiological Collection of the Estonian University of Life Sciences, Rannu (EMHC). Taxonomic delimitation of species, hybrid formulas and nomenclature of all taxa follow Wiegleb & Kaplan (1998) except for *P. pusillus* agg., which was later refined and follows Kaplan & Štěpánek (2003).

Molecular analyses

The taxonomic assessment of the plants studied was based on the sequencing of selected nuclear and chloroplast regions of putative hybrids and potential parental taxa. In a second step, a set of molecular characters generated using AFLPs (Amplified Fragment Length Polymorphism; Vos et al. 1995) was analysed for individuals of the putative hybrid and the parental species earlier defined in a sequencing analysis, for testing the genetically intermediate character of the taxon between *P. friesii* and *P. rutilus*, in a population genetic framework.

Total DNA was extracted from ca 10 mg of dried plant material using Mixer Mill 300 (Retsch) and the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol (final elution step was carried out using 2 × 50 µL elution buffer). DNA quality and concentration were estimated against λ-DNA on 1% agarose gel stained with ethidium bromide. Two regions of the nuclear ribosomal DNA were examined by direct sequencing: the internal transcribed spacer (ITS) and the 5S non-transcribed spacer (5S-NTS). Additionally, the chloroplast intergenic spacer *rpl*32-*trn*L (Shaw et al. 2007), the most variable cpDNA region according to previous analyses of *Potamogeton* (Zalewska-Gałosz et al. 2009, 2010), was analysed. PCR amplification of the ITS region and the chloroplast intergenic spacer *rpl*32-*trn*L and sequencing of all the DNA fragments investigated were done as previously described (Zalewska-Gałosz et al. 2009, 2010). The nuclear ribosomal 5S non-transcribed spacer region was amplified using the primers PI and PII (Cox et al. 1992). This region was previously used for species-level studies in plants (Cox et al. 1992) and a molecular phylogenetic *Potamogeton* study (Lindqvist et al. 2006). The following reaction composition was applied in a total volume of 25 µl: 1× concentration of PCR Taq Buffer (Roche Diagnostics), 0.18 mM of each dNTP (Roche Diagnostics), 1 µM of each primer, 1 µg of bovine serum albumin (BSA), 1 U of the Taq DNA Polymerase (Roche Diagnostics) and 2 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. PCR reaction was carried out twice and the PCRs were pooled for the final reaction volume of 50 µl. Multiple amplification products (different sizes of nrDNA repeats) were isolated by electrophoresis in 2% agarose gel (Prona Agarose Plus, Belgium) in 1×TBE buffer with addition of ethidium bromide (0.5 µg/ml). 50 µl of PCR mixture were separated for 1 h at 10 $V \cdot cm^{-1}$ and visualized using an ultraviolet transilluminator. Repeats ca 350 bp long, isolated from adjacent contaminated fragments, were cut from the gel and purified using High Pure PCR Product Purification Kit (Roche Diagnostics). Sequences were manually edited, aligned in BIOEDIT v.5.0.9. (Hall 1999) and submitted to GenBank under the following numbers: HQ850984–HQ850994 for 5S-NTS, HQ850995–HQ851005 for *rpl*32-*trn*L and HQ851006– HQ851017 for ITS.

The AFLP analysis followed the procedure of Vos et al. (1995) with modifications as described in detail by Ronikier et al. (2008). Double-digestion of DNA was performed using *Eco*RI and *Mse*I enzymes. Subsequently, double-stranded *Eco*RI/*Mse*I adapters were ligated to the digested DNA using T4 DNA ligase. Polymerase chain reaction (PCR) amplifications were performed on a GeneAmp 9700 thermal cycler (Applied Biosystems). Preselective PCR used *Eco*RI-A and *Mse*I-C primers. Subsequently, selective PCR reactions were performed using 5'-fluorescence-labelled *Eco*RI selective primers (6-FAM). Selective amplification products were separated using 36-cm capillaries and POP 4 polymer (Applied Biosystems) with Genescan-500 ROX (Applied Biosystems) internal size standard on an ABI PRISM 3100-*Avant* sequencer (Applied Biosystems). At the preliminary screening step, 12 selective primer pair combinations were tested and evaluated for clarity of profiles (i.e. prevalence of well separated markers), and number and repeatability of polymorphic markers. Two primer pairs were selected for the complete analysis: *Eco*-ACC/*Mse*-CAG and *Eco*-ACA/*Mse*-CAT.

AFLP fragments were manually scored using Genographer 2.1 (http://sourceforge.net/projects/genographer). To assess the quality and repeatability of AFLP profiles, six samples were replicated (based on single DNA extractions) and carried in parallel through all reaction steps. Only markers that scored unambiguously (i.e. were well separated) and repeatedly in the duplicates were considered. AFLP fragments in the size range of 50–500 bp were scored and assembled in a binary presence/absence matrix. The specific bands characteristic for *P. friesii* and *P. rutilus*, and those of hybrid individuals and shared with either of the parental taxa were calculated. Genetic relationships among individual genotypes were estimated using a Principal Coordinate Analysis (PCoA) based on Jaccard's similarity coefficient and performed using FAMD 1.25 software (Schlüter & Harris 2006). Genetic composition of individuals was also examined using Bayesian inference, applying the recessive allele model for dominant markers, as implemented in STRUCTURE 2.2 (Falush et al. 2007). To assess the influence of parental taxa on the genetic structure of the hybrid's genome, the admixture model with correlated allele frequencies was used. The number K = 2 (for two parental taxa) followed by 3–10 were tested with 10 replicates per K. 1×10^6 Markov Chain Monte Carlo repetitions were applied with a burn-in period of 200,000. Outputs of all STRUCTURE runs were analysed using R-script Structure-sum (Ehrich 2006). Average similarity coefficients among runs were calculated for each K analysed to verify the consistency of replicated runs. The following values were observed in order to assess the most appropriate number of clusters: (i) the lnP(D) values, estimates of posterior probabilities provided in STRUCTURE outputs, examined as a function of increasing K; (ii) ΔK values, estimating the change of the likelihood function with respect to K and estimated as an indicator of the most reliable clustering structure (Evanno et al. 2005).

Morphological analysis

The description of *Potamogeton* ×*maëmetsiae* (= *P. friesii* × *P. rutilus*) was based on specimens collected from two localities: Lake Skaidrys in Lithuania (one herbarium sheet deposited in KRA) and Lake Soitsjärv in Estonia (one herbarium sheet deposited in EMHC). Morphological characters of stem, leaves, stipules, inflorescences, peduncles and flowers were measured or qualitatively described. For each of 15 individuals identified on the two herbarium sheets, up to 25 features were examined.

Results

Variation in the ITS sequences and analysis of hybrid individuals

The ITS sequences obtained from the samples varied between 666 and 682 bp. Nine polymorphisms were detected in the total data set, including seven nucleotide substitutions and two insertions/deletions (1 bp and 15 bp; Table 2). Samples from the same species had identical sequences and did not have any additive, intra-individual polymorphism. The sequence of *P. berchtoldii* was the most divergent as it differed from the remaining samples at almost all polymorphic sites (compare Table 2). Sequences of *P. friesii*, *P. pusillus*, *P. rutilus* and the putative hybrid were identical. Samples of *P. obtusifolius* differed from this group by three substitutions (Table 2). While the morphologically intermediate plants were shown to be closely related to the three above-mentioned species, it was impossible, based on the ITS sequences, either to confirm or exclude that they were hybrid taxa. Therefore, in the next step another DNA region, the rapidly evolving nuclear ribosomal 5S non-transcribed spacer (5S-NTS), was used to differentiate *P. friesii*, *P. pusillus* and *P. rutilus*.

Variation in the 5S non-transcribed spacer and analysis of hybrid individuals

5S-nrDNA repeats of at least four different sizes were produced in the PCR amplification. The banding pattern consisted of fragments ca 350, 750, 1150 and 1500 base-pairs long and was similar for each taxon analysed. The sequences obtained from the shortest intergenic spacer were 298 bp long and covered almost the whole NTS region. Alignments were highly polymorphic and the polymorphic sites were prevalently species-specific. Altogether, 21 polymorphic sites (substitutions) were detected in the alignments of *P. friesii*, *P. pusillus* and *P. rutilus* sequences. Two examined accessions of the putative hybrid, from both populations, had identical sequences and displayed a rigorously additive polymorphism at 11 diagnostic positions between *P. friesii* and *P. rutilus*, revealing these two species as parental taxa (Table 3).

Variation in cpDNA and identification of the maternal species

The sequences of the *rpl*32-*trn*L intergenic spacer varied between 732 and 747 bp. Among samples of all the taxa studied, seven polymorphic sites (substitutions) were identified. All species had diagnostic polymorphisms and showed no intraspecific variation. *Potamogeton friesii* differed from *P. rutilus* in all seven substitutions and from *P. pusillus* in only one (alignment position 208). *Potamogeton pusillus* differed from *P. rutilus* in six polymorphisms (Table 4). Based on this polymorphism pattern *P. friesii* was designated as the maternal species of the hybrid in both samples.

AFLP inference of the hybrid genetic structure

AFLP fingerprinting of *Potamogeton friesii*, *P. rutilus* and the hybrid individuals resulted in good quality profiles; reproducibility of markers, based on evaluation of entire duplicated profiles, averaged out at 96.85%. Two selective primer combinations yielded 81 good quality markers, 76 (93.83%) of which were polymorphic. Profiles of all taxa consisted of comparable numbers of markers (*P. friesii* – 49; *P. rutilus* – 52; hybrid plants – 54).

Table 2. – Polymorphism of the ITS sequences from *Potamogeton berchtoldii*, *P. friesii*, *P. obtusifolius*, *P. pusillus*, *P. rutilus* and hybrid individuals of *P.* ×*maëmetsiae* (with DNA sample reference numbers).

		Position in the alignment							
Taxon	DNA sample no.	47	135	233	244	434	455	584	586–600
P. berchtoldii	GQ247403.1	C	C	G	A	C	T	C	TGGGCATCTTCGTCC
P. friesii	85, 143,145	T	A	T	–	T	C	C	———————
P. obtusifolius	216	T	A	G	–	T	T	T	———————
P. pusillus	287, 288, 289	T	A	T	–	T	C	C	———————
P. rutilus	93, 153, 158	T	A	T	–	T	C	C	———————
P. ×*maëmetsiae*	67, 218	T	A	T	–	T	C	C	———————

Table 3. – Polymorphism of the 5S-NTS sequences from *Potamogeton friesii*, *P. pusillus*, *P. rutilus* and hybrid individuals of *P.*×*maëmetsiae* (with DNA sample reference numbers). Polymorphic nucleotide sites are coded using the IUPAC code. Diagnostic positions are marked in bold.

		Position in the alignment										
Taxon	DNA sample no.	10	25	28	30	33	40	56	61	88	119	123
P. friesii	85, 145, 177	C	A	T	S	Y or C	S	G	S or C	T	R or G	R or G
P. pusillus	287, 288, 289	S	W	G	G	T	C	A	C	W	A	G
P. rutilus	93,136, 158	C	A	G	G	T	C	A	C	A	A	G
P. ×*maëmetsiae*	67, 218	C	A	K	G	Y	C or S	R	C	W	R	G

		Position in the alignment									
Taxon	DNA sample no.	137	138	**151**	155	**156**	162	184	197	**243**	257
P. friesii	85, 145, 177	C	R or G	G	T	T	T	R or G	G	G	T
P. pusillus	287, 288, 289	Y or C	G	C or S	Y or C	T	T	G	C	G	T
P. rutilus	93,136, 158	Y or C	G	B	C	Y	Y or C	R or G	C	C or S	A
P. ×*maëmetsiae*	67, 218	Y	G	B	Y	Y	Y	R or G	S	S	W

Table 4. – Sequence variation in the *rpl*32-*trn*L intergenic spacer from *Potamogeton friesii*, *P. pusillus*, *P. rutilus* and hybrid individuals of *P.* × *maëmetsiae* (with DNA sample reference numbers). The position diagnostic for *P.* × *maëmetsiae* is marked in bold.

		Position in the alignment						
Taxon	DNA sample no.	124	146	**208**	213	277	562	598
P. friesii	85, 143,145	C	A	G	C	A	A	T
P. pusillus	287, 288, 289	C	A	T	C	A	A	T
P. rutilus	93, 153, 158	G	C	T	A	G	C	G
P. × *maëmetsiae*	67, 218	C	A	G	C	A	A	T

Considering parental species, *P. friesii* and *P. rutilus* were characterized by 29 and 32 unique markers (i.e. those present only within a given taxon), respectively. Among them, 11 and 13 markers were fixed for either *P. friesii* or *P. rutilus.* All hybrid individuals had the same profile, suggesting clonal origin. Hybrid genotypes did not have any private markers. They shared 17 markers with both parental species and had 17 specific markers of *P. rutilus* and 20 of *P. friesii*. Accordingly, the PCoA analysis demonstrated an intermediate

character of the hybrid individuals between the groups formed by parental taxa (differentiation of species along the 1st axis accounted for over 70% of the variation; Fig. 1). Bayesian admixture analysis of the genetic structure at K = 2 assigned all individuals of two parental taxa to their respective groups while the intermediate character of hybrid plants of *Potamogeton ×maëmetsiae* was clearly supported by their nearly equal contributions from each of the parental groups (Fig. 2). The likelihood of the data strongly increased for K = 2. It showed a slight further increase until K = 5 but the average similarity coefficient for each K was high only for K = 2 (0.9995) and was substantially less (below 0.5) for all remaining K values. Accordingly, the modal value of the ΔK distribution clearly indicated K = 2 as the best configuration (Fig. 2A).

Morphological description of the hybrid plants

Stem up to 0.40 m long, compressed, with few, short axillary branches near the base; nodal glands well-developed or absent. Submerged leaves sessile, linear 44–52 mm long, 0.7–1.5 mm wide, 27.6–49.0 times as long as wide, bright green or olive-green, entire and plane at margin, bordered by a marginal vein, cuneate at the base, a part of gradually tapering to a very finely pointed apex (bristle like), the others obscurely mucronate, midrib prominent occupying 15–30% of the leaf width near the base, not bordered by lacunae or bordered by a narrow band restricted to the base of the leaf, the lateral veins two on each side, distinct, the outer one reaching 2/3 to 3/4 of the leaf length, secondary veins infrequent. Floating leaves absent. Stipules 11–14 (–17) mm long, tubular when young but splitting with age, milky white and translucent when fresh, white and opaque when dry, persistent but the apex eroding to fibrous strands, with a green rib along each side. Generative organs were not seen.

Selected diagnostic morphological features of *Potamogeton ×maëmetsiae* are compared with adequate characters of *P. friesii*, *P. pusillus* and *P. rutilus* (Table 5).

Table 5. – Comparison of selected morphological characters of *Potamogeton friesii*, *P. pusillus*, *P. rutilus* and *P. ×maëmetsiae* (after Preston 1995, Wiegleb & Kaplan 1998).

	P. friesii	*P. pusillus*	*P. rutilus*	*P. ×maëmetsiae*
Branching pattern	sparingly branched; axillary branches short	richly branched; axillary branches mainly long	not branched to sparingly branched; axillary branches short	sparingly branched; axillary branches short
Nodal glands	well-developed	absent or poorly developed	absent	absent or well-developed
Number of lateral veins on one side of the midrib	2	1 (–2)	1 (–2)	2 (the outer one reaches up to 3/4 of the leaf length)
Secondary veins	infrequent	absent	absent	infrequent
Leaf apex	mucronate	variable but mostly acute	acuminate, bristle-like	acuminate, bristle-like, obscurely mucronate
Veins on stipules	prominent when dry	inconspicuous when dry	prominent when dry	prominent when dry

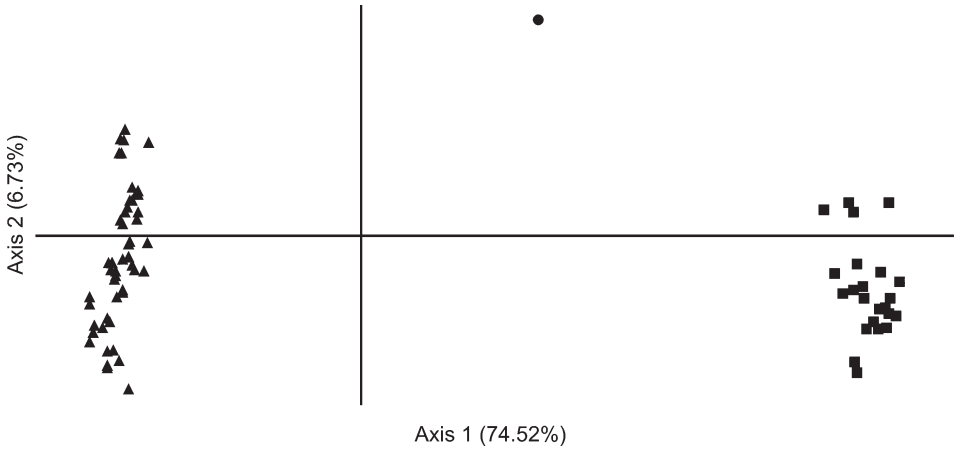

Fig. 1. – Principal coordinate analysis (PCoA) of 25 samples of *P. friesii* (■), 59 samples of *P. rutilus* (▲) and 11 samples of *P.* ×*maëmetsiae* (●) based on Jaccard's similarity among AFLP phenotypes.

The new hybrid taxon and its diagnostic features

Morphological and molecular features provide evidence for the hybrid origin of the plants discovered in Lithuania and Estonia, with *Potamogeton friesii* and *P. rutilus* as parental species. As this taxon has not previously been validly described (see Introduction), it is described here based on the data presented, as a new hybrid according to ICBN rules. Two hybrid clones express marked morphological difference between each other. Plants from Lithuania are slender, with narrow leaves (up to 1.25 mm) and more bristle-like leaf apices, which put it closer to *P. rutilus*, but unlike this species they have well-developed nodal glands. Estonian hybrid individuals are more similar to *P. friesii* but lack the nodal glands always present in *P. friesii*. Diagnostic features of the new hybrid taxon include: habit and branching pattern like *P. rutilus* but sometimes with well-developed nodal glands; the lateral veins strong, the outer one reaching up to 3/4 leaf length, some of leaf apices, especially those on the main stem are acuminate and bristle-like.

Potamogeton ×maëmetsiae Zalewska-Gałosz et M. Ronikier **nothosp. nova** (Figs 3, 4) **(=** *P. friesii* × *P. rutilus*)

D i a g n o s i s: Planta hybrida, proprietatibus intermediis taxoni parentalis i.e. *Potamogetonis friesii* et *P. rutili*; a *P. rutili* praesentia nodulorum in articulis, paribus glandulis in nodulis caulis ad pediculum foliorum et interiore nervo laterali tres partes longitudinis folii habente, et a *P. friesii* angustioribus foliis et subuliformibus apicibus folii differt.

T y p e: Lithuania, Lake Skaidrys, W from Visaginas, N 55°35'59.9" E 26°21'16.7", in water ca 0.7 m deep, NW part of the lake, 13 July 2007, coll. J. Zalewska-Gałosz (holotype: KRA 359167). Paratype: Estonia, Lake Soitsjärv, N 58°33'" E 26°41' 00", 14 July 1972, coll. Aime Mäemets (EMHC).

E t y m o l o g y: The taxon's epithet honours Aime Mäemets, a skilled Estonian taxonomist of the genus *Potamogeton*.

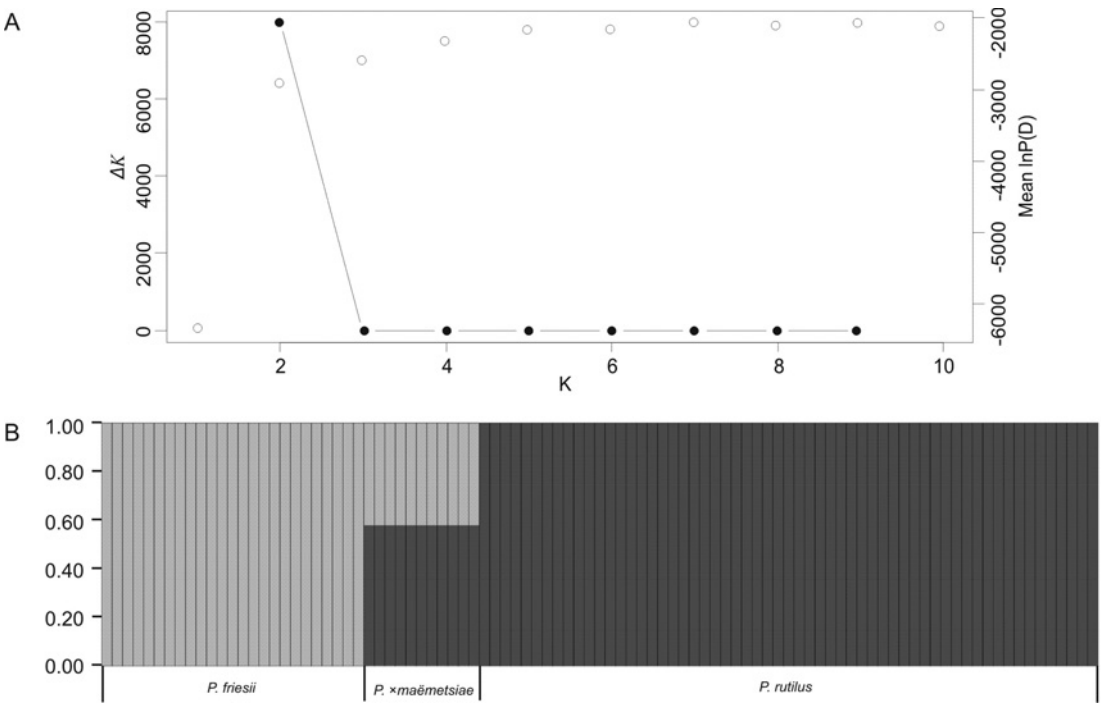

Fig. 2. – Results of the Bayesian analysis of the genetic structure of *P. friesii*, *P. rutilus* and *P.* ×*maëmetsiae*. Average log-probability of data – lnP(D) (○) and *ΔK* (●) values in function of K (A). Diagram of cluster distribution among individuals of *P. friesii*, *P. rutilus* and *P.* ×*maëmetsiae* inferred at K = 2 (B)

H a b i t a t s : Population in Lake Skaidrys was scattered and in shallow (up to 0.7 m deep) places mostly along the north-western edges of the lake. Besides *P.* ×*maëmetsiae*, no other linear-leaved pondweeds grew in the lake, only the broad-leaved *P.* ×*angustifolius* J. Presl was recorded. Lake Skaidrys is mesotrophic and surrounded by pine forests. Lake Soitsjärv is very shallow and characterized by hard water (Mäemets & Freiberg 2007).

Discussion

The majority of the linear-leaved hybrids currently recognized, such as *Potamogeton* ×*pseudofriesii*, *P.* ×*sudermanicus* or *P.* ×*grovesii*, are extremely rare (Preston 1995). Only recently, a more common occurrence of a hybrid from this group, *P.* ×*bambergensis* Fisch., was demonstrated (Zalewska-Gałosz & Ronikier 2010). Also *Potamogeton* ×*maëmetsiae*, described here, is only known from two distant localities. Common co-occurrence of its parental taxa, however, a factor potentially facilitating hybridization, could indicate a more common occurrence and likely discoveries of further localities.

Morphological identification of some linear-leaved *Potamogeton* hybrids is not always possible (Whittall et al. 2004, Les et al. 2009). In the case of *P.* ×*maëmetsiae*, even though it does not show any well defined morphological characters distinguishing it, its morpho-

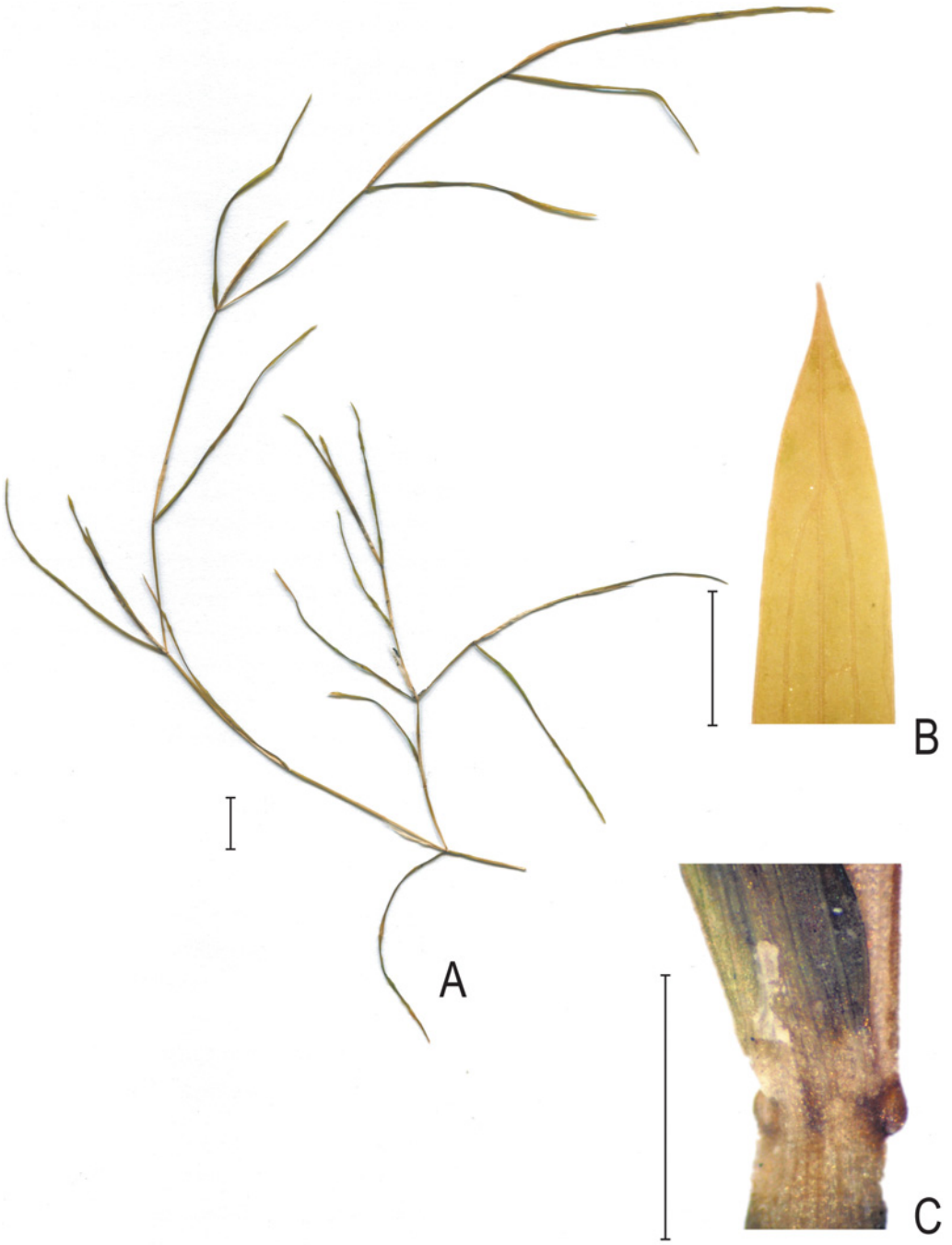

Fig. 3. – Habit (A), leaf apex (B) and leaf base (C) of a plant of *P.* ×*maëmetsiae* from Lake Skaidrys (holotype, KRA 359167). Scale bar 10 mm (A), 1 mm (B, C).

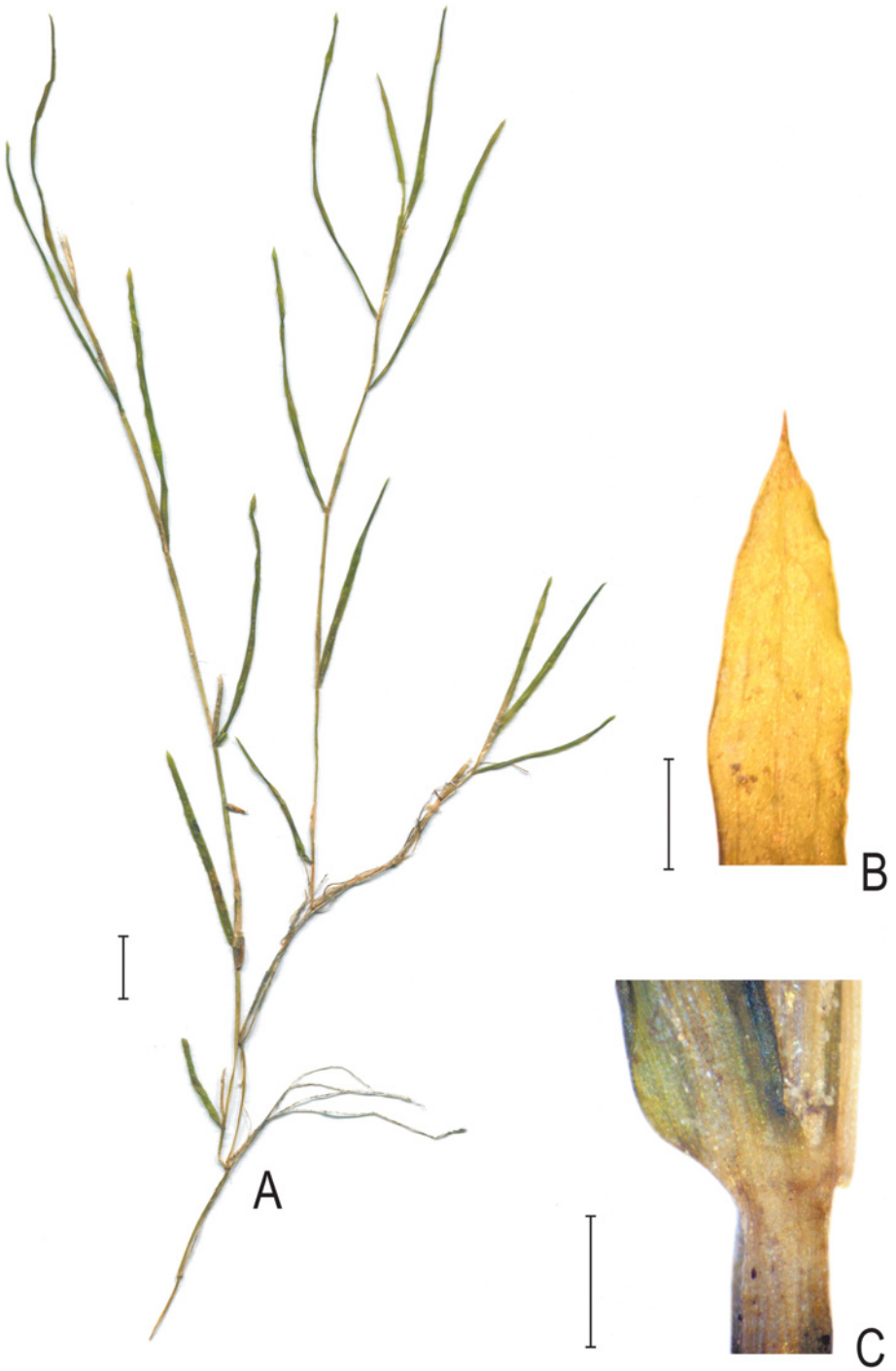

Fig. 4. – Habit (A), leaf apex (B) and leaf base (C) of a plant of *P.* ×*maëmetsiae* from Lake Soitsjärv (paratype, EMHC). Scale bar 10 mm (A), 1 mm (B, C).

logical identification is possible based on a mixed set of parental characters occurring in a single, hybrid plant. The AFLP population genetic analysis of *P.* ×*maëmetsiae* from Lake Skaidrys demonstrated that this population resulted from a single hybridization event and represented a single vegetatively spreading clone. No trace of potential backcrossing with parental taxa was found, as all plants were almost exactly intermediate from the genetic point of view (as shown by the Bayesian analysis).

The limited number of morphological differences between linear-leaved species may be due to their close genetic relationships and/or morphological convergence. In the three species studied, *Potamogeton friesii*, *P. pusillus* and *P. rutilus*, sequences of the ITS region were identical, which suggests their close phylogenetic affinities. This result is somewhat surprising considering there is set of well defined morphological characters differentiating each of these taxa. In contrast, *P. berchtoldii*, previously grouped together with *P. pusillus* (Wiegleb & Kaplan 1998, Haynes & Hellquist 2000), appeared to be far more genetically distant from that species as already observed by Kaplan & Štěpánek (2003) and lately confirmed by Les et al. (2009). These results indicate that morphological similarities among some linear-leaved pondweed taxa can reflect adaptations to the same kind of ecological conditions rather than their close phylogenetic relationships, and complicate the inference of evolutionary relationships in this group of plants.

In this study, AFLP fingerprinting and sequencing of 5S non-transcribed spacer (NTS) were used to differentiate closely related linear-leaved pondweeds. Both methods appeared useful and provided sufficient resolution for analysing closely related linear-leaved taxa. AFLPs are widely used in intra-specific population genetic studies as informative, variable and reliable markers. They are also used at higher taxonomic levels to support phylogenetic inference, especially when sequencing of particular DNA regions fails to reveal variability (e.g. Després et al. 2003). Successful application of AFLP analysis in the weakly diversified group of *Potamogeton* confirms their utility in such difficult cases, although this potential is only rarely explored (for an exception see Whittall et al. 2004). 5S nrDNA is organized in tandemly repeated arrays, which consist of a 120 base-pair genic region and a non-transcribed spacer of variable length between them (Dvorak et al. 1998). While the genic region is highly conserved, the intergenic spacer sequence can vary widely and therefore can provide useful taxonomic information on the intra- and inter-specific genetic variation in plants (Cox et al. 1992). In *Potamogeton*, 5S NTS appeared to be more polymorphic than ITS. As demonstrated in the analysis of *P.* ×*maëmetsiae*, the analysis of the 5S-NTS region efficiently overcame the limitations of using ITS in studies on linear-leaved pondweeds. Moreover, in *Potamogeton*, the presence of polymorphic sites in a pure species indicates that 5S nrDNA occurs at several loci. Such 5S unit divergence with uncompleted intralocus homogenization can be useful in phylogenetic studies. Display of consistent additive patterns in hybrid sequences and generally low intra-specific variation makes this region a valuable taxonomical tool. Together with the variable AFLP markers, it can be employed in studies of the variation in closely related *Potamogeton* species, where ITS does not provide sufficient resolution, and stimulate further advances in the appraisal of linear-leaved pondweed diversity.

Acknowledgements

Our thanks are due to Helle Mäemets (Estonia) for her agreement to extract DNA from the herbarium specimen of *Potamogeton* ×*maëmetsiae* collected by her mother Aime Mäemets and designated here as a paratype. We are also grateful to Krzysztof Pawłowski (Poland) for his translation of the Latin diagnosis and to two anonymous reviewers for their critical comments on a previous version of the manuscript. The freely accessible Bioportal at the University of Oslo (http://www.bioportal.uio.no) is acknowledged for the Bayesian analysis computation using STRUCTURE software. The study was financially supported by the following grants N303 098 32/3404 and N303 564439 from the Polish Ministry of Science and Higher Education.

Souhrn

Rozlišování a určování kříženců mezi úzkolistými druhy rdestů (*Potamogeton* sect. *Graminifolii*) na základě morfologických znaků je obtížné a často i sporné. V důsledku toho je dnes přijímána existence jen několika takových kříženců a mnoho hybridních kombinací popsaných v minulosti není uznáváno. Molekulární metody však nyní umožňují efektivní test morfologicky podezřelých rostlin a vystopování výskytu hybridizačních událostí. V tomto příspěvku je popsán nový kříženec *Potamogeton* ×*maëmetsiae* Zalewska-Gałosz & M. Ronikier, vzniklý z hybridizace úzkolistých druhů *P. friesii* a *P. rutilus*. Studované rostliny byly nalezeny v litevském jezeře Skaidrys a estonském jezeře Soitsjärv. Hybridní původ těchto rostlin byl stanoven na základě morfologického srovnání s rodičovskými druhy a nezávisle potvrzen za použití sekvencí jaderné (ITS, 5S-NTS) a chloroplastové (*rpl*32-*trn*L) DNA a analýzy AFLP.

References

Ascherson P. & Graebner P. (1907): Das Pflanzenreich IV.11 (Heft 31). *Potamogetonaceae*. – Wilhelm Engelmann, Berlin.

Cox A. V., Bennett D. B. & Dyer T. A. (1992): Use of the polymerase chain reaction to detect spacer size heterogeneity in plant 5S-rRNA gene clusters and to locate such clusters in wheat (*Triticum aestivum* L.). – Theor. Appl. Genet. 83: 684–690.

Després L., Gielly L., Redoutet B. & Taberlet P. (2003): Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability. – Mol. Phylogen. Evol. 27: 185–196.

Dvorak J., Zhang H-B., Kota R. S. & Lassner M. (1998): Organisation and evolution of the 5S ribosomal RNA gene family in wheat and related species. – Genome 32: 1003–1016.

Ehrich D. (2006): AFLPdat: a collection of R functions for convenient handling of AFLP data. – Mol. Ecol. Notes 6: 603–604.

Evanno G., Regnaut S. & Goudet J. (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Mol. Ecol. 14: 2611–2620.

Falush D., Stephens M. & Pritchard J. K. (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. – Mol. Ecol. Notes 7: 574–578.

Galinis V. (1963): Keletas naujų *Potamogeton* formų ir hibridų Lietuvos TSR Floroje [Several new forms and hybrids of *Potamogeton* in flora of Lituania]. – Lietuvos TSR Aukđtųjų Mokyklų Mokslo Darbai, Biologija 3: 97–108.

Hagström J. O. (1916): Critical researches on the potamogetons. – Kungl. Svenska Vetenskapsakad. Handl. 55/5: 1–281.

Hall T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucl. Acids Symp. Ser. 41: 95–98.

Haynes R. R. & Hellquist C. B. (2000): *Potamogetonaceae* Dumortier. – In: Flora of North America Editorial Committee (eds), Flora of North America north of Mexico 22 [*Magnoliophyta*: *Alismatidae*, *Arecidae*, *Commelinidae* (in part), and *Zingiberaceae*]: 47–74, Oxford Univ. Press, New York.

Kaplan Z. (2002): Phenotypic plasticity in *Potamogeton* (*Potamogetonaceae*). – Folia Geobot. 37: 141–170.

Kaplan Z. (2010): Hybridization of *Potamogeton* species in the Czech Republic: diversity, distribution, temporal trends and habitat preferences. – Preslia 82: 261–287.

Kaplan Z. & Fehrer J. (2006): Comparison of natural and artificial hybridization in *Potamogeton*. – Preslia 78: 303–316.

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

Kaplan Z., Fehrer J. & Hellquist C. B. (2009): New hybrid combinations revealed by molecular analysis: the unknown side of North American pondweed diversity (*Potamogeton*). – Syst. Bot. 34: 625–642.

Kaplan Z. & Štěpánek J. (2003): Genetic variation within and between populations of *Potamogeton pusillus* agg. – Plant Syst. Evol. 239: 95–112.

Kaplan Z. & Wolff P. (2004): A morphological, anatomical and isozyme study of *Potamogeton* ×*schreberi*: confirmation of its recent occurrence in Germany and first documented record in France. – Preslia 76: 141–161.

Les D. H., Murray N. M. & Tippery N. P. (2009): Systematics of two imperiled pondweeds (*Potamogeton vasneyi*, *P. gemiparus*) and taxonomic ramifications for subsection Pusilli (*Potamogetonaceae*). – Syst. Bot. 34: 643–651.

Lindqvist Ch., De Laet J., Haynes R. R., Aagesen L., Keener B. R. & Albert V. A. (2006): Molecular phylogenetics of an aquatic plant lineage, *Potamogetonaceae*. – Cladistics 22: 568–588.

Mäemets A. (1984): Sugukond penikeelelised *Potamogetonaceae* Dumort. [*Potamogetonaceae* Dumort.]. – *Potamogetonaceae* Dumort. – In: Eichwald K., Kukk E., Kuusk V., Lellep E., Mäemets A., Rebassoo H., Sander E., Talts S., Trei T. & Viljasoo L. (eds), Eesti NSV Floora [Flora of Estonia] 9: 46–139, Valgus, Tallin.

Mäemets H. & Freiberg L. (2007): Coverage and depth limit of macrophytes as tools for classification of lakes. – Proc. Eston. Acad. Sci. Biol. Ecol. 56: 124–140.

McNeill J., Barrie F. R., Burdet H. M., Demoulin V., Hawksworth D. L., Marhold K., Nicolson D. H., Prado J., Silva P. C., Skog J. E., Wiersema J. H. & Turland N. J. (eds) (2006): International Code of Botanical Nomenclature (Vienna Code): adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. – Regnum Vegetabile 146, Gantner Verlag, Ruggell.

Preston C. D. (1995): Pondweeds of Great Britain and Ireland. – Bot. Soc. British Isles, London.

Preston C. D., Hollingsworth P. M. & Gornall R. J. (1998): *Potamogeton pectinatus* L. × *P. vaginatus* Turcz. (*P.* ×*bottnicus* Hagstr.), a newly identified hybrid in the British Isles. – Watsonia 22: 69–82.

Ronikier M., Cieślak E. & Korbecka G. (2008): High genetic differentiation in the alpine plant *Campanula alpina* Jacq. (*Campanulaceae*): evidence for glacial survival in several Carpathian regions and long isolation between the Carpathians and the Alps. – Mol. Ecol. 17: 1763–1775.

Schlüter P. M. & Harris S. A. (2006): Analysis of multilocus fingerprinting data sets containing missing data. – Mol. Ecol. Notes 6: 569–572.

Shaw J., Lickey E. B., Schilling E. E. & Small R. L. (2007): Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in Angiosperms: the tortoise and the hare III. – Am. J. Bot. 94: 275–288.

Trei T., Gavrilova Ģ., Suško U. & Sinkevičienë Z. (2003): CXI. *Potamogetonaceae* Dumort. – In: Kuusk V., Tabaka L. & Jankevičienë R. (eds), Flora of the Baltic countries, p. 197–208, Estonian Agricultural University, University of Latvia, Institute of Botany, Tartu.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. & Zabeau M. (1995): AFLP: a new technique for DNA fingerprinting. – Nucl. Acids Res. 23: 4407–4414.

Whittall J. B., Hellquist C. B., Schneider E. L. & Hodges S. A. (2004): Cryptic species in an endangered pondweed community (*Potamogeton*, *Potamogetonaceae*) revealed by AFLP markers. – Am. J. Bot. 91: 2022–2029.

Wiegleb G. & Kaplan Z. (1998): An account of the species of *Potamogeton* L. (*Potamogetonaceae*). – Folia Geobot. 33: 241–316.

Wiegleb G., van de Weyer K., Bolbrinker P. & Wolff P. (2008): *Potamogeton*-hybriden in Deutschland. – Feddes Rep. 119: 433–448.

Zalewska-Gałosz J. (2002): Occurrence and distribution of *Potamogeton* hybrids in Poland. – Feddes Rep. 113: 380–393.

Zalewska-Gałosz J. (2010): *Potamogeton* ×*subrufus* Hagstr.: a neglected *Potamogeton* hybrid. – Ann. Bot. Fennici 47: 257–260.

Zalewska-Gałosz J. & Ronikier M. (2010): Are linear-leaved *Potamogeton* hybrids really so rare? Molecular evidence for multiple hybridizations between *P. acutifolius* and *P. compressus* in central Europe. – Nord. J. Bot. 28: 257–261.

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.

Zalewska-Gałosz J., Ronikier M. & Kaplan Z. (2010): Discovery of a new, recurrently formed *Potamogeton* hybrid in Europe and Africa: molecular evidence and morphological comparison of different clones. – Taxon 59: 559–566.

Received 29 September 2010
Revised 11 January 2011
Accepted 13 January 2011