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Present Status of Genetic Diversity of *Potamogeton praelongus* Populations in the Czech Republic

By

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With 4 Figures

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Summary

Kitner M., Prausova R. & Adamec L. 2013. Present status of genetic diversity of *Potamogeton praelongus* populations in the Czech Republic. – Phyton (Horn, Austria) 53(1): 73–86, with 4 figures.

According to the European Red list, *Potamogeton praelongus* Wulfen (*Potamogetonaceae*) is a Least Concern species predominantly distributed throughout northern Europe, Asia and North America. It is considered a critically endangered species in the Czech Republic, with the last natural population in a standing oxbow in the Orlice river floodplain at Malšova Lhota, near Hradec Králové in E Bohemia. In this study, the genetic diversity of plants from natural and introduced Czech populations as well as a rescue culture was determined using AFLP analyses. At these sites, despite prolific flowering and seed setting, it propagates only vegetatively as seed germination is apparently extremely rare if any. The analyses revealed a very low genetic diversity in existing Czech micropopulations suggesting a predominant clonal propagation within the natural populations. This may also be a

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result in the course of ongoing repatriation of in vitro micropropagated plants. However, we observed differences in the genetic profiles and higher genetic diversity in plant individuals raised from seeds (obtained from rescue cultures) produced by sexual reproduction. Trying to obtain seeds could be utilized to increase the genetic diversity within natural and newly established populations. Other aspects of successful conservation of this species are also discussed.

Zusammenfassung

Kitner M., Prausova R. & Adamec L. 2013. Present status of genetic diversity of *Potamogeton praelongus* populations in the Czech Republic. [Gegenwärtiger Stand der genetischen Diversität von *Potamogeton praelongus*-Populationen in Tschechien]. – Phyton (Horn, Austria) 53(1): 73–86, mit 4 Abbildungen.

Nach der Europäischen Roten Liste ist *Potamogeton praelongus* Wulfen (*Potamogentonaceae*) eine in Europa zur Zeit nicht gefährdete Art, die hauptsächlich in Nordeuropa, Asien und Nordamerika vorkommt. In Tschechien wird sie als stark gefährdet angesehen. Die letzte natürliche Population gedeiht in Ost-Böhmen in einem Altwasser in der Orlice-Flussniederung bei Malšova Lhota nahe Hradec Kralové. Untersucht wurde die genetische Diversität von Pflanzen aus natürlichen und in Tschechien eingebürgerten Populationen ebenso wie aus einer Erhaltungskultur mittels AFLP-Analyse. An diesen Standorten vermehrt sich die Art trotz reicher Blüte und Samenansatzes nur vegetativ weil Samenkeimung, falls überhaupt vorkommend, extrem selten ist. Die Analysen ergaben eine sehr geringe genetische Diversität in den tschechischen Kleinpopulationen, was auf Vorherrschen klonaler Vermehrung in den natürlichen Populationen hinweist. Das kann auch das Resultat laufender Wiedereinbürgerungen von in vitro-vermehrten Pflanzen sein. Wir beobachteten aber Unterschiede im genetischen Profil und höhere genetische Diversität in Pflanzen, die aus sexuell produzierten Samen (aus der Erhaltungskultur) erhalten wurden. Eine Möglichkeit, die genetische Diversität in natürlichen und neu etablierten Populationen zu erhöhen, ist die Vermehrung über Samen. Weitere Aspekte der Erhaltung der genannten Art werden diskutiert.

1. Introduction

Potamogeton praelongus Wulfen (*Potamogetonaceae*) is a rare species having a nordic, partly suboceanic and circumpolar distribution. It occurs predominantly throughout northern Europe, Asia and North America and is rare throughout its entire distribution area (Vöge 1992). In the Czech Republic, this species has historically inhabited between 18 and 20 sites at the middle and lower reaches of the Orlice, Vltava, Otava and Ploučnice rivers. The number of recent Czech sites of *P. praelongus* has considerably decreased during the last 30 years and today, it only exists in two small micropopulations in the floodplain of the Orlice river (Kaplan 2010, Prausová & al. 2011). One of these is a natural one in a standing oxbow in the Orlice river floodplain at Malšova Lhota in East Bohemia (TPA, Transient protected area "Rameno u Stříbrného rybníka") while the other site was established nearby as a part of the official rescue programme for *P. praelongus* in 2008 (Fig. 1).

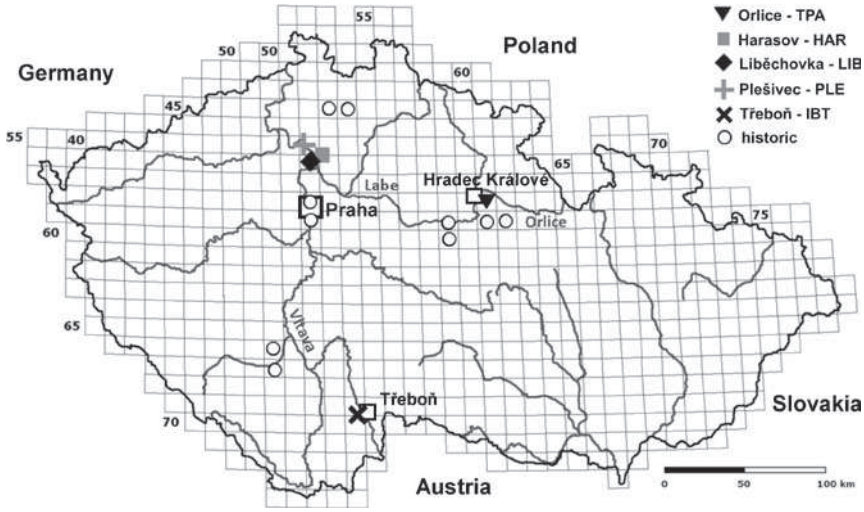

Fig. 1. Historic and recent (2009–2011) spread of *Potamogeton praelongus* in the Czech Republic.

Regular monitoring of all Czech *P. praelongus* micropopulations and selected abiotic factors (e.g. water and sediment chemistry) started in 2005. Simultaneously, repatriation of the species to the Orlice river floodplain has been conducted, using plants grown in an outdoor rescue culture at the Institute of Botany at Třeboň (IBT), the Czech Republic (Husák & Adamec 1998, Prausová & al. 2011). There are two recent rescue cultures of *P. praelongus,* established using plants originating from the last native site in the Czech Republic (TPA): 1) the IBT culture in Třeboň (maintained since 1988, Husák & Adamec 1998) and 2) a semi-natural rescue population in restored backwater pools at Liběchovka, Harasov and Plešivec in the Kokořínsko region in Central Bohemia (maintained since 2001–2005) (Fig. 1). It can be deduced from its habitat and cultivation requirements that *P. praelongus* prefers deeper, colder, streaming or standing waters which are rich in calcium, medium-hard and mesotrophic (Husák & Adamec 1998, Prausová & al. 2011). At its sites, despite prolific seed setting, it propagates only vegetatively as seed germination is apparently extremely rare (Prausová & al. 2011) in the Czech Republic. Using seed sterilisation by NaClO, an in-vitro culture of *P. praelongus* was prepared from seeds coming from the IBT. The plants were subsequently grown in the IBT. Six of them were used for AFLP analysis in this study. Seed germination tests were an important part of the official rescue programme for *P. praelongus*. The goal of the achene germination tests was to determine the best method for breaking the achene dormancy caused by the achene's hard outer shell – the pericarp and seed coat (Prausová & al. 2011, 2013).

Amplified Fragment Length Polymorphism (AFLP) has become one of the widely used methods of molecular biology in population genetics. AFLP analysis allows to describe the level of genetic diversity and discriminate between closely related genotypes or clones within a species (Vos & al. 1995; Barker & al. 1998, Lombard & al. 2000, Meud & Clarke 2007, Kitner & al. 2012). AFLP has become the ideal means in situations where there is neither an a priori-sequence information nor suitable established markers (such as microsatellites) (Meud & Clarke 2007). The main advantage of AFLP is that large numbers of genetic markers can be typed relatively quickly and effectively at low cost (Evanno & al. 2005, Nicolè & al. 2007). The main disadvantages of AFLP are its dominant (heterozygotes cannot be distinguished from dominant homozygotes) and biallelic character (for a given size, the fragment is either present or absent) and homoplasy of bands (Vekemans & al. 2002, Bensch & Åkeson 2005, Meud & Clarke 2007, Paris & al. 2010).

The aims of this study were to assess the level of genetic diversity within and between populations in the Czech Republic using AFLP markers, and to detect possible differences in the genetic profiles of samples taken from natural habitats (propagated clonally) and samples obtained by sexual reproduction in rescue cultures. It may be expected that the latter samples shall be genetically more diverse.

2. Material and Methods

2.1. Plant Sampling, DNA Extraction and AFLP Analysis

Altogether 71 *P. praelongus* individual plants at four Czech microsites [Orlice (TPA; N50°12′35.6", E15°53′18.4"), Harasov (HAR; N50°24′48.7", E14°34′46.7"), Liběchovka (LIB; N50°26′2.1", E14°28′3.4"), Plešivec (PLE; N50°32′15.5", E14°34′12.2")] and a *P. praelongus* rescue cultivation at Třeboň (IBT; N49°0′19.7", E14°46′23.7"), were used for initial investigation in 2009. At the microsites, the distance of collected plants from each other ranged from 5 to 50 m. Subsequently, due to the successful propagation of six plants raised from seed in 2011 in IBT, we compared their AFLP profiles with six samples from previously investigated Czech sites (PLE and HAR) as well as with one *P. praelongus* sample collected from lake Latinalahti in Scandinavia (SCA; N68°50′33.87", E 21°08′35.89").

Two leaves from each individual were placed in plastic bags and transported in a cooled box to the laboratory, treated with liquid nitrogen and stored at -80 °C. Genomic DNA was extracted using the CTAB method (Doyle & Doyle 1987). The number of sampled individuals per microsite is given in Table 2.

AFLP analysis was performed according to the original procedure by Vos & al. 1995, with the modification by Kitner & al. 2008. Primer pairs for the selective amplification were selected after testing various primer combinations on a subset of four samples. A total of eight selective primer combinations with *Eco*RI primers carrying three selective nucleotides and *Mse*I primers carrying three, four or five selective nucleotides were chosen for the final analysis. Primer sequences with detailed information on the number of scored bands as well as the number of polymorphic bands are present in Table 1. Detailed information on core primer sequenc-

Table 1. A summary of AFLP primer combinations (PC) used for amplification profiles of *Potamogeton praelongus* populations in the Czech Republic (N_{SB}, number of bands scored; N_{Pol}, number of polymorphic bands; PLP, percentage of polymorphisms).

PC		N_{SB}	N_{Pol}	PLP (%)
a	*Eco*RI-AGC / *Mse*I-CGATC	59	14	23.7
b	*Eco*RI-AGC / *Mse*I-CAACG	59	16	27.1
c	*Eco*RI-ACT / *Mse*I-CAAC	35	6	17.1
d	*Eco*RI-ACA / *Mse*I-CAACG	13	2	15.4
e	*Eco*RI-ACT / *Mse*I-CGA	23	2	8.69
f	*Eco*RI-ACT / *Mse*I-CAG	45	1	2.22
g	*Eco*RI-ACT / *Mse*I-CTT	35	3	8.57
h	*Eco*RI-AGC / *Mse*I-CGATG	18	2	11.1
	Total	287	46	16.0

Table 2. Genetic variability indices of five populations of *Potamogeton praelongus* in the Czech Republic tested by means of AFLP (n, number of samples; N_{Pol}, number of polymorphic bands; N_{FPri}, number of fixed private bands; PLP, proportion of polymorphic loci; H_e, estimated heterozygosity with standard error, SE).

Parameters	Harasov (HAR)	Liběchovka (LIB)	Orlice (TPA)	Plešivec (PLE)	Třeboň (IBT)
n	7	14	22	21	7
N_{Pol}	48	24	5	19	39
N_{FPri}	0	0	0	0	0
PLP (%)	16.7	8.4	1.7	6.6	13.6
H_e	0.0985	0.0518	0.0183	0.0292	0.0714
SE	0.0065	0.0038	0.0016	0.0027	0.0047

es is published elsewhere (Kitner & al. 2012). Products and amplification were separated on a 6 %, 0.4 mm thick denaturing polyacrylamide gel (0.5× TBE buffer) using the T-REX (Thermo Scientific Owl Separation Systems, Rochester, NY, USA) sequencing gel electrophoresis apparatus. Subsequent silver staining was used for visualization of AFLP patterns.

2.2. Data Analysis of AFLP

To verify the reproducibility of amplification, all plant samples were separated and visualized in duplicates. Only intense and unambiguous bands were scored visually as present (1) or absent (0) and translated into a binary matrix for subsequent evaluation of basic variability indices [N_{Pol}, PLP, number and percentage of polymorphic loci at the 0.95 criterion; N_{FPri}, number of fixed private bands; H_t, the total

gene diversity; the gene diversity within (H_e) and among (H_b) populations level following Nei 1987; F_{ST}, Wright's fixation index] these were calculated in AFLP-SURV (Bayesian method, Vekemans & al. 2002) and analysis of molecular variance (AMOVA) using GenAlEx 6.4 (Peakall & Smouse 2006). Three methods visualizing genetic structure and relationships among individuals were applied: a) Neighbor-joining (NJ) phylogenetic tree [Dice coefficient, 1000 bootstrap replicates (Felsenstein 1985), Freetree (Pavlíček & al. 1999) and TreeView (Page 1996) software]; b) Principal coordinate analysis [PCoA; FAMD ver. 1.23 beta (Schlüter & Harris 2006)]; c) Bayesian clustering approach implemented in BAPS 3.2 software (Corander & al. 2006).

3. Results

Altogether, 287 AFLP markers were detected with eight primer combinations, of which 134 (46.7 %) were segregating and only 46 (16.0 %) exhibited polymorphism at the 0.95 criterion. The percentage of polymorphic loci over all populations ranged from 1.7 % (TPA) to 16.7 % (HAR). Overall ge-

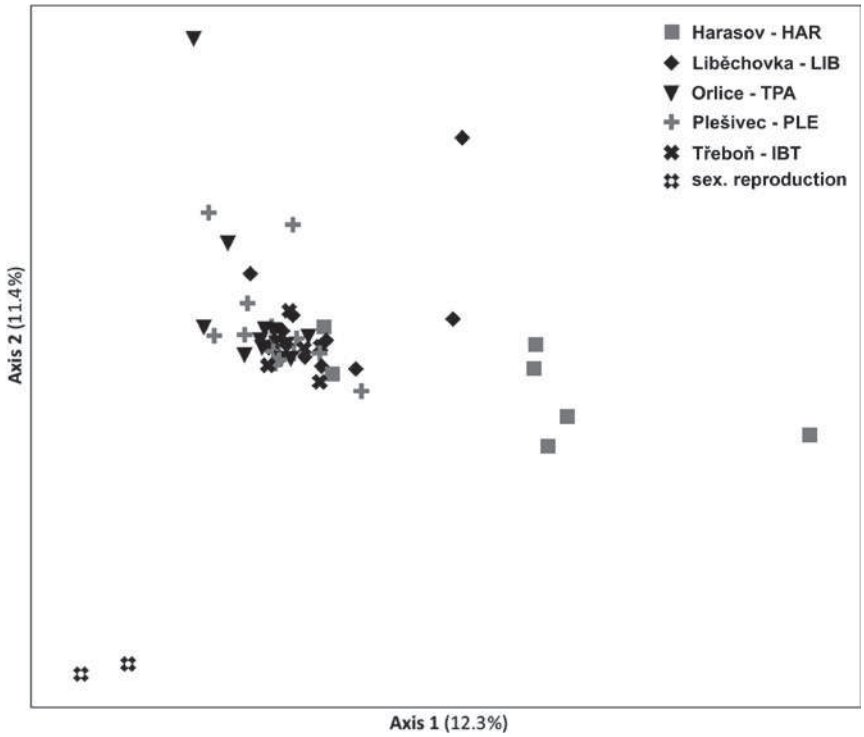

Fig 2. Principal coordinate analysis (PCoA) plot of 71 investigated individuals of *Potamogeton praelongus* based on 287 AFLP loci (Jaccard's similarity coefficient). The first two PCoA axes together accounted for 23.7 % of the overall variability.

netic diversity was very low (H_t = 0.0548). Genetic diversity (H_e) across all populations was 0.0539, ranging from 0.0183 (TPA) to 0.0985 (HAR) (Table 2).

No fixed-private bands were present in any of the investigated populations. Also, the overall population differentiation (F_{ST} = 0.018) indicates a very low genetic differentiation among investigated populations. This was also confirmed by the AMOVA results (Φ_{pt} = 0.167 at P < 0.001) showing that only 17.0 % of the variation refers to differences between populations, while the majority (83.0 %) represents the intra-population variation. Neighbor-joining analysis failed to find any relevant structure among investigated populations with no bootstrap support for the tree topology (figure not shown). Also, on the PCoA plot (Fig. 2), no clear pattern of population structure can be observed, with only one noticeable separation of two IBT samples, having their origin in sexual reproduction during the 2010 season.

Analysis of the second sample set was performed using a five primers combination (c, e, f, g, *Eco*RI-ACG/*Mse*I-CAAC; Table 1) which gave 162 unambiguous AFLP fragments (45 polymorphic at the 0.95 criterion). PCoA analysis clearly separated a highly uniform "micropopulation" group of samples PLE and HAR from more variable samples from the rescue culture (CUL) and the sample from Scandinavia (SCA, Fig. 3).

A similar view was also suggested in Neighbor-joining and BAPS analysis (Fig. 4), with 100 % bootstrap support for separation of the Czech sites PLE and HAR and the group from the rescue culture (CUL), as well as with significant bootstrap support for separation of the sample from Scandinavia (SCA). Furthermore, no differences were observed when comparing AFLP profiles of two plants at PLE sampled in 2009 and 2011 (Fig. 4).

4. Discussion

4.1. Genetic Structure of *P. praelongus* Populations

Genetic diversity of the plant species reflects their breeding systems (Hamrick & Godt 1996). Moreover, growth and genetic diversity of aquatic plant populations may be strongly influenced by clonal growth (Waycott 1995). Generally, species which propagate asexually exhibit low levels of population genetic diversity, whereas species reproducing sexually show high levels of genetic diversity (Loveless & Hamrick 1984). Additionally, low values of intra-population variability are exhibited by long-lived perennial, endemic, gymnosperm taxa with a middle to late successional status (Nybom & Bartish 2000). In the present study, a very low level of intra- and inter-population genetic diversity was detected in Czech populations of *P. praelongus* (PLP = 16.0 %, H_e = 0.054). A similar level of genetic diversity (PLP = 13.0 %, H_e = 0.042, based on 130 ISSR markers) has recently been reported for an aquatic macrophyte *Ottelia alismoides* (L.) Pers. from lakes in China (Chen & al. 2008) or for two critically endangered west-arctic species *Sagina caespitosa* and *Arenaria humifusa* (H_e = 0.05 and 0.08, respectively), using

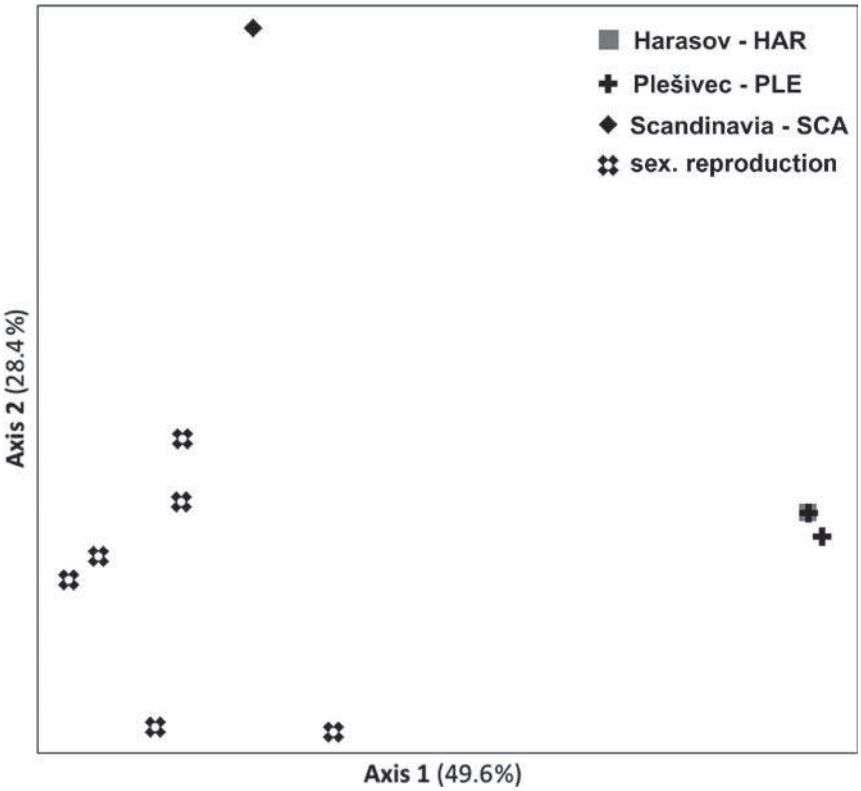

Fig 3. Principal coordinate analysis (PCoA) plot of 13 investigated individuals of *Potamogeton praelongus* based on 162 AFLP loci (Jaccard's similarity coefficient). The first two PCoA axes together accounted for 78.0 % of the overall variability.

AFLP markers (Westergaard & al. 2011). A moderate level of genetic diversity of *P. malaianus* populations (PLP = 70.5 %, H_e = 0.163) was reported by Chen & al. 2009, while Li & al. 2004 reported a high level of genetic diversity (I = 0.414, based on RAPD markers) for *P. maackianus* A. Benn. from the identical area in China along the Yangtze river. Very high levels of H_e (0.527 and 0.452) were reported from 12 lake and 14 Baltic Sea populations of *P. pectinatus* using microsatellite markers (Nies & Reusch 2005).

In contrast, data available from allozyme studies present low or no intra-population genetic variation of *P. cheesemanii* A. Benn., *P. crispus* L., *P. coloratus* Hornem., *P. epihydrus* Raf., *P. ochreatus* Raoul, *P. pectinatus* L. and *P. pusillus* L. (Hofstra & al. 1995, Hollingsworth & al. 1995, 1998, van Wijk & al. 1988, Kaplan & Štěpánek 2003). A very low intra-species genetic variation in *P. clystocarpus* was also revealed by AFLP markers by Whittall & al. 2004. The low levels of genetic diversity in *Potamogeton* populations

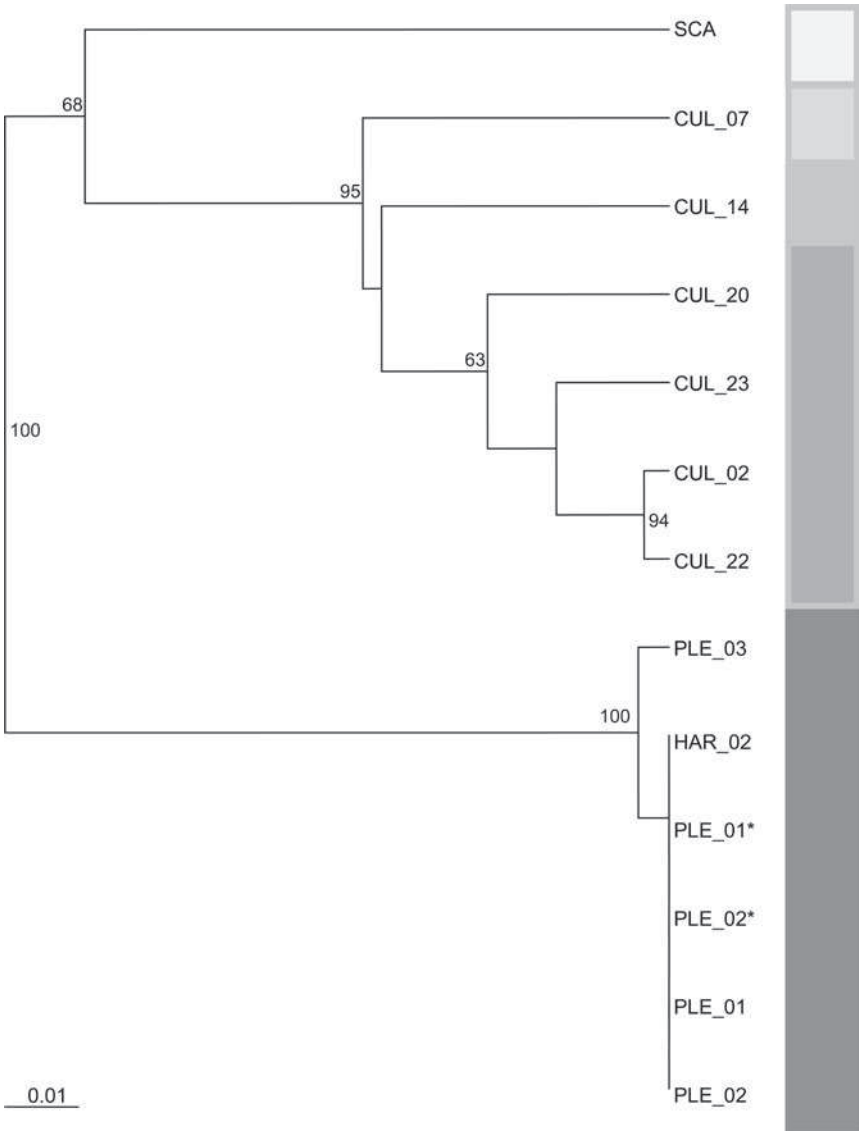

Fig. 4. Phylogenetic tree based on Jaccard's similarity coefficient and UPGMA clustering method among 13 *Potamogeton praelongus* samples obtained from 162 AFLP loci. The designation of samples is present on tips of branches, while numbers on branches indicates significant bootstrap support (1000 replications). Colored boxes on the right side corresponds to BAPS mixture clustering graphical output. (CUL, rescue culture; HAR, Harasov; PLE, Plešivec; SCA, Scandinavia; * designation of samples taken in 2011)

were attributed to a combination of bottlenecks, founder effects, inbreeding and clonal growth.

Most of the observed diversity among Czech populations of *P. praelongus* in this study is due to the individual genetic variation within populations rather than between populations. This emphasizes that the recorded absence of population structure among investigated sites can be regarded as a consequence of the ongoing rescue programme during which the plants collected from the last autochthonous *P. praelongus* site TPA "Rameno u Stříbrného rybníka" were transferred, clonally propagated and grown in the rescue culture at IBT Třeboň. From this stock rescue culture, repatriation to all investigated sites was done (Prausová & al. 2011). Mallón & al. 2009 and Valladares & al. 2006 used RAPD markers to demonstrate that the in vitro propagation did not induce genetic changes in regenerated individuals of the endangered endemic perennial herb *Centaurea ultreiae* or the forest tree *Quercus robur*. Thus, the present status of genetic variation and structure in Czech populations of *P. praelongus* reflects the degree of variation of the last natural population in TPA. However, present results indicate that the genetic variability of micropopulations can be increased considerably by sexual reproduction in rescue cultures.

4.2. Implications for Conservation

The last native Czech micropopulation of *P. praelongus* (TPA) is endangered by the deposition of a nutrient-rich sediment in the standing oxbow reach which covers the leaf surfaces with fine particles (Prausová & Janová 2010, Prausová & al. 2011). High concentrations of mineral nutrients in the water also lead to the growth of metaphytic filamentous green algae (mainly the genera *Oedogonium* and *Vaucheria*), and the disturbance of plants and micropopulations during flood events is also of potential concern. It is neccessary to carry out strict conservation with appropriate habitat management and monitoring of the current *P. praelongus* populations to ensure the long-term protection (e. g. cutting of inshore tree stands, excessive sediment removal, building of a sedimentation tank and/or reduction of nutrient ritch water inflow; see Prausová & al. 2011).

There are general concerns about the risks associated with the introduction of maladapted genotypes originating from different sites, long-distance transplantation or from crosses between plants from different populations that may result in heterosis or breakdown of coadapted gene complexes. Local adaptation may also result in fitness reduction through outbreeding depression (Templeton 1986, Tallmon & al. 2004, Edmands 2007, Crémieux & al. 2010). Nevertheless, experimental studies reported the rapid spread of immigrant genomes within inbred populations due to heterosis (Richards 2000, Ebert & al. 2002, Saccheri & Brakefield 2002) and that a small amount of artificial gene flow into natural populations quickly re-

duced inbreeding depression and fitness reduction from fixed genetic loads (Westemeier & al. 1998, Madsen & al. 1999, Stokstad 2005).

However, recent results of Raabová & al. 2008 proved that increasing genetic diversity by hybridisation experiments between plants from different populations does not lead to immediate outbreeding depression. There are also reports that the consequences of the negative effects of outbreeding depression are unlike those in small populations and when the source of the genetic material comes from populations within the same region (Willi & al. 2007).

Proper management practice would lead to an increase in population fitness as well as the adaptive potential of the population to changing enviromental pressures and stochastic factors (Frankham & al. 2002, Reed & Frankham 2003, Willi & al. 2007, Holmes & al. 2008). We believe that these goals can be reached by increasing the effective population size by repatriation of micropropagated plants, as well as by increasing its genetic variation by planting new genotypes raised from seed in vitro into the native sites of *P. praelongus* in the Czech Republic. In line with this measure, recent germination tests revealed near-natural methods of breaking the achene dormancy such as anaerobic conditions, stratification, UV-A radiation etc. (Prausová & al. 2011, 2013).

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6. References

Barker J. H. A., Matthes M., Arnold G. M., Edwards K. J., Åhman I., Larsson S. & Karp A. 1998. Characterization of genetic diversity in potential biomass willows (*Salix* spp.) by RAPD and AFLP analyses. – Genome 42: 173–183.

Bensch S. & Åkeson M. 2005. Ten years of AFLP in ecology and evolution: why so few animals? – Molec. Ecol. 14: 2899–2914.

Chen Y. Y., Li X. L., Yin L. Y. & Li W. 2008. Genetic diversity of the threatened aquatic plant *Ottelia alismoides* in the Yangtze River. – Aquat. Bot. 88: 10–16.

Chen Y. Y., Li X. L., Yin L. Y. & Li W. 2009. Genetic diversity and migration patterns of the aquatic macrophyte *Potamogeton malaianus* in a potamolacustrine system. – Freshwater Biol. 54: 1178–1188

Corander J., Marttinen P. & Mäntyniemi S. 2006. Bayesian identification of stock mixture from molecular marker data. – Fish B-Noaa 104: 550–558.

Crémieux L., Bischoff A., Müller-Schärer H. & Steinger T. 2010. Gene flow from foreign provenances into local plant populations: Fitness consequences and implications for biodiversity restoration. – Am. J. Bot. 97: 94–100.

Doyle J. J. & Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochem. Bull. 19: 11–15.

Ebert D., Haag C., Kirkpatrick M., Riek M., Hottinger J. W. & Pajunen V. I. 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. – Science 295: 485–488.

Edmands S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. – Molec. Ecol. 16: 463–475.

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

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. – Evolution 39: 783–791.

Frankham R., Ballou J. D., Briscoe D. A. 2002. Introduction to conservation genetics. – Cambridge University Press, Cambridge, U. K.

Hamrick J. L. & Godt M. J. W. 1996. Conservation genetics of endemic plant species. – In: Avise J. C. & Hamrick J. L. (eds.), Conservation genetics: Case histories from nature, pp. 281–304. – Chapman and Hall, Melbourne.

Hofstra D. E., Adam K. D. & Clayton J. S. 1995. Isozyme variation in New Zealand populations of *Myriophyllum* and *Potamogeton* species. – Aquat. Bot. 52: 121–131.

Hollingsworth P. M., Gornall R. J. & Preston C. D. 1995. Genetic variability in British populations of *Potamogeton coloratus* (*Potamogetonaceae*). – Plant Syst. Evol. 197: 71–85.

Hollingsworth P. M., Preston C. D. & Gornall R. J. 1998. Lack of detectable isozyme variability in British populations of *Potamogeton epihydrus* (*Potamogetonaceae*). – Aquat. Bot. 60: 433–477.

Holmes G. D., James E. A. & Hoffmann A. A. 2008. Limitations of reproductive output and genetic rescue in population of rare shrub *Grevillea repens* (*Proteaceae*). – Annals Bot. 102: 1031–1041.

Husák Š. & Adamec L. 1998. Záchranné kultivace ohrožených druhů vodních a mokřadních rostlin v Botanickém ústavu AV ČR v Třeboni. – Příroda (Prague) 12: 7–26.

Kaplan Z. 2010. *Potamogeton* L. – rdest. – In: Chrtek J. (jun.), Kaplan Z. & Štěpánková J. (eds.), Květena České republiky 8 (1): 330–378. – Academia, Prague.

Kaplan Z. & Štěpánek J. 2003. Genetic variation in *Potamogeton pusillus* agg. – Plant Syst. Evol. 239: 95–112.

Kitner M., Lebeda A., Doležalová I., Maras M., Křístková E., Beharav A., Nevo E., Pavlíček T. & Meglic V. 2008. AFLP analysis of *Lactuca saligna* germplasm collections from four European and three Middle East countries. – Isr. J. Plant Sci. 56: 185–193.

Kitner M., Majeský Ľ., Gillová L., Vymyslický T. & Nagler M. 2012. Genetic structure of *Artemisia pancicii* populations inferred from AFLP and cpDNA data. – Preslia 84: 97–120.

Lombard V., Baril C. P., Dubreuil P., Blouet F. & Zhang D. 2000. Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: Consequences for varietal registration. – Crop Sci. 40: 1417–1425.

Li W., Xia L. Q., Li J. Q. & Wang G. X. 2004. Genetic diversity of *Potamogeton maackianus* in the Yangtze River. – Aquat. Bot. 80: 227–240.

Loveless M. D. & Hamrick J. L. 1984. Ecological determinants of genetic structure in plant populations. – Annual Rev. Ecol. Syst. 15: 65–69.

Madsen T., Shine R., Olsson M. & Wittzell H. 1999. Conservation biology: restoration of an inbred adder population. – Nature 402: 34–35.

Mallón R., Rodríguez-Oubiña J. & González M. 2010. In vitro propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. – Plant Cell Tissue Organ Cult. 101: 31–39.

Meudt H. M. & Clarke A. C. 2007: Almost forgotten or latest practice? AFLP applications, analysis and advances. – Trends Plant Sci. 12: 106–117.

Nei M. 1987. Molecular evolutionary genetics. – Columbia University Press, New York, USA.

Nicolè F., Tellier F., Vivat A. & Till-Bottraud I. 2007. Conservation unit status inferred for plants by combining interspecific crosses and AFLP. – Conserv. Genet. 8: 1273–1285.

Nies G. & Reusch T. B. H. 2005. Evolutionary divergence and possible incipient speciation in post-glacial populations of a cosmopolitan aquatic plant. – J. Evol. Biol. 15: 19–26.

Nybom H. & Bartish I. V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. – Perspect. Plant Ecol. 3: 93–114.

Page R. D. M. 1996. TreeView: An application to display phylogenetic trees on personal computers. – Comput. Appl. Biosci. 12: 357–358.

Paris M., Bonnes B., Ficetola G. F., Poncet B. N. & Despres L. 2010. Amplified fragment length homoplasy: in silico analysis for model and non-model species. – BMC Genomics 11: 287.

Pavlíček B. A., Hrda S. & Flégr J. 1999. FreeTree – Freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia*. – Folia biol. (Prague) 45: 97–99.

Peakall R. & Smouse P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. – Molec. Ecol. Notes 6: 288–295.

Prausová R. & Janová J. 2010. Současný stav výskytu rdestu dlouholistého (*Potamogeton praelongus*) v České republice. – Příroda (Prague) 27: 155–168.

Prausová R., Janová J. & Adamec L. 2011. Rescue of the critically endangered longstalked pondweed (*Potamogeton praelongus*) in the Czech Republic. – Acta biol. slovenica 54: 43–54.

Prausová R., Janová J. & Šafářová L. 2013. Testing achene germination of *Potamogeton praelongus* Wulfen. – Central europ. J. Biol. 8(1): 78–86.

Raabová J., Münzbergová Z. & Fischer M. 2009. Consequences of near and far between-population crosses for offspring fitness in a rare herb. – Plant Biol. 11: 829–836.

Reed D. H. & Frankham R. 2003. Correlation between fitness and genetic diversity. – Conserv. Biol. 17: 230–237.

Richards C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. – Am. Naturalist 155: 383–394.

Saccheri I. J. & Brakefield P. M. 2002. Rapid spread of immigrant genomes into inbred populations. – Proc. roy. Soc., B, biol. Sci. 269: 1073–1078.

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

Stokstad E. 2005. 'Genetic rescue' helps panthers but puts researchers on the spot. – Science 309: 1162–1162.

Tallmon D. A, Luikart G. & Waples R. S. 2004. The alluring simplicity and complex reality of genetic rescue. – Trends Ecol. Evol. 19: 489–496.

Templeton A. R. 1986. Coadaptation and outbreeding depression. – In: Soulé M. E. (ed.), Conservation biology: The science of scarcity and diversity, pp. 105-121. – Sinauer Assoc., Sunderland, MA.

Valladares S., Sánchez C., Martínez M. T., Ballester A. & Vieitez A. M. 2006. Plant regeneration through somatic embryogenesis from tissues of mature oak trees: tru-to-type conformity of plantlets by RAPD analysis. – Plant Cell Rep. 25: 879–886.

Vekemans X., Beauwens T., Lemaire M. & Roldan-Ruiz I. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. – Molec. Ecol. 11: 139–151.

Vöge M. 1992. Die Entwicklung von *Potamogeton praelongus* im Großensee bei Hamburg. – Tuexenia 12: 275–284.

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.

Waycott M. 1995. Assessment of genetic variation and clonality in the seagrass *Posidonia australis* using RAPD and allozyme analysis. – Marine Ecol. Progr. Ser. 116: 289–295.

Westemeier R. L., Brawn J. D., Simpson S. A., Esker T. L., Jansen R. W., Walk J. W., Kershner E. L., Bouzat J. L. & Paige K. N. 1998. Tracking the long-term decline and recovery of an isolated population. – Science 282: 1695–1698.

Westergaard K. B., Alsos I. G., Popp M., Engelskjøn T., Flatberg K. I. & Brochmann C. 2011. Glacial survival may matter after all: nunatak signatures in the rare European populations of two west-arctic species. – Molec. Ecol. 20: 376–393.

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.

Wijk R. J. van, Goor E. M. J. van & Verkley J. A. C. 1988. Ecological studies on *Potamogeton pectinatus* L. II. Autecological characteristics, with emphasis on salt tolerance, intraspecific variation and isoenzyme patterns. – Aquat. Bot. 32: 239–260.

Willi Y., Kleunen M. van, Dietrich S. & Fischer M. 2007. Genetic rescue persists beyond first-generation outbreeding in small populations of a rare plant. – Proc. roy. Soc., B, biol. Sci. 274: 2357–2364.