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**DISTRIBUTION AND GENETIC VARIATION OF INVASIVE
CRAYFISH OF THE GENUS *ORCONECTES***

(VÝSKYT A GENETICKÁ VARIABILITA INVAZNÍCH RAKŮ RODU *ORCONECTES*)

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ABSTRACT

Crayfish are an important part of European fauna, but since the 19th century native crayfish species have been largely influenced by biological invasions, when large number of their populations was dramatically reduced due to the introduction of the pathogen of the crayfish plague (oomycete *Aphanomyces astaci*) to Europe. Several North American crayfish species were then brought to the European continent to substitute lost populations of native crayfish, the most widespread being the spiny-cheek crayfish (*Orconectes limosus*), the signal crayfish (*Pacifastacus leniusculus*) and the red swamp crayfish (*Procambarus clarkii*). However, these crayfish can carry pathogen of the crayfish plague and therefore represent a serious threat to the native species.

My work focused mostly on the spiny-cheek crayfish (*O. limosus*). Available literature data suggest that the species was brought to Europe only once, and all European individuals may be descendants of the founder population. However, other cases of introduction may not have been documented, and cannot be ruled out.

The first aim of my thesis was to evaluate the haplotype variation of the spiny-cheek crayfish populations from Europe and North America. Mitochondrial gene for cytochrome c oxidase subunit 1 (COI) of selected *O. limosus* individuals from several European countries and from a part of its American range (Maine, Pennsylvania) was sequenced to obtain data about haplotype variation of the examined populations and to get more information about the possible origin of the European individuals of the species. Our results showed that the founder population for European spiny-cheek crayfish came most likely from the northern part of its American range. Differences in distributions of haplotypes found in studied populations in America were most likely connected with anthropogenic origin of populations in the northern part of the range or with the location of refugia during the last glaciation and the subsequent recolonisation of the territory.

After assembling detailed data on the distribution of *O. limosus* in the Czech Republic, we analysed genetic variability of selected Czech populations of the species using allozyme electrophoresis in order to test whether enough variability was maintained during the introduction of the species to Europe. Our results show, that although the founding population was relatively small, allozyme variability was not dramatically reduced. No correlation between genetic and geographic distances among populations suggest that the distribution of the species was influenced by translocations of crayfish by people, followed by random drift in allele frequencies.

Last aim of my study was to analyse individuals of another North American crayfish, the virile crayfish (*Orconectes virilis*), which has been discovered several years ago in London (UK). We tried to assess their position within the lineages of the *O. virilis* species complex known from a part its American range by sequencing of the mitochondrial gene for COI. As the analysis shows, London individuals (and also one sample from Iowa, USA) represent new lineages of the *O. virilis* complex.

ABSTRAKT (in Czech)

Raci tvoří již po staletí nedílnou součást evropské fauny. V minulosti byli v přírodě loveni a využíváni na konzumaci. V posledních letech však po celém světě dochází stále častěji k invazím živočišných i rostlinných druhů. Následky těchto invazí zasáhly také původní evropské raky, jejichž populace byly z velké části zdecimovány původcem račího moru (oomycetou *Aphanomyces astaci*, Saprolegniales). Ve snaze nahradit tyto ztracené populace bylo od roku 1890 do Evropy dovezeno několik severoamerických raků, kteří v Evropě dobře prosperují, pro původní druhy ale představují vážné nebezpečí - jsou přenašeči patogenu račího moru a v případě kontaktu s původními druhy je mohou dále infikovat.

Ve své diplomové práci jsem se zaměřila na jeden z těchto invazních druhů, raka pruhovaného (*O. limosus*). Na základě dostupných literárních dat byl *O. limosus* do Evropy úspěšně introdukovan pouze jednou, všichni evropští raci pruhovaní by tedy měli být potomky těchto dovezených jedinců, zcela není ale vyloučena ani možnost dalších, v literatuře nezaznamenaných pokusů o introdukci tohoto raka do Evropy. S cílem zjistit, odkud mohla pocházet zdrojová populace evropských jedinců tohoto druhu jsem sekvenovala mitochondriální gen pro podjednotku I cytochrom c oxidázy (COI) raků pruhovaných z Evropy a Severní Ameriky. Počet haplotypů nalezených v evropských populacích byl výrazně nižší než v Severní Americe, což ukazuje, že v minulosti došlo zřejmě k jediné introdukci tohoto druhu do Evropy. Haplotyp, který byl dominantní v Evropě, se dále vyskytoval také v severní části amerického areálu (severní Pensylvánie, Maine), v populacích z jižní Pensylvánie byl dominantní odlišný haplotyp. Je tedy pravděpodobné, že evropští raci pruhovaní pochází spíše ze severní části areálu v USA. Rozdíly ve složení haplotypů v populacích raka *O. limosus* v severní a jižní části jeho amerického areálu souvisí pravděpodobně s jeho nedávnou introdukcí do severní části areálu či s existencí více refugií tohoto druhu během poslední doby ledové.

Dále jsem se zabývala výskytem raka *O. limosus* na území České Republiky a genetickou variabilitou jeho českých populací. Pomocí alozymové elektroforézy jsem analyzovala vybrané populace raka *O. limosus* s cílem zjistit, do jaké míry jsou tyto populace variabilní. Výsledky ukazují, že i přes relativně malý počet zakládajících jedinců, byla během introdukce raka pruhovaného do Evropy zachována dostatečná variabilita na úrovni alozymů. Nebyl zjištěn vztah mezi genetickou a geografickou vzdáleností, což napovídá, že se na šíření tohoto druhu podílel člověk a při sekundárních introdukcích docházelo k náhodným posunům ve frekvenci alel.

Součástí práce byla také genetická analýza jedinců jiného severoamerického druhu, raka *Orconectes virilis*, z Velké Británie pomocí sekvenace COI. Na základě porovnání s liniemi tohoto druhového komplexu známými z jeho areálu v Americe se ukázalo, že se jedná o novou linii, odlišnou od těch, které byly dosud detekovány v Severní Americe. Další dosud neznámá linie byla zjištěna i u jedince z lowy (USA).

INTRODUCTION

Crayfish, with about 600 species in the whole world, represent an important part of freshwater ecosystems (Sinclair *et al.*, 2004). Several native crayfish species, belonging to genera *Astacus* and *Austropotamobius*, can be found in Europe (Souty-Grosset *et al.*, 2006). Since the 1860s, when crayfish plague was introduced to Europe, its pathogen (oomycete *Aphanomyces astaci*) has caused mass mortalities of many native crayfish populations and it still presents a serious threat for them (Vogt, 1999). In an attempt to replace lost populations, several species of non-indigenous crayfish were introduced to Europe (Souty-Grosset *et al.*, 2006).

In my study I focused especially on the spiny-cheek crayfish, *Orconectes limosus*. The species was first brought to Europe from North America in 1890 (Kossakowski, 1966; McDonald, 1893), which has most probably been the only case of its introduction to Europe (Chapter 1). From the place of its release, the species has spread to at least 17 European countries (Souty-Grosset *et al.*, 2006). Its presence in the Czech Republic was first recorded in 1988 (Hajer, 1989), but the species has most likely been observed in our country already in 1960s (Matouš, 1995). The spiny-cheek crayfish has probably invaded the territory by upstream migration in the river Elbe from Germany (Kozák *et al.*, 2004) and it has quickly spread over the western part of the country. During the first part of my studied, I contributed to assembling the detailed data on the distribution of *O. limosus* in the Czech Republic (Petrušek *et al.*, 2006; Filipová *et al.*, 2006 – see Appendix).

The main objective of the present thesis, apart from summarising the distribution of the spiny-cheek crayfish in the Czech Republic, was to learn more about its genetic variation, both in Czech populations and abroad. In the different parts of my work, I used allozyme electrophoresis and analysis of mitochondrial DNA variation.

Although in several publications the source region of the founder population of European *O. limosus* was supposed to be the watershed of the Delaware River (northeastern USA), in some of the recent papers (e.g., Holdich and Black, 2007; Souty-Grosset *et al.*, 2006) their authors expressed doubts about its real origin. We have therefore tried to identify the possible source area by sequencing the mitochondrial gene for COI of European and American individuals of the species. Also the origin of another North American crayfish, *Orconectes virilis*, was unknown, the only information available being that it came from an aquarium trade. Our aim was to analyse its population from Great Britain and compare it with known lineages of *O. virilis* species complex in America.

Results, which I obtained during the last four years of research, are presented as three manuscripts (Chapters 1-3), one chapter in a Czech monograph on invasive species, of which I am the first author (Appendix), and one published paper which I co-authored (Appendix). Each of these parts can be read independently, having its own introduction providing the necessary background information.

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CHAPTER 1

HAPLOTYPE VARIATION OF EUROPEAN AND AMERICAN POPULATIONS OF THE SPINY-CHEEK CRAYFISH, *ORCONECTES LIMOSUS*

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HAPLOTYPE VARIATION OF EUROPEAN AND AMERICAN POPULATIONS OF THE SPINY-CHEEK CRAYFISH, *ORCONECTES LIMOSUS*

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Abstract

According to available literature, the North American spiny-cheek crayfish, *Orconectes limosus*, was introduced to Europe once, in 1890 when 90 individuals were released in Poland. The exact origin of these founders remains unknown, although some sources suggested the watershed of the Delaware River (eastern USA) as the source area. In our study we tested whether all European populations of *O. limosus* come from a single source in North America and we also tried to identify the possible source of these invasive populations. We analysed diversity of the mitochondrial gene for cytochrome c oxidase subunit I of *O. limosus* individuals from Europe (8 countries, 22 populations, 67 individuals) and North America (eastern USA, 15 populations, 74 individuals), including the Delaware watershed. In European populations, two haplotypes were found, one widespread, the other very rare (4 individuals in 1 population). Six haplotypes were detected in the USA, two of them common, the first of them mostly in southern Pennsylvania, the second prevailing in the northern part of *O. limosus* present range (Maine, northern Pennsylvania). The latter one was identical with the dominant European haplotype, suggesting that the source of the European stock was located in northern parts of the species distribution in the late 19th century. Low haplotype variation in introduced populations supports the scenario of a single introduction of *O. limosus* to Europe.

Key words:

Orconectes limosus, haplotype variation, introduction, origin, Europe, North America

Introduction

Several non-indigenous crayfish species, mostly from North America, were introduced to Europe since the 19th century in order to replace lost populations of native species decimated by crayfish plague, which was accidentally introduced to Europe in 1860s (Vogt, 1999). Although the presence of introduced crayfish in Europe can be economically beneficial, they have negative impact on the local environments, in particular, they directly endanger native crayfish. Apart from interspecific competition, the North American species crayfish transmit the crayfish plague pathogen, the oomycete *Aphanomyces astaci*, to indigenous species; this results in mass mortalities and further reduces their numbers in areas invaded by American species (Holdich, 1999).

The colonisation process of European waters by three most widespread American invasive crayfish species differs substantially. Two of them, the signal crayfish (*Pacifastacus leniusculus*) and the red swamp crayfish (*Procambarus clarkii*), were brought to European continent several times and in large numbers (Souty-Grosset *et al.*, 2006). The most important introductions took place in the 1960s, when more than 100 thousand individuals of the signal crayfish from California were introduced into Sweden (Skurdal *et al.*, 1999) and in 1973, when about 40 thousand individuals of the red swamp crayfish from Louisiana were released in Spain (Henttonen and Huner, 1999).

However, colonisation by the third of the most widespread species, the spiny-cheek crayfish (*Orconectes limosus*) has been different. It was first brought to Europe in 1890, when 90 individuals of a batch, sent by the United States Commission of Fish and Fisheries, survived transport to Germany (McDonald, 1893) and were released to a fishpond near Barnòwko (Berneuchen) in Pomerania (currently western Poland) (Kossakowski, 1966). This seems to be the only known successful introduction of this species to Europe (Kulmatycki, 1935), as another recorded attempt to introduce it from New York to France in 1895 failed (Kossakowski, 1966).

Exact origin of the European spiny-cheek crayfish is unknown. Its North American range is on the eastern coast of the USA and Canada but has also been affected by human activities. Since 1970, it was introduced to Maine, New Hampshire and the watershed of the St. Lawrence River (Souty-Grosset *et al.*, 2006) and in 2005

non-native population of the species has been found in Nova Scotia in Canada (Lambert *et al.*, 2007). Several publications (e.g., Kossakowski, 1966; Henttonen and Huner, 1999; Holdich, 2003) claimed that the crayfish introduced to Europe came from the Delaware River in the northeastern USA, but in some recent papers authors doubt about the real origin of the stock (e.g., Holdich and Black, 2007; Souty-Grosset *et al.*, 2006). However, this information seem to be only an overinterpretation of Schikora (1916), who supposed that the possible source locality might have been in the Delaware watershed. Unfortunately, the report of the US Commission confirming the overseas transport of crayfish in late 1889 does not mention their origin (McDonald, 1893).

During the century after its introduction to Europe, the spiny-cheek crayfish has rapidly spread to neighbouring countries, both naturally and by secondary human-mediated introductions (Souty-Grosset *et al.*, 2006). Nowadays, it can be found in at least 17 European countries, and is likely to appear in the River Danube in Bulgaria, Romania and Ukraine (Souty-Grosset *et al.*, 2006). It has also been introduced to Morocco in North Africa (Holdich, 2003). The present distribution of the species in Europe and the USA is summarised in Fig. 1. Interestingly, although in Europe this species is considered invasive pest, it becomes endangered in its native range, being itself threatened by other aggressively spreading species of cambarid crayfish (Bouchard *et al.*, 2007).

The successful spread of *O. limosus* in Europe can be explained by its ecological plasticity, tolerance to deteriorated environmental conditions, and reduction of competition with native crayfish populations through transmission of crayfish plague (Lindqvist and Huner, 1999); its disjunct distribution then by long-range transport by humans, and secondary introductions. However, we cannot completely rule out an alternative scenario that undocumented introduction(s) from the original distribution area increased not only colonised range in Europe but also the species genetic diversity, reducing the potentially negative effect of introduction bottleneck. Our preliminary analyses of intrapopulation genetic variation of the species in the Czech Republic, based on allozyme markers (Chapter 2), suggested that *O. limosus* populations are diverse even within a small area for which introduction scenario is supposed to be relatively simple (Petrusek *et al.*, 2006).

To test whether all European populations come from a single source, and in an attempt to locate the source area within the species native range, we compared

sequences of cytochrome *c* oxidase I (COI) gene fragment of randomly selected individuals from different European populations with those from its North American range, including the supposed source region for the 1890 introduction. Other studies on cambarid crayfish showed substantial divergences of mitochondrial lineages among various geographic regions or watersheds in their native range (Fetzner and Crandall, 2003; Mathews *et al.* 2008) as well relatively high mtDNA variation in introduced populations (Barbaresi *et al.*, 2007). We therefore tested whether American *O. limosus* populations show similar patterns. If so, multiple introductions from different sources could result in presence of divergent haplotypes in invaded area in Europe. Additionally, knowledge of the level of intraspecific geographic variation may be important for conservation measures in the native range of this crayfish.

Materials and methods

Sampling

Overall, 138 individuals of *Orconectes limosus* from 34 localities were sequenced (Table 1, Fig. 1). The populations came from the European range of its distribution: the Czech Republic, France, Great Britain, Germany, Belgium, Italy, Hungary (overall 65 individuals from 20 populations) and from several localities in Pennsylvania and Maine in its American range (73 individuals from 14 populations) (Fig. 1). In our samples we also included populations from a potential source region of the animals introduced to Europe in 1890, from several brooks of the Delaware River watershed. Three extra sequences, two from Poland (AF517105, Soroka *et al.*, unpublished data; DQ882096, Costa *et al.*, 2007) and one from New York (AY701199, Taylor and Knouft, 2006) were obtained from Genbank.

An effort was made to collect crayfish from representative localities in its invasive range in Europe. We included more localities from the Czech Republic, selected to cover populations and individuals showing differences in allozyme markers (Chapter 2), therefore maximising the chance that divergent mtDNA haplotypes, if present, would be detected.

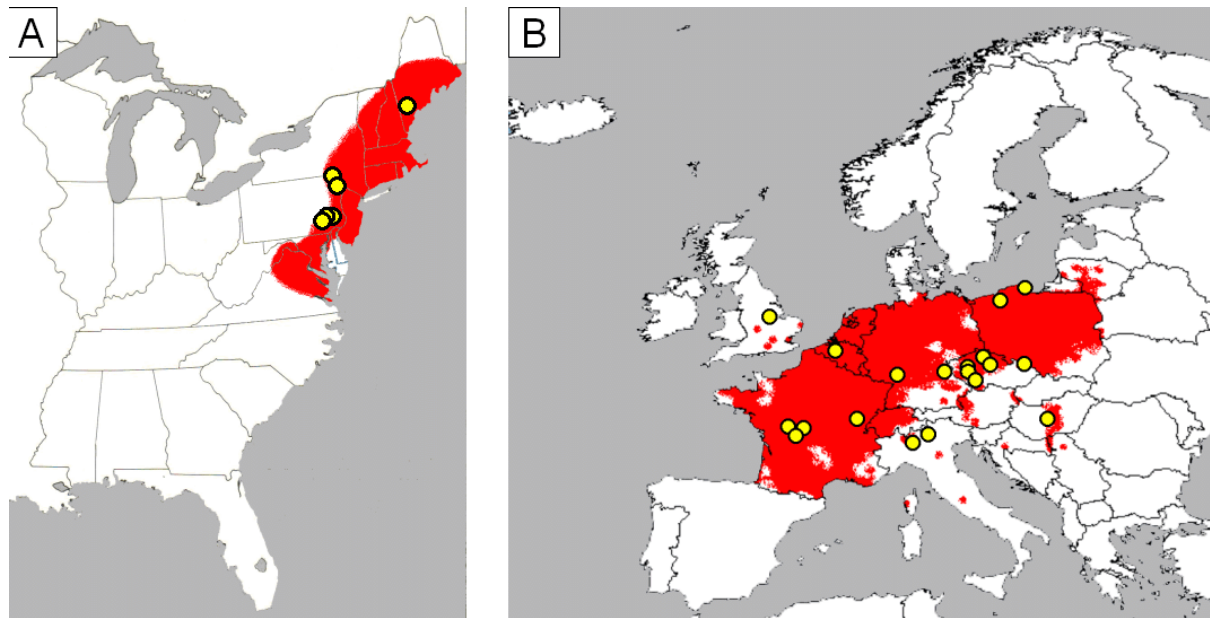
Table 1.

Summary of sampled localities of *O. limosus*, numbers of analysed individuals (n) and detected haplotypes. Numbers in parentheses at haplotype codes indicate number of individuals with the particular haplotype, if no number is provided, all individuals from the respective population carried the same haplotype. Localities from North and South Pennsylvania are distinguished.

		locality	area	n	sampling date	latitude	longitude	haplotype codes
NORTH AMERICA	Maine	Stroudwater River	Cumberland County	9	28.7.2003	43° 40' N	70° 22' W	N1
	New York	East Branch Delaware River	Delaware County	1	1.10.2002	41° 58' N	75° 11' W	S1
	Pennsylvania	West Branch Delaware River	Wayne County	1	10.3.-13.7.05	41° 57' N	75° 17' W	S1
	Pennsylvania	Raymondskill creek	Pike County	6	10.3.-13.7.05	41° 17' N	74° 50' W	N1(5), S1(1)
	Pennsylvania	Dingmans creek	Pike County	5	10.3.-13.7.05	41° 14' N	74° 54' W	N1
	Pennsylvania	Hornbecks creek	Pike County	10	10.3.-13.7.05	41° 12' N	74° 54' W	N1
	Pennsylvania	Manatawny Creek	Berks County	6	25.5.2006	40° 19' N	75° 44' W	S1(5), N2(1)
	Pennsylvania	Stony Run	Chester County	3	25.5.2006	40° 10' N	75° 35' W	S1
	Pennsylvania	Valley Creek	Chester County	2	10.5.2006	39° 59' N	75° 40' W	S1
	Pennsylvania	Ridley Creek	Delaware County	6	11.5.2006	39° 57' N	75° 27' W	S1(4), S2(2)
	Pennsylvania	Buck Run	Chester County	6	10.5.2006	39° 56' N	75° 50' W	S1
	Pennsylvania	West Branch Chester Creek	Delaware County	6	11.5.2006	39° 53' N	75° 30' W	S1(5), S3(1)
	Pennsylvania	Brandywine Creek	Delaware County	6	10.5.2006	39° 52' N	75° 36' W	S1(5), N3(1)
	Pennsylvania	East Branch White Clay Creek	Chester County	6	12.5.2006	39° 52' N	75° 47' W	S1
Pennsylvania	Big Elk Creek	Chester County	1	11.5.2006	39° 44' N	75° 51' W	N3	
EUROPE	Great Britain	Clifton Pond, Attenborough	Nottinghamshire	3	April 2006	52° 54' N	1° 14' W	N1
	Belgium	Zonhoven	Flemisch Region	5	January 2008	50° 59' N	5° 22' E	N1
	France	Auxances, Migné-Auxances	Poitou-Charentes	2	13.7.2006	46° 37' N	0° 18' E	N1
	France	Vouneuil sous Biard	Poitou-Charentes	1	13.7.2006	46° 34' N	0° 16' E	N1
	France	Jazeneuil	Poitou-Charentes	3	13.7.2006	46° 27' N	0° 4' E	N1
	France	Lac d'Illay, La Chaux-du-Dombief	Franche-Comté	3	7.8.2007	46° 37' N	5° 54' E	N1
	Germany	Rhine, Breisach	Baden-Württemberg	3	10.8.2005	48° 1' N	7° 34' E	N1
	Germany	Naab, Pielenhofen	Bayern	2	29.6.2004	49° 4' N	11° 57' E	N1
	Italy	Ticino, Pavia	Lombardia	1	March 2008	45° 10' N	9° 9' E	N1
	Italy	Cherio, Borgo di Terzo	Lombardia	1	March 2008	45° 43' N	9° 53' E	N1
	Czech Republic	Záluží u Litvínova	Ústí nad Labem Region	4	June 2007	50° 33' N	13° 36' E	N1
	Czech Republic	Cítov, Mělník	Central Bohemia	3	12.10.2005	50° 21' N	14° 26' E	N1
	Czech Republic	Lhota	Central Bohemia	4	July 2005	50° 15' N	14° 40' E	N1
	Czech Republic	Smečno	Central Bohemia	2	9.4.2006	50° 12' N	14° 2' E	N1
	Czech Republic	Hracholusky, Čerňovice	Plzeň Region	2	25.6.2006	49° 48' N	13° 6' E	N1
	Czech Republic	Starý Klíčov, Domažlice	South Bohemia	4	23.10.2005	49° 24' N	12° 58' E	N1
	Czech Republic	Soběslav	South Bohemia	1	23.8.2007	49° 15' N	14° 43' E	N1
	Czech Republic	Malše, České Budějovice	South Bohemia	7	12.9.2005	48° 58' N	14° 29' E	N1
	Czech Republic	Prudník, Osoblaha	Silesia	12	27.10.2006	50° 18' N	17° 43' E	N1(8), N4(4)
	Poland	Lake Spore, Szczecinek	Zachodniopomorskie	1	September 2000	53° 47' N	16° 42' E	N1
Poland	Vistula Lagoon, Elbląg	Warmińsko-Mazurskie	1	September 2001	54° 16' N	19° 20' E	N1	
Hungary	Bóni-fok, Bogyiszló	Tolna	2	14.10.2006	46° 22' N	18° 47' E	N1	

Figure 1.

Distribution of *O. limosus* in the USA (A) and Europe (B) (Souty-Grosset *et al.*, 2006; Bouchard *et al.*, 2007; Fetzner, 1999-2006) with highlighted source localities of individuals included in our study.



DNA analysis

One segment of leg of captured crayfish was dissected to obtain muscle tissue, from which the genomic DNA was subsequently extracted following the Chelex extraction protocol: approx. 1 mm³ of the muscle tissue was placed in a solution of 175 µl of distilled water and 5 µl of proteinase K (20 mg/ml), and homogenised; subsequently, 175 µl of H₂O were added and the content was homogenised again. Finally, a small amount (about 50 µl) of Chelex 100 beads was added, the Eppendorf tubes were vortexed gently and incubated at 56°C for four hours, followed by incubation at 100°C for 8 minutes to denature proteins. Chelex resin and undigested solids were removed from the suspension by centrifugation for 4 minutes at 12,000 rpm, and the supernatant was stored at -20°C.

PCR reaction mixtures of the volume 25 µl contained 5x PCR buffer (1.5 mM MgCl₂) (Promega), 200 µM dNTP, 25 µM each primer, 0.625 units Taq polymerase (Promega), 0.5 µl of the template (about 100 ng DNA). HCO 2198 and LCO 1490

primers (Folmer *et al.*, 1994) were used to amplify the COI gene fragment. The amplification program consisted of an initial denaturation step of 5 min at 95°C, 35 cycles of 50 s at 95°C, 50 s at 55°C and 50 s at 72°C, and a final extension for 5 min at 72°C. PCR products were purified using Exonuclease I (New England Biolabs) and Shrimp Alkaline Phosphatase (Fermentas), with an incubation for 1 hour at 37°C followed by 20 min at 80°C. Purified products were then sequenced using LCO primers and BigDye v. 3.1 Terminator kit on a capillary sequencer (ABI PRISM 3130). The length of obtained sequences was mostly about 627 bp.

Results of the cytochrome *c* oxidase I (COI) sequencing were analysed using Mega 4.0 (Tamura *et al.*, 2007). Newly obtained 138 sequences together with three sequences from GenBank could unambiguously be aligned by eye. Haplotype network was constructed using the program TCS v. 1.21 (Clement *et al.*, 2000).

Results

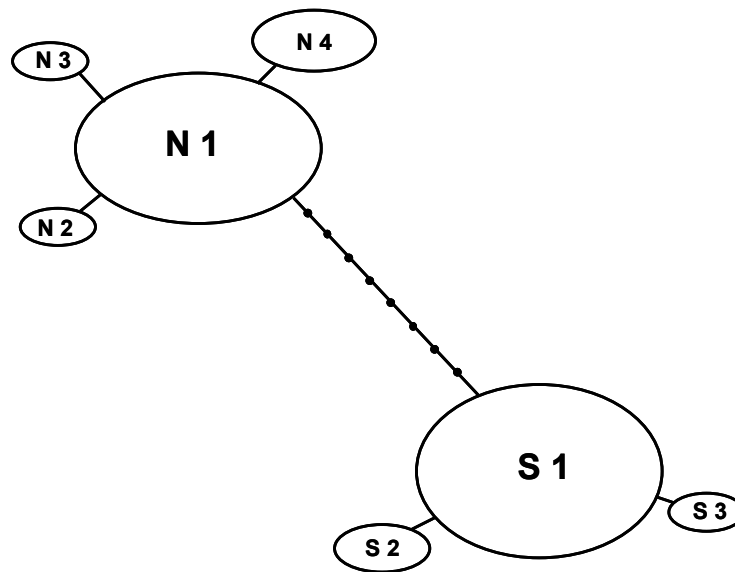
European populations of *O. limosus* show very low variability of the analysed COI fragment. A vast majority of analysed individuals carried an identical haplotype (N1 in Figure 2). Only in four individuals from a single locality (Prudník, Czech Republic), another haplotype (N4) was present, differing by a single point mutation from the common haplotype N1. On the other hand, we observed higher COI variation in populations of the spiny-cheek crayfish from the USA; however, most of the variation was concentrated in samples from southern Pennsylvania.

In total, seven haplotypes were detected in tested populations of the spiny-cheek crayfish from the USA and Europe, split into two well-separated clusters (Fig. 2), labelled S (south) and N (north) according to the prevailing distribution of the two most common haplotypes, central to the clusters, in the USA (Fig. 3). One or two haplotypes were observed in each of studied American populations. Haplotype N1, which was dominant in Europe (in 63 out of 67 tested individuals), was also present in Maine (all 9 ind.) and northern Pennsylvania (in 20 out of 22 individuals). The second most common haplotype, S1, was found especially in southern Pennsylvania (in 36 out of 42 individuals) but occurred also in northern Pennsylvania (in 2 out of 22 ind.). These two major haplotypes differed by 8 mutations (1.3%) from each other. Five other haplotypes, each differing by a single point mutation from the central

haplotypes (Figure 2), were detected: haplotype N4 only in Europe and the remaining four haplotypes (S2, S3, N2, N3) in southern Pennsylvania, each in one or two localities (Table 1, Fig. 3).

Figure 2.

Network of COI haplotypes detected in studied individuals of the spiny-cheek crayfish from Europe and North America. Haplotypes are labelled according to their prevailing distribution in America: northern part of the range (N) or southern Pennsylvania (S).



Discussion

Our study showed that haplotype variation of invasive European populations of *O. limosus* are significantly lower in comparison with American populations of the species, which is consistent with the scenario of a colonisation from a single source. Nevertheless, multiple introductions from an area with the presence of the same dominant haplotype cannot be excluded. Presence of a few individuals with a different haplotype recorded at a single locality in Europe suggests that individuals carrying more than one haplotype could have been brought to Europe during the initial introduction; however, this single point mutation could have also arisen after the introduction. If this haplotype is eventually found in the species native range, and shows only a limited distribution, it might help us identify the source area of European spiny-cheek crayfish.

In another crayfish species introduced from North America to Europe, *Procambarus clarkii*, invasive populations were also less variable than those from its native range (Barbaresi *et al.*, 2007). However, COI haplotype variability found in European populations of this species was much higher (6 haplotypes in 53 individuals from 10 populations) than the variation detected in our study of European *O. limosus* populations. Barbaresi *et al.* (2007) therefore suggested that *P. clarkii* had been introduced to Europe several times from different source localities.

Our results show, that all samples from Maine and most from northern Pennsylvania had the same haplotype as European *O. limosus* individuals, while the dominant haplotype in southern Pennsylvania was different from the dominant haplotype in Europe. Therefore, the distribution pattern of haplotypes present in the USA suggests that the source population for the European spiny-cheek crayfish was more likely from the northern part of the species range.

However, substantial differences in the haplotype variation of populations in Maine and northern Pennsylvania compared to southern Pennsylvania, might also be due to the fact that spiny-cheek crayfish are not native in at least some parts of the northern half of their present American range (Lambert *et al.*, 2007; McAlpine *et al.*, 1991; Souty-Grosset *et al.*, 2006). The haplotype composition and variation in newly colonised areas could have been affected by introduction bottlenecks and founder effects.

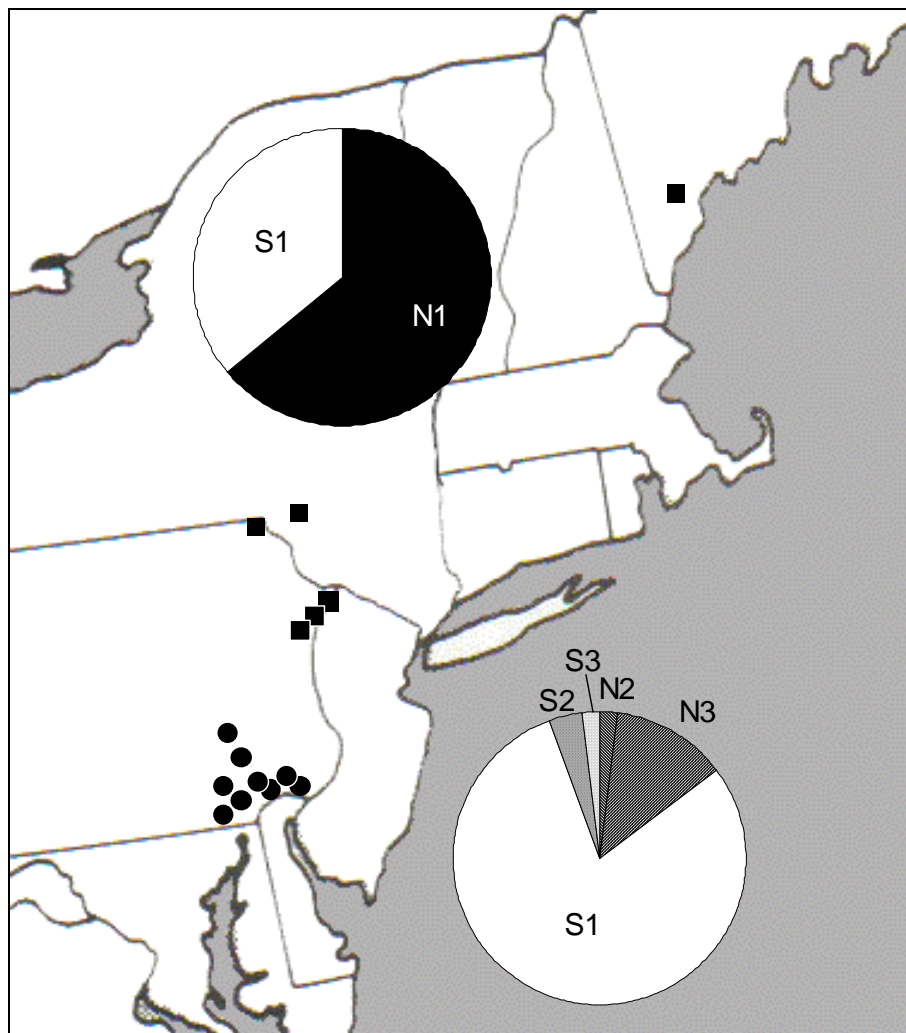
Northern Pennsylvania itself could have been colonised by the spiny-cheek crayfish from the south recently, through artificial canals or with the assistance of people. Bouchard *et al.* (2007) suppose that individuals of *O. limosus* in the Upper Delaware River system could have arrived to the area from the south via the Delaware and Hudson Canal, which could also explain the apparent absence of the species in the central part of the river. Maine is certainly considered as a region colonised in the recent decades (Souty-Grosset *et al.*, 2006; McAlpine, 1991). Given the conspicuous sharing of the dominant haplotype between invaded areas in Europe and North America, we might presume that their source had been in a similar region.

However, genetic differences of spiny-cheek crayfish populations from northern and southern Pennsylvania could also reflect more ancient processes, in particular, recolonisation after the last ice age. The existence of at least two glacial refugia of *O. limosus* is supposed: one in northeastern Pennsylvania and lower Hudson Rivers (Rhoades, 1962; Ortmann, 1906), the other in southeastern

Pennsylvania and Chesapeake Bay (Ortmann, 1906). Survival of different haplotypes, and possibly reduction of the haplotype diversity in the northern refugium, could then lead to the patterns observed today.

Figure 3.

Distribution of studied *O. limosus* populations in the Northeastern United States with pie charts of haplotypes (based on weighted average) detected in two examined areas: northern Pennsylvania, New York and Maine (squares, upper chart) and southern Pennsylvania (circles, bottom chart).



The two star-shaped clusters in the haplotype network suggest recent fast expansions. However, the distributions of the two groups, as well as the two dominant haplotypes, overlap, showing that both haplogroups got into contact. Further sampling is needed to assess more detailed overview on the mtDNA diversity of the spiny-cheek crayfish in its American range, and reconstruction of its recent recolonisation history.

More detailed data would be important also for the conservation – the species is endangered in the USA by other invasive crayfish, such as *O. virilis* and probably also *O. rusticus* and *O. obscurus* (Bouchard et al., 2007). Additional work may therefore target most important areas for conservation to preserve the gene pool of the species.

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CHAPTER 2

**ALLOZYME VARIATION OF POPULATIONS OF THE SPINY-CHEEK CRAYFISH,
ORCONECTES LIMOSUS (CAMBARIDAE), IN THE CZECH REPUBLIC**

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ALLOZYME VARIATION OF POPULATIONS OF THE SPINY-CHEEK CRAYFISH, *ORCONECTES LIMOSUS* (CAMBARIDAE), IN THE CZECH REPUBLIC

FILIPOVÁ L., KOZUBÍKOVÁ E., PETRUSEK A.

Abstract

The North American spiny-cheek crayfish, *Orconectes limosus*, was most probably introduced to Europe only once, in 1890. The size of the founding population was just 90 individuals. As a consequence of a bottleneck effect during the introduction, a low genetic variability of the European spiny-cheek crayfish populations could be supposed; on the other hand, the fast spread of *O. limosus* in Europe, and colonisation of various habitats suggest that this species does not suffer from inbreeding depression due to the introduction bottleneck. We analysed selected *O. limosus* populations from the Czech Republic using allozyme electrophoresis to evaluate the level of intra- and among-population genetic variation. Our results revealed several variable allozyme loci in this species, suggesting that enough variability was maintained during the first introduction. Genetic differentiation of its populations was relatively low and comparison of the genetic and geographic distance among populations did not reveal any significant relationship.

Key words

Orconectes limosus, allozyme electrophoresis, genetic variation, Czech Republic

Introduction

With an increasing number of species introduced to new territories, there arises a need to understand the process of colonisation and factors influencing the distribution potential of the studied species. Apart from other factors, the success of invasive taxa may depend on the genetic variability of their populations on the new territory. High genetic variability is supposed to be advantageous in invading new areas, because in sexual species it allows adaptation to changing environmental

conditions. However, even organisms with very low genetic variability of their invasive populations can be excellent colonisers. In an extreme case, a widespread invading species can be represented by a single clone, such as the tropical alga *Caulerpa taxifolia*, invading a very large area of the North-western Mediterranean (Jousson *et al.*, 1998), or an asexual American water flea (hybrid *Daphnia "pulex" × D. pulicaria*) in Africa (Mergeay *et al.*, 2006). In these cases, low genetic diversity is no obstruction for the invader's spread and competition with genetically diverse indigenous species.

Prominent among successful invasive animal groups in European waters are several species of North American crayfish, which have been brought to the continent since the end of the 19th century. They were introduced in an attempt to replace lost native crayfish populations decimated by crayfish plague. Three of these invasive crayfish – the signal crayfish (*Pacifastacus leniusculus*), the red swamp crayfish (*Procambarus clarkii*), and the spiny-cheek crayfish (*Orconectes limosus*) – are extremely widespread in Europe (Henttonen and Huner, 1999). They inhabit variable types of habitats and successfully compete with native species, moreover, they serve as a vector of the oomycete *Aphanomyces astaci*, pathogen of the crayfish plague. If infected American crayfish get into a contact with native species, they may cause their mass mortalities (Holdich, 1999).

In our study, we have focused on the genetic variation of the third mentioned species, the spiny-cheek crayfish *O. limosus*. It was first introduced to Europe in 1890, when 90 individuals were released into a pond in Pomerania (currently western Poland; for details see Chapter 1), which has most probably been the only case of its introduction to Europe (Kossakowski, 1966). This is supported also by the analysis of mitochondrial gene for cytochrome c oxidase subunit I of European and American populations of the species (see Chapter 1). From the point of its first introduction *O. limosus* has spread to at least 17 European countries (Souty-Grosset *et al.*, 2006), both naturally and by human-mediated translocations. Among those who spread non-native crayfish to both standing and running waters are anglers, owners of waterbodies or recreational scuba divers (Petrušek *et al.*, 2006); usually being unaware of the negative impact of such activities on native ecosystems.

Orconectes limosus is the most widespread invasive North American crayfish in the Czech Republic. Its presence in the country was first confirmed in 1988 close to the border with neighbouring Germany. However, the species has most probably been observed in the country already in the 1960s. *O. limosus* has most likely

invaded the territory by upstream migration in the river Elbe from Germany (Petrušek *et al.*, 2006, Kozák *et al.*, 2004). Since the late 1980s, these crayfish have quickly spread over a large area of the Czech Republic, especially its western part (Petrušek *et al.*, 2006). *O. limosus* can now be found mostly in large watercourses, lower reaches of their tributaries and in isolated standing waters, such as flooded quarries, sandpits or ponds. Detailed distribution of the spiny-cheek crayfish in the Czech Republic has been described by Petrušek *et al.* (2006) and Filipová *et al.* (2006) (see Appendix). However, new localities with the presence of this species in the country are still being discovered. In July 2006, *O. limosus* was recorded in the Lipno Reservoir (Beran and Petrušek, 2006), in October 2006, it was first found in the north-eastern part (Silesian region) in the brook Prudník, close to the border with Poland (Ďuriš and Horká, 2007; Kozubíková *et al.*, 2008).

The aim of this study was to assess the level of genetic variability of chosen populations of this species in the Czech Republic, using allozyme electrophoresis. We tested the hypothesis that sufficient genetic variation was maintained during the introduction of the species to Europe, so that allozyme markers could be used for analysis of the genetic structure of these populations. Although we suppose a large influence of long-range translocations of the spiny-cheek crayfish within the Czech Republic in contrast to the stepping-stone model of population structure, we wanted to verify it by comparing genetic and geographic distances of studied populations.

Material and methods

Sampling

Overall, 222 individuals of *Orconectes limosus* from 14 populations were analysed. Crayfish were sampled during the years 2004 to 2007 from various types of localities – brooks, sandpits, lakes, reservoirs, and flooded quarries (Fig. 1, Table 1). Nearly all the samples came from the western part of the country, with one exception, locality in Prudník (Silesia), which was colonised by individuals from a different region, by upstream or downstream migration from Poland (Ďuriš and Horká, 2007). Crayfish were mostly captured by hand or while scuba-diving. After a transport in cooling boxes, individuals were stored in a deep freezer (in -80°C). The tissue for the analyses was then dissected from leg or claw of the captured crayfish.

Figure 1.

Distribution of *O. limosus* in the Czech Republic (empty circles) and localities where individuals were sampled for the present study (red circles with numbers, corresponding to the codes of localities in the Table 1).

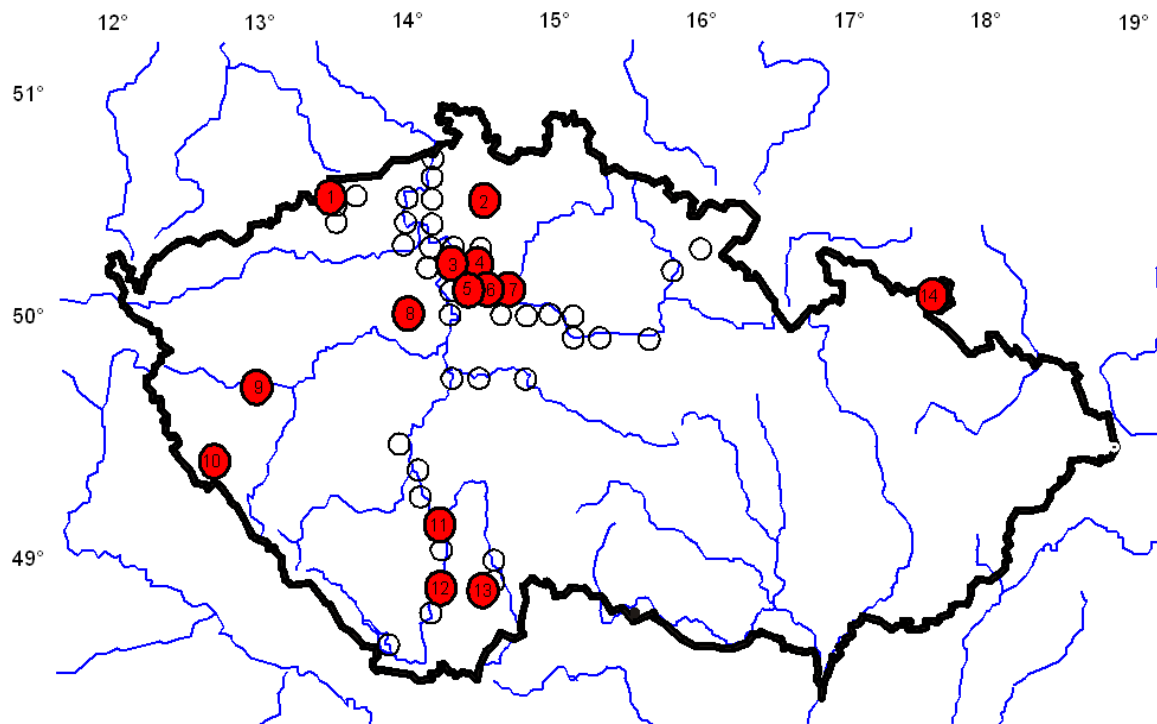


Table 1.

Summary of localities from where the analysed individuals of the spiny-cheek crayfish were collected (codes correspond to numbers in the map in Fig. 1), character of the locality, numbers of captured individuals (n), date of sampling.

code	name of locality	closest settlement	locality character	n	date of sampling	latitude (N)	longitude (E)
1	Záluží	Litvínov	retentive reservoir	22	June 2007	50°33'	13°36'
2	Stará pískovna	Provodín	sandpit	10	13.9.2004	50°37'	14° 36'
3	Cítov	Horní Počaply	sandpit	8	12.10.2005	50°21'	14°26'
4	Pšovka	Lhotka	brook	10	4.6.2005	50°23'	14° 33'
5	Kojetice - quarry	Kojetice	flooded quarry	20	20.7.2005	50°14'	14°31'
6	Proboštská jezera	Stará Boleslav	sandpit	17	3.9.2005	50°12'	14°39'
7	Lhota	Lhota	sandpit	35	July 2005	50°14'	14°40'
8	Smečno - pond	Smečno	pond	21	9.4. 2006	50°11'	14° 2'
9	Hracholusky	Přovany	reservoir	11	25.6.2006	49°47'	13° 6'
10	Klíčov	Mrákov	flooded quarry	20	23.10.2005	49°23'	12°57'
11	Kořensko	Neznašov	reservoir	11	26.4.2004	49°14'	14°22'
12	Malše	České Budějovice	river	12	12.9.2005	48°58'	14°29'
13	Zlatá Stoka	Třeboň	brook	14	19.7.2006	49°0'	14°46'
14	Prudník	Slezské Pavlovice	brook	11	27.10.2006	50°17'	17°43'

Methods

Horizontal cellulose acetate electrophoresis was used for the genetic analyses as described in Hebert and Beaton (1993). Overall, seventeen enzymes were tested. Some of them did not show sufficient activity and were therefore excluded from analyses: α -amylase (**AMY**, EC 3.2.1.1), fumarate hydratase (**FUM**, EC 4.2.1.2), hexokinase (**HEX**, EC 2.7.1.1), xanthine dehydrogenase (**XDH**, EC 1.1.1.204), alcohol dehydrogenase (**ADH**, EC 1.1.1.1), and α,α -trehalase (**TRE**, EC 3.2.1.28), isocitrate dehydrogenase (**IDH**, EC 1.1.1.42), malate dehydrogenase NADP⁺ (**ME**, EC 1.1.1.40) and adenylate kinase (**AK**, EC 2.7.4.3). Further, we did not include aspartate amino transferase (**AAT**, EC 2.6.1.1), although it scored well, as it showed very low migration speed under the conditions used for other enzymes.

Eight enzyme loci were finally selected for further analyses: glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 5.4.2.2), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), malate dehydrogenase (two loci, MDH 1; MDH 2, EC 1.1.1.40), arginine kinase (ARK, EC 2.7.3.3), lactate dehydrogenase (LDH, EC 1.1.1.27) and aldehyde oxidase (AO, EC 1.2.3.1). The most common allele for each locus was designated M (medium). Other alleles were labelled corresponding to their relative mobility to the M-allele: F (fast), S (slow), S⁻ (very slow). Tissue of one crayfish individual was used as a standard in all analyses, to simplify the scoring.

A small amount of tissue was dissected from crayfish legs or claws and homogenised with a plastic rod in about 10 μ l of distilled water. Allozyme electrophoresis was carried out in the Tris-Glycine buffer system (pH=8.5) on 76x76 mm cellulose acetate plates (Titan III, Helena Laboratoires). In each run, eleven animals and one standard, loaded in one row, were analysed. In some cases the number of individuals analysed together was twenty-two with two standards in two rows on the same gel.

Data analysis

Allelic frequencies, observed and expected heterozygosities, F statistics (Weir and Cockerham, 1984) and genetic distances were calculated in Genetix 4.03 (Belkhir *et al.*, 1996). GenAIEx 6.1 (Peakall and Smouse, 2006) was used to test

whether genotypic frequencies at studied loci are consistent with Hardy-Weinberg expectations. Nei's genetic distance (Nei, 1978) was calculated to estimate levels of genetic distance between tested populations. Based on these results, the UPGMA (Unweighted Pair Group Method with Arithmetic mean) dendrogram was created using Statistica 6.1 (Stasoft, Inc.), to depict graphically similarity between studied populations.

To test the relationship between genetic and geographic distances among populations, Mantel test for dependent variables (Mantel, 1967; software by Bonnet and Van de Peer, 2002) was used. Orthodromic distances were calculated from geographic coordinates to obtain distances between studied localities, being afterwards transformed in a logarithmic scale. These were then compared with a pairwise matrix of Nei's genetic distances.

Results

Out of eight loci used in our analyses, two enzymes (LDH and AO) showed no variability, and six revealed to be polymorphic, i.e., with more than one detected allele (GPI, PGM, MPI, MDH 1, MDH 2, ARK). However, in MDH 1 one of the two detected alleles was very rare (1%). The most variable locus was PGM with 4 different alleles detected, the slowest of them, S⁻, being relatively rare (5%). In four enzymes (GPI, MDH 2, MPI, ARK) three different alleles could be distinguished.

The summary of population characteristics is shown in the Table 3.

Genetic variability within studied populations

All 14 populations analysed in our study were polymorphic on at least two loci. The highest average number of alleles per locus was in the population from Lhota (2.25 alleles/locus) and in populations from Cítov, Kojetice and Klíčov (2 alleles/locus). The lowest average number of alleles per locus (1.375) was in the population from the Malše River.

Observed heterozygosity of populations was in most cases consistent with expected values (Table 3). The studied loci were in good agreement with Hardy-Weinberg expectations in most populations. However, two of the studied

populations, Zlatá stoka and Záluží, exhibited significant deviations of the Hardy-Weinberg equilibrium.

Table 2.

Allele frequencies [%] observed in eight studied loci in 222 individuals of the spiny-cheek crayfish from the Czech Republic.

locus	Allele frequency [%]			
	F	M	S	S-
GPI	39	20	41	
PGM	19	11	64	5
MPI	0	84	16	
MDH 1		99	1	
MDH 2	48	18	34	
ARK	1	96	3	
LDH		100		
AO		100		

Genetic differentiation among populations

Spiny-cheek crayfish populations in the Czech Republic were significantly genetically structured, with the mean F_{ST} value for all loci being 0.160. Nei's genetic distance between populations varied from 0.003 (between populations from Lhota and Malše) to 0.20 (between populations from Zlatá Stoka and Pšovka). The geographically distant population from Prudník (Silesia) did not markedly differ from other populations, and it was genetically closest to population from Záluží, located more than 290 km far away.

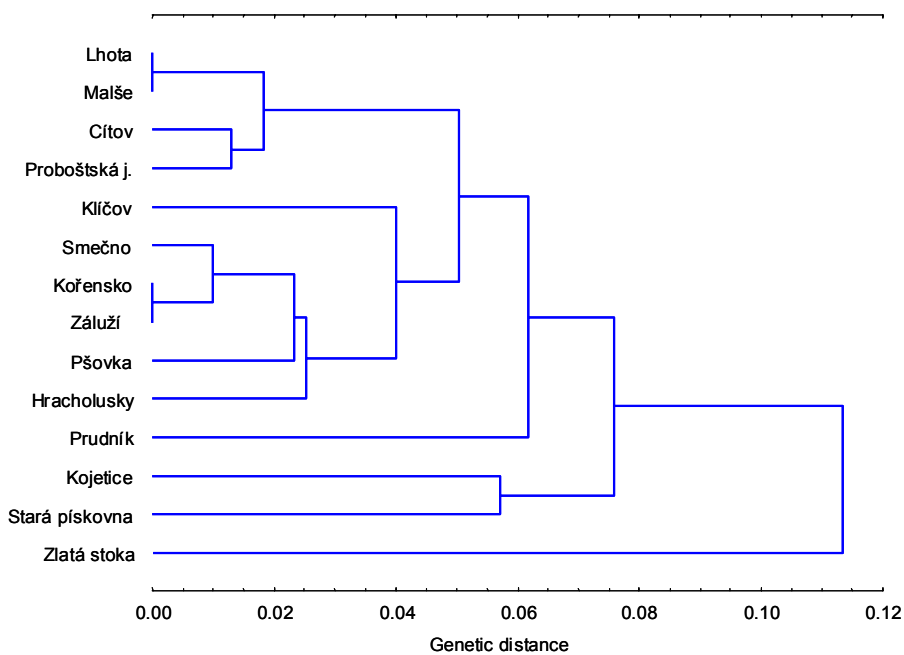
The UPGMA dendrogram (Fig. 2) does not show any apparent clustering. In some cases, even very distant localities revealed to be similar, such as a pair Kořensko and Litvínov (160 km) or Lhota and Malše (147 km). Relationship between logarithm of geographic distance and Nei's genetic distance revealed to be non-significant for all tested population (Mantel test, $p=0.126$). The result was non-significant whether Prudník population (Silesian region) was included or not.

Some populations could have been expected to be more similar to each other than the rest of the studied Czech populations of *O. limosus*, as one was the source of crayfish for the other: the population in Klíčov was founded by individuals coming

from the Hracholusky Reservoir, and the sandpit Proboštská jezera were supplied with crayfish from the sandpit Lhota. However, we did not observe any substantially higher similarity between these populations in comparison to the others.

Figure 2.

UPGMA dendrogram, using Nei's genetic distance (Nei, 1978), showing genetic similarity of *O. limosus* populations included in our study.



Discussion

As Dlugosch and Parker (2008) showed, in invasions where a single introduction occurred, allelic richness was generally lower in introduced populations than in the native ones. Moreover, reductions in genetic diversity tend to be inversely correlated with the size of the founder population (Merilä *et al.*, 1996). Although the European populations of the species were founded just once and by a relatively small number of individuals, presence of several variable enzyme loci in the studied populations suggests that the bottleneck effect was not very dramatic and enough variation was retained during the introduction of the species to Europe. This is also supported by quick spread of the species and its presence in variable types of habitats.

Table 3.

Summary of population characteristics: numbers of examined individuals (n), heterozygosity expected non-biased (Hexp. n.b.) [%] and heterozygosity observed (Hobs.) [%], mean number of alleles per locus and Hardy-Weinberg exact probability.

	Lhota	Čítov	Klíčov	Hracholusky	Smečno	Malše	Kojetice	St. pískovna	Proboštská j.	Zlatá stoka	Kořensko	Pšovka	Záluží	Prudník
n	35	8	20	11	21	12	20	10	17	14	11	10	22	11
Hexp n.b.	1.90	2.99	3.19	2.17	2.55	1.30	2.74	2.10	2.00	1.13	2.36	2.30	2.50	2.79
Hobs.	1.41	2.59	2.88	1.71	2.62	1.04	2.38	1.38	2.28	1.25	2.50	1.50	2.67	1.82
alleles/locus	2.25	2	2	1.875	1.75	1.375	2	1.75	1.75	1.625	1.75	1.75	1.875	1.875
H-W exact probability	0.162	0.216	0.131	0.293	0.128	0.133	0.172	0.257	0.420	0.007	0.249	0.437	0.007	0.181

Table 4.

Orthodromal distances [km] (below the diagonal) and Nei's genetic distances (above the diagonal; Nei, 1978) between studied populations of the spiny-cheek crayfish from the Czech Republic.

	Lhota	Čítov	Klíčov	Hracholusky	Smečno	Malše	Kojetice	St. Pískovna	Proboštská j.	Zlatá stoka	Kořensko	Pšovka	Záluží	Prudník
Lhota	X	0.022	0.082	0.056	0.078	-0.003	0.086	0.057	0.011	0.07	0.051	0.065	0.038	0.062
Čítov	20.1	X	0.038	0.038	0.045	0.024	0.096	0.075	0.013	0.071	0.014	0.046	0.014	0.062
Klíčov	154.3	151.2	X	0.081	0.027	0.094	0.051	0.13	0.071	0.113	0.031	0.037	0.024	0.055
Hracholusky	120.2	113.2	44.6	X	0.028	0.068	0.061	0.067	0.016	0.15	0.026	0.024	0.023	0.086
Smečno	45.7	36.0	116.0	77.3	X	0.096	0.042	0.09	0.036	0.15	0.012	0.011	0.008	0.071
Malše	147.1	159.4	120.8	134.3	142.5	X	0.104	0.064	0.016	0.059	0.059	0.082	0.046	0.066
Kojetice	12.0	15.2	143.8	108.7	33.7	144.6	X	0.057	0.065	0.182	0.093	0.034	0.068	0.076
Provošín	41.2	29.4	177.9	137.8	62.0	187.4	43.0	X	0.031	0.101	0.107	0.107	0.081	0.09
Proboštská j.	5.7	23.4	150.0	116.8	43.9	141.5	11.3	46.6	X	0.085	0.024	0.036	0.016	0.061
Zlatá stoka	139.2	153.7	138.2	145.9	141.8	23.1	138.5	180.3	133.7	X	0.082	0.204	0.08	0.125
Kořensko	114.9	126.1	104.4	108.0	108.6	33.9	111.5	154.5	109.2	38.1	X	0.058	0.044	0.053
Pšovka	17.0	7.3	157.6	120.1	42.9	161.4	17.1	26.1	21.6	154.6	128.4	X	0.025	0.058
Záluží	82.6	63.5	135.7	91.1	51.1	190.3	72.9	69.8	84.0	191.7	156.8	69.8	X	0.09
Prudník	218.0	233.3	355.6	332.0	263.4	279.2	229.9	226.1	219.5	256.9	267.5	226.2	294.4	X

In populations of the spiny-cheek crayfish from the Czech Republic, moderate allozyme variation was detected, relatively low in comparison to other crayfish, such as *Astacus astacus* (Fevolden and Hessen, 1989), *Parastacoides tasmanicus* (Hansen *et al.*, 2001), or *Austropotamobius pallipes* (Largiadèr *et al.*, 2000; Lörtscher *et al.*, 1998); this was most likely due to the introduction of the species to the continent. However, the variation was still higher than in several other crayfish, in which a complete absence of variation was recorded, supposedly due to introduction bottlenecks (Agerberg, 1990; Brown, 1981; Busack, 1989).

Despite gradual bottlenecks during the colonisation of Czech waters by *O. limosus*, genetic variability has been maintained also in the terminal populations. Interestingly, the observed allozyme variation was usually higher in isolated populations in comparison to those from rivers or brooks (Table 3), which could be explained by random changes in allele frequencies during colonisation.

Results of the present study show that populations of *O. limosus* in the Czech Republic are structured but their differentiation is not very strong; this is in agreement with a recent colonisation of the territory from one direction (apart from the population from Prudník, which nevertheless did not differ from the others).

High levels of F_{ST} in other crayfish were detected in populations of the taxa now regarded as species complexes. For example, $F_{ST} = 0.925$ was observed among *Austropotamobius pallipes* populations in the Alps, showing that analysed clades were genetically isolated (Largiadèr *et al.*, 2000). High F_{ST} (0.384) was also found in the virile crayfish (*Orconectes virilis*) from midwestern USA, showing a remarkable subdivision of the studied populations (Fetzner *et al.*, 1997). However, *O. virilis* was recently discovered to be a cryptic species complex with several lineages in the USA (Mathews *et al.*, 2008). Czech populations of *O. limosus* studied by us certainly belong to a single biological species, so it is not surprising that the values of among-population differentiation are lower than in *A. pallipes* or *O. virilis*. Interestingly, despite a very short history on the Czech territory, F_{ST} in *O. limosus* was still higher than that found among populations of another undisputed species, *Astacus astacus*, in Norway ($F_{ST}=0.059$; Fevolden and Hessen, 1989).

Our analyses didn't show any obvious correlation between geographic distance and genetic similarity of studied populations. This can be explained by human-mediated translocations of the spiny-cheek crayfish on the territory of the Czech Republic. We supposed that population from Silesia could differ from the

remaining Czech populations, because the individuals originated from a different region, and due to the stochastic events could carry other alleles, or in different proportions. However, the allozyme variation in this population was similar to the others, suggesting that most alleles brought to Europe got into the newly established populations of the species. Nevertheless, analysis of the mitochondrial gene for COI of European *O. limosus* showed that the Prudník population differed from the rest of the analysed populations by presence of a rare haplotype found uniquely in several individuals from this population (Chapter 1). Our results did not prove that pairs of populations, where one was founded by individuals from the other, were genetically closer to each other than to the rest of the studied populations, suggesting that the founder effects could have led to changes of allele frequencies during introduction.

Our analysis showed, that in populations which have been founded once and by a relatively small founding population, genetic variability can be maintained, even in terminal populations. No correlation between genetic and geographic distances supports the influence of crayfish translocations by people on the genetic structure of the species in our country. Moreover, the data on allozyme variation were useful in the selection of individuals and populations for analysis of the variation of the mitochondrial gene for COI of European spiny-cheek crayfish (see Chapter 1) and also for testing microsatellite markers in this species (currently in progress).

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CHAPTER 3

NEW LINEAGES OF THE VIRILE CRAYFISH (*ORCONECTES VIRILIS*) SPECIES COMPLEX FROM EUROPE AND THE USA

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NEW LINEAGES OF THE VIRILE CRAYFISH (*ORCONECTES VIRILIS*) SPECIES COMPLEX FROM EUROPE AND THE USA

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Abstract

The virile crayfish (*Orconectes virilis*) represents a cryptic species complex with several lineages present in the USA. In Europe, two populations of this invasive North American crayfish are established, one in Great Britain (London), another in the Netherlands. We assessed the position of *O. virilis* individuals from Great Britain within the complex by sequencing part of the mitochondrial gene for cytochrome *c* oxidase subunit 1 (COI). Tested individuals of the virile crayfish from London population do not belong to any of the mitochondrial lineages found in the USA so far, but form a separate clade with a similar level of divergence as other members of the complex. Additionally sequenced individual of the virile crayfish from Iowa (USA) also represents a new clade, suggesting that lineage variation within *O. virilis* is much higher than presently known.

Key words:

Orconectes virilis, cryptic species complex, COI, introduction, origin, Europe, North America

Introduction

Several North American crayfish species have been brought to Europe since 1890 to substitute lost populations of native crayfish, decimated by the pathogen of the crayfish plague (oomycete *Aphanomyces astaci*). Three of these non-indigenous

crayfish have spread over a large part of the European continent: the spiny-cheek crayfish (*Orconectes limosus*), the signal crayfish (*Pacifastacus leniusculus*) and the red swamp crayfish (*Procambarus clarkii*) (Henttonen and Huner, 1999). Besides the ability of these invasive species to compete with the native European crayfish, all of them can transmit crayfish plague and cause mass mortalities of the indigenous species (Holdich, 1999). Although the negative effect of the North American crayfish on indigenous species is well-known, several other crayfish species have been introduced to Europe during the last few years. Some of them have most probably got to European waters through an aquarium trade, such as *Orconectes immunis*, which can be found in southwestern Germany since 1997, marbled crayfish (*Procambarus* sp.) discovered in 2003 in Germany and in 2004 in the Netherlands, and *Orconectes rusticus* first recorded in 2005 from France (Souty-Grosset *et al.*, 2006).

Another North American crayfish introduced to Europe through an aquarium trade is the virile crayfish, *Orconectes virilis*. The range of the virile crayfish in North America is very wide. It can be found in many regions, in Canada (from Alberta to New Brunswick) to the north, Texas to the south, Utah and Montana to the west and New York to the east and it has also been recorded from California, Arizona and New Mexico (Fig. 1) (McAlpine *et al.*, 1999; McAlpine *et al.*, 2007; Souty-Grosset *et al.*, 2006). On a large part of this territory the species is considered as invasive (Global Invasive Species Database; www.invasivespecies.net). Besides its introduction to Europe, the species has also been introduced to Chihuahua in Mexico. High abundance of *O. virilis* specimens may have a substantial impact on submerged macrophytes and increase the turbidity of the water (Souty-Grosset *et al.*, 2006).

Recent data have shown that populations identified in the USA as the virile crayfish represent, together with additional related species, a highly diversified cryptic complex (Mathews *et al.* 2008). Apart from already recognised taxa *O. deanae* and *O. nais*, at least four divergent lineages exist within *O. virilis*, well separated at mitochondrial (16S rRNA, cytochrom *c* oxidase subunit 1) as well as nuclear (glyceraldehyde-3-prosphate dehydrogenase) markers. At least three of them exhibit also morphological differences (Mathews *et al.*, 2008). However, samples of the virile crayfish analysed in this study originated from a relatively small area within the entire range of the taxon distribution in North America (Figure 1). It might be therefore expected that even more distinct lineages exist within this complex. Allozyme data

published by Fetzner *et al.* (1997) also suggest that reproductive isolation among geographically distant populations of the virile crayfish is common.

At least two unsuccessful attempts to introduce *O. virilis* to Europe were made in the past. First the crayfish were released in France in 1897 (Arrignon *et al.*, 1999) and later, in 1960, into Swedish waters (Skurdal *et al.*, 1999). First established European population of the virile crayfish was recorded in 2005 in the Netherlands at Vinkeveen near Amsterdam from where it has been quickly spreading to neighbouring waters (Souty-Grosset *et al.*, 2006). This population is supposed to come from an aquarium trade and apparently, it has been present in the area already several years before (Pöckl *et al.*, 2006). In the future, the virile crayfish will probably significantly influence the ecology of Dutch waters (Souty-Grosset *et al.*, 2006).

In 2004, a population of non-indigenous crayfish was discovered within the River Lee system of North London in the United Kingdom. The individuals were first believed to be the spiny-cheek crayfish, *Orconectes limosus*. However, after a detailed re-examination of their morphology, the specimens were identified as *O. virilis*. The possible source of this population seems to be the contents of an aquarium tank tipped into a pond in Enfield by a local resident (Ahern *et al.*, 2008). However, the exact origin of the population stays unknown.

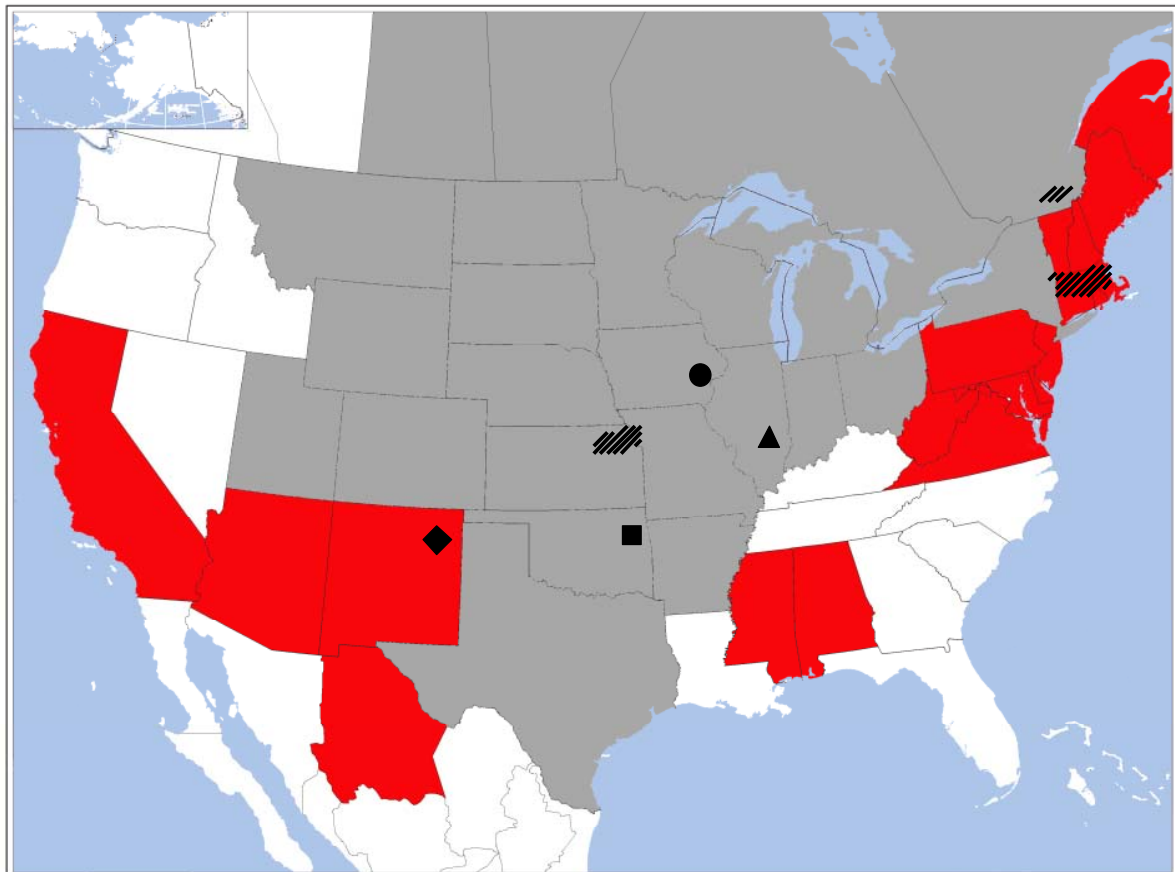
Neither genetic data nor data on origin exist for populations of *O. virilis* introduced to Europe. In this study, we sequenced mitochondrial gene for the cytochrome *c* oxidase subunit I (COI) of selected *O. virilis* individuals from London population to assess their position within the complex. Additionally, we included in the analysis one individual sampled in Iowa (USA), outside the regions from which virile crayfish have been studied genetically.

Material and methods

Three individuals of *Orconectes* cf. *virilis* were collected from the River Lee system of North London (Great Britain; 51°36' N, 0°2' W). An additional male individual was sampled in Squaw Creek near Cedar Rapids in Iowa (USA; 41°58' N, 91°40' W). Sampled material was preserved in ethanol.

Figure 1

North American range of *Orconectes virilis* (after Global Invasive Species Database; www.invasivespecies.net, colours show presence of *O. virilis* in the state, grey – native, red - alien) with the distribution of areas from where individuals were collected for previous and present studies: Mathews *et al.* (2008) (hatching: *O. cf. virilis*, diamond: *O. deanae*, square: *O. nais*), Taylor and Hardman (2002) (triangle), *O. cf. virilis* from Iowa (circle).



The same methods of DNA extraction, amplification and sequencing was used as described in Chapter 1. DNA was extracted from a leg muscle following the Chelex protocol. Mitochondrial gene for the cytochrome *c* oxidase subunit I was amplified using the pair of universal primers LCO 1490 and HCO 2198 (Folmer *et al.*, 1994). PCR products were purified by Exonuclease I (New England Biolabs) and Shrimp Alkaline Phosphatase (Fermentas).

Sequences representing divergent haplotypes and various geographic regions from all known lineages of the *O. virilis* complex (usually 2-3 per clade), including *O. nais* and *O. deanae*, analysed previously by Mathews *et al.* (2008), and *O. virilis* from Illinois analysed by Taylor and Hardman (2002), were obtained from GenBank. Accession numbers of these sequences are provided in Figure 2. Sequence of *Orconectes limosus* (GenBank acc. no. EU442747) was used as an outgroup in subsequent analyses.

Sequences were aligned in the software Mega 4.0 (Tamura *et al.*, 2007), and truncated to length available for all individuals (486 bp). In the same program, we subsequently constructed a neighbour-joining tree based on Kimura 2-parameter model to assess the diversity of the *O. virilis* complex and position of UK and Iowa individuals within it, and calculated the sequence divergences among lineages.

Results

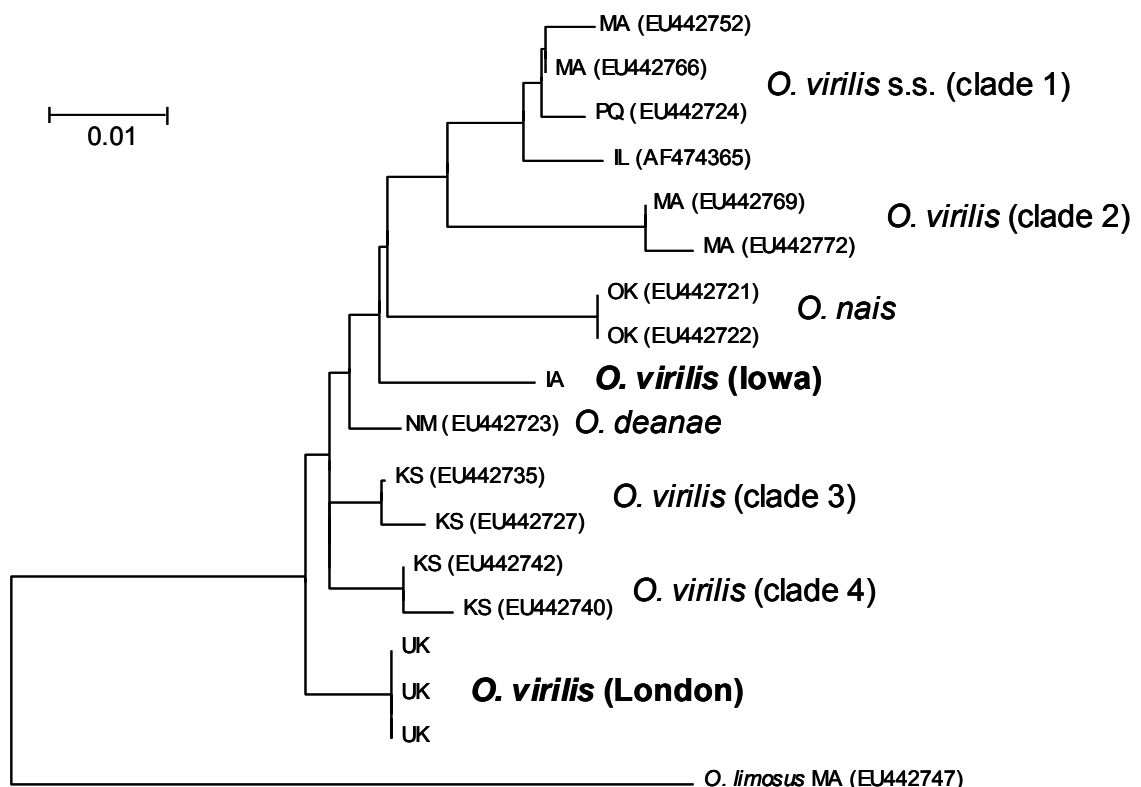
Position of the newly analysed samples within the *O. virilis* complex is shown in Figure 2. All three tested samples of *O. virilis* from London population shared the same haplotype, apparently basal to so far known lineages of the complex. The average COI divergence of the UK haplotype from other clades of the complex ranged from 1.26% (to *O. deanae*) to 3.9% (to clade 2). The value of its divergence to *O. deanae* was very low, however, *O. deanae* itself differed equally (1.26%) from clade 3.

The sample from Iowa represented an apparently new lineage as well, its average divergence from other clades of the complex varied between 2.11% (to *O. deanae*) and 3.85% (to clade 2).

The average pairwise divergence among clades of the *O. virilis* complex sampled in North America (including *O. nais* and *O. deanae*), was 2.86% (uncorrected divergence 2.78%), while their divergence to the London population was 2.47% (uncorrected divergence 2.40%).

Figure 2

Neighbour-joining tree of the *Orconectes virilis* species complex from North America and Great Britain. GenBank accession numbers of individual sequences are listed in parentheses. Clades within the complex are labeled after Mathews *et al.* (2008), newly analysed lineages are marked by bold font. State and country abbreviations: MA – Massachusetts, PQ – Quebec, IL – Illinois, IA – Iowa, NM – New Mexico, OK – Oklahoma, KS – Kansas, UK – United Kingdom. Scale represents 1% divergence (Kimura 2-parameter distance).



Discussion

Individuals of *O. virilis* from London and Iowa analysed in this study represent new lineages of the *O. virilis* complex, substantially divergent from other clades of the species complex known from the examined part of its range in North America. The native distribution of this lineage therefore remains unknown, as no information is available on the origin of the London population.

The divergence of the London population was lowest to *O. deanae*, but comparison of average divergences among studied clades showed that the divergence of American clades to *O. deanae* (2.36%) is even smaller than to the London population, nevertheless, it is considered a distinct species.

The average COI divergences among all currently known members of the species complex, including *O. nais* and *O. deanae*, did not exceed 3%. In a number of animal groups, such levels of divergences could represent intraspecific variation (Hebert *et al.*, 2003). Costa *et al.* (2007) actually showed that among crustaceans, the average divergence of congeneric species was about 17% (Kimura 2-parameter distance), the highest value detected so far in animals. However, divergences between many *Orconectes* species are much lower. Taylor and Knouft (2006) provided sequences of 86 species or subspecies of the genus. We computed Kimura 2-parameter distances of several sister pairs of described species used in their study. The lowest value was 3.3% (between *O. peruncus* and *O. quadruncus*). Although this is still higher than the divergence of American lineages of *O. virilis* to London population (2.47%) or to *O. deanae* (2.36%), it is apparent that many currently recognised *Orconectes* species are very closely related.

Two species of another crayfish genus, European *Austropotamobius pallipes* and *A. torrentium*, showed deep phylogeographic structure, with COI divergences between major geographic clades (uncorrected values 5.9% and 4.1%, respectively; Trontelj *et al.*, 2005) exceeding values observed among lineages within the *O. virilis* complex. However, existence of differences in morphology of some *O. virilis* clades, and differences between lineages detected in its nuclear markers (Mathews *et al.*, 2008; Fetzner *et al.*, 1997) suggest that the complex is young, recently diversifying and already consists of distinct biological species. Mathews *et al.* (2008) suppose that the complex has undergone radiation since the late Pleistocene, with the divergences among clades originating within the last 2 millions years. Such diversification may be a result of a substantial range fragmentation during Pleistocene glaciations.

Our results support the hypothesis of Mathews *et al.* (2008) that the lineage variation of the *O. virilis* complex is very high. Until now, genetic data on virile crayfish were mostly collected from a limited part of their American range, especially from Massachusetts and Kansas. However, the entire range of *O. virilis* in North

America is rather extensive; more sampling from remaining regions is therefore needed to get more detailed picture about the variation within the complex. On the European continent, sampling and genetic analyses of the virile crayfish from the Netherlands will answer the question whether the Dutch population belongs to the same clade as the UK population (possibly even originating from the same source) or whether these represent different invading species of the complex.

Our finding of a new member of the species complex in an invasive population outside its native range is not the only case of a discovery of unknown crayfish distributed through aquarium trade. The most famous is another cambarid species, the parthenogenetically-reproducing marbled crayfish, which is widespread among aquarists but not known from North American waters (Scholtz *et al.*, 2002). However, its wild populations are already established in Europe (Souty-Grosset *et al.*, 2006). Clearly, genetic analyses of invaders' populations can improve knowledge about biodiversity of some taxa in their original distribution areas.

Acknowledgements

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CONCLUSIONS

The spiny-cheek crayfish (*Orconectes limosus*) can be found in the Czech Republic in various types of localities, especially in the western part of the country, but new localities are still being discovered.

Although the European *O. limosus* have been established by relatively small number of individuals, allozyme variability in its populations has been maintained. Comparison of genetic and geographic distances among populations has not shown any correlation, suggesting that human-mediated translocations have played an important role in the distribution of the species.

The study of haplotype variation in the spiny-cheek crayfish from Europe and North America showed that the species has most likely been introduced to Europe once, from the northern part of its American range. However, more sampling in North America is needed to help us better identify the source region.

The distribution of haplotypes in *O. limosus* from North America is most probably partly connected with anthropogenic origin of its populations in the northern part of the range but also with more ancient processes, in particular distribution Pleistocene refugia and postglacial recolonisation of the presently occupied watersheds.

As the species is endangered in its native range, information on the distribution of rare genotypes will be valuable for its conservation.

Analysis of the mitochondrial gene for cytochrome c oxidase subunit 1 has shown that the virile crayfish (*O. virilis*) from Great Britain and Iowa (USA) represent new lineages of the *O. virilis* species complex, which have not yet been recorded in North America. This shows that the lineage diversity of the complex is very high, and still poorly known.

The *O. virilis* species complex seems to be relatively young, but consists of distinct species. More sampling from its extensive American range is needed to detect other lineages of the complex and to identify the source region of the British population.

APPENDIX

Filipová L., Kozubíková E. and Petrusek A., 2006. Rak pruhovaný (*Orconectes limosus*). In: Mlíkovský J., Stýblo P. (eds): Nepůvodní druhy fauny a flóry ČR [Alien species in fauna and flora of the Czech Republic]. Praha, ČSOP, 237-239 (in Czech)

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Polícar T., Štambergová M. and Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present. Bulletin Français de la Pêche et de la Pisciculture, 380-381: 903-918

RAK PRUHOVANÝ (*ORCONECTES LIMOSUS*)

(in Czech)

FILIPOVÁ L., KOZUBÍKOVÁ E. A PETRUSEK A.

Taxonomické zařazení druhu

třída:	Malacostraca	rakovci
řád:	Decapoda	desetinožci
čeleď:	Cambaridae	

Popis druhu

Menší rak (délka těla obvykle nepřevyšuje 10 cm) s charakteristickými trny vpředu po stranách havohrudi (na „lících“) a červenohnědými příčnými proužky na zadečku.

Rozšíření

Původní rozšíření:

Nearktická oblast: východní pobřeží USA (státy Connecticut, Delaware, Washington, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia a West Virginia)³.

Nepůvodní rozšíření ve světě:

Do Evropy byl rak pruhovaný záměrně dovezen v roce 1890, kdy bylo 100 jedinců vysazeno do rybníka u vesnice Barnowko v Pomořanech (nyní na území záp. Polska)¹⁰, odkud se tito raci přirozeně či s pomocí člověka rozšířili do dalších zemí. Další úspěšná introdukce z Ameriky do Evropy není známa. V současnosti se tento druh vyskytuje na území Polska, Německa, Francie, České republiky, Rakouska, Švýcarska, Maďarska, Lucemburska, Holandska, Belgie, Itálie, Litvy, Běloruska, Chorvatska, západního Ruska, Anglie, pravděpodobně také Ukrajiny^{3,8}. Kromě Evropy byl *O. limosus* introdukován také do Maroka^{3,9}.

Rozšíření v Česku:

První, avšak neúspěšný, pokus o vysazení raka pruhovaného do České republiky proběhl již na přelomu 19. a 20. století¹⁷. Do České republiky se znovu *Orconectes limosus* dostal pravděpodobně přirozenou migrací proti proudu Labe²⁰. Poprvé byl výskyt tohoto druhu dokumentován v roce 1988, kdy byl spatřen v Labi u Ústí nad Labem⁴. Zřejmě se však u nás rak pruhovaný vyskytoval už v 60. letech 20. století, kdy amatérský rybář spatřil v labských tůních poblíž Štětí velká množství raků, jejichž charakteristika odpovídala tomuto druhu^{6,18}.

Zatím se rak pruhovaný vyskytuje pouze v povodí Labe, především ve velkých řekách (Labe, Vltava) a ve spodních částech jejich přítoků. Můžeme ho nalézt také na mnoha pískovnách nebo zatopených lomech – výskyt na těchto lokalitách je důsledkem zásahů člověka. Z některých takto osídlených míst se pak raci mohou šířit také do přilehlých menších toků²⁰.

V Labi byl po roce 2000 výskyt raka *Orconectes limosus* prokázán na mnoha lokalitách od Hřenska po Pardubice (5151 - 5960). Mezi větší přítoky Labe (6. řád toku a vyšší), kde se tento druh alespoň v dolním toku vyskytuje, patří Ohře (5450, 5550), Vltava (viz níže), Jizera (5854), Mrlina (5856), Cidlina (5857), Doubrava (5958), Metuje (5661) a Úpa (5661, 5562, 5462). V případě posledně dvou jmenovaných řek se však jedná o populaci vysazenou rybáři. Ve Vltavě byl rak pruhovaný nalezen ve Vrbně u Mělníka (5652), Klecanech (5852) a v Praze (5852) a dále na mnoha lokalitách od Zvíkovského Podhradí (přehrada Orlík) po České Budějovice (6551 - 7052). Nejsou známy lokality s výskytem tohoto druhu v přehradních nádržích Štěchovice, Slapy a Kamýk, je proto možné, že populace ve vyšší části toku Vltavy jsou důsledkem záměrné introdukce²⁰. Většími přítoky Vltavy s výskytem tohoto druhu v dolní části (obvykle v bezprostřední blízkosti ústí, v oblasti vzduť přehradní nádrží či jezem) jsou Otava (6551), Lužnice (6752), Sázava (6152) a Malše (7052)²⁰.

Raci pruhovaní byli nalezeni také v mnoha menších tocích (o 5. nebo nižším řádu toku), obvykle však pouze v blízkosti ústí do některé z výše uvedených větších řek. V povodí Labe se jedná o potok Kamenička (Boletice nad Labem) (5251), Poustka (Dobkovice) (5251), Lužecký potok (Povrly) (5351), Luční potok (Malé Březno) (5351), Modla (Lovosice) (5450), Pšovka (Střemy) (5653), Vlkava (Kostomlaty nad Labem) (5855), Výrovka (Písty) (5856), Liduška (Nymburk) (5956) a Bačovka (Velký Osek) (5857). V povodí Vltavy je to Janovický potok (Krusičany)

(6153), Vlkančický potok (Pyskočely) (6155), Jickovický potok (Jickovice) (6551), Hrejkovický potok (Vůsí) (6551), Velký a Novosedlský potok (Strouhy) (6651), Chřešřovický potok (Chřešřovice) (6651) a Bílinský potok (Vesce) (6752)²⁰. Občasný výskyt raka pruhovaného lze však předpokládat i v dalších přítocích Labe, Vltavy a dalších velkých řek osídlených tímto druhem.

Mezi stojaté vody, kde se v současné době prokazatelně rak pruhovaný vyskytuje, patří zatopené lomy Kojetice (u Neratovic) (5753) a Starý Klíčov (u Mrákova na Domažlicku) (6543), zatopený povrchový důl Barbora (u Oldřichova u Teplic) (5348), nádrže Modlany (5349) a Kateřina (u Soběchleb) (5349), dále pískovny Cítov (u Mělníka) (5652), Mlékojedy (u Neratovic) (5753), Proboštská „jezera“ (5753) a pískovna u letiště Borek (u Staré Boleslavi) (5754), Lhota (5754), Ovčáry (5753), Ostrá (5855), Píšťany (5450) a Stará pískovna (v Provodíně) (5353), veslařský kanál v Račicích (poblíž Štětí) (5552), rybník na Říčanském potoce (v Praze – Dubči) (5953), rybník ve Smečně (5850), rybník Koclířov (u Lomnice nad Lužnicí) (6954), rybníky Štampach a Velký rybník (Střemy) (5653), zatopená důlní propadlina u Černic (5447) a další pískovny a mrtvá ramena v okolí Labe²⁰. Pravděpodobně budou osídleny i jiné jihočeské rybníky a nádrže či zatopené plochy po povrchové těžbě v severních Čechách.

V 90. letech byli raci pruhovaní údajně spatřeni také na několika dalších lokalitách poměrně vysoko proti proudu příslušných řek⁵ – v Ohři v Žatci (5647), v Lužnici v Táboře (6554), v Sázavě v Havlíčkově Brodě (6359) a v přítocích Berounky v Plzni (6245-6), jejich výskyt tam ale nebyl v současnosti potvrzen. Koncem 90. let však byl výskyt tohoto druhu potvrzen v přehradní nádrži Hracholusky na Mži (6244)²⁰.

Nároky na prostředí

Rak pruhovaný je dobře přizpůsobený životu ve stálých tekoucích i stojatých vodách⁷. Daří se mu také na lokalitách s bahnitým dnem, které jsou pro evropské druhy raků méně vhodné. Oblast původního výskytu raka pruhovaného je značně rozsáhlá, což napovídá, že se jedná spíše o generalistu s dobrými předpoklady osídlit nové typy lokalit. V porovnání s původními druhy raků vykazuje vyšší toleranci ke snížené koncentraci kyslíku a k eutrofním a znečištěným vodám, lépe se vyrovnává s výraznými změnami prostředí¹⁶. V Česku lze tento druh nalézt jak ve větších řekách, tak v zatopených lomech a pískovnách.

Charakter české populace

Raci pruhovaní, kteří se vyskytují na území České republiky, jsou pravděpodobně všichni potomky jedinců, kteří se do naší republiky dostali přirozenou migrací proti proudu řeky Labe. Populace tohoto druhu, která se vyskytuje v Labi, je tedy napojená na populace v německé části této řeky. Na osidlování dalších lokalit, nejčastěji stojatých vod (pískovny, zatopené lomy), se pak významně podílí člověk. Vznikají tak izolované populace bez kontaktu se zakladatelskou populací v povodí Labe. Oddělené se zdají být i populace ve středním toku Vltavy a jejích přítocích.

Zajímavé je, že v posledních letech (v období 2002-2005) došlo na řadě lokalit k výraznému snížení početnosti raků pruhovaných^{1,2,15}. Částečně se může jednat o vliv katastrofálních povodní z roku 2002, ale úbytek raků byl pozorován i v oblastech povodní nezasažených (např. v severovýchodním Polsku)¹. Příčina tohoto fenoménu zatím není známa.

Interakce

Rak pruhovaný je přenašečem původce račího moru, oomycety *Aphanomyces astaci*, sám je však vůči akutnímu průběhu tohoto onemocnění téměř imunní. Jestliže dojde k přenosu patogenu na evropské raky, následují jejich masové úhyny. Račí mor vyhubil většinu původních račích populací na českém území na přelomu 19. a 20. století¹⁴. Od té doby byl jeho výskyt hlášen pouze sporadicky^{21,22}, v posledním desetiletí se však objevují nové masové úhyny raků s podezřením na račí mor^{11, 12, 13}.

Minimálně v jednom případě, v potoce Pšovka v CHKO Křivoklátsko, byl přenos *A. astaci* z raků pruhovaných na evropské druhy raků nejpravděpodobnější příčinou úhynu¹³. V dolní části toku byl do rybníka vysazen rak pruhovaný, ačkoli se výše po proudu vyskytovali raci říční a bahenní. V letech 1998-1999 zde došlo k masovému úhynu obou druhů s příznaky odpovídajícími račímu moru, raka pruhovaného se přitom nemoc nedotkla¹¹. Ve vzorcích tohoto druhu odebraných v roce 2004 byla prokázána přítomnost *Aphanomyces astaci* molekulárními metodami¹³. Přítomnost patogenu račího moru byla testována u jedinců raka *O. limosus* z několika dalších lokalit v České republice: Labe, lom Kojetice, Jickovický potok, přehradní nádrž Orlík, Stará pískovna u Provodína. Pouze v případě poslední jmenované populace nebyla přítomnost *A. astaci* potvrzena¹³.

Pokud by populace raka pruhovaného nebyla infikovaná patogenem račího moru, mohlo by dojít k dlouhodobější kompetici tohoto druhu s některým z původních raků. Taková situace zatím nebyla v České republice zaznamenána. Rak pruhovaný je však známý svou agresivitou, vysokou plodností, tolerancí ke sníženým hodnotám kyslíku a k znečištění, v kompetici s evropskými raky by proto mohl uspět¹⁶.

Rak pruhovaný je všežravec, živí se například rostlinami, řasami, bezobratlými, rybami, ale také živočišnými a rostlinnými zbytky. Ožíráním makrovegetace a predací na bentických býložravých bezobratlých může nepřímo měnit prostředí ostatních organismů¹⁹. Sám se stává potravou některých ryb (např. úhořů) a dalších predátorů lovcích ve vodách (volavky, vydry, norci aj.).

Rak pruhovaný je menší než rak říční, má malá klepeta a vyskytuje se často v značně znečištěných vodách, nepatří proto (na rozdíl např. od raka říčního nebo signálního) mezi vyhledávané gastronomické pochoutky.

Analýza rizika

Rak pruhovaný může přenášet račí mor, je proto nutné zabránit jeho šíření na další lokality. Bohužel není známa žádná metoda, která by vedla k eliminaci nepůvodních druhů raků, ale která by byla zároveň šetrná k ostatním organismům, zejména původním druhům raků.

Rak pruhovaný se šíří jak přirozenou migrací, tak za přispění člověka (např. rybářů nebo potápěčů)²⁰. Velmi důležitou prevencí jeho dalšího šíření je proto dobrá informovanost veřejnosti o výskytu tohoto druhu u nás a jeho nebezpečí pro evropské raky. Manipulace s rakem pruhovaným by měla být pod pokutou zakázána.

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DISTRIBUTION OF THE INVASIVE SPINY-CHEEK CRAYFISH (*ORCONECTES LIMOSUS*) IN THE CZECH REPUBLIC – PAST AND PRESENT

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Abstract

The American spiny-cheek crayfish, *Orconectes limosus*, was first introduced into European waters in 1890. The first literature record about the occurrence of *O. limosus* on the territory of the Czech Republic was published almost 100 years later – in 1989. The presence of this species in Czechia, however, was first recorded already in the 1960s, when crayfish were observed in the dead arms and pools adjacent to the river Elbe (Labe) in Central Bohemia. In the following few decades the spiny-cheek crayfish has spread into several larger rivers of the Elbe watershed and some of their smaller tributaries. The eastern part of the country (mostly belonging to the watershed of the river Morava) has not yet been colonised by this species. *O. limosus* can be found in lower reaches of a number of watercourses of a low stream order, but does not seem to penetrate far upstream in such localities. Its distribution in standing waters is largely the result of intentional human-mediated translocations. The long-term coexistence of *Orconectes* and native crayfish species has not yet been recorded, although both introduced and native crayfish at least occasionally come into contact. As *O. limosus* is a major carrier of the crayfish plague on the Czech territory, and crayfish plague outbreaks have been recently recorded, the dynamics of *Orconectes* invasion deserves careful monitoring in the future.

Key words

Orconectes limosus, spiny cheek crayfish, invasive species, distribution, crayfish plague, Czech Republic

Introduction

The crayfish plague, caused by the oomycete *Aphanomyces astaci*, was accidentally introduced to Europe in the 1860's (VOGT, 1999). The disease quickly spread over a large part of European water bodies and the numbers of native crayfish were dramatically reduced. The attempts to replace the lost populations, and to substitute sensitive species with those resistant to the disease, led to a number of intentional introductions of non-native crayfish to Europe (HENTTONEN and HUNER, 1999). The introduced species were *Astacus leptodactylus* (native to the eastern part of Europe and the Near East, sensitive to the plague) and the North American species *Orconectes limosus*, *Pacifastacus leniusculus* and *Procambarus clarkii*. The presence of non-native species on the continent caused, however, a number of unexpected additional problems. The crayfish from North America often carry the crayfish plague pathogen or other diseases and may transmit them to the native populations (VOGT, 1999). Therefore, with the spread of non-native species in recent decades, the crayfish plague outbreaks are again gaining in intensity (HOLDICH, 2003).

Furthermore, the indigenous crayfish can also be displaced through direct interactions or competition for resources with the non-native species (HOLDICH, 1999). American crayfish are usually characterized by high growth rates, early maturity and large amounts of offspring, but also short life spans and high mortality rates. They are often aggressive and tolerate deteriorated habitat conditions (LINDQVIST and HUNER, 1999). Some of them (especially *Procambarus clarkii*, in Britain also *Pacifastacus leniusculus*) can cause large damages in agricultural and recreational areas by burrowing (HOLDICH, 1999).

Out of five species of crayfish present in the wild in Czechia (POLICAR and KOZÁK, 2000), only two are native: the noble crayfish, *Astacus astacus*, and the stone crayfish, *Austropotamobius torrentium*. Both of them are protected by law as critically endangered species. The rest of the crayfish species present in the country have been either intentionally introduced (*Astacus leptodactylus*, *Pacifastacus leniusculus*) or colonised the area naturally from another region (*Orconectes limosus*). The narrow-clawed crayfish, *Astacus leptodactylus*, was introduced to Czechia at the end of the 19th century in order to replenish the reduced native populations of the noble crayfish. Since then, it became an integral part of the local fauna, and its populations are scattered all over the country (ĎURIŠ and HORKÁ, 2001).

The signal crayfish, *Pacifastacus leniusculus*, was brought to the Czech territory from Sweden in the 1980s for aquaculture purposes. Its acclimation at most localities was unsuccessful, but the population near the town Velké Meziříčí has established itself successfully and specimens from that area have been later transferred to several other places in the country (POLICAR and KOZÁK, 2000). The distribution of this species still remains restricted to only a few localities.

The most widespread non-native species in the Czech Republic is the spiny-cheek crayfish, *Orconectes limosus*. It was first intentionally introduced to Europe in 1890, when about 100 specimens from Pennsylvania were released to a fishpond in Barnowko (Berneuchen, Western Pommern; currently in Poland close to the German border) (KOSSAKOWSKI, 1966; HAMR, 2002). In 1895, an additional but unsuccessful attempt was made to introduce this crayfish from New York to France (KOSSAKOWSKI, 1966). The original locality in Poland therefore seems to be the only source of *Orconectes limosus* populations on the European territory. Since the first introduction, this crayfish has spread naturally or by secondary translocations to over 15 European countries, including the Czech Republic and its neighbours – Poland, Germany, and Austria (HENTTONEN and HUNER, 1999; HOLDICH, 2003). Its presence in Slovakia has not been confirmed so far (STLOUKAL and HARVÁNEKOVÁ, 2005).

A brief overview of the distribution of *Orconectes limosus* in the Czech Republic has already been included in the study of KOZÁK *et al.* (2004); however, no detailed information on the localities was given. The aim of the present study is to summarise available records on the history of the invasion of *Orconectes limosus* on the territory of the Czech Republic, present up-to-date data on its current distribution, and analyse types of water bodies this species occupies.

Methods

The review of the distribution of *Orconectes limosus* on the territory of the Czech Republic in the past (up to 2000) is based on available literature data, mostly published in local journals inaccessible to the international scientific community.

The present distribution of this species was assessed mainly from the long-term activities aimed at collecting data on the distribution of all crayfish species in the country. During the last five years, a number of localities with *Orconectes* presence reported in the past were repeatedly re-sampled.

Additionally, we present here the data on *Orconectes limosus* from an intensive mapping of crayfish distribution, which was carried out by the Agency for Nature Conservation and Landscape Protection of the Czech Republic in 2004-5. The mapping covered running waters in most of the country (over 90 % of the surface area, and over 90 % of all watercourses except of the smallest ones). Small to medium water courses were examined for crayfish presence every 3 kilometres, starting 100 m above the confluence with the higher-order stream, and continuing upstream up to the stream source. At each locality, an approximately 100 m long stretch of the stream was inspected, and shelters suitable for crayfish were searched. Larger rivers were sampled every 4-7 kilometres (depending on the habitat suitability) using the baited traps. At least three traps containing fresh fish meat as bait were left overnight at each site, in a slow-flowing part of the river in deeper water close to the river bank. Traps were collected in the morning and checked for crayfish presence.

Altogether, over 9000 profiles on small streams, and over 350 on large rivers, were inspected during the mapping project.

We also used information provided by the general public (e.g. scuba divers) in cases when the identification of the species was unambiguous or photodocumentation was available. For each locality, we present the date (if available) or year(s) of observation and the character of the habitat (stagnant/running water, type of the water body). Additionally, we include the literature data on observations of this species from 2001 to present.

For populations from running waters, we calculated the distance from the mouth of the river/stream and the stream order, using the GIS data on the hydrological network of the Czech Republic (T.G.M. WRI Hydroecological Information System, <http://heis.vuv.cz>). To be able to compare the position of localities and tributaries of the river Elbe, we use the distance from the German border rather than standard river kilometres, as there are several overlapping kilometric systems in use on the Czech stretch of this river. A distribution map, using a standard grid for faunistic mapping recommended for the territory of the Czech Republic (BUCHAR, 1982), was compiled using the data from all accessible sources. Recent records (after 2000) and pre-2000 historical localities with *Orconectes* not recorded later are distinguisher by colour.

Results

Historical data

Published records of *Orconectes limosus* on the territory of the Czech Republic prior to 2001 are listed in Table I. The first effort to introduce this species to the region had been already made at the turn of the 20th century but with no success (MATĚNA, 1995). Its presence was first reported in the literature by HAJER (1989). The spiny-cheek crayfish was repeatedly observed in the river Elbe (Labe) in the surroundings of the town Ústí nad Labem, approximately 40 km upstream from the German border, since at least the mid-1980s. By that time it had probably also penetrated to the river Bílina (left-side tributary of the Elbe). Between 1987 and 1988, the crayfish were also found in Čelákovice, 146 km upstream from the border (SAMEK, pers. comm.). Since then, the species has been recorded in a wide range of localities in the western part of the country (HAJER, 1990, 1994; BERAN, 1995, 2003; KOZÁK *et al.*, 2004).

The spiny-cheek crayfish had most likely immigrated into the region naturally through the river Elbe (KOZÁK *et al.*, 2004). It seems, however, that the *Orconectes* invasion had gone undetected for a long time. According to the record of an amateur fisherman (MATOUŠ, 1995), already in the 1960s small crayfish were present in high densities in dead arms and pools adjacent to the river near Štětí, about 80 km upstream from the border with Germany. The identity of these crayfish as *O. limosus* is beyond doubt for several reasons: the author described them as small (no more

than 8 cm long) aggressive crayfish often exhibiting daytime activity, and these crayfish had been apparently able to tolerate heavily polluted water of the Elbe in the 1960s (MATOUŠ, 1995). *Orconectes* is still present in the same area (Tables I, II).

Following his first report on *Orconectes* presence in Czech waters, HAJER (1994) reported the occurrence of the spiny-cheek crayfish in a number of rivers of the Elbe watershed. The indicated populations were often located far from the confluence of the particular river with the Elbe or Vltava (see Table I). The most significant are records of the presence of *Orconectes* in higher reaches of the rivers Ohře (km 90), Sázava (km 162), Berounka (km 139) and its tributaries, or Vltava (km 240) (HAJER, 1994). Apart from the latter, the presence of *O. limosus* in such distant parts of these rivers has not been confirmed later. Such areas are marked in Figure 1 by open circles.

Present status

The localities where *Orconectes limosus* was recorded since 2001 are listed in Table II, and shown in Figure 1. Although the list certainly does not include all water courses and bodies with the presence of this species on the territory of the Czech Republic, it gives a reasonable picture of the current distribution of the species. The spiny-cheek crayfish is mainly present in the western part of the country (Elbe watershed). The core of its distribution still remains in the river Elbe (including various adjacent oxbows and pools). It occurs in the whole navigable part of this river (which is almost completely canalised) from the border with Germany to the town Pardubice (240 km from the German border) but probably in lower densities also further upstream. The population of *O. limosus* was recorded also in the town Jaroměř (289 km upstream from the border) in the Elbe and two of its tributaries (Úpa and Metuje). However, according to local fishermen as well as the river management authorities (ŠPAČEK, pers. comm.), the spiny-cheek crayfish had been intentionally stocked in this area.

Larger watercourses (of 6th or higher stream order) with the certain presence of the spiny-cheek crayfish include Ohře, Vltava, Jizera, Mrlina, Cidlina, Doubrava, Metuje, and Úpa (all tributaries of the Elbe), and Otava, Lužnice, Sázava, and Malše (tributaries of the Vltava). The presence of this species may be presumed also in the Lomnice and other rivers and streams partially flooded by Vltava reservoirs, and in various larger tributaries of the Elbe.

In most of the above-mentioned rivers, the crayfish presence was confirmed in their lower reaches but recent data from upper reaches are lacking. The notable exceptions are: 1) the Vltava where the spiny-cheek crayfish is present in its lower part (from Prague downstream to the river Elbe), in several reservoirs in its middle section, and reaches upstream to the town České Budějovice (240 km from the confluence); 2) the Sázava, a tributary of the Vltava, where the crayfish seem to be present at least 50 km upstream from its confluence with the Vltava. Another such

river is the Úpa in north-east Bohemia, with *Orconectes* over 30 km upstream from the Elbe. The latter case is known, however, to be a result of human introduction (ŠPAČEK, pers. comm.).

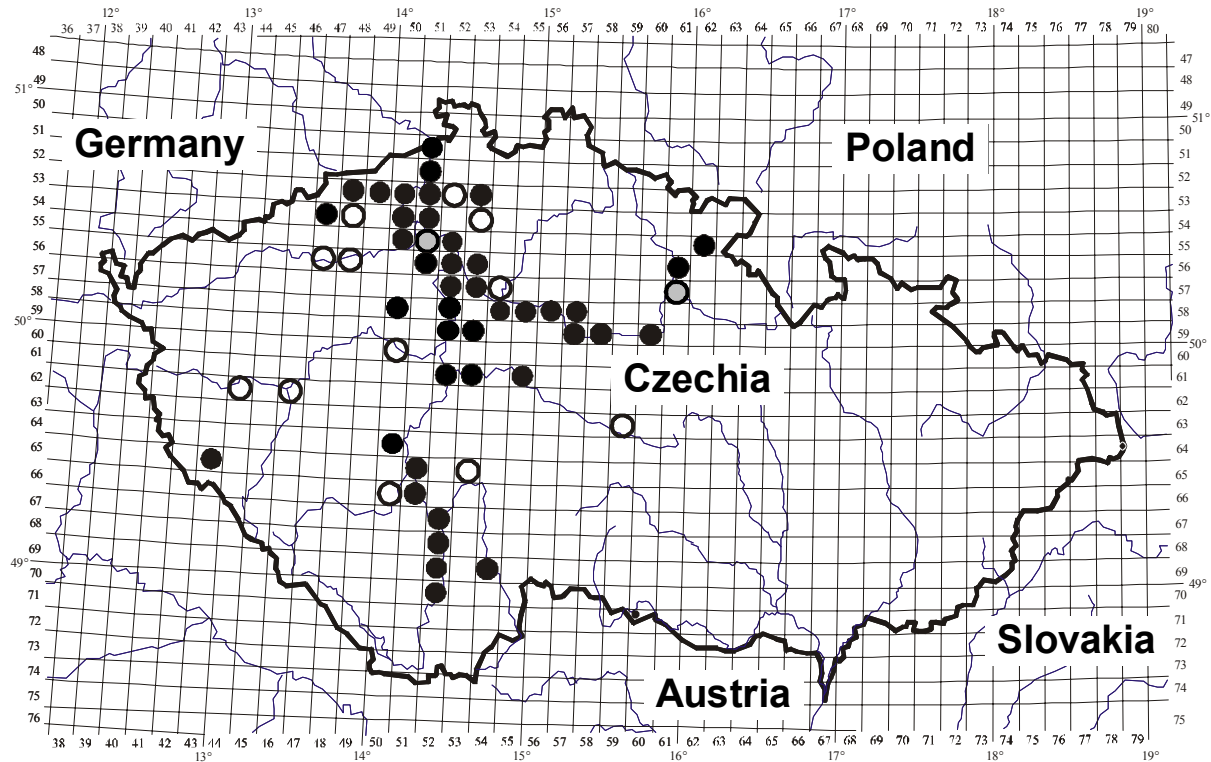


Figure 1

Currently known and previously recorded localities of *Orconectes limosus* marked on a standard grid used for the faunistic mapping in Czechia (each square corresponds to 10' of longitude and 6' of latitude, i.e. approx. 11 x 11 km).

black circle: presence in the square confirmed after 2000

grey circle: historical record (up to 2000) with very likely continuous presence

open circle: records up to 2000 not confirmed later

Figure 1

Sites de présence d'*Orconectes limosus*, passée ou actuelle, localisés sur une grille standard utilisée pour la cartographie faunistique en Tchéquie (chaque carré correspond à 10' de longitude et 6' de latitude, i.e. approximativement 11 x 11 km).

rond noir : présence dans le carré confirmée après 2000,

rond gris : présence ancienne (jusqu'à 2000) avec présence actuelle très vraisemblable,

cercle vide : présence jusqu'à 2000, non confirmé ultérieurement.

Orconectes limosus was found also in a number of smaller streams (i.e. those with a low stream order), which join larger rivers. In these cases the crayfish usually stay very close to the confluence with the major watercourse (less than 3 km, usually

only a few hundred meters), and do not penetrate far upstream. The relationship between the stream order and the maximum distance of recorded *Orconectes* presence from the stream mouth is shown in Figure 2. (In cases of small streams joining other watercourse first, the total distance to the nearest larger river with presumed or confirmed *Orconectes* presence was calculated.)

Additionally, this species is found in several standing waters. Some of them, especially flooded sandpits and quarries, have no surface inflow or outflow. Several other standing waters are connected to watercourses, and crayfish may penetrate into them. This has certainly happened at three places (the flooded surface coal mine Barbora and its outflow; the mining water reservoirs Kateřina and Modlany and their connecting channel; and the large fishpond Velký rybník on the stream Pšovka). At some other places similar relationships between *Orconectes* populations in standing water bodies and adjacent streams may be suspected. All such cases belong among the exceptions where the spiny-cheek crayfish is present in a watercourse of a low stream order (1-4) relatively far (more than 10 km) from the confluence with a major river. These cases are marked in Table II by “+” followed by a superscript numerical code, which identifies corresponding records from running and standing waters.

Discussion

According to the results of the intensive mapping effort, the spiny-cheek crayfish on the territory of the Czech Republic is as yet present only in the Elbe watershed. The area drained by the Morava, covering most of the eastern part of the country, has not been colonised yet, nor the upper part of the watershed of the Odra (Oder), which extends into the Czech territory. There is a certain likelihood that the spiny-cheek crayfish may naturally reach lower reaches of the Morava by upstream migration from the Danube. Not only this species is present in the Danube in Austria (PÖCKL, 1999) and in Hungary where it is actively spreading (PUKY, 2000; PUKY *et al.*, 2005), but it has been already sparsely found directly in the lower reaches of the Morava in Austria, approximately 30 km downstream from the Czech border (PÖCKL and PEKNY, 2002). Other potential sources from where this species might be transferred to the Morava watershed are located in watersheds of the Odra (Oder) and the Wisla (Vistula) in Poland (KOZÁK *et al.*, 2004), or of the Elbe. In those cases the natural spread of the crayfish is unlikely as they would have to cross the watershed divide. However, the spiny-cheek crayfish may be stocked by humans intentionally or transferred unintentionally from any other locality.

The occurrence of this species in a number of isolated standing water bodies, especially flooded quarries or sandpits, is certainly, at least in some cases, caused by human-mediated translocations (especially by fishermen, recreational scuba divers etc., who admit to the stocking, being usually unaware of the potential danger of transferring non-indigenous species). Some populations present in running waters

are also of anthropogenic origin. Apart from above-mentioned upstream section of the Elbe and its tributaries (Úpa, Metuje), one more recent case of crayfish stocking into a stream is confirmed (Drnovský stream, Central Bohemia; stocked in 2005 from a nearby village pond, most individuals were removed later during the year). Additionally, crayfish penetrate to inflows or outflows of standing waters, into which they may have been previously stocked artificially.

It is also possible that artificial stocking allowed this species to colonise reservoirs on the river Vltava, as the distribution pattern in this river (Fig. 1, southern part of the country) does not seem to confirm the natural upstream spread of crayfish, and the reservoir dams (some of them over 50 m high) are significant migration barriers.

Scuba divers are certainly responsible for stocking of the spiny-cheek crayfish into the flooded quarry “Na skále” near Starý Klíčov (Mrákov, western Bohemia), which is a popular diving site. Currently it is inhabited by a very dense population of *O. limosus*, which is even supplied food by the local diving club. Additionally, a single specimen of the noble crayfish *Astacus astacus* was recorded in the same locality in December 2002, and several individuals of the narrow-clawed crayfish *Astacus leptodactylus* were found there between December 2002 and November 2003 (KOZÁK, unpublished). This confirms that various crayfish had been illegally released to the quarry multiple times.

The quarry Klíčov contains an isolated population of *Orconectes* relatively far from other localities of this species, and it is situated only about 10 km away from a stream where a population of the critically endangered stone crayfish *Austropotamobius torrentium* is found (KOZÁK *et al.*, 2002). The presence of *Orconectes* in the region is therefore highly undesirable, as there is a danger of potential transmission of the crayfish plague. The eradication of *Orconectes* population is virtually impossible; however, every effort should be made not to spread the species further.

Although *Astacus* spp. and *Orconectes* have been spotted at the above-mentioned locality, so far there is no evidence of the long-term coexistence of American and native species on the Czech territory – unlike some other countries where this was occasionally reported, e.g. in Finland where *Astacus astacus* and *Pacifastacus leniusculus* occurred in the same lake (NYLUND and WESTMAN, 2000) or in Austria, with *O. limosus* and *A. leptodactylus* in the same stretch of the river Morava (PÖCKL and PEKNY, 2002).

On the other hand, there is evidence for at least one case of crayfish plague transmission from *Orconectes* to the native species in recent years (KOZUBÍKOVÁ *et al.*, 2006). In the Pšovka brook (Central Bohemia, north of Prague) three crayfish species were found in the 1990s, each of them in a different part of the river – *Orconectes limosus* in the lower reach, *Astacus leptodactylus* further upstream and the native *Astacus astacus* in higher reaches of this brook (BERAN, 1995). According to a local fisherman, the spiny-cheek crayfish had been intentionally stocked to the

fishpond Velký rybník near Střemy. An outbreak of a disease with typical crayfish plague symptoms in 1998-1999 practically eradicated the populations of both *Astacus* species but did not affect *Orconectes*. The spiny-cheek crayfish is still abundant in the brook, and the presence of the crayfish plague pathogen *Aphanomyces astaci* was detected in several specimens (KOZUBÍKOVÁ *et al.*, 2006).

Additional evidence of the presence of *Astacus astacus* and *Orconectes limosus* in the same water body is in the reservoir Orlík on the river Vltava. The American species is rather abundant in the reservoir but a specimen of the noble crayfish burrowing in the mud in the depth of several meters was photographed by a scuba diver in October 2003 (PETRUSEK, unpublished). However, it is not possible to conclude for how long such a coexistence may have lasted.

Czech populations of *Orconectes limosus* seem to be widely infected by the crayfish plague pathogen (KOZUBÍKOVÁ *et al.*, 2006). Fortunately, the fact that the species does not seem to penetrate far upstream in small watercourses may protect some populations of native species (*A. astacus* and especially *Austropotamobius torrentium*) from the transmission of the plague by direct contact with its carriers. This is especially important, for example, for the Central Bohemian population of *A. torrentium*, which is present approximately 11 km upstream from the mouth of a stream directly joining an *Orconectes*-inhabited section of the river Elbe. A similar situation has also been recorded in Hungary in the streams of the Danube Bend region (PUKY and SCHÁD, 2006).

The limited ability of *O. limosus* to penetrate into small streams can be documented by the fact that in spite of an intensive monitoring effort, records of this species from streams up to the 5th stream order are usually limited to less than three kilometres from their confluence with a larger river (Figure 2, Table II). All exceptions from this pattern can be associated with a potential source population in a close-by standing water connected to the watercourse, or are known to be originally stocked by humans (indicated in Table II and Figure 2),

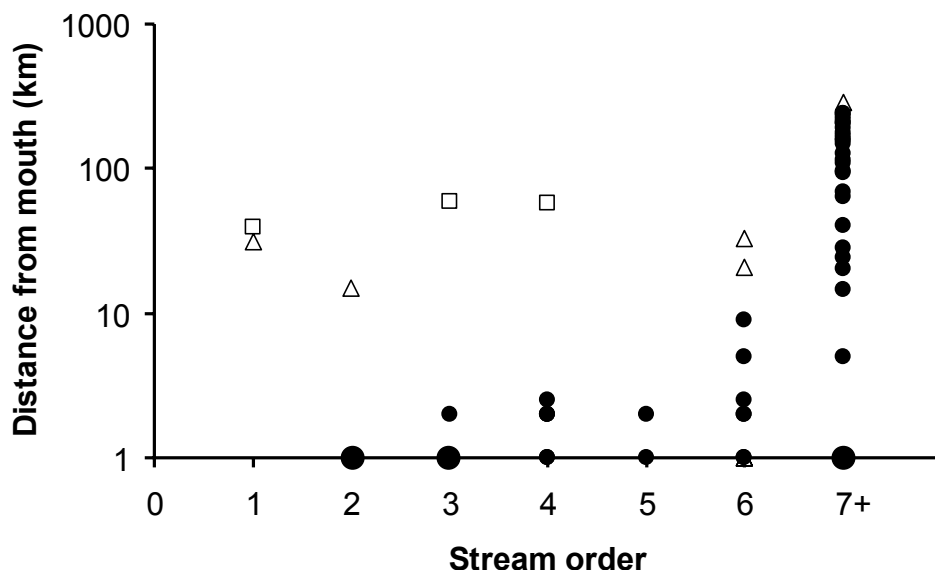


Figure 2

The relationship between the stream order and the distance of recent Czech *Orconectes* populations from the stream mouth (or nearest larger watercourse with presumed or confirmed presence of the species); the distance from the German border is used for the river Elbe. Large dots represent four or more sites. Populations originating with certainty from human stocking are marked by triangles, those spreading from adjacent standing waters by squares.

Figure 2

Relation entre l'ordre du cours d'eau et la distance des populations récentes d'*Orconectes* de l'embouchure du cours d'eau (ou du grand cours d'eau le plus proche avec présence supposée ou confirmée de l'espèce); la distance à la frontière allemande est utilisée pour l'Elbe. Les gros points représentent 4 sites ou plus. Les populations introduites avec certitude par l'homme sont indiquées par des triangles; celles qui se sont propagées à partir de plans d'eaux adjacents sont indiquées par des carrés.

Table I

Data on the presence of *Orconectes limosus* in the Czech streams and water bodies up to 2000. The river kilometres are given from the mouth, in case of the Elbe (Labe) from the border with Germany.

Parentheses after the name of a watercourse give the name of the river which it joins (marked by an arrow), and the river kilometre where the confluence is located. The sources are abbreviated as follows: H89, H90, H94 – HAJER, 1989, 1990, 1994; B95, B03 – BERAN, 1995, 2003; M95 – MATĚNA, 1995; AOPK – archive of the Agency for Nature Conservation and Landscape Protection, ZD – unpublished data of Z. Ďuriš.

Tableau I

Données sur la présence d'*Orconectes limosus* dans les rivières et les plans d'eau tchèques jusqu'en 2000. Les « kilomètres-rivière » sont indiqués depuis l'embouchure, et dans le cas de l'Elbe depuis la frontière avec l'Allemagne.

Entre parenthèses après le nom des cours d'eau sont indiqués les noms des cours d'eau dans lesquels ils se jettent (repérés par une flèche), et le kilomètre rivière de la

confluence. Les références bibliographiques sont abrégées comme suit : H89, H90, H94 – HAJER, 1989, 1990, 1994 ; B95, B03 – BERAN, 1995, 2003; B06 – BERAN, sous presse; M95 – MATĚNA, 1995; AOPK – archive de l'agence pour la conservation de la nature et la protection des Paysages, ZD – données non publiées de Z. Ďuriš.

Table II

Localities where the presence of *Orconectes limosus* in the Czech Republic was recorded or reconfirmed between 2000 and 2005. River kilometres are given as in Table 1, the value in parentheses gives the effective distance to the nearest watercourse with presumed or confirmed presence of *O. limosus*.

The column "source" refers either to initials of one of the authors (AP, LF, ZD, PK, TP, MS), literature data (V03 – VRZAL, 2003; B03 – BERAN, 2003), data of the Czech Union for Nature Conservation from Nymburk (CSOP), mapping programme of the Agency for Nature Conservation and Landscape Protection (AOPK), or to reliable personal communications by those listed. An asterisk in the "distance from mouth" column indicates a population with known origin by human stocking, "+" followed by a number in superscript after the locality name indicates potential source populations in standing waters and the corresponding records of *Orconectes* in running waters.

Tableau II

Sites où la présence d'*Orconectes limosus* en République tchèque a été notée ou confirmée entre 2000 et 2005. Les « kilomètres-rivière » sont indiqués comme dans le

tableau I, les valeurs entre parenthèses donnent la distance effective au cours d'eau le plus près où la présence d'*O. limosus* est supposée ou confirmée.

Les données de la colonne "source" se réfèrent soit aux initiales abrégées de l'un des auteurs (AP, LF, ZD, PK, TP, MS), à des données de la littérature (V03 – VRZAL, 2003; B03 – BERAN, 2003; B06 – BERAN, *in press*), aux travaux non publiés de Luboš Beran (LB), aux données de l'Union tchèque pour la Conservation de la Nature de Nymburk (CSOP), aux données du programme de cartographie de l'Agence pour la Conservation de la Nature et de la Protection des Paysages (AOPK), ou à des communications personnelles fiables des auteurs listés. Un astérisque dans la colonne « distance à l'embouchure » indiquent que la population a été avec certitude introduite par l'homme, « + » suivi par un nombre en exposé après le nom du site signale les populations potentielles d'origine dans les eaux calmes, et les populations correspondantes d'*Orconectes* dans les eaux courantes.

Tableau I

Watercourse / body	Nearest settlement	Latitude (N)	Longitude (E)	Date of observation	Stream order	Distance from mouth (km)	Source
Labe (Elbe): between Ústí nad Labem and Pardubice canalised (slow flow or standing), distances calculated from the border with Germany							
Labe	Hřensko	50°52'	14°14'	pre-1995	8	1	M95
Labe	Děčín	50°46'	14°13'	1991-2	8	15	H94
Labe	Ústí nad Labem	50°39'	14°03'	1988-92	8	38	H89, H94
Labe	Brná nad Labem	50°37'	14°05'	7.-9.05.88	8	43	H89, H90
Labe	Litoměřice	50°32'	14°08'	1991-2	8	65	H94
Labe	Roudnice	50°26'	14°16'	1991-2	8	82	H94
Labe	Račice	50°30'	14°22'	1991-2	8	93	H94
Labe	Mělník	50°22'	14°28'	1991-2	8	109	H94
Labe (adjacent pools)	Křivenice	50°25'	14°25'	05.06.98	8	100	B03
Labe	Neratovice	50°16'	14°32'	1991-2	7	122	H94
Labe	Čelákovice	50°10'	14°45'	pre-1995	7	146	M95
Labe	Nymburk	50°11'	15°03'	1991-2	7	168	H94
Labe	Poděbrady	50°08'	15°07'	1991-2	7	176	H94
Labe	Pardubice	50°03'	15°46'	1991-2	7	240	H94
Labe	Hradec Králové	50°12'	15°49'	1991-2	7	268	H94
tributaries in the watershed of Elbe: running waters							
Ploučnice (→ Elbe, km 14)	Benešov nad Ploučnicí	50°44'	14°19'	24.05.91	6	11	H94
Dolský potok (→ Fojtovický p., km 0.5; → Ploučnice, km 12; → Elbe, km 14)	Heřmanov	50°44'	14°18'	1991	2	< 1?	H94
Luční potok (→ Elbe, km 28)	Malé Březno	50°40'	14°10'	1991-2	4	< 1	H94
Homolský potok (→ Elbe, km 30)	Velké Březno	50°40'	14°08'	1991-2	3	< 1	H94
Bílina (→ Elbe, km 39)	Bílina,	50°33'	14°46'	1990?	6	< 5	H90
Bílina	Bílina, confluence with Syčivka	50°33'	14°46'	29.05.91	6	36	H94
Průčelský potok (→ Elbe, km 43)	Brná nad Labem	50°37'	14°05'	May 1988	2	< 1	H90
Tlučenský potok (→ Elbe, km 47)	Sebuzín	50°36'	14°05'	1991-2	2	< 1	H94
Ohře (→ Elbe, km 65)	Litoměřice	50°32'	14°08'	1991-2	6	< 1	H94
Ohře	Terezín	50°31'	14°09'	14.07.97	6	3	B03
Ohře	Bohušovice nad Ohří	50°30'	14°09'	1992, 1997	6	5	H94, B03
Ohře and its dead arm	Doksany	50°27'	14°10'	2000	6	9	B03
Ohře	Budyně nad Ohří	50°25'	14°07'	05.06.91	6	18	H94
Ohře	Louny	50°22'	13°48'	11.05.92	6	54	H94
Ohře	Žatec	50°20'	13°33'	1991-2	6	90	H94
Pšovka (→ Elbe, km 107)	Střemy	50°23'	14°33'	1994	2	15	B95
Jizera (→ Elbe, km 141)	Benátky nad Jizerou	50°17'	14°50'	20.10.91	6	20	H94
Orlice (→ Elbe, km 268)	Hradec Králové	50°12'	15°49'	02.11.92	6	< 1	H94
river Vltava (→ Elbe, 109 km): conditions vary from running to standing water (headwaters of weirs, reservoirs)							
Vltava	Mělník	50°21'	14°29'	1991, 1999	8	< 1	H94, B03
Vltava	Vepřek	50°18'	14°20'	20.06.98	8	14	B03
Vltava	Kralupy	50°15'	14°19'	11.05.91	8	22	H94
Vltava	Praha (Prague)	50°07'	14°28'	1991-2, 1998-9, 2000	8	47	H94, P00, B05
Vltava (reservoir Orlík)	<i>not specified</i>	~49.5°	~14°	1994, 1998-9, 2000	8	145 - 180	M95, P00, H00
Vltava (reservoir Kořensko)	<i>not specified</i>	~49.2°	~14.4°	1994, 1998-9	7-8	200 - 209	M95, P00
Vltava (reservoir Hněvkovice)	<i>not specified</i>	~49°	~14.5°	1994	7	210 - 225	M95
Vltava	České Budějovice	49°58'	14°28'	26.10.92	7	240	H94
tributaries in the watershed of Vltava: mostly running waters							
Berounka (→ Vltava, km 63)	Beroun	49°58'	14°05'	14.10.92	7	35	H94
Berounka	Plzeň	49°45'	13°23'	27.10.91	7	139	H94
Úslava (→ Berounka, km 136)	Plzeň	49°45'	13°24'	27.10.91	5	< 5	H94
Mže (→ Berounka, km 139)	Plzeň	49°45'	13°22'	27.10.91	6	< 5	H94
Mže (reservoir Hracholusky)	Plešnice	49°47'	13°09'	1999	reservoir	24	AOPK
Radbuza (→ Berounka, km 139)	Plzeň	49°43'	13°23'	27.10.91	6	< 5	H94
Úhlava (→ Radbuza, km 5)	Plzeň	49°45'	13°23'	27.10.91	6	< 5	H94
Sázava (→ Vltava, km 78)	Havlíčkův Brod	49°36'	15°35'	15.10.92	6	162	H94
Otava (→ Vltava, km 169)	Písek	49°18'	14°08'	26.10.91	7	26	H94
Lužnice (→ Vltava, km 202)	Bechyně	49°17'	14°28'	1992	7	11	M95
Lužnice	Tábor	49°25'	14°40'	02.09.92	7	40	H94
Malše (→ Vltava, km 240)	České Budějovice	49°58'	14°28'	16.07.92	6	< 1	H94
other standing waters							
sandpit Lhota	Lhota	50°15'	14°40'	12.07.97			B05
Probošský rybník + other sandpits	Stará Boleslav	50°12'	14°39'	01.05.95			B05
sandpit Ovcáry	Ovcáry	50°15'	14°37'	23.04.95			ZD
Klíčov (quarry "Na skále")	Mrázov	49°24'	12°58'	1998			ZD
Máchovo jezero	Doksy	50°35'	14°39'	1991-2	fishpond		H94
unspecified fishponds	Česká Lípa (region)	~50.5°	~14.5°	1991-2	fishponds		H94

Tableau II

Watercourse / body	Nearest settlement	Latitude (N)	Longitude (E)	Date of observation	Stream order	Distance from mouth (km)	Source
Labe (Elbe): between Ústí nad Labem and Pardubice canalised (slow flow or standing), distances calculated from the border with Germany							
Labe	Hřensko	50°52'	14°14'	2001-4	8	1	J. Špaček
Labe	Děčín	50°47'	14°12'	08.09.05	8	15	LF
Labe	Nebočady	50°43'	14°11'	02.07.03	8	20	ZD
Labe	Těchlovice	50°42'	14°12'	02.07.03	8	24	ZD
Labe	Malé Březno	50°40'	14°10'	16.07.03	8	28	ZD
Labe	Ústí nad Labem (Střekov)	50°39'	14°03'	02.07.03	8	40	ZD
Labe	Litoměřice	50°32'	14°09'	2002-3	8	64	B05, ZD
Labe (confluence with Luční potok)	Třeboutice	50°31'	14°12'	03.07.03	8	69	ZD
Labe	Štětí	50°27'	14°22'	03.07.03	8	94	ZD
Labe	Hněvice	50°27'	14°22'	01.10.04	8	95	ZD
Labe	Mělník	50°22'	14°28'	13.09.04	7	108	MS
Labe	Obříství	50°18'	14°29'	2001-5	7	115	ZD, PK, LF
Labe	Kostelec nad Labem (Jiřice)	50°14'	14°34'	22.05.04	7	126	ZD
Labe	Čelákovice	50°10'	14°45'	2001-4	7	146	AP
Labe (confluence with Farský potok)	Ostrá	50°10'	14°54'	22.05.04	7	155	ZD
Labe	Hradištko	50°10'	14°56'	2001, 2005	7	159	ZD, LF
Labe	Nymburk	50°11'	15°03'	2002	7	168	CSOP
Labe	Poděbrady	50°09'	15°06'	09.10.03	7	176	ZD
Labe	Kolín	50°02'	15°13'	04.07.03	7	193	ZD
Labe	Týnec nad Labem	50°02'	15°22'	20.04.02	7	205	B05
Labe	Valy	50°02'	15°37'	06.09.05	7	227	LF
Labe	Pardubice (Polabiny)	50°03'	15°46'	04.07.03	7	239	ZD
Labe	Jaroměř	50°03'	15°46'	2004	7	287 *	J. Špaček
tributaries of the river Elbe: running waters							
Kamenička (→ Elbe, km 10)	Boletice nad Labem	50°45'	14°11'	17.09.05	3	< 1	AOPK
Poustka (→ Elbe, km 18)	Dobkovice	50°43'	14°12'	26.09.04	3	< 1	AOPK
Lužecký potok (→ Elbe, km 28)	Povrly	50°40'	14°10'	26.09.04	3	2	AOPK
Luční potok (→ Elbe, km 28)	Malé Březno	50°40'	14°10'	17.09.05	4	< 1	AOPK
Modla (→ Elbe, km 63)	Lovosice	50°30'	14°04'	03.10.04	4	2	AOPK
Ohře (→ Elbe, km 65)	Bohušovice nad Ohří	50°30'	14°09'	2004, 2005	6	5	ZD, AOPK
Ohře	Doksany	50°27'	14°09'	2003, 2005	6	9	ZD, AOPK
Stará Ohře (→ Elbe, km 67) (side channel of Ohře)	České Kopisty	50°31'	14°10'	21.09.04	N/A	1	AOPK
Pšovka (→ Elbe, km 107)	Mělník	50°21'	14°30'	2002	3	< 1	V03
Pšovka [†]	Střemy	50°23'	14°33'	1995-2005	2	14-15	V03, AP
Pšovka: fishpond Velký rybník [†]	Střemy	50°23'	14°33'	1995-2005	fishpond	15 *	ZD, AP
Jizera (→ Elbe, km 141)	Nový Vestec	50°11'	14°44'	22.05.04	6	1	ZD
Vlkava (→ Elbe, km 159)	Kostomlaty nad Labem	50°10'	14°56'	22.09.05	4	< 2	AOPK
Výrovka (→ Elbe, km 163)	Písty	50°10'	15°00'	2002	5	< 2	CSOP
Liduška (→ Elbe, km 166)	Nymburk	50°10'	15°02'	22.09.05	2	< 1	AOPK
Mřína (→ Elbe, km 168)	Nymburk	50°11'	15°04'	2002	6	< 2	CSOP
Cidlina (→ Elbe, km 180)	Libice nad Cidlinou	50°07'	15°11'	20.05.04	6	2,5	ZD
Bačovka (→ Elbe, km 182) and surrounding dead arms	Velký Osek	50°06'	15°11'	2002-4	4	2,5	CSOP, AOPK
Doubrava (→ Elbe, km 203)	Záboří nad Labem	50°01'	15°21'	20.05.04	6	1	ZD
Metuje (→ Elbe, km 287)	Jaroměř	50°20'	15°55'	22.05.05	6	< 1 *	AOPK
Úpa (→ Elbe, km 289)	Jaroměř	50°21'	15°56'	04.09.04	6	< 1 *	AOPK
Úpa	Slatina nad Úpou	50°27'	16°02'	04.09.04	6	21 *	AOPK
Úpa	Úpice	50°30'	16°01'	2004	6	33 *	J. Špaček
river Vltava (→ Elbe, km 109): most localities located in reservoirs (standing water)							
Vltava	Vrbno u Mělníka	50°19'	14°27'	01.10.04	8	5	ZD
Vltava	Klečany	50°10'	14°24'	2001	8	37	ZD
Vltava	Praha - Suchdol	50°08'	14°24'	26.08.05	8	41	LF
Vltava (reservoir Orlík, confluence with Otava)	Zvíkovské Podhradí	49°26'	14°12'	11.07.01	8	169	PK
Vltava (reservoir Orlík)	Vůsí	49°24'	14°15'	2001-4	8	177	PK
Vltava (reservoir Orlík)	Temešvár	49°21'	14°16'	10.07.01	8	182	PK
Vltava (reservoir Orlík)	Strouhy	49°20'	14°17'	15.07.03	8	184	PK
Vltava (reservoir Orlík)	Jehnědno	49°18'	14°20'	15.07.03	8	191	PK
Vltava (reservoir Kořensko, confluence with Lužnice)	Neznašov	49°14'	14°23'	2001-4	8	202	PK, B05
Vltava	Týn nad Vltavou	49°13'	14°25'	06.05.02	7	205	B05
Vltava (reservoir Hněvkovice)	Purkarec	49°08'	14°27'	2001	7	217	PK
Vltava	Hluboká nad Vltavou	49°03'	14°27'	30.08.04	7	228	AOPK
Vltava (confluence with Malše)	České Budějovice	48°58'	14°28'	12.09.05	6-7	240-241	AOPK

Tableau II (continued)

Watercourse / body	Nearest settlement	Latitude (N)	Longitude (E)	Date of observation	Stream order	Distance from mouth (km)	Source
tributaries in the watershed of Vltava: running waters							
Sázava	Davle	49°53'	14°24'	30.08.05	7	< 1	AOPK
Sázava	Luka pod Medníkem	49°52'	14°27'	30.08.05	7	5	AOPK
Janovický potok (→ Sázava, km 19; → Vltava, km 78)	Krusičany	49°48'	14°36'	2004	5	< 1	V. Horálek
Vlkančický potok (→ Sázava, km 49; → Vltava, km 78)	Pyskočely	49°53'	14°53'	2004	4	2	V. Horálek
Otava (→ Vltava, km 169)	Zvíkovské Podhradí	49°26'	14°12'	11.07.01	7	< 1	PK
Jickovický potok (→ Vltava, km 171)	Jickovice	49°27'	14°13'	15.09.04	2	< 1	PK
Hřejkovický potok (→ Vltava, km 177)	Vůsí	49°24'	14°16'	2001-5	4	< 1	PK
Velký potok (→ Vltava, km 184)	Strouhy	49°21'	14°18'	2001-5	2	< 1	PK
Novosedlský potok (→ Vltava, km 184)	Strouhy	49°21'	14°18'	05.09.04	2	< 1	TP
Chřešřovický potok (→ Vltava, km 185)	Chřešřovice	49°20'	14°18'	05.09.04	2	< 1	TP
Lužnice (→ Vltava, km 202)	Neznašov	49°14'	14°24'	06.05.02	7	1	B05
Bílinský potok (→ Lužnice km 5; → Vltava, km 202)	Vesce	49°16'	14°26'	14.10.04	3	< 1	AP
Maše (→ Vltava, km 240)	České Budějovice	48°58'	14°29'	12.09.05	6	< 2	PK, AOPK
running waters not directly connected to main areas of <i>Orconectes</i> distribution							
Loupnice ⁺² (→ Bílina, km 56; → Elbe, km 39)	Záluží (below the pond)	50°33'	13°35'	23.10.04	4	< 1 (57)	AOPK
Loupnice ⁺²	Horní Jiřetín (below the pond Vítěz)	50°34'	13°34'	22.10.04	3	3 (59)	AOPK
outflow from the quarry Barbora ⁺³	Oldřichov u Teplic	50°38'	13°45'	16.07.03	1	< 1 (upstream) 39 (downstream)	ZD
artificial channel connecting water bodies Kateřina and Modlany ⁺⁴	Modlany	50°39'	13°53'	25.09.05	N/A	< 1	AOPK
Drnecký potok (→ Šternberský p., km 4; → Červený, km 14; → Bakovský, km 10; → Vltava, km 14)	Drnek	50°12'	13°59'	21.08.05	1	4 (31) *	AOPK
Zlatá stoka ⁺⁵ (artificial channel connecting fishponds)	Třeboň	49°00'	14°46'	02.07.01	N/A	N/A	PK
other standing waters					character of the locality		
Klíčov (quarry "Na skále")	Mrákov	49°24'	12°58'	2002-4	flooded quarry		PK
Kojetice	Kojetice u Neratovic	50°14'	14°30'	2004-5	flooded quarry		AP, LF
Černice ⁺²	Černice	50°34'	13°32'	2005	undermined depression		M. Holzer
Barbora ⁺³	Oldřichov u Teplic	50°38'	13°45'	2003-5	flooded surface coalmine		ZD, LF
Kateřina ⁺⁴	Soběchleby	50°40'	13°54'	25.09.05	mining water reservoir		AOPK
Modlany ⁺⁴	Modlany	50°39'	13°53'	30.09.05	mining water reservoir		AOPK
sandpit Lhota	Lhota	50°15'	14°40'	2004-5	sandpit		ZD, AP, LF
sandpit Cítov - Baraba	Mělník	50°22'	14°27'	2004-5	sandpit		ZD, LF
sandpit Ovčáry	Ovčáry	50°15'	14°37'	27.10.04	sandpit		ZD
Račice "channel" (rowing stadium)	Račice	50°27'	14°21'	2004, 2005	sandpit		ZD, AOPK
Stará pískovna	Provoďín	50°37'	14°35'	13.09.04	sandpit		MS
sandpit near the airport Borek	Stará Boleslav	50°12'	14°40'	21.08.04	sandpit		ZD
Proboštský rybník + other sandpits	Stará Boleslav	50°12'	14°39'	2004-5	sandpits		ZD, LF
sandpit Ostrá	Ostrá	50°11'	14°54'	2001-2	sandpit connected to the Elbe		B05, CSOP
sandpit Pišťany	Pišťany	50°32'	14°04'	14.09.05	sandpit connected to the Elbe		AOPK
village pond	Smečno	50°11'	14°02'	July 2005	small pond		AOPK
fishpond Koclířov ⁺⁵	Lomnice nad Lužnicí	49°04'	14°41'	October 2005	fishpond		LF
fishpond by Říčanský potok	Praha - Dubeč	50°03'	14°35'	2004	fishpond		AOPK

The presence of the spiny-cheek crayfish close to the mouth of most small streams, however, does not necessarily indicate a viable population, as the source population may be in the adjacent larger river or reservoir, and crayfish may penetrate to the small watercourse only temporarily. This is apparent from the fact that *Orconectes* has not been found in a number of small tributaries of the Elbe where historical records exist, but could be found in similar streams in close vicinity (Tab. I, II).

There are more discrepancies between older published distribution data of *O. limosus* and our present results. A study on the distribution of the species in

Czechia in the 1990s (HAJER, 1994) reported the occurrence of this crayfish in middle or upper reaches of various rivers where it has not been confirmed after 2000 (most open circles in Figure 1). Moreover, *Astacus astacus* currently lives in one location (the river Ploučnice in the town Benešov nad Ploučnicí; 50°44' N, 14°19' E) where *Orconectes limosus* had been reported in the 1990s. Similarly, the noble crayfish was found downstream of alleged *O. limosus* localities in the river Ohře. HAJER (1994) reported the spiny-cheek crayfish in the river Ohře from the town Litoměřice (at the confluence with the Elbe) to Žatec (88 km upstream from the Elbe). However, *A. astacus* was more recently found in Počedělice (44 km from the Elbe; ĎURIŠ, unpublished), and the presence of *Orconectes* was confirmed only downstream, in the village Doksany (9 km from the Elbe).

It is not clear whether the discrepancy of recent and older data has been caused by misidentification in the past, the downstream retreat of *Orconectes*, fragmentation of its populations during the last few years, or a reduction of *Orconectes* population density which subsequently prevented its detection. The combination of all these factors could play a role. For example, after extensive floods in 2002, the *Orconectes* density dropped considerably in the Elbe (ĎURIŠ *et al.*, 2006; ŠPAČEK, pers. comm.) as well as in adjacent flooded sandpits and dead arms, and the floods may have similarly affected the crayfish populations in large rivers elsewhere.

Conclusions

Orconectes limosus is the most widespread crayfish of non-European origin in the Czech Republic. It has occupied a large part of the Elbe watershed and due to human-mediated translocations, it can also be found in a number of isolated water bodies and some small streams. However, the monitoring of its distribution revealed that the species usually stays only in the lower reaches of smaller streams or rivers and that there are significant differences between the recent distribution and the historical data on its occurrence.

The species is able to carry the crayfish plague pathogen and transmit it to native species. Therefore, it is important to monitor its invasion in the Czech Republic also in the future.

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